Effects of compounding and storage conditions on stability of pergolide mesylate

Jennifer L. Davis, DVM, PhD, DACVIM, DACVCP;
Loren Madden Kirk; Gigi S. Davidson, RPh; Mark G. Papich, DVM, MS, DACVCP

Objective—To determine the effects of temperature and light over a 35-day period on stability of pergolide mesylate after compounding in an aqueous vehicle.

Design—Evaluation study.

Procedures—Pergolide was compounded into a formulation with a final target concentration of 1 mg/mL. Aliquots of the formulation were then stored at –20°, 8°, 25°, or 37°C without exposure to light or at 25°C with exposure to light for 35 days. Samples were assayed in triplicate by means of high-pressure liquid chromatography immediately after compounding and after 1, 7, 14, 21, and 35 days of storage.

Results—Mean ± SD concentration of pergolide in the formulation immediately after compounding was 1.05 ± 0.086 mg/mL. Samples exposed to light while stored at 25°C had undergone excessive degradation by day 14, samples stored at 37°C had undergone excessive degradation by day 21, and samples stored at 25°C without exposure to light had undergone excessive degradation by day 35. The decrease in expected concentration corresponded with the appearance of degradation peaks in chromatograms and with a change in color of the formulation.

Conclusions and Clinical Relevance—Results indicated that pergolide mesylate was unstable after compounding in an aqueous vehicle and that storage conditions had an effect on stability of the compounded formulation. Compounded pergolide formulations in aqueous vehicles should be stored in a dark container, protected from light, and refrigerated and should not be used > 30 days after produced. Formulations that have undergone a color change should be considered unstable and discarded. (*J Am Vet Med Assoc* 2009;234:385–389)

Pergolide mesylate is an orally administered ergot-derived dopamine agonist used to treat Parkinson's disease in humans and pituitary pars intermedia dysfunction (Cushing's syndrome) in horses. In March 2007, the US FDA announced a voluntary withdrawal of human products containing pergolide following reports of a possible association between pergolide use and development of adverse cardiac effects, including valvular dysfunction.[1] Because there currently are no commercial products containing pergolide approved for use in horses, the withdrawal of human products has created a dilemma for veterinarians treating horses with this drug.

To address concerns about the availability of pergolide products for treatment of pituitary pars intermedia dysfunction in horses, the FDA Center for Veterinary Medicine has been working with the drug's manufacturers to make the drug available.[2] In particular, the FDA has issued a limited exemption allowing pergolide products to be compounded from bulk substance.[2] Although this compounding is currently allowed, the FDA has indicated that compounded products will be closely monitored for development of any adverse or unexpected side effects.

According to the US Pharmacopeial Convention,[3] individuals compounding drug formulations are responsible for producing preparations of acceptable strength, quality, and purity with appropriate packaging and labeling in accordance with good compounding practices, official standards, and relevant scientific data and information. This includes performing appropriate stability evaluations or obtaining necessary published information to ensure that compounded preparations maintain expected strength, quality, purity, and other characteristics at least until the labeled date beyond which the product should not be used.[3]

To our knowledge, there currently is no published information on the stability of compounded pergolide formulations. Importantly, although it is known that the chemical form of pergolide mesylate is unstable when exposed to light,[4,5] it is not known how long liquid pergolide formulations prepared with vehicles commonly used by compounding pharmacies will be stable when stored under various conditions. The purpose of the study reported here, therefore, was to determine the effects of temperature and light over a 35-day period on stability of pergolide mesylate after compounding in an aqueous vehicle.

Materials and Methods

Study protocol—A liquid pergolide formulation for oral administration prepared by a licensed pharmacist

From the Departments of Clinical Sciences (Davis), Clinical Pharmacy (Kirk, Davidson), and Molecular and Biomedical Sciences (Papich), College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606.

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Address correspondence to Dr. Davis.

