Stability of three commonly compounded extemporaneous enrofloxacin suspensions for oral administration to exotic animals

Olivia A. Petritz, DVM; David Sanchez-Migallon Guzman, LV, MS, DACZM; Valerie J. Wiebe, Pharm D; Mark G. Papich, DVM, MS, DACVCP

Objective—To evaluate the stability of 3 extemporaneous oral suspensions of enrofloxacin mixed with readily available flavoring vehicles when stored at room temperature (approx 22°C).

Design—Evaluation study.

Samples—3 commonly compounded oral suspensions of enrofloxacin.

Procedures—On day 0, commercially available enrofloxacin tablets were compounded with a mixture of distilled water and corn syrup (formulation A) or cherry syrup (formulation B) flavoring vehicles to create suspensions with a nominal enrofloxacin concentration of 22.95 mg/mL, and 2.27% enrofloxacin injectable solution was compounded with a liquid sweetener (formulation C) to create a suspension with a nominal enrofloxacin concentration of 11.35 mg/mL. Preparations were stored in amber-colored vials at room temperature for 56 days. For each preparation, the enrofloxacin concentration was evaluated with high-performance liquid chromatography at prespecified intervals during the study. The pH, odor, and consistency for all suspensions were recorded at the start and completion of the study.

Results—Relative to the nominal enrofloxacin concentration, the enrofloxacin concentration strength ranged from 95.80% to 100.69% for formulation A, 108.44% to 111.06% for formulation B, and 100.99% to 103.28% for formulation C. A mild pH increase was detected in all 3 suspensions during the study.

Conclusions and Clinical Relevance—Results indicated that, when stored in amber-colored vials at room temperature for 56 days, the enrofloxacin concentration strength in all 3 formulations was retained within acceptance criteria of 90% to 110%. Subjectively, cherry syrup flavoring was better at masking the smell and taste of enrofloxacin than were the other mixing vehicles. (J Am Vet Med Assoc 2013;243:85–90)

Enrofloxacin belongs to the fluoroquinolone family, a group of synthetic antimicrobials derived from nalidixic and oxolinic acid.1 As a group, fluoroquinolones have excellent activity against gram-negative bacteria including Pseudomonas aeruginosa; moderate activity against staphylococci, chlamydiae, and mycoplasmas; and little to no activity against streptococci and anaerobic bacteria.1,2 Following administration, fluoroquinolones are widely distributed throughout the body, and high concentrations are generally detectable in nasal secretions and mucosa, bronchial epithelium,3 prostatic fluid,4 myometrium, cervix, fallopian tubes,5 and bone.6 In the United States, enrofloxacin is commercially available in tablet forms (film coated and chewable) that are approved by the FDA for use in cats and dogs as well as in 2 injectable forms, a solution (22.7 mg/mL) labeled for IM use in dogs and a more concentrated solution (100 mg/mL) labeled for use in cattle and pigs only.7 Unfortunately, there is currently no commercially available oral suspension of enrofloxacin.

In addition to its FDA-approved use in dogs and cats, enrofloxacin has been used in many exotic animal species, including psittacines,8,9 ferrets,10 rabbits,11–14 rodents,15 and reptilian species.16–19 Despite the widespread use of enrofloxacin, there is still a paucity of pharmacokinetic data available for its oral or parenteral use in many nontraditional species. Intramuscular administration of enrofloxacin has resulted in injection-site irritation in psittacine birds20 and local irritation and soft tissue necrosis in several reptilian species18,19; therefore, oral administration is often preferred over IM administration. Commercially available enrofloxacin tablets cannot be administered to many exotic animal species because of their small size; thus, for oral administration of enrofloxacin to those ani-
mals, the practitioner must use either a compounded oral suspension or the injectable (22.7 mg/mL) solution, which anecdotally has a bitter taste. Oral administration of the large animal injectable solution of enrofloxacin (100 mg/mL) is not recommended for any species because it has been associated with severe oral ulceration.24

