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Sterility and Stability of Diluted Carprofen in a Multidose Vial in the Laboratory Animal Setting

Gregory D Simonek,[1,*] Gwendolyne G Alarcio,[2] and Laurie L Brignolo[3]

Using compounded multidose vials (cMDV) is a common practice in the laboratory animal setting, where medications often are diluted to provide appropriate doses to rodents. However, bacterial contamination of MDV has been well established in both the human and veterinary medical literature. For this study, we created 14 cMDV by diluting carprofen into sterile water (dilution, 1:10) and stored 6 cMDV each at 5 and 24 °C. The stoppers of the cMDV were not cleaned with alcohol, and all were punctured twice daily for 28 d. The sterility of the diluted carprofen was evaluated by assessing bacterial growth on days 0, 7, 14, 21, and 28 and by testing for bacterial endotoxin on days 0 and 28. We used liquid chromatography–tandem mass spectrometry to assess the stability of 2 cMDV, with each cMDV being divided into the 2 storage-temperature subsets for days 0, 7, 14, 21, and 28. Neither bacterial contamination nor endotoxin was detected, and drug stability was stable over the 28 d. We suggest that with pragmatic techniques, such as secondary containment and consistent use of new needles, the contents of cMDV can remain sterile and stable for 28 d.

Abbreviation: cMDV, compounded multidose vial

Using compounded multidose vials (cMDV) is a common and widespread practice for drug combination or dilution in the laboratory animal setting. Common commercially available medications are generally formulated for use in much larger animals than laboratory species and therefore require dilution to provide appropriate drug concentrations and volumes for small animals, such as rodents. In addition, cMDV often are created when multiple rodents need to be treated, such as during perioperative care.

Iatrogenic contamination and thus the safety of MDV has been studied in human medicine. Nosocomial pathogens have been demonstrated to proliferate in MDV and are a source of infections in human patients.[1,2,5,9,11] Potential risk factors for contamination include number of withdrawals performed, poor aseptic technique, injection of ambient air into the vial, compromised rubber stoppers, and lack of bacteriostatic preservatives.[16,20]

Reports regarding the use and contamination of MDV are sparse and controversial in veterinary medicine and even more so in the laboratory animal setting. In one study, 18% of multiple-dose saline bottles and medications at a veterinary teaching hospital showed bacterial contamination.[18] Because bags containing saline, sterile water, or Lactated Ringer solution are often used for diluting medications, they can be considered to be MDV and can be chronically maintained and remain sterile for 30 d.[14]

In human medicine, standard practice is to use a new MDV for each patient and to discard any remaining drug. In laboratory animal medicine, the volumes used in rodents are small, so that sharing an MDV among multiple animals is more pragmatic and cost-effective. However, in these circumstances, the length of time that an MDV can be considered safe and stable is unknown. Guidelines include visually inspecting the vial for cloudiness and particulates.[21] Many institutions have developed policies outlining accepted practices for how long an MDV can be used that are based on few scientific data. The stability of compounded medications, especially oral formulations, has been evaluated, albeit infrequently, in both human and veterinary medicine,[7,15] and that of compounded injectable medications has been reviewed even less often. Ketamine–acepromazine–xylaxine, a common anesthetic combination in the laboratory setting, reportedly remains safe and stable for 180 d, but neither the use of this drug combination over time nor the number of withdrawals from the vial were evaluated.[19]

The objective of the current study was to determine how long the contents of a cMDV remain sterile and stable when pragmatic clinical practices and techniques are used. We hypothesized that, under these conditions, a cMDV that is used daily can remain sterile and stable for as long as 28 d.

Materials and Methods

We created 14 cMDV by combining carprofen (Zoetis, Florham Park, NJ) from new unopened bottles with sterile water (dilution, 1:10) in additive-free serum tubes. We placed the cMDV in secondary containers (Ziplock, Racine, WI). The cMDV were split into 2 storage groups of 6 cMDV each; one group was refrigerated at 5 °C, and the other group stored at room temperature (24 °C). Each cMDV was removed from secondary containment and punctured with a sterile 23-g needle to withdraw 0.2 or 0.5 mL twice daily for 28 d; the rubber stopper of the cMDV was not swabbed with alcohol prior to sampling, and the syringe contents were discarded, except on days of culture. On days 0, 7, 14, 21, and 28 d, 0.5 mL of drug was withdrawn and inoculated into 5.0 mL of tryptic soy broth, which was then incubated at 37 °C for a maximum of 3 d. Every 24 h, the broth was visually inspected for turbidity; when turbidity was present, the broth was inoculated onto blood agar plates. For consistency, the same person performed all assessments, handling, and sampling.

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[1]School of Veterinary Medicine, [2]California Animal Health and Food Safety Laboratory System, and [3]Teaching and Research Animal Care Services, University of California, Davis, California.
[*]Corresponding author. Email: gdsimonek@ucdavis.edu

In addition, 6 cMDV (3 from each storage group) were tested for endotoxin on days 0 and 28 (Pyrosate kit, Associates of Cape Cod, East Falmouth, MA) according to manufacturer's instructions. For this assay, 0.5 mL was withdrawn from the cMDV by using a sterile needle, transferred to a sample tube, and gently mixed until the contents of the tube were dissolved. By using a new sterile needle, 0.25 mL of sample was transferred to the positive product control tube and gently mixed until dissolved. The tubes were incubated at 37 °C for 29 min. A clot in the product control tube indicated the presence of endotoxin at a concentration of 0.25 U/mL or greater.

To determine the stability of the carprofen concentration in the cMDV over the course of the study, 2 cMDV were submitted to the toxicology laboratory at the California Animal Health and Food Safety Laboratory System (CAHFS, Davis, CA) for analysis. The concentration of carprofen solution was measured by liquid chromatography–tandem mass spectrometry on days 0, 7, 14, 21, and 28. To analyze carprofen in the linear range of detection of the mass spectrometer, the sample was diluted 5000-fold in methanol:water (1:1, v/v). On day 0, the diluted sample was analyzed in duplicate against a 6-point calibration curve (range, 0.025 µg/mL to 2 µg/mL) of carprofen standard (Sigma, St Louis, MO). After this initial analysis, the contents of each of the 2 original cMDV were divided, with one from each vial stored in a refrigerator at a temperature range of 2 to 5 °C, and the other stored at room temperature (24 °C) on the laboratory benchtop. On days 7, 14, 21, and 28, each sample was diluted as described earlier after the addition of 0.1 mL of 10 µg/mL carprofen-d3 (Toronto Research Chemicals, Ontario, Canada) in methanol:water (1:1,v/v) as an isotopically labeled internal standard. Samples were analyzed in triplicate against calibration curves and reagent blanks fortified with 0.1 mL of 10 µg/mL carprofen-d3 in methanol:water (1:1, v/v). Standard deviations between replicates were less than 20%. The ratio of chromatographic peak area units of carprofen:carprofen-d3 was plotted against concentration and best-fit linear equations (R^2 values greater than 0.99) were used to calculate the sample concentrations.

Chromatography was achieved with HPLC (Michrom BioResources, Auburn, CA). Briefly, an analytical Luna C18 column (20 mm × 2 mm × 3 µm particle size; Phenomenex, Torrance, CA) was used with mobile phases consisting of (A) 0.01 M ammonium acetate in 0.1% formic acid in water and (B) 0.01 M ammonium acetate in 0.1% formic acid in methanol at a flow rate of 0.2 mL/min under a linear gradient of 50% B to 95% B over 7 min.

The HPLC system was coupled with a hybrid triple-quadrupole linear ion-trap mass spectrometer (model 4000 QTrap, AB Sciex, Concord, Ontario, Canada) equipped with a heated electrospray ionization probe. The mass spectrometer was operated in negative mode by using the enhanced product ion scan function. The transitions of ions m/z 272 $[M-H]^-$ to m/z 226 for carprofen and m/z 277 $[M-H]^-$ to m/z 233 for carprofen-d3 were monitored and used for quantitation with the following MS parameters: declustering potential, –25; collision energy, –44; spray source temperature, 600 °C; and ion spray voltage, –4500 V. Analyst version 1.5 software was used for data analysis.[10]

The limit of quantitation for this assay was calculated as the lowest carprofen concentration (0.025 µg/mL) multiplied by the dilution factor (5000) of the sample, which is 125 µg/mL (or 0.125 mg/mL).

Linear regression was used to analyze the data within the storage groups. Two-way ANOVA was used to compare the data between the 2 storage groups. Both analyses were performed by using XLSTAT software (Addinsoft, New York, NY). A P value less than 0.05 was considered significant.

Results

Bacterial contamination was not identified in any of the cMDV at the 2 storage conditions at any time point. Endotoxin assays were negative for all of the cMDV tested on days 0 and 28.

The concentration of carprofen in the cMDV remained stable over the course of the study at both the refrigerated and room-temperature storage conditions for both cMDV. The initial concentration for cMDV 1 was 3.2 mg/mL. The concentration of the solutions on day 28 was 3.4 mg/mL for the refrigerated sample and 3.2 mg/mL for the solution stored at room temperature (Table 1). There was no significant difference between the initial and final concentrations for both storage conditions (P = 0.49 for 24 °C and P = 0.62 for 5 °C) or between the 2 different storage conditions (P = 0.45).

The initial concentrations of the samples from cMDV 2 were 5.2 and 5.3 mg/mL (5 and 24 °C, respectively). The concentrations on day 28 were 4.5 mg/mL for the solution stored refrigerated and 5.1 mg/mL for the one at room temperature. There was no significant difference between the initial and final concentrations for both storage conditions (P = 0.62 for 5 °C and P = 0.75 for 24 °C) or between the 2 storage groups (P = 0.14). No statistical significance is found by using 2-way ANOVA (P = 0.9).

Discussion

This study determined that over 28 d, solutions of diluted carprofen in cMDV remained sterile by using drug withdrawal techniques commonly used in laboratory animal settings. In addition, the concentrations of diluted carprofen solutions stored at room temperature or refrigerated remained equivalent over the 28-d period.

Bacterial contamination was not identified in any of the cMDV. Cleaning the stoppers of MDV with alcohol is recommended prior to withdrawing the medication. However, in this study, the rubber stoppers were not cleaned with alcohol or other disinfectants prior to puncturing and sampling, to simulate practices commonly used in laboratory animal settings. However, we did place the cMDV in secondary containment, thus limiting the amount of time that the rubber stoppers of the cMDV were exposed to the environment and reducing the risk of environmental contamination. In addition, we used a new sterile needle at each sampling. Using needles multiple times dulls them and creates irregular burrs that can compromise the rubber stopper, whereas using a new needle each time helps maintain the integrity of the stopper and prevents bacterial contamination.

Because only viable bacteria can be isolated in culture, we tested for the presence of endotoxin to rule out any by products from nonviable bacteria. Endotoxin (bacterial LPS) is a molecule derived from the outer membrane of gram negative bacteria. Release of LPS into the bloodstream can induce a profound inflammatory response that results in multiple organ failure and even death.[13,22] For this reason, the FDA mandates that veterinary products and devices have a maximum of 0.5 endotoxin units/mL. We were unable to detect endotoxin in any of the samples in this study. This result combined with the negative culture findings confirms that neither viable nor nonviable bacteria products were a concern for the 28 d of the study.

The expected carprofen concentration of the cMDV was 5 mg/mL. However, liquid chromatography–mass spectrometry analysis revealed that the concentration in MDV1 was lower

Table 1. Concentration (mg/mL) of diluted carprofen over 28 d of storage

	cMDV 1		cMDV 2	
	5 °C	24 °C	5 °C	24 °C
0	3.2	3.2	5.2	5.3
7	2.9	3.7	4.8	4.6
14	3.2	3.0	5.8	5.6
21	3.0	3.2	5.1	4.9
28	3.4	3.2	4.5	5.1

Drug concentrations were determined by liquid chromatography–tandem mass spectrometry and are reported as the average of duplicate runs. Linear regression analysis of data within each storage group and *t*-tests of data between the 2 storage groups revealed no significant differences between MDV.

than expected and lower than that in MDV2. This result likely reflects a dilution error, but it has no effect on our analysis.

Carprofen, a NSAID, was selected because it is frequently used to provide analgesia to rodents.[6,8,12] NSAID are preferred over opioids, another commonly used class of analgesics, because NSAID have fewer effects on behavior physiologic parameters.[3,4,17] In addition, NSAID are not controlled substances, a feature that might otherwise limit their use in some facilities.

The results of our current study may guide IACUCs as they develop policies concerning the use of cMDV, particularly for those containing carprofen. A carprofen cMDV may be useful longer than was previously thought or permitted, thus increasing the cost-effectiveness of medications in rodent studies. The knowledge that a cMDV is free of contamination and that the carprofen concentration is stable for at least 28 d increases confidence that animal welfare needs are being met. In conclusion, our results show that despite multiple withdrawals from a cMDV, diluted carprofen can remain sterile and stable for 28 d when stored either at room temperature or refrigerated.

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