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by means of standard compounding pharmacy protocols was used in the study. Pergolide mesylate[a] powder (purity, 99.1%) used for preparation of the formulation was purchased from a commercial supplier and refrigerated at 8°C until used. In initial testing, the formulation was compounded by means of a mortar and pestle technique. However, a substantial proportion of the suspending vehicle was lost during the grinding process, which resulted in initial drug concentrations that were 20% higher than expected. Thus, a syringe technique was used to prepare the pergolide formulation. In brief, two 35-mL syringes that had been calibrated with a volumetric pipette[b] were attached to a fluid-dispensing connector.[c] The plunger of 1 syringe was removed and set aside, and 10 mL of the vehicle for oral suspension[d] (**Appendix 1**) that had been drawn up into a 12-mL Luer-lock syringe was added to this open syringe. Twenty milligrams of pergolide mesylate powder that had been weighed on a calibrated and balanced digital scale was then added to the vehicle for oral suspension in the open syringe. The powder was mixed with the suspending agent by replacing the plunger in the 35-mL syringe and depressing the plunger to force the mixture through the fluid-dispensing connector into the empty 35-mL syringe. This was repeated for a total of 50 depressions until a uniformly suspended mixture was achieved. Ten milliliters of a vehicle for oral solution[e] (**Appendix 2**) was then added in a similar manner to create a 20-mL suspension containing 1 mg of pergolide/mL.

The stock pergolide formulation was dispensed into a 250-mL glass beaker, and aliquots (500 µL) of the formulation were dispensed with a 500-µL calibrated pipette into thirty 5-mL storage tubes.[f] Tubes were placed in brown, light-protective, ziplock bags and stored in a warm environment (37°C [98.6°F]), at room temperature (25° to 27°C [77° to 80.6°F]), in a refrigerator (8°C [46.4°F]), or in a freezer (–20°C [–4°F]). Additional tubes were placed in a clear, ziplock bag and stored at room temperature where they were exposed to light.

Pergolide concentration was measured immediately after compounding (day 0) and after 1, 7, 14, 21, and 35 days of storage. For determination of pergolide concentration, a single tube from each storage environment was brought to room temperature for analysis. Samples were visually inspected prior to analysis to detect any changes in color.

Determination of pergolide concentration—Pergolide concentration was determined by means of high-pressure liquid chromatography with UV detection. The apparatus consisted of a pump and autosampler,[g] UV detector,[h] and computer for data collection and analysis.[i] A C18 reverse-phase column and a guard column[j] were used for separations. The mobile phase consisted of 50% acetonitrile and 50% 0.01M octane sulphonate buffer (pH adjusted to 2.2 with glacial acetic acid). The UV detection was performed at 223 nm. The injection volume was 25 µL, and the retention time of pergolide mesylate was between 3.6 and 4.0 minutes.

Calibration curves were prepared with a pergolide mesylate reference standard[k] each day prior to analysis of test samples. Drug standard was dissolved in 100% methanol to a concentration of 1 mg of pergolide mesylate/mL. This solution was further diluted with mobile phase to create a calibration curve that was linear ($R^2 > 0.99$) for pergolide concentrations ranging from 1 to 20 µg/mL. Bulk powder used in the compounded formulation was tested for potency by dissolving the powder in an identical manner to the reference standard and further diluting it to a concentration of 10 µg/mL. The lower limit of quantification for the assay was 0.1 µg/mL. The accuracy and precision of the assay were determined at concentrations of 20, 5, and 1 µg/mL and reported as mean ± SD. Accuracy of the HPLC assay was within 1.95 ± 1.56% of the true value, and precision was within 2.01 ± 1.05% of the mean.

Test samples were prepared by diluting the 500-µL aliquot of the pergolide formulation with 4.5 mL of 50% methanol and 50% 0.01N HCl (pH, 2.4) and sonicating the mixture for 5 minutes. This mixture was further diluted by adding 100 µL of the resulting solution to 900 µL of mobile phase, resulting in a nominal pergolide concentration of 10 µg/mL. The final solution was injected directly onto the liquid chromatography apparatus. All samples were run in triplicate, and values are reported as the mean and SD of the 3 replicates.

