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Strength and Sterility of Stock and Diluted Carprofen Over Time

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Carprofen, a nonsteroidal antiinflammatory drug, is a commonly used analgesic for laboratory animals. The manufacturer labeling indicates the stock bottle may be stored and used for up to 28 d under refrigeration. However, institutional guidelines may vary in how long diluted carprofen solutions can be stored before they must be discarded. When administered to laboratory rodents, small volumes of the stock solution are diluted to provide accurate dosing and ease of administration. Because carprofen is formulated for use in companion animals (for example, dogs) and comes in larger volume multidose vials, the majority of carprofen at our institution is discarded before it can be used. In this study, we evaluated the amount of target ingredient present (strength), sterility, and endotoxin levels of both stock and diluted carprofen when stored in a variety of containers and at multiple temperature settings for up to 180 d, mimicking facets of typical use in laboratory animal research and medicine. We demonstrated that when refrigerated and stored in sterile vials, stock and diluted carprofen can be kept and used for up to 180 d while retaining strength and sterility. For short-term use, diluted carprofen can also be stored for up to 60 d at room temperature in conical tubes. These results will help establish scientifically justified storage conditions for carprofen to ensure that these agents remain appropriately potent and sterile for therapeutic use in laboratory animals.

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Preclinical research involving animal models must be designed and performed to maximize animal welfare and the benefit to harm ratio while minimizing pain, distress and research variability. A common component of most preclinical research involves evaluating novel test articles for use in treating disease or using experimental compounds to either modify an animal's pathophysiology or induce disease. In addition to the experimental compounds, therapeutic drugs may be administered for anesthesia, analgesia, or treatment of disease. Experimental test articles and therapeutic drugs are generally administered to laboratory animals (primarily rodents) by a parenteral route (subcutaneous, intraperitoneal, intravenous, intrathecal) rather than orally. The use of Food and Drug Administration (FDA)-approved drugs manufactured under Good Manufacturing Practice conditions is recommended, especially if the drugs can be used without dilutions or modifications. During product development, manufacturers determine the compound's chemical and physical stability, packaging integrity, sterility, and expiration date based on recommended storage conditions. However, FDA approved drugs are not routinely labeled for small animals, rodents, or exotic species; thus, dilutions of manufactured drugs are often necessary to achieve the correct dose and an appropriate volume for safe administration. The Guide for the Care and Use of Laboratory Animals (8th ed.)[7] and USDA policies state that whenever possible, pharmaceutical grade drugs should be used to minimize unwanted side effects or toxicities. According to the Guide, "if a non-pharmaceutical grade chemical or substance must be used to meet scientific goals or if a human or veterinary pharmaceutical grade product is unavailable then consideration should be given to the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and pharmacokinetics of the chemical or substance to be administered, as well as animal welfare and scientific issues relating to its use."[7] The United States Pharmacopeia (USP) publishes standards, guidelines for tests and procedures, monographs and reference standards for drugs, biologics, and compounding preparations (sterile and nonsterile) in their USP-National Formulary used by the FDA and state pharmacy boards to enforce and regulate nonsterile and sterile preparation compounding, adulteration, and misbranding of drugs. The USP National Formulary (USP-NF) is composed of general chapters; chapter numbers below 1000 are legally enforceable standards referenced by the FDA; and chapters above 1000 are generally used for informational guidance although they may be enforced in some countries.[4,20]

Regarding sterile pharmaceutical compounding in human and veterinary medicine, the USP General Chapter <797> Pharmaceutical Compounding – Sterile Preparations (a legally enforceable standard) must be followed when enforced by regulatory agencies or professional boards. The provisions include strict engineering processes regarding clean room requirements, airborne particle sampling, microbial monitoring, cleaning and disinfectant procedures, training, and development of quality assurance program. Per USP <797>, a compounded sterile preparation must state a beyond-use date (BUD) after which the preparation must not be stored, transported, or administered and therefore, must be discarded. A BUD is different from an expiration date, which is assigned by the manufacturer and is based upon rigorous analytical stability testing, specific for the formulation, dosage container, and storage conditions.[21] Although USP General Chapter <797> standards are not a mandatory requirement for all preclinical research (unless required by the state board of pharmacy or other regulatory agency), the chapter's components align with the use of nonpharmaceutical

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grade compounds as described in the Guide. The use of product-specific quantitative strength assays (e.g. HPLC), validated stability-indicating methods, sterility testing per USP <71> and bacterial endotoxin testing per USP <85> can be used to best determine optimal storage or beyond-use dating for compounded or diluted preparations. For instance, the USP <71> sterility criteria require a minimum use quantity depending on container size, and a 2-wk incubation of sample in growth media to determine if contamination is present. Utilization of such practices will ensure that laboratory animals receive experimental compounds and clinical therapeutics that are free from contamination and have the required potency to achieve a therapeutic clinical response until the storage or beyond-use date is reached.

Carprofen, a nonsteroidal antiinflammatory drug, is one of the most commonly used analgesic compounds at our institution. When administered to mice, the stock solution is diluted from 50 mg/mL to 0.5 to 1 mg/mL to allow accurate dosing. Per USP guidelines for multidose vials, manufacturer labeling for injectable carprofen indicates that the stock vial should be maintained under refrigeration for up to 28 d once the vial stopper is punctured. Because carprofen is formulated for use in companion animals (for example, dogs) and comes in larger unit volumes (20 mL), the majority of carprofen become outdated before it can be used, requiring disposal and thus leading to waste and reduced cost-efficiency. In efforts to promote chemical and physical stability and reduce contamination in pharmaceutical formulations or preparations, research institutes should develop guidelines or policies for storing diluted aliquots of carprofen, including the amount of time that aliquots may be kept under various storage conditions (refrigeration, room temperature, or freezing). However, product-specific scientific data to support such recommendations is often sparse and must be extrapolated from methods outlined in USP <795>, or empirical beyond-use dates as indicated in USP <797> may be necessary. Moreover, default beyond-use dates in absence of quantitative or stability-indicating testing can be very restrictive, as indicated in USP <797>, requiring disposal of larger quantities of an unused drug that is normally manufactured in larger volume containers, yet must be diluted or used in smaller volumes in an animal research setting.

This study was designed to evaluate carprofen (undiluted and diluted) for strength and sterility when stored in a variety of sterile containers and at multiple temperature settings over time. This storage was performed in conjunction with frequent manipulation intended to mimic common laboratory animal research and medicine usage. Results from this study will provide veterinarians and institutional animal care and use committees with additional information for determining appropriate beyond-use dates for this commonly used analgesic to ensure that the compound remains potent and sterile for therapeutic use.

Materials and Methods

Experimental setup. Four variables were evaluated in this study: 1) dilution (diluted carprofen [1 mg/mL] compared with stock [50 mg/mL]), 2) storage container (sterile vial with rubber stopper [SV] compared with conical tube [CT]), 3) storage temperature (room temperature [RT], refrigerated [RF], and frozen [FZ]), and 4) time point (time since dilution, 0 to 180 d). In limited sampling, we recorded the temperatures of the different storage conditions as follows: RT, 21.7 to 22.1 °C (mean = 21.9 °C); RF, 4.1 to 5.2 °C (mean = 4.8 °C), and FZ, -7.5 to -20.5 °C (mean = -12.5 °C).

Diluted samples were stored in either CT (15 mL polypropylene conical tube, Falcon, Corning, NY) or SV (Sterile empty vials, ALK Laboratories, Hørsholm, Denmark) containers at the 3 storage conditions (RT, RF, FZ) for up to 180 d. Stock solution was stored either 1) consistently at RF, or 2) at FZ for a period, then thawed and stored at RF for 30 d before sending out for testing. We will refer to the second stock storage condition as FT (freeze-thaw).

Samples were manipulated as described below. At their designated time points, the samples were sent out for testing in triplicate (3 samples per unique dilution/storage container/storage temperature/time point combination). Strength was evaluated at each time point for each sample. Sterility was pooled for each triplicate (samples combined, tested once), at each time point. Endotoxin was tested for each sample, but only during the baseline (BL) and final time points (variable – 90 d, 120 d, or 180 d).

The number of samples per condition are shown below (Table 1).

Mixing and aliquoting. Injectable carprofen vials (50 mg/mL) (Dechra Veterinary Products, Overland Park, KS [formerly Putney]) used for diluted and stock samples were all from the same lot (GI90055 – FDA approved, manufactured under cGMP).

Samples. Twelve mL of 50 mg/mL stock carprofen (600 mgs) were mixed with 588 mL of 0.9% sterile nonbacteriostatic saline (Hospira, Lake Forest, IL) in a 1000 mL sterile saline evacuated container (Hospira, Lake Forest, IL). The final mixture contained 600 mL of 1 mg/mL diluted carprofen. Seven mL of diluted carprofen were aliquoted into either CTs ($n = 39$) or SVs ($n = 63$). Seven mL of stock solution were aliquoted into each SV. There was no CT condition for the stock solution.

Manipulation. Samples were manipulated every 7 to 10 d until 120 d, and then every 10 to 20 d after 120 d. A manipulation consisted of 1) wiping down counter (Super Sani-Cloth, PDI, Temple, TX), 2) swabbing SV samples with an alcohol pad (Webcol 2 ply Alcohol prep, Covidien, Dublin, Ireland) if indicated, 3) withdrawing 1 mL of carprofen from the container, withdrawing the needles/syringe/drug from the container, replacing the volume back into the container, 4) repeating step 3 twice, for a total of 3 withdrawals. The manipulation procedure was intended to simulate the puncture and withdrawal of drugs in laboratories. While normally drugs that are withdrawn would not be replaced back into the vial, in this experiment we did so to avoid wasting limited experimental material. The manipulator wore gloves, and samples were collected with a 22G needle (Monoject 22g 3/4 "veterinary needle, Covidien) and 3 mL syringe (BD 3mL luer-lock tip syringe, Becton, Dickenson and Company, Franklin Lakes, NJ). Samples were manipulated on a clean benchtop surface, rather than inside a HEPA filtered laminar flow hood or biological safety cabinet.

Shipping and testing. All samples were tested by Compounder's International Analytical Laboratory (CIAL), (Castle Rock, CO). For shipping, SVs were wrapped in paper towels for padding, and CTs were wrapped with Parafilm (Bemis Company, Neenah, WI) during later shipments to prevent leakage. Samples were shipped in an insulated Styrofoam container with an ice pack overnight.

CIAL tested strength (concentration of drug in compounded sample) using ultra-high-performance liquid chromatography (UHPLC) and strength was considered acceptable when results were 90% to 110% of the labeled claim. Sterility was tested in compliance with United States Pharmacopeia (USP) <71> and <797> standards and was considered to be acceptable when no organic growth was detected after 14 d of culture. Endotoxin

Table 1. Experimental set up of all drug samples tested.

Container	Dilution	Temp	BL	30 d	60 d	90 d	120 d	180 d	TOTAL SAMPLES
CT	1/50 (1 mg/mL)	RT	3#	3	3	3	N/A	3#	15
		RF	N/A	3	3	3	N/A	3#	12
		FZ	N/A	3	3	3	N/A	3#	12
SV	1/50 (1 mg/mL)	RT	N/A	3	3	3	N/A	3#	12
		RF	N/A	3	3	3	N/A	3#	12
		FZ	N/A	3	3	3	N/A	3#	12
SV	Stock (50 mg/mL)	RF	3#	3	3	3	N/A	3#	15
		FT	N/A	N/A	3	3+ 3*#	3*#	N/A	12

CT = conical tube, SV = sterile vial, BL = baseline. RT = room temperature. RF = refrigerated. FZ = frozen, FT = freeze-thaw, N/A = not applicable. Numbers indicate number of 7 mL samples during each time point. * = sample was taken out of freezer 30 d prior. # = in addition to sterility and potency, the sample was also tested for endotoxin.

was tested in compliance with <85> Bacterial Endotoxins Test and was deemed acceptable when endotoxin levels were < 25 EU/mL.

Results

Diluted solution. Regarding strength, all RF samples fell within 90% to 110% of the labeled claim (1 mg/mL) at all time points up to 180 d. FZ samples showed large variability, with samples outside of the 90% to 110% range for SV and CT storage conditions, at 30 d (CT 69%, SV 112%) and 180 d (SV 79%, CT 84%). For RT samples, all SV samples stayed within the 90% to 110% range, but CT samples dropped out of range at 90 d and continued to drop until the end of testing at 180 d (Table 2, Table 3).