With some exceptions, the compounding of medications with a sweet or sugar-based flavoring often improves palatability and patient compliance, particularly that of exotic species.22,23 In addition to patient palatability considerations, it is also important to consider potential adverse interactions between the drug and flavoring vehicle when compounding medications. Fluoroquinolones can bind divalent cations and form insoluble complexes that are not absorbed orally.24,25 Many of the commonly used flavoring agents do not contain divalent cations; however, some liquid vitamin solutions, which contain considerable concentrations of iron, copper, and calcium, can significantly reduce oral absorption of fluoroquinolones.26 Alteration of an original formulation of a drug to make a more convenient or palatable oral suspension may compromise the stability of that drug. For certain drugs, even a slight change in the pH of the solution can cause a significant reduction in efficacy.27 The US Pharmacopeial Convention states that individuals who compound drug formulations are responsible for the production of solutions with acceptable strength, quality, and purity in accordance with good compounding practices and relevant scientific data.28 To comply with those principles, knowledge of the compounded medication’s stability and the storage requirements necessary to ensure its efficacy is essential. Several formulas for the in-clinic compounding of enrofloxacin into an oral suspension have been published.7,9,23 Investigators of a study29 reported results for the stability of enrofloxacin after it was mixed with each of 4 formulations of small animal ear cleaner for the treatment of otitis. However, to our knowledge, a study to evaluate the stability of enrofloxacin formulations compounded for oral administration to veterinary patients has not been performed. The objective of the study reported here was to evaluate the stability of 3 commonly compounded extemporaneous oral suspensions of enrofloxacin when stored at room temperature (approx 22°C).

Materials and Methods

Suspension preparation—Three extemporaneous oral suspensions of enrofloxacin were prepared (Appendix). Formulation A was made with commercially available, film-coated enrofloxacin tablets and a vehicle of corn syrup in distilled water. The corn syrup and distilled water were measured and mixed together in a graduated cylinder. The enrofloxacin tablets were placed in the bottom of a small mortar and allowed to sit for 15 minutes in 30 mL of the corn syrup–distilled water solution (1:1). Once the film coating was dissolved, the tablets were pulverized in the mortar with a pestle until a homogeneous paste was formed. The mixture was carefully transferred into a plastic amber-colored 8-oz dispensing vial. Another 30 mL of the corn syrup–distilled water mixture was poured into the mortar and carefully stirred to facilitate the transfer of any remaining enrofloxacin residue to the mortar to the vial. A sufficient quantity of the corn syrup–distilled water vehicle was then added to the vial to bring the final volume of the suspension to 80 mL. This resulted in a suspension with an enrofloxacin concentration of 22.95 mg/mL. Formulation B was made with commercially available, film-coated enrofloxacin tablets and a vehicle of cherry syrup and distilled water in a 50:30 volume ratio by means of the same method as that described for formulation A. Formulation B also had an enrofloxacin concentration of 22.95 mg/mL. Formulation C was prepared with enrofloxacin injectable solution and a liquid sweetener. Equal volumes (40 mL) of the enrofloxacin injectable solution and liquid sweetener were mixed in a plastic amber-colored 8-oz dispensing vial, resulting in a suspension with an enrofloxacin concentration of 11.35 mg/mL.

Sample collection and monitoring—The suspensions were stored at room temperature in sealed plastic amber-colored vials that were exposed to fluorescent room light. On days 0 (compounding), 3, 7, 14, 21, 28, and 56, three 2-mL samples were obtained from each vial for determination of enrofloxacin concentration. Briefly, each vial was shaken until the suspension appeared homogeneous, then a plastic 3-mL oral dosing syringe was used to transfer three 2-mL samples of the suspension to 3 sterile additive-free blood collection tubes. On days 0 and 56, the pH of the suspension in each vial was measured, and 5 individuals subjectively assessed the odor, taste, and uniformity of each suspension. The same researcher (VJW) prepared, sampled, and measured the pH of all suspensions. All samples were stored at 8°C and then were shipped on dry ice overnight (approx 12 hours) for analysis by another researcher (MGP).

Sample analysis—The samples were analyzed by means of HPLC in accordance with guidelines published by the US Pharmacopeia.30 Each sample was thawed and vortexed to resuspend the mixture. For the suspensions that contained corn or cherry syrup, 0.125 µL of each sample was mixed with 2.744 mL of distilled water. For the suspension that contained the liquid sweetener, 250 µL of each sample was mixed with 2.588 mL of distilled water. Then for all samples, 10 µL of each respective mixture was mixed with 990 µL of a mixture of 0.1% trifluoroacetic acid and methanol (85:15). For each formulation, the nominal concentration of enrofloxacin was used to calculate the respective volumes of diluent added to each sample to theoretically yield a diluted sample with an enrofloxacin concentration of 10 µg/mL. An analytic reference standard of enrofloxacin was also diluted with a mixture of 0.1% trifluoroacetic acid and methanol (85:15) to yield a sample with an enrofloxacin concentration of 10 µg/mL. Finally, all samples were filtered to remove any remaining solids and transferred to HPLC-injection vials. For HPLC, 20 µL of each sample was analyzed. The enrofloxacin concentration in each sample was measured by the use of a reverse-phase HPLC column (4.6 mm × 15 cm) and a fluorescence detector set at
an excitation wavelength of 280 nm and an emission wavelength of 300 nm. The isocratic mobile phase consisted of 77% deionized water, 23% acetonitrile, and 0.1% trifluoroacetic acid at a flow rate of 1 mL/min. The retention time for enrofloxacin was approximately 4.0 minutes. Chromatographs were integrated with a commercially available software program.