Data analysis—Initial concentration of the pergolide formulation was calculated as the mean concentration of 4 samples tested immediately after compounding and was designated as 100%. Owing to the small sample size, the 95% confidence interval was calculated for reported sample concentrations, and excessive degradation was defined as a sample concentration > 10% lower than the lower confidence limit for the initial concentration.

Linear regression was performed with standard software[l] to determine the slope and intercept of the concentration-versus-time curve, and for each storage condition, the day on which the pergolide concentration was 90% of the lower confidence limit for the initial concentration was calculated.

Monte Carlo simulations were also performed with standard software[m] to estimate, for each storage condition, the day on which pergolide concentration was 90% of the lower confidence limit of the initial concentration. Each Monte Carlo run included 1,000 simulations, representing 1,000 random formulations of pergolide mesylate, and mean and range of number of days when the formulation would undergo excessive degradation were calculated for each storage condition.

Results

Initial concentration of the bulk powder used to compound the formulation was within the expected range, compared with the reference standard. Mean ± SD per-

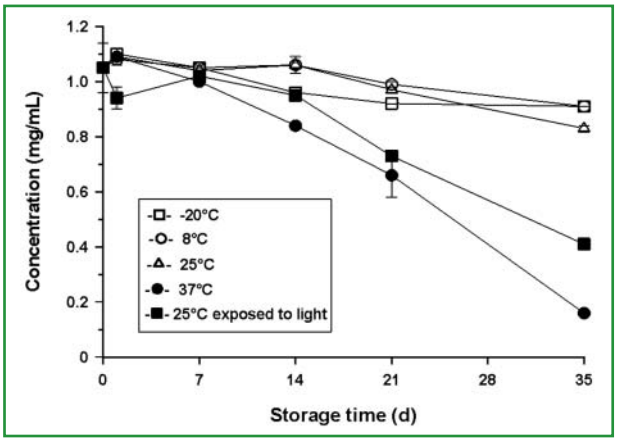

Figure 1—Pergolide concentrations in samples of a pergolide formulation compounded in an aqueous vehicle that had been stored under various conditions. Concentrations were determined by means of high-pressure liquid chromatography, and data represent mean and SD of 3 replicate determinations for each sample.

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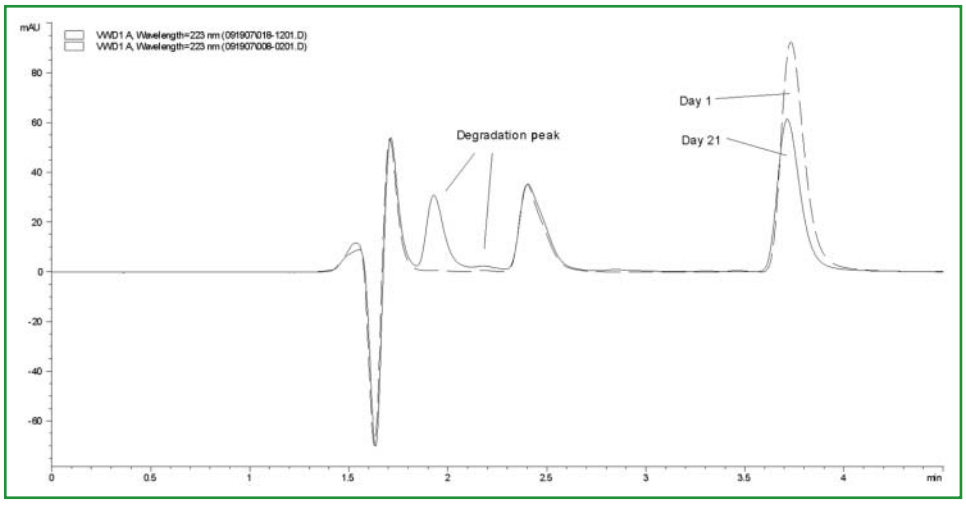

Figure 2—Sample chromatograms of samples of a compounded pergolide formulation that had been stored at room temperature (approx 25° to 27°C [77° to 80.6°F]) and exposed to light for 1 (dashed line) and 21 (solid line) days. For the sample that had been stored 21 days, notice the decrease in the peak height for pergolide and the presence of degradation peaks.

