PEER REVIEWED

Effect of elevated storage temperatures on the concentration of active ingredients in 5 commonly used large animal pharmaceuticals

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Abstract

Most veterinary pharmaceuticals are labeled to be stored at or below 77°F (25°C) or 86°F (30°C). Previous work showed that temperatures in ambulatory veterinary practice vehicles frequently exceeded those temperatures. The objective of this study was to determine the effect of higher storage temperatures on the active ingredient concentration of drugs commonly used in large animal practice. Five bottles of dinoprost, flunixin meglumine, gonadorelin, tulathromycin, and xylazine were maintained at room temperature (controls) and 5 additional bottles of each product were maintained in a programmable chamber set to mimic temperatures previously recorded in a veterinary practice vehicle. Samples were collected from all bottles on days 0, 40, 80, and 120, and were analyzed in duplicate by liquid chromatography/mass spectrometry. Changes in active ingredient concentration were assessed by linear regression, and t-tests were performed to compare slopes of time:concentration curves for control and treatment drugs. Slopes of drug concentrations over 120 days for all 5 drugs were less than 0.04, and there was no statistically significant difference between concentration slopes over time for control vs treatment bottles. No significant effect of elevated storage temperatures on product active ingredient was found in this study. However, due to the limited conditions of this study, practitioners are still advised to follow label recommendations when storing pharmaceuticals.

Key words: drug storage, elevated temperatures, large animal practice, dinoprost, flunixin, gonadorelin, tulathromycin, xylazine

Résumé

Selon les directives de l'étiquette, la plupart des médicaments vétérinaires devraient être entreposés à ou sous 77°F (25°C) ou 86°F (30°C). Des travaux antérieurs ont démontré que la température dans les véhicules de service ambulatoire vétérinaire excédait souvent ces normes. L'objectif de cette étude était de déterminer l'effet d'une température d'entreposage plus élevée sur la concentration des ingrédients actifs dans des médicaments utilisés couramment en pratique des grands animaux. Cinq contenants de dinoprost, de flunixine, de méglumine, de gonadoréline, de tulathromycine et de xylazine ont été maintenus à température ambiante (témoin) et cinq autres contenants de ces mêmes produits ont été maintenus dans une enceinte climatique programmable afin de simuler les températures rapportées préalablement dans des véhicules de service ambulatoire vétérinaire. Des échantillons ont été recueillis de tous les contenants aux jours 0, 40, 80 et 120 et ont été analysés en double avec la chromatographie liquide/spectrométrie de masse. Les changements dans la concentration des ingrédients actifs ont été analysés avec la régression linéaire. Des tests de t ont servi à comparer la pente de la relation entre la concentration et le temps pour les médicaments dans les contenants témoins et traités. La pente de la relation entre la concentration des ingrédients actifs et le temps sur une période de 120 jours était moins de 0.04 pour tous les médicaments. De plus, il n'y avait pas de différence statistiquement significative au niveau de la pente sur cette période dans les contenants témoins et traités. Aucun effet significatif d'une température d'entreposage plus élevée sur la concentration des ingrédients actifs dans des médicaments n'a été mis en évidence dans cette étude. Toutefois, en raison des conditions limitées de cette étude, on recommande aux praticiens de continuer à suivre les recommandations sur les étiquettes pour l'entreposage des médicaments.

Introduction

Guidelines from the US Pharmacopeia (USP) about potency, stability, and storage standards are applicable to all aspects of drug handling, from manufacture to the point of use, including transport by shipping and emergency service vehicles.[15] USP guidelines state that 'temperature is one of the most important conditions to control',[15] with light, air, and

humidity also affecting storage stability.[14] In order to establish stability of a pharmaceutical dosage form, manufacturers must propose a stability schedule prior to drug approval. The Food and Drug Administration Center for Veterinary Medicine (FDA-CVM) recommends that drugs be tested at the defined normal room temperature of 77°F (25°C) and at elevated temperature of 98.6 to 104°F (37 to 40°C) and sampled every 2 to 3 months through the proposed expiration date.[14]