Statistical analysis—For each formulation on each sample collection day, the mean ± SD enrofloxacin concentration and percentage concentration strength of enrofloxacin were calculated. The percentage concentration strength of enrofloxacin in a sample was calculated as follows: (RU/RS) × (CU/CS) × (P/1,000) × 100, where RU is the response factor for the sample solution, RS is the response factor for the reference standard solution, CU is the nominal enrofloxacin concentration of the formulation from which the sample was obtained, CS is the enrofloxacin concentration of the reference standard solution, and P is the potency (µg of enrofloxacin/mg) of the enrofloxacin reference standard.

Results

Enrofloxacin stability—The mean ± SD enrofloxacin concentration and percentage concentration strength of enrofloxacin, pH, and homogeneity of the 3 formulations during the course of the study were summarized (Table 1). For formulation A, the measured enrofloxacin concentration was slightly less than the nominal enrofloxacin concentration (22.95 mg/mL) on all sample collection days, whereas for formulations B and C, the measured enrofloxacin concentrations were slightly higher than the nominal enrofloxacin concentrations (22.95 mg/mL and 11.35 mg/mL, respectively) on all sample collection days. The percentage concentration strength of enrofloxacin ranged from 95.80% to 100.69% for formulation A, 108.44% to 111.06% for formulation B, and 100.99% to 103.28% for formulation C. At day 56, the enrofloxacin concentrations were > 90% of the respective nominal concentrations of enrofloxacin for all 3 formulations.

Sample monitoring—Within a collection day, variability among the triplicate samples obtained from each formulation was minimal, as evidenced by the fact that all SDs were < 0.28 mg/mL for enrofloxacin concentration and < 1.2% for percentage concentration strength of enrofloxacin. During the 56-day observation period, the pH of all 3 formulations increased slightly (Table 1). Immediately following compounding on day 0 as well as on day 56, formulation B was the most homogeneous suspension. Conversely, formulation C rapidly separated into 2 distinct layers immediately after compounding on day 0 and was completely separated into 2 layers on day 56. Subjectively, the sweet odor of all 3 formulations decreased over time, and the cherry syrup–flavoring vehicle was better than were the other flavoring vehicles at masking the smell and taste of the enrofloxacin.

Discussion

Results of the present study indicated that 3 commonly prepared extemporaneous oral suspensions of enrofloxacin compounded with distilled water and 1 of 3 flavoring agents (corn syrup, cherry syrup, or liquid sweetener) maintained an enrofloxacin concentration strength > 90% for 56 days when stored at room temperature in amber-colored vials. The US Pharmacopeia defines compounding as the preparation, mixing, assembling, packaging, and labeling of a drug or device in accordance with a licensed practitioner's prescription. According to the FDA, compounding of a medi-

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Variable</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Enrofloxacin concentration (mg/mL)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Strength (%)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Enrofloxacin concentration (mg/mL)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Strength (%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Enrofloxacin concentration (mg/mL)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Strength (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homogeneity</td>
<td></td>
</tr>
</tbody>
</table>

— = Not determined.

Table 1—Mean ± SD enrofloxacin concentration and enrofloxacin concentration strength (relative to the nominal enrofloxacin concentration), pH, and homogeneity for 3 commonly compounded enrofloxacin oral suspensions that were stored in plastic amber-colored vials at room temperature (approx 22°C) for 56 days.
cation is acceptable when it is necessary to adminis-
ter a concentration of a drug that is not commercially
available or to improve palatability and, consequently,
patient compliance—2 factors that often arise during
the treatment of exotic animal patients. In veterinary
medicine, compounding is considered legal as long as
certain conditions listed in the AMDUCA extralabel
drug use regulations (21 CFR 530.13) are followed.
The US Pharmacopeia outlines the requirements for
the compounding of nonsterile drug formulations, 2 of
which are that the compounded preparation will con-
tain an acceptable range (≥90% and ≤110%) of the the-
oretically calculated and labeled quantity of the active
ingredient and the assigned do-not-use-beyond date for
the compounded preparation is calculated on the basis
of available data or defaulted to the US Pharmacopeia’s
standards.

