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Sterility and Stability of Diluted Meloxicam in Compounded Multi-dose Vial after 365 Days

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Meloxicam is a common analgesic for rodents. Because meloxicam is only formulated commercially for companion animals, it requires dilution to achieve doses appropriate for small, laboratory species. Compounded multidose vial (cMDV) are often created to dilute and store a diluted drug. However, chronic cMDV use runs the risk of contamination and becoming a potential source of nosocomial infection. In this study, we created 15 cMDV by diluting meloxicam with sterile water (dilution, 1:10). cMDV were punctured once daily for 30 d. To determine the sterility of the diluted meloxicam, we assessed 8 cMDV for bacterial growth on days 0, 10, 20, 30, and 365 and tested them for endotoxin on days 0, 30, and 365. In addition, the stability of the remaining 7 cMDV was assessed on days 0, 10, 20, 30, and 365, by using liquid chromatography–diode assays. No bacterial growth or endotoxin was detected at any time point, and the drug concentrations remained stable over 365 d. Given the results this study, we believe that cMDV of diluted meloxicam can remain sterile and stable for 365 d.

Abbreviations: cMDV, compounded multidose vial; COX, cyclooxygenase

Meloxicam is an NSAID that has been widely used in veterinary medicine. NSAID work by inhibiting the cyclooxygenase (COX) enzyme that is responsible for producing prostaglandins, thus resulting in inflammation. Inhibition of COX1 causes adverse effects, and an advantage of meloxicam is that it preferentially inhibits COX2, so adverse effects are limited.[6] Unlike COX2-selective inhibitors, COX2-preferential inhibitors like meloxicam may inhibit some degree of COX1, especially at higher doses.[15] Nonetheless, meloxicam has been used to treat osteoarthritic pain and to provide postoperative analgesia in rodents.[4,7,10]

However, meloxicam is labeled and formulated for companion animals. For use in smaller species, such as laboratory rodents, meloxicam must be diluted to achieve appropriate drug concentrations and practical administration volumes. In the current study, a 1:10 dilution of meloxicam was used, which provides an appropriate drug concentration and a practical volume for dosing an average, adult male C57BL/6 mouse.

Often compounded multidose vial (cMDV) are used to make and store diluted medications.[11,12] In the laboratory setting, MDV are used frequently, because they are an efficient and cost-effective way to create a diluted medication to be used repeatedly for multiple cohorts of animals. However, concerns regarding the chronic use of MDV include the risk of contamination and the instability of the compound over time.[2,9]

The shelf life of extemporaneously compounded injectable meloxicam is unknown, and according to the United States Pharmacopeia, an experimental sterility and stability study is the only valid method for estimating how long a compounded drug can be used.[14] Only limited scientific evidence is available to help guide institutions regarding when to discard cMDV. The studies that have evaluated the shelf life of drugs in cMDV used

in laboratory animal settings found that ketamine–acepromazine–xylazine cocktail and buprenorphine were sterile and stable for 180 d;[5,13] however, neither of these studies evaluated the number of withdrawals from the vials. A study assessing the sterility of fluid bags punctured at different frequencies revealed that the fluid bags that were punctured most the often were contaminated with *Acinetobacter lwoffi* and *Staphylococcus* spp., thus demonstrating the increased risk of bacterial contamination with usage.[9] Given the lack of studies determining the shelf life of chronically used cMDV, it is imperative to experimentally test the sterility and stability of meloxicam given its frequent use in laboratory animal medicine.

The objective of this current study was to determine the effects of daily use of cMDV on the sterility of diluted, injectable meloxicam for 30 d. We also analyzed the stability of diluted, injectable meloxicam that has been stored for 365 d. We hypothesized that the solution would remain stable and sterile for 30 d and that the solution will become unstable after a year.

Materials and Methods

We made 15 cMDV by combining injectable meloxicam (Ostilox, VetOne, Boise, ID) from a new bottle of sterile water with the target diluted concentration of 0.5 mg/mL (dilution, 1:10) in 30 mL amber vials. No gloves were worn and the stoppers of cMDV were not swabbed with alcohol, to simulate common drug-withdrawal techniques that are used in laboratory animal settings. The cMDV were stored in the dark at room temperature. Eight cMDV were tested for sterility, and the remaining 7 cMDV were tested for stability.

Once daily for 30 d, 8 cMDV were punctured with a sterile 23-g needle to withdraw 0.1 mL. On days 0, 10, 20, and 30, all 8 cMDV were tested for bacterial growth and for the presence of endotoxins: 0.5 mL of the drug was withdrawn by using a sterile needle and then inoculated into 3.0 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

occurred, the broth was inoculated onto blood agar plates. On days 0 and 30, all 8 cMDV were tested for the presence of endotoxins: by using a sterile needle, 0.5 mL of each solution was transferred to a sample vial (Pyrosate kit, Associates of Cape Cod, East Falmouth, MA) and mixed for 20 s. Sample vials and a positive control vial were made according to the manufacturer's instruction. A clot in a sample vial or the positive product control that withstood an inversion indicated that the solution contained an endotoxin concentration of 0.125 U/mL or greater.[1] On day 365, 4 of the 8 cMDV were randomly chosen to be further analyzed for bacterial growth and endotoxin.

The remaining 7 cMDV were submitted to the toxicology laboratory at the California Animal Health and Food Safety Laboratory System (Davis, CA) for analysis to determine the stability of the meloxicam concentration throughout the study. The concentration of meloxicam solution was measured by liquid chromatography–diode array detector. A total of 3 cMDV were analyzed on days 0, 10, 20, and 30; 4 cMDV were analyzed on day 365. The samples were diluted 50-fold in acetonitrile:water (1:1, v/v) to analyze meloxicam in the linear range of detection of the diode array. The diluted samples were analyzed in triplicate against a 6-point calibration curve (range, 1.0 to 20 μg/mL) of meloxicam standard (Sigma, St Louis, MO). Standard deviations between replicates were less than 20%. The ratio of chromatographic peak area units of meloxicam:meloxicam (standard) 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 by using a model 1100 HPLC system (Agilent). We used an analytical Zorbax Eclipse XDB-C8 column (4.6 mm × 150 mm × 5 μm particle size; Agilent) with mobile phases consisting of (A) 0.1% formic acid in water and (B) acetonitrile at a flow rate of 0.4 mL/min under isocratic conditions of 30% A and 70% B over 10 min of injecting 20 μL.

The HPLC system was coupled with a diode array detector (model G1315B, Agilent 1100 Diode Array). The diode array was operated at a wavelength of 360 nM, with a 450 nM reference wavelength. Chemstation Rev B.03.01 software (Agilent) was used for data analysis. The limit of quantitation for this assay was calculated as the lowest amount of meloxicam (20 ng) injected divided by the amount of sample injected (0.4 mg on column), thus yielding 50 μg/mL. Linear regression was used to determine any significant difference between the concentrations of diluted meloxicam over 365 d. Both analyses were performed by using Analysis ToolPak (Microsoft, Redmond, WA). A P value less than 0.05 was considered significant.

Results

Bacterial contamination was not detected in any of the vials at any time point. For all 8 cMDV tested, endotoxin assays were negative at all time points.

The concentration of meloxicam was stable throughout the study. On day 0, the mean concentration of meloxicam was 0.624 mg/mL; on day 30, the mean concentration was 0.603 mg/mL (Figure 1). There was no significant difference ($P = 0.37$) between the initial (day 0) and the final (day 30) concentration of diluted meloxicam over 30 d. On day 365, the mean concentration was 0.598 mg/mL. There was no significant difference ($P = 0.19$) between the initial and final concentrations of diluted meloxicam over 365 d.

Discussion

The results from this study demonstrated that injectable meloxicam in cMDV remained sterile for 30 d under drug
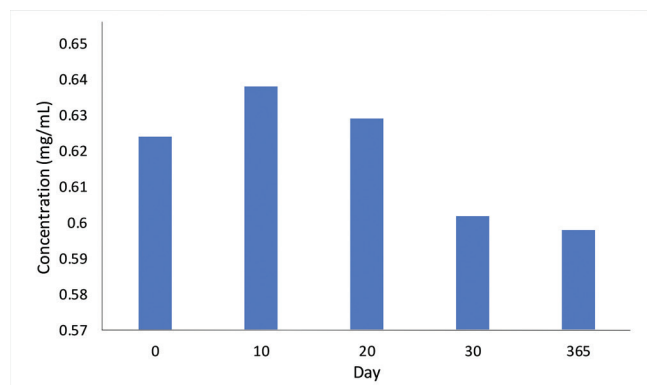

Figure 1. Mean concentration (mg/mL) of diluted meloxicam over 365 d of storage.

withdrawal techniques used in clinical setting. In addition, the concentration of meloxicam remained stable for 30 d. Furthermore, cMDV punctured daily for 30 d remained stable and sterile for 365 d.

The mean concentration of meloxicam was approximately 0.6 mg/mL, which was slightly higher than the targeted diluted concentration of 0.5 mg/mL. This result was likely due to a dilution error. However, the concentrations of diluted meloxicam throughout the study were consistent with no significant differences, indicating the absence of drug degradation during the experiment.

In addition to bacterial culture, which detects viable bacteria in the solution at the time of sampling, the Pyrosate kit was used to identify bacterial components, such as LPS, that are released when the bacteria die. Because these endotoxins in the bloodstream can cause septic shock and organ failure, the FDA mandates a maximum of 0.5 endotoxin units/mL for veterinary products and devices.[8] We did not detect endotoxins in any of the cMDV tested. With the lack of endotoxin and bacterial growth, we infer of the absence of bacterial contamination at all points during the experiment.

To prevent contamination, studies have recommended cleaning the stopper of the MDV with alcohol before withdrawing the drug.[3,11] In a study where *Staphylococcus* spp. were inoculated on the rubber stopper of MDV, disinfecting the top with alcohol greatly reduced bacterial growth. However, we have observed when vials are handled appropriately, there is minimal risk of contamination. In a previous study, the stability of a cMDV of carprofen remained sterile with chronic use without cleaning the stopper.[12] To obviate the need to clean the stopper, the cMDV should be stored away from the patient treatment area to limit contact with potentially contaminated surface and equipment.[16] In addition, sterile needles should be used to make and withdraw solutions.

We conclude that diluted meloxicam in a cMDV that is punctured daily for 30 d can remain stable and sterile for 365 d. The results of this study could be used as a guide for institutions regarding the long-term use of diluted meloxicam prepared in a cMDV. We suggest that in the laboratory setting, cMDV of meloxicam can remain sterile and stable for as long as 365 d, with the caveat that increased withdraw may increase the risk of contamination.

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