Pharmaceuticals are routinely carried in vehicles outfitted to provide veterinary care for large animals on the farm, where the drugs are stored within the vehicle or in after-market inserts for truck beds or a chassis mount storage unit. Regardless of storage method, these pharmaceuticals are subject to the effects of environmental temperatures on the interior of the vehicle or insert. Medical emergency service vehicles,[1,2,6,8] medical helicopters,[13] and medical bags[11] have all been studied with regards to drug storage temperature, with container temperatures frequently falling outside the label range. Emergency medications, including lorazepam,[1,6] epinephrine, lidocaine, diltiazem, dopamine, and nitroglycerine,[5] are known to be unstable at real-world ambulance temperatures, some experiencing greater than 10% reduction in concentration in correlation with thermal exposure time.[5] Responding to concerns over the impact of extra-label storage, USP added a section to the Good Storage and Distribution Practices for Drug Products addressing emergency medical service vehicles and other road vehicles used to transport drug products, suggesting the addition of temperature monitoring devices to drug storage areas for monitoring during seasonal extremes.[15]

In veterinary medicine, few studies have evaluated storage temperatures for pharmaceuticals in veterinary vehicles. In Europe, where veterinary vehicles are more typically cars or other enclosed vehicles, 2 studies measuring the temperature in drug storage compartments showed temperatures frequently varied outside the label range for storage of most veterinary drugs.[7,12] In an earlier study performed by the authors in the US, 11 of 12 study vehicles were trucks with after-market bed inserts in which summertime temperatures in drug compartments rose above the common label upper temperature limit of 77°F (25°C) in up to 95% of total temperature logger readings.[10]

The objectives of this project were therefore to: 1) determine the effect of storage temperatures encountered in veterinary practice vehicles on concentrations of the active ingredient present in commercial preparations of xylazine, gonadorelin, flunixin meglumine, tulathromycin, and dinoprost, and 2) predict the effect of elevated storage temperatures on tested commercial preparations on drug efficacy. Our hypothesis was that pharmaceuticals exposed to temperatures typically encountered in ambulatory or mobile veterinary practice vehicles during the summer months would cause a clinically significant change in the concentration of active ingredients.

Materials and Methods

Five pharmaceutical products were selected for inclusion in the study based on the lack of available information regarding the effect of high storage temperatures on active ingredient concentration in the product, and the authors' experiences that these products are routinely stored in ambulatory veterinary practice vehicles. Ten bottles of each commercially manufactured product, xylazine,[a] gonadorelin,[b] flunixin meglumine,[c] tulathromycin,[d] and dinoprost,[e] were purchased with identical lot numbers and expiration dates for each product. Five bottles of each product were randomly assigned to the control group. These bottles of medication were maintained at room temperature (65° to 75°F; 18.3°C to 23.9°C) in a closed, insulated shipping box on a countertop in a laboratory without windows or external walls, with thermostatically controlled air temperatures for the duration of the study. The remaining 5 bottles of each product were placed in a closed cardboard box and stored in a programmable incubator[f] in the laboratory for the duration of the study.

The incubator had a 1-time memory capacity of 10 programs consisting of 12 steps per program; 1 program equaled 1 day and each step represented a 2-h portion of the day. The temperature settings were assigned to the programs and steps to mimic actual temperatures recorded in a practice vehicle which most frequently had the highest temperatures of vehicles in a previous study.[10] This truck was located in south-central Texas with an after-market medication and equipment compartment.[10] This was accomplished by averaging the temperature readings for each 2-h interval of vehicle data and using this average temperature as a 2-h step in the program. In the previous study performed, the test vehicle's storage area temperature was recorded every 15 minutes during the summer of 2012, with the temperature ranging from 69.3°F (20.7°C) to 116.9°F (47.2°C). The percent of readings >77°F (25°C) and >86°F (30°C) were 98.1% and 74.7%, respectively, and the percent of days with at least 1 reading >77°F (25°C) and >86°F (30°C) were 100% and 98%, respectively.[10]

All bottles of medication were sampled on d 0, 40, 80, and 120. On each sampling day, a 2 mL aliquot was drawn from each bottle using a 3 mL syringe with a 16 gauge × 1" (2.54 cm) needle, and placed in a sterile cryovial labeled "A." Then, a second aliquot was drawn in the same manner and placed in a separate cryovial labeled "B." After all samples were collected on each sampling day, they were placed in an ultracold freezer (-112°F; -80°C) until the completion of the study.

After the final samples were collected on d 120 and frozen, samples labeled "A" were placed in an insulated container with dry ice and delivered by overnight shipping to an analytical laboratory for analysis. Samples labeled "B" were left in the ultracold freezer as backup samples in case of loss or damage to samples labeled "A".

Samples were analyzed in triplicate by liquid chromatography/mass spectrometry (LC/MS/MS). High performance liquid chromatography tandem mass spectrometry was performed on an Agilent 1200 HPLC system coupled with an Agilent 6410 triple quadrupole mass spectrometer.[g] Analytes were separated on an Ascentis Express C18 column (10 cm x 2.1mm, 2.7 µm)[h] using a mobile phase of H_2O with 0.1% formic acid/acetonitrile with 0.1% formic acid at a constant flow rate of 0.5 mL/min. The LC/MS/MS was run in positive and negative electrospray ionization mode.

Changes in active ingredient concentration were assessed by linear regression. As recommended[4], t-tests[i] were performed to compare slopes of time:concentration curves for room temperature and environmental chamber-stored drugs.

Results

Average slopes of drug concentrations over the 120 d study period are shown in Table 1. Slopes of drug concentrations over 120 d for all 5 drugs were less than 0.04, and there was no statistically significant difference between the slopes of concentrations over time for room temperature (control) vs environmental chamber-stored bottles for any of the drugs. Concentration slopes for individual drugs are shown in Figures 1 thru 5.

Drug efficacy modeling was not performed due to the nonsignificant impact of the environmental chamber temperatures on active ingredient concentrations.

Discussion

Responsible use of veterinary drugs includes proper storage to maintain efficacy. Previous studies have demonstrated elevated temperatures in veterinary vehicles during summer months,[7,10,12] raising concerns that drug efficacy may be compromised. Because the major concern in the present study was temperature, we did not conduct all tests recommended by the FDA CVM[4] to evaluate oxidation, photolysis or pH ranges since the purpose of this study was not to evaluate those potential changes or to establish an expiration date. We also chose to mimic storage conditions for a limited period of time since we did not expect high summer temperatures to exceed 4 months.

The concentrations of dinoprost were outliers at some time points as the concentrations did not follow the expected pattern, as shown in Figure 1. Because the coefficient of variation (ratio of average concentrations from the 3 replicates for each sample) was fairly consistent across all samples and drugs (1 to 8% for most samples; data not shown), and because all but 1 of the lower concentrations were not in the 120 d samples, it is likely there was an error in sample handling or preparation rather than a true decrease in concentration of the active ingredient. The variability across replicates represented by coefficient of variation was also similar for the other drugs tested, but because the concentration of these products were higher, the variability was less obvious in Figures 2 thru 5.

Based on results of this study, we concluded that there was no significant effect on the concentration of active ingredients in the 5 products tested under storage conditions mimicking summer temperatures in a veterinary practice vehicle. Although it is unlikely that drug efficacy would be affected, additional studies are recommended to determine stability of animal drugs once they are opened and in use.[3] It is possible that an increased number of needle punctures

Table 1. Average slope of active pharmaceutical concentrations over 120 d (n=5 bottles/drug/storage condition) based on linear regression of the time:concentration observations.
[Two-sample 2-tailed t-tests were performed to test if there was a difference among slopes from the 2 storage conditions of room temperature and environmental chamber with elevated temperatures; they were not statistically different between the 2 conditions, $P>0.05$]

Drug	Condition	Average slope
Dinoprost	Control	-0.002
	Chamber	-0.002
Flunixin	Control	-0.013
	Chamber	0.000
Gonadorelin	Control	-0.030
	Chamber	-0.024
Tulathromycin	Control	-0.018
	Chamber	0.024
Xylazine	Control	0.013
	Chamber	0.032