Because all compounded medications should be
made from commercial sources of drugs rather than
from bulk-source drugs, which are defined as active
ingredients used in the manufacture of commercial-
ly available drugs, we used commercially available
film-coated enrofloxacin tablets and the small animal
enrofloxacin injectable solution (22.7 mg/mL) for
the compounded suspensions of the present study.
The chewable liver-flavored enrofloxacin tablets that
are labeled for use in dogs were not evaluated in the
present study because we presumed they would be
unpalatable to many exotic animal patients. The
large animal enrofloxacin injectable solution (100
mg/mL) was not used because oral administration
of that product has been associated with mucosal
membrane irritation and oral ulcers, even when it
was diluted and mixed with a flavoring vehicle. The
flavoring vehicles used in the compounded formula-
tions of the present study were chosen on the basis
of the investigators’ prior experience with formulat-
ing palatable medications for exotic animals and the
case of flavoring vehicle availability to veterinarians
or compounding pharmacists.

We chose a 56-day observation period for the pres-
ent study because levofloxacin, a fluoroquinolone la-
beled for human use, has stability for up to 57 days
when compounded to a concentration of 50 mg/mL in
a 1:1 mixture of an oral-suspending vehicle and straw-
berry syrup. The stability of a medication is defined
as the extent to which a product retains, within speci-
fied limits, the same properties and characteristics that
it possessed at the time of its manufacture. Accord-
ing to the US Pharmacopeia, when no data are avail-
able, aqueous formulations prepared from solid-form
ingredients should be refrigerated and the do-not-use-
beyond date must be set at 14 days after manufacture.

To our knowledge, prior to the present study, data on
the stability of compounded oral suspensions of enro-
foxacin were lacking, and it was unknown whether
those suspensions were stable beyond the US Phar-
macopeia’s empirical 14-day period. The US Phar-
macopeial Convention describes 5 types of stability:
chemical, physical, microbiological, therapeutic, and
toxicological. In the present study, only chemical and
physical attributes of the compounded enrofloxacin
suspensions were evaluated.

To ensure chemical stability, the concentration
strength of the active drug in a compounded medica-
tion should not be <90% or >110% of the labeled drug
concentration. In the present study, all 3 formulations
retained >90% of their respective original enroflo-
xacon concentrations at day 56. In fact, the percentage
concentration strength, or recovery, of enrofloxacin from
formulations B and C was >100% at all sample col-
collection days. Several factors may account for a >100%
recovery of enrofloxacin from the formulations of the
present study, including variability in the enrofloxacin
concentration within the commercial tablets or inject-
able solution, insufficient dilution of the suspensions to
a final volume to 80 mL, measurement error that result-
ed in the inclusion of excessive amounts of enrofloxa-
cin tablets or solution in the formulations, or random
error associated with the HPLC method. Storage tem-
perature and pH can also substantially affect the chemi-
cal stability of a drug because of hydrolysis or oxidative
reactions. A change in pH of only 1 can decrease the
chemical stability of a drug by a factor of ≥10 times. In
the present study, the pH of all 3 formulations be-
came more alkaline over the 56-day observation period;
or however, the chemical stability of the formulations
was not substantially affected.

All formulations were stored at room temperature
throughout the present study to mimic conditions un-
der which drugs are commonly stored at a veterinary
hospital or the home of a veterinary patient. Refrigera-
tion may have affected the stability of the 3 formulations
evaluated in this study and warrants further research.
Exposure to UV light may cause oxidation and lysis of
covalent bonds, which reduces the stability of certain
drugs. Fluoroquinolones are sensitive to degradation by
UV or natural light, particularly when they are in an
aqueous suspension; only 36% of the fluoroquinolone
oraloxacin was recovered from an aqueous suspension
that was subjected to UV light for 24 hours. In the
present study, storage of the enrofloxacin suspensions
in amber-colored vials appeared to provide sufficient
protection against chemical degradation of the suspen-
sions secondary to fluorescent light exposure for the
entire 56-day observation period.

For aqueous suspensions of medications, physi-
cal signs of drug instability include precipitation or
cloudiness, formation of a solid phase that cannot be
resuspended, crystallization, or evidence of microbial
growth such as discoloration, turbidity, or gas forma-
tion. None of these signs were detected in the 3 enro-
foxacin formulations of the present study. On day 56,
formulation C had completely separated into 2 distinct
layers; however, the suspension became homogenous
after minimal shaking. Microbiological cultures of the
enrofloxacin suspensions were outside the scope of the
present study but may have provided further informa-
tion as to their long-term stability. Bacterial growth can
affect stability as well as the appearance, pH, and palat-
ability of compounded aqueous medications. Because
enrofloxacin is a potent antimicrobial and relevant
changes in the suspensions with regard to enrofloxa-
cin concentration, pH, and homogeneity were not de-
tected, it was unlikely that substantial bacterial growth
developed during the 56-day observation period.
The lack of a commercially available oral enrofloxacin suspension has resulted in a variety of compounded formulations that use various enrofloxacin products mixed with flavoring agents and vehicles to facilitate oral enrofloxacin administration to exotic animals. To our knowledge, prior to the present study, the chemical and physical stability of most of those suspensions had not been evaluated. Results of the present study indicated that the 3 extemporaneous enrofloxacin oral suspensions assessed were chemically and physically stable for at least 56 days when stored in amber-colored vials at room temperature. Because fluoroquinolones are susceptible to chemical inactivation by chelation with divalent cations and exposure to UV or natural light, proper precautions should be taken during and after compounding enrofloxacin to ensure its stability. Further research on the stability of compounded medications used in veterinary practice is warranted.

References


Continued on next page.
Appendix

Formulations for 3 extemporaneous oral suspensions of enrofloxacin mixed with readily available flavoring vehicles that were stored in plastic amber-colored vials at room temperature (approx 22°C) for 56 days.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Component</th>
<th>Amount</th>
<th>Nominal enrofloxacin concentration in the formulation (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Enrofloxacina film-coated 68-mg tablets</td>
<td>27 tablets 40 mL</td>
<td>22.95</td>
</tr>
<tr>
<td></td>
<td>Corn syrup¹</td>
<td>40 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>40 mL</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Enrofloxacina film-coated 68-mg tablets</td>
<td>27 tablets 50 mL</td>
<td>22.95</td>
</tr>
<tr>
<td></td>
<td>Cherry syrup¹</td>
<td>50 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>30 mL</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Enrofloxacina injectable solution</td>
<td>40 mL</td>
<td>11.35</td>
</tr>
<tr>
<td></td>
<td>Liquid sweetener²</td>
<td>40 mL</td>
<td></td>
</tr>
</tbody>
</table>

From this month's AJVR

Effects of chemical restraint on electroretinograms recorded sequentially in awake, sedated, and anesthetized dogs
Kate S. Freeman et al

Objective—To quantitatively and qualitatively compare electroretinography (ERG) recordings in awake, sedated, and anesthetized dogs.

Animals—Six 6-month-old Beagles.

Procedures—A brief ERG protocol for dogs was used. Following 1-minute and subsequent 5-minute dark adaptation, ocular mixed rod-cone responses were recorded bilaterally with a handheld multispecies ERG device with dogs in each of 3 states of consciousness: awake, sedated (dexmedetomidine and butorphanol), and anesthetized (atropine and hydromorphone, followed by propofol and midazolam and anesthetic maintenance with isoflurane). Low- and high-frequency noise levels were quantified via Fourier analysis, and the effect of consciousness state on signal amplitude, implicit time, and noise was analyzed via repeated-measures ANOVA. In addition, 13 veterinary ophthalmologists who were unaware of the dogs’ consciousness states subjectively graded the ERG recording quality, and scores for each tracing were compared.

Results—ERG amplitudes were highest in awake dogs and lowest in anesthetized dogs. Implicit times were shortest in alert dogs and longest in anesthetized dogs. Differences in b-wave amplitudes and a-wave implicit times were significant. Neither low- nor high-frequency noise levels differed significantly among consciousness states. Furthermore, no significant differences were identified among observers’ scores assigned to ERG tracings.

Conclusions and Clinical Relevance—Anesthesia and sedation resulted in significant attenuation and delay of ERG responses in dogs. Chemical restraint of dogs had no consistently significant effect on low- or high-frequency noise levels or on observer perception of signal quality. (Am J Vet Res 2013;74:1036–1042)