None of the submitted sample showed growth after a 14 d incubation. All endotoxin samples were < 1 EU/mL, and below the 25 EU/mL threshold considered acceptable.

Stock solution. All samples at all storage temperatures (FZ, RF, and FT) were within 90% to 110% of the labeled claim (50 mg/mL). Both the FT subset that was tested immediately after thawing and RF samples showed a downward trend with time. The greatest variability between triplicates was seen in the 90d + 30d FT group (stored at FZ for 90 d, then RF for 30 d, before testing, 101% to 109%) (Table 4).

None of the submitted samples showed growth after a 14 d incubation. Baseline endotoxin samples were < 2 EU/mL, 60 d to 180 d samples were < 5 EU/mL. All samples were below the 25 EU/mL threshold considered acceptable.

Discussion

This study showed that despite fluctuations and decreases in potency, both stock and diluted carprofen maintained strength, remained sterile, and stayed within acceptable endotoxin limits for well beyond the 28 d labeled for the stock carprofen,[3] and the previously described 28 d for diluted carprofen.[13]

In general, the strength of the diluted solution fell more quickly in RT conditions than in the RF conditions and had the greatest variability in the FZ condition. Cold temperature storage is known to decrease microbial growth, yet colder temperatures can cause variation in chemical or physical stability in some formulations and active pharmaceutical ingredients. SV storage maintained strength better than CT storage. All samples remained sterile for the duration of testing (up to 180 d). While endotoxin levels remained under the 25 EU/mL limit for duration of the study, levels were higher in the stock solution (< 2 to 5 EU/mL) than in the diluted solution (< 1 EU/mL) and increased with time.

This study used clean, but not sterile, techniques in creating, aliquoting and manipulating samples. The surfaces in contact were wiped down and sanitized, but masks and hair bonnets were not worn, and the samples were manipulated on a benchtop setting compared with an ISO 5 laminar flow hood or biologic safety cabinet. In discussions with colleagues and research personnel, we chose these techniques to reflect how researchers in academic institutions prepare dilutions while minimizing contamination. Thus, the results of this study are applicable to the research setting.

Strength and stability testing may differ depending on whether a stability-indicating method was defined to separate intact drug from coeluting degradation products.[1] A previous publication demonstrated carprofen diluted 1:10 could be stored for up to 28 d while retaining stability and sterility.[13] Another previous publication demonstrated carprofen stability in reverse-osmosis water when stored under ambient light, ambient dark, and 4 °C conditions for up to 7 d.[6] To provide additional quality assurance and method validation, our study used a FDA registered compounding analytical laboratory specializing in USP standards for pharmaceutical compounding - nonsterile

Table 2. Concentration (reported as % labeled claim [%LC]) of diluted carprofen samples stored in sterile vials (SV) at various conditions.

	FZ			RF			RT		
	%LC	M ± SD	WLC	%LC	M ± SD	WLC	%LC	M ± SD	WLC
BL	99.2 100.8 101.3	100.4 ± 1.1	3/3	99.2 100.8 101.3	100.4 ± 1.1	3/3	99.2 100.8 101.3	100.4 ± 1.1	3/3
30 d	111.7 96.6 108.5	105.6 ± 8.0	2/3	101.8 99.8 99.8	100.5 ± 1.2	3/3	99.3 98.6 99.2	99.0 ± 0.4	3/3
60 d	100.5 97.7 104.7	101.0 ± 3.5	3/3	100.3 103.5 100.7	101.5 ± 1.7	3/3	99.1 98.3 99.6	99.0 ± 0.7	3/3
90 d	98.6 98.3 98.3	98.4 ± 0.2	3/3	97.7 98.6 98.2	98.1 ± 0.5	3/3	95.4 95.5 96.1	95.7 ± 0.4	3/3
180 d	96.6 78.9 91.6	89.0 ± 9.1	2/3	95.9 98 98.2	97.4 ± 1.3	3/3	95.8 95.5 94	95.1 ± 1.0	3/3

M ± SD = mean ± SD. WLC = within 90% to 110% of labeled claim. FZ = frozen. RF = refrigerated. RT = room temperature. BL = baseline.

Table 3. Concentration (reported as % labeled claim [%LC]) of diluted carprofen samples stored in conical tubes (CT) at various conditions.

	FZ			RF			RT		
	%LC	M ± SD	WLC	%LC	M ± SD	WLC	%LC	M ± SD	WLC
BL	99.2 100.8 101.3	100.4 ± 1.1	3/3	99.2 100.8 101.3	100.4 ± 1.1	3/3	99.2 100.8 101.3	100.4 ± 1.1	3/3
30 d	97.9 102.2 69.0	89.7 ± 18.1	2/3	97 97.9 97.9	97.6 ± 0.5	3/3	99.5 97.9 98.5	98.6 ± 0.8	3/3
60 d	95.4 100.2 100.5	98.7 ± 2.9	3/3	97.2 97.9 102.6	99.2 ± 2.9	3/3	98.1 96.2 97.4	97.2 ± 1.0	3/3
90 d	106.7 108.3 97.9	104.3 ± 5.6	3/3	98.5 98.3 97.5	98.1 ± 0.5	3/3	90.2 89 90.6	89.90 ± 0.8	2/3
180 d	97.6 83.7 96.5	92.6 ± 7.7	2/3	96.7 96.3 96.2	96.4 ± 0.3	3/3	79.6 83.6 78.2	30.5 ± 2.8	0/3

M ± SD = mean ± SD. WLC = within 90% to 110% of labeled claim. FZ = frozen. RF = refrigerated. RT = room temperature. BL = baseline.

Table 4. Concentration (reported as % labeled claim [LC]) of stock carprofen stored in sterile vials (SV) at various conditions.

	FZ			RF		
	%LC	M ± SD	WLC	%LC	M ± SD	WLC
BL	102 102.4 102.5	102.3 ± 0.3	3/3	102 102.4 102.5	102.3 ± 0.3	3/3
30 d	N/A	N/A	N/A	102.3 104.8 105.8	104.3 ± 1.8	3/3
60 d	102.3 100.6 103.5	102.1 ± 1.5	3/3	102 101.7 103	102.2 ± 0.7	3/3
60 d + 30 d	98.7 100.9 101.2	100.3 ± 1.4	3/3	N/A	N/A	N/A
90 d	100.4 98.4 99.4	99.4 ± 1.0	3/3	98 98.1 98.8	98.3 ± 0.4	3/3
90 d + 30 d	109.3 101 101.2	103.8 ± 4.7	3/3	N/A	N/A	N/A
180 d	N/A	N/A	N/A	95.3 97.5 98.7	97.1 ± 1.7	3/3

M ± SD = mean ± SD. WLC = within 90% to 110% of labeled claim. FT = freeze-thaw. RF = refrigerated, N/A = not applicable. For FT samples, 30 d, 60 d, 90 d, and 180 d samples were stored in the frozen (FZ) condition, and then directly tested. "60 d + 30 d" and "90 d + 30 d" refer to samples that were stored in the FZ condition for 60 or 90 d respectively, and then in the RF condition for an additional 30 d.

compounding <795>,[19] pharmaceutical compounding - sterile compounding <797>,[21] validation of compendial procedures <1225>,[16] chromatography <621>,[15] mass spectrometry <736>,[18] sterility tests <71>[14] and bacterial endotoxin test <85>[17] to ensure reproducible results that conform with industry and USP standards. Although USP General Chapters <795> and <797> were revised and published in 2019, the revised General Chapters are currently under appeal via Article VII, Section 7 of USPs bylaws. As a result, revision dates for General Chapters <795> and <797> are postponed until further notice, with the USP <797> chapter standards (last revised 2008) remaining official as of the date of our research and manuscript submission. In addition, we used triplicate samples and minimum volumes per USP guidelines.

Based on our findings, we recommend that stock or diluted carprofen not be frozen, as freezing does not appear to increase lifespan and may subject the compound to freeze-thaw artifacts such as crystallization or container damage. If kept for long periods of time, stock or diluted carprofen should be stored at RF conditions in SVs. Under these conditions, stock or diluted carprofen can be effectively stored for up to 180 d while maintaining strength and sterility. For short-term use, diluted carprofen can be stored for up to 60 d at RT conditions in CT containers. The storage and use dating recommended by our findings is consistent with several aspects of the current USP <797> criteria (2008) for determining beyond-use dates, including starting with sterile components, maintaining storage under colder temperatures, and passing sterility testing. Our study also evaluated effects of repeated container manipulations, adding breadth to our recommendation.

Increased endotoxin in the stock solution should be interpreted in the context of drug concentration, and the dose given to the animal.[2,9] Endotoxin limits are difficult to interpret, ranging from 0.25 EU/mL for sterile water to 5 EU/kg/h for drugs injected intramuscularly or intravenously.[5] In this study, we used the endotoxin limit of 5 EU/kg/h, based on with how carprofen is given in rodents (usually subcutaneously).[10] Based on our results of < 1 EU/mL for dilute carprofen samples and < 5 EU/mL for stock carprofen samples, the upper endotoxin limit of our samples is 0.1 to 1 EU/mg (from the extremely conservative assumption that < 1 EU/mL in the dilute carprofen sample is at or around 1 EU/mL). At typical doses of carprofen in laboratory animals (5 mg/kg),[12] this level is at or below the 5 EU/kg recommended endotoxin limit. Several inconsistences were noticed in our samples that may explain the variation in FZ and CT samples, as compared with other storage conditions. One dilute sample (30 d SV FZ) was initially tested to be above the 90% to 110% range (112%), but on subsequent days during the same week, retested to be within range (95%), then out of range again (77%). In this paper, we reported this sample as its original sample (112%). Precipitation or crystal formation may have caused these out-of-range results, although we do not know why the other 2 manipulated samples that were stored and shipped concurrently did not have the same problem. Another inconsistency was that 2 of 3 dilute samples (60 d CT RT) arrived at the testing facility with small cracks. The cracks may have been caused by lack of padding in SVs, resulting in more damage to the container, or increased susceptibility of plastic to altitude related pressure changes (either during air flight, or between Michigan and Colorado) To prevent potential leakage, CTs were wrapped with Parafilm after this incident. CIAL performed additional testing and validation to rule out any potential interference of Parafilm with analytical testing.

This study is limited by evaluating strength commonly used for product release testing and by not using a stability-indicating or force degradation testing method that was validated per USP <1225>, which is the gold standard for optimizing beyond-use dates in conjunction with sterility and endotoxin testing. If a published study indicates that stability was assessed to determine optimal beyond-use dates, then the stability-indicating analytical methods and validation should be described and delineated from quantitative potency or strength methods.[8,11] In addition, we did not perform container-closure, particulate,

pH, and formal physical stability assays, and our study did not include in-vivo testing (therapeutic stability) to demonstrate analgesic efficacy in animals. However, we do not anticipate measurable changes in therapeutic response based upon strength levels. We did not perform pH and osmolarity testing for stock solutions, as these solutions were not diluted or mixed with additives. Calculated osmolarity for diluted solutions approximated the osmolarity for 0.9% normal saline. Visual examination of stored solutions during manipulation and prior to shipping did not reveal gross signs of color change, homogeneity, precipitation, or instability.

Because formulation and hydrolysis breakdown between different carprofen products may vary, and in the absence of a formal stability-indicating study, the results of this study should be limited to the drug-specific materials and procedures outlined in the study, with judicious empirical extrapolation or inferred beyond-use dating to other formulations. However, the experimental results of this study may be used to guide considerations for determining drug storage and future studies.

In conclusion, our study has demonstrated that the sterility and strength of diluted carprofen stored in conical tubes can be maintained up to 60 d at RT conditions. Likewise, the sterility and strength of stock or diluted carprofen stored at RF conditions in sterile vials were maintained for up to 180 d. However, we cannot confirm true stability as formal stability assays were not performed. These results will aid in the establishment of appropriate storage guidelines for diluted carprofen in laboratory animals.

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References

1. **Allen LV Jr.** 2013. Basics of compounding: potency and stability testing. Int J Pharm Compd **17:**220–224.
2. **Brito LA, Singh M.** 2011. Acceptable levels of endotoxin in vaccine formulations during preclinical research. J Pharm Sci **100:**34–37. https://doi.org/10.1002/jps.22267.
3. **Dechra.** [Internet]. 2017. Carprofen [Package Insert]. Dechra veterinary products. [Cited 14 July 2020]. Available at: https://www.dechra-us.com/Files/Files/ProductDownloads/us/Carprovet-FlavoredTablets-Package-Insert.pdf.
4. **DeStefano A.** [Internet]. 2020. USP 2010–2015 council of experts: Expert committee orientation. General chapters overview. [Cited 30 September 2020]. Available at: https://www.usp.org/sites/default/files/usp/document/get-involved/stakeholder-forums/4c-usp-general-chapters-overview-2012-11-09.pdf.
5. **FDA.** [Internet]. 2014. Endotoxin-Guidelines. [Cited 14 July 2020]. Available at: https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/bacterial-endotoxinspyrogens.
6. **Ingrao JC, Johnson R, Tor E, Gu Y, Litman M, Turner PV.** 2013. Aqueous stability and oral pharmacokinetics of meloxicam and carprofen in male C57BL/6 mice. J Am Assoc Lab Anim Sci **52:**553–559.
7. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals. Washington, (DC): National Academies Press.
8. **Kirk LM, Brown SD.** 2015. Beyond-use date determination of buprenorphine buccal solution using a stability-indicating high-performance liquid chromatographic assay. J Feline Med Surg **17:**1035–1040. https://doi.org/10.1177/1098612X15569329.
9. **Malyala P, Singh M.** 2008. Endotoxin limits in formulations for preclinical research. J Pharm Sci **97:**2041–2044. https://doi.org/10.1002/jps.21152.
10. **McCullough KZ**. [Internet]. 2018. Calculating endotoxin limits for drug products. [Cited 14 July 2020]. Available at: https://www.americanpharmaceuticalreview.com/Featured-Articles/353977-Calculating-Endotoxin-Limits-for-Drug-Products/
11. **Papich MG.** 2015. How to (properly) determine the beyond-use date and stability of a compounded formulation for pets. J Feline Med Surg **18:**364–365. https://doi.org/10.1177/1098612X15622518.
12. **Plumb DC.** 2018. Plumb's veterinary drug handbook, 9th ed. Stockholm (WI): Pharma Vet.
13. **Simonek GD, Alarcio GG, Brignolo LL.** 2017. Sterility and stability of diluted carprofen in a multidose vial in the laboratory animal setting. J Am Assoc Lab Anim Sci **56:**296–298.
14. **US Pharmacopeia.** 2013. Chapter 71 Sterility Tests (Official Prior to 2013). United States Pharmacopeia and National Formulary (USP 43-NF 38). Rockville (MD): US Pharmacopeia.
15. **US Pharmacopeia**. 2017. Chapter 621 Chromatography (Official 8/1/2017). United States Pharmacopeia and National Formulary (USP 43-NF 38). Rockville (MD): US Pharmacopeia.
16. **US Pharmacopeia**. 2017. Chapter 1225 Validation of Compendial Procedures (Official 8/1/2017). United States Pharmacopeia and National Formulary (USP 43-NF 38). Rockville (MD): US Pharmacopeia.
17. **US Pharmacopeia**. 2018. Chapter 85 Bacterial Endotoxin Test (Official 5/1/2018). United States Pharmacopeia and National Formulary (USP 43-NF 38). Rockville (MD): US Pharmacopeia.
18. **US Pharmacopeia**. 2018. Chapter 736 Mass Spectrometry (Official 5/1/2018). United States Pharmacopeia and National Formulary (USP 43-NF 38). Rockville (MD): US Pharmacopeia.
19. **US Pharmacopeia**. 2020. Chapter 795 Pharmaceutical Compounding – Nonsterile Preparations (Official 5/1/2020 - Revision Bulletin). United States Pharmacopeia and National Formulary (USP 43-NF 38). Rockville (MD): US Pharmacopeia.
20. **US Pharmacopeia.** [Internet]. 2020. Legal recognition of USP standards. Available at: https://www.usp.org/about/legal-recognition.
21. **US Pharmacopeia**. 2020. Chapter 797 Pharmaceutical Compounding – Sterile Preparations (Official 5/1/2020 - Revision Bulletin). United States Pharmacopeia and National Formulary (USP 43-NF 38). Rockville (MD): US Pharmacopeia.