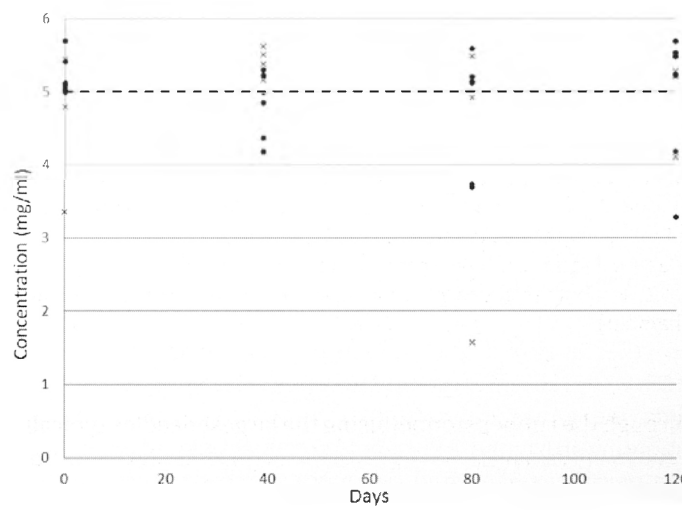

Figure 1. Concentrations of dinoprost after storage in original bottles at room temperature or in an environmental chamber programmed to mimic temperatures found in a veterinary practice vehicle.
[Dashed line is the nominal concentration of dinoprost from the drug label; ● = bottles at room temperature; x = bottles in environmental chamber]

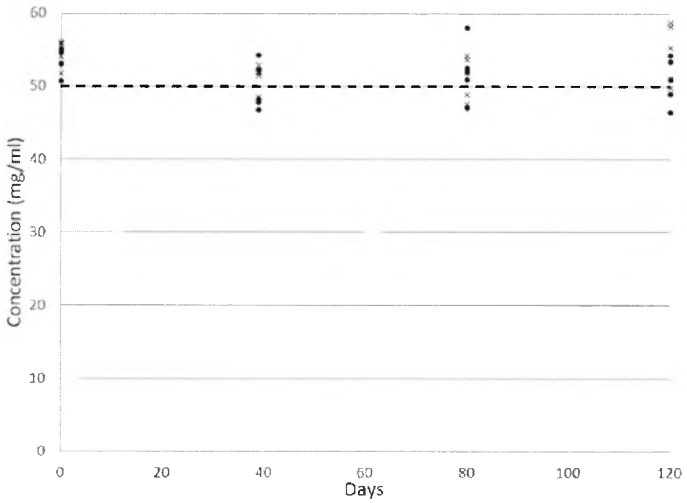

Figure 2. Concentrations of flunixin after storage in original bottles at room temperature or in an environmental chamber programmed to mimic temperatures found in a veterinary practice vehicle.
[Dashed line is the nominal concentration of flunixin from the drug label; ● = bottles at room temperature; x = bottles in environmental chamber]

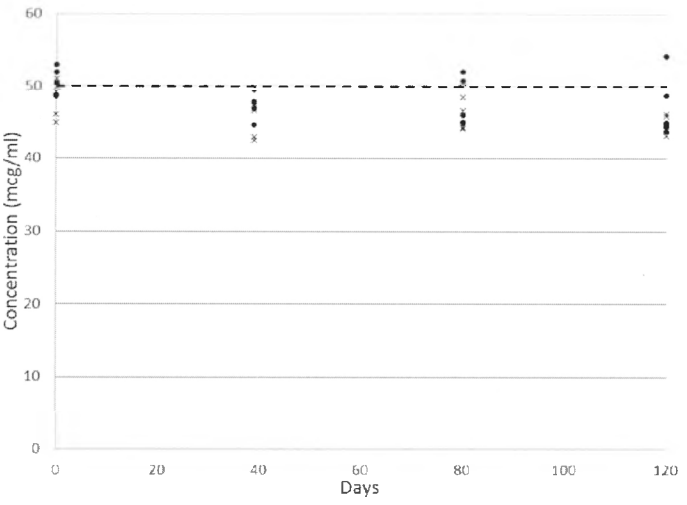

Figure 3. Concentrations of gonadorelin after storage in original bottles at room temperature or in an environmental chamber programmed to mimic temperatures found in a veterinary practice vehicle.
[Dashed line is the nominal concentration of gonadorelin from the drug label; ● = bottles at room temperature; x = bottles in environmental chamber]

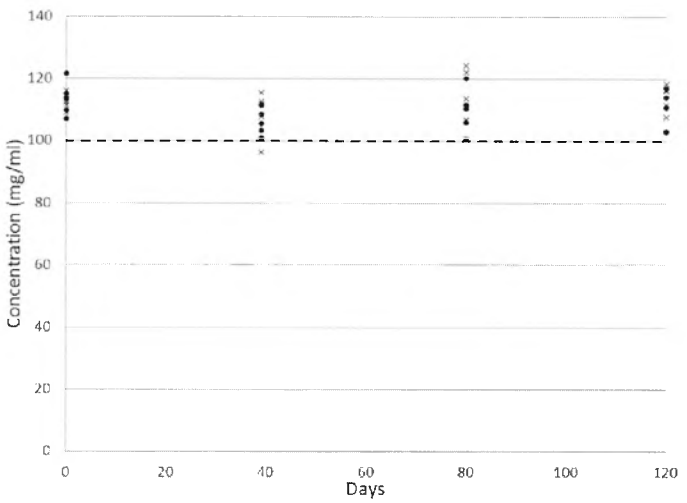

Figure 4. Concentrations of tulathromycin after storage in original bottles at room temperature or in an environmental chamber programmed to mimic temperatures found in a veterinary practice vehicle.
[Dashed line is the nominal concentration of tulathromycin from the drug label; ● = bottles at room temperature; x = bottles in environmental chamber]

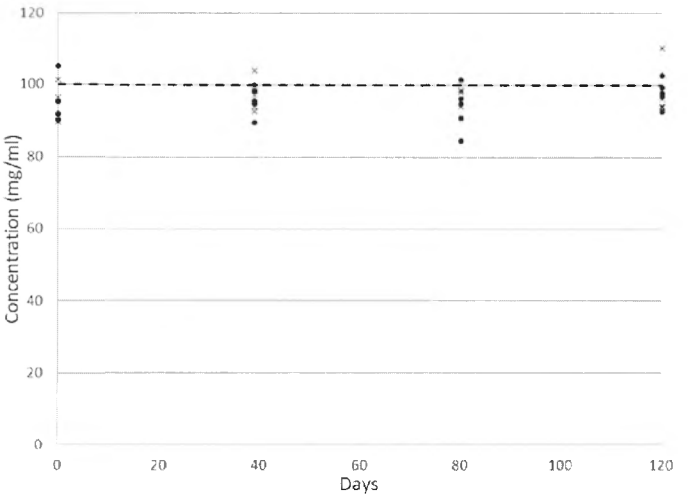

Figure 5. Concentrations of xylazine after storage in original bottles at room temperature or in an environmental chamber programmed to mimic temperatures found in a veterinary practice vehicle.
[Dashed line is the nominal concentration of xylazine from the drug label; ● = bottles at room temperature; x = bottles in environmental chamber]

through the rubber stopper using the largest needles typically used in veterinary practice until the expiration date could affect product stability. In the present study, drugs were stored for 4 months, but we only performed needle punctures to collect aliquots of the drugs for analysis. In addition, we did not analyze for breakdown products for each of the drugs, since that was outside of the scope of the study. Therefore, we cannot make conclusive recommendations about stability after storage under the conditions studied here.

Veterinarians providing veterinary service to clients under conditions where label storage recommendations cannot be maintained are encouraged to take measures to protect the integrity of medications, as concentration is only 1 factor that might affect efficacy. A recent study used computer modeling of the thermal performance of various types of drug storage containers, and recommendations were made to optimize thermal protection of medications during short-term transport and use of veterinary drugs.[9]

Conclusions

Elevated storage temperatures did not significantly impact the product active ingredient concentrations in this study. This study utilized a limited number of products for only 120 days, therefore practitioners are advised to protect all pharmaceuticals from elevated storage temperatures.

Endnotes

[a]Rompun™, Bayer Healthcare, Animal Health Division, Shawnee Mission, KS
[b]Cystorelin®, Merial Limited, Duluth, GA
[c]Banamine®, Merck Animal Health, Madison, NJ
[d]Draxxin®, Zoetis, Parsippany NJ
[e]Lutalyse®, Zoetis, Parsippany, NJ
[f]Panasonic MIR-554-PA, Panasonic Healthcare Company of North America, Wood Dale, IL
[g]Agilent 1200 HPLC and Agilent 6410 mass spectrometer, Agilent Technologies, Santa Clara, CA
[h]53823-U, Supelco Inc., Bellefonte, PA
[i]Microsoft Excel 2016, 16.0.4639.1000, Redmond, WA

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