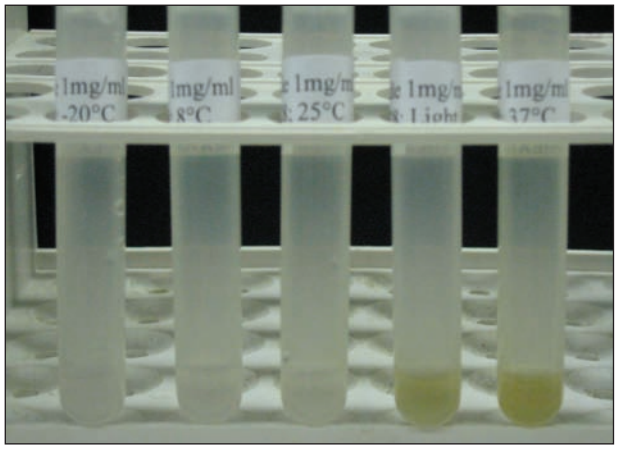

Figure 3—Appearance of samples of a compounded pergolide formulation that had been stored at various temperatures for 35 days.

golide concentration of the formulation immediately after compounding was 1.05 ± 0.086 mg/mL. The 95% confidence interval for the initial concentration was 0.96 to 1.14 mg/mL. Thus, given the lower confidence limit of 0.96 mg/mL, any sample that had a concentration ≤ 0.869 mg/mL was considered to have undergone excessive degradation. On the basis of this criterion, samples exposed to light while stored at room temperature (25° to 27°C) had undergone excessive degradation by day 14 (**Figure 1**), samples stored at 37°C had undergone excessive degradation by day 21, and samples stored at room temperature without exposure to light had undergone excessive degradation by day 35. Samples stored at –20°C or at 8°C had concentrations > 0.869 mg/mL throughout the 35-day study period. By day 35 of the study, the formulation had degraded by 7.2%, 6.6%, 15.1%, and 57.7%, respectively, when stored without exposure to light at –20°, 8°, 25°, and 37°C and by 83.5% when stored at 25°C and exposed to light. Stated another way, concentrations in the formulation were 92.8%, 93.4%, 84.9%, and 42.3% of the initial concentration, respectively, after 35 days of storage without exposure to light at –20°, 8°, 25°, and 37°C and 16.5% of the initial concentration after 35 days of storage at 25°C and exposure to light. Degradation peaks were evident on the chromatograms at times corresponding to the decreased concentrations (**Figure 2**). A change in color from opaque white to brown was also evident at times corresponding to decreased concentrations (**Figure 3**). Results of linear regression analysis of concentration-versus-time curves to determine the time needed for excessive degradation to occur under each of the storage conditions and of Monte Carlo simulations of the time needed for excessive degradation to occur were similar, except that time calculated by means of linear regression analysis for storage at 8°C was substantially longer than mean time calculated by use of Monte Carlo simulations (**Table 1**).

Discussion

Results of the present study indicated that pergolide mesylate was unstable after compounding in an aqueous vehicle

Table 1—Projected times for excessive degradation (ie, concentration < 90% of the lower confidence limit for the initial concentration) to occur in samples of a compounded pergolide formulation stored at various temperatures.

Storage temperature (°C)	Linear regression (d)	Monte Carlo simulations		
		Mean (d)	90% CI (d)	Range (d)
–20	41	32	25–49	20–62
8	59	39	35–50	26–60
25	33	29	24–34	21–42
37	9	11	6–15	3–21
25 (exposed to light)	8	10	8–12	7–14

Projected times for excessive degradation to occur were determined by means of linear regression analysis of sample concentrations or Monte Carlo simulations. All samples were stored in light-protective bags, except samples stored at 25°C that were exposed to light.
CI = Confidence interval.

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and that storage conditions had an effect on stability of the compounded formulation. Compounded pergolide formulations in aqueous vehicles should be stored in a dark container, protected from light, and refrigerated and should not be used > 30 days after produced. Compounded pergolide formulations that have undergone a color change should be considered unstable and should be discarded, owing to the potential for drug degradation and a corresponding lack of therapeutic effect.

The US Pharmacopeial Convention describes 5 types of stability: chemical, physical, microbiologic, therapeutic, and toxicologic.[6] Types of stability evaluated in the present study consisted of chemical stability, including labeled potency, and physical stability, including appearance.

Concentration of the initial formulation in the present study (mean ± SD, 1.05 ± 0.086 mg/mL) was slightly higher than the target concentration of 1 mg/mL. However, it was within limits established by the US Pharmacopeial Convention that compounded preparations not be less than 90% or more than 110% of the labeled concentration.[7]

In the present study, we tested stability of pergolide after compounding in 2 aqueous vehicles that meet standards of the national formulary.[3] These vehicles[d,e] were chosen because they are commonly used in compounding pharmacies as suspending and flavoring agents. The vehicle for oral suspension[d] contains 97% purified water, and the vehicle for oral solution[e] contains as much as 37% water. Our results indicated that pergolide was not stable when compounded in these vehicles, and a potential mechanism for this instability would be hydrolysis of the mesylate ester portion of the molecule.[8] Other drugs that commonly undergo hydrolysis after mixing with aqueous vehicles include β-lactam antimicrobials, particularly cephalosporins.[9] An oxidation reaction may have occurred as well, which resulted in the color change that was evident in the degraded samples.[8]

We also found in the present study that the stability of our pergolide formulation varied greatly depending on the storage conditions. Photodegradation has previously been reported to occur with pergolide,[4,5,10] and exposure to light had a substantial effect on drug stability in the present study, with samples exposed to light while stored at room temperature having undergone excessive degradation by day 14. The most common degradation products following exposure of pergolide to light are pergolide sulphoxide and pergolide sulfone.[4] In rodents, these metabolites have dopamine agonist activity similar to that of the parent compound.[11] However, no studies exist on the activity of these compounds in horses, and because of a lack of reference standards, we were unable to specifically determine degradation products present in samples in the present study. A previous study[4] demonstrated the presence of up to 16 other degradation products following photodegradation, none of which were identified or known to have any dopamine agonist activity.

Photodegradation of pergolide has been reported to be concentration dependent, with higher concentrations in the initial formulation causing the product to degrade faster.[4] A target concentration of 1 mg/mL was used in the present study because this is a common concentration for compounded formulations of pergolide used in horses. Because use of higher concentrations may result in faster degradation, we do not recommend the use of higher concentrations when compounding pergolide formulations.

Storing the pergolide formulation at 37°C also had a profound effect on the drug's stability in the present study. This temperature was chosen to simulate conditions that might be found in horse barns during the summer months in warmer climates. Examination of sample concentrations indicated that samples stored at this temperature had undergone excessive degradation between 14 and 21 days after compounding. By contrast, linear regression analysis and Monte Carlo simulations of the formulation predicted much shorter times to degradation (9 and 11 days, respectively) when stored at this temperature. This discrepancy most likely represented a mathematical artifact caused by errors associated with the fact that the degradation curve was more sigmoidal than linear. Pergolide degradation products are hypothesized to catalyze the degradation reaction, causing a faster degradation rate as time progresses and a sigmoidal degradation curve.[4] To the author's knowledge, instability of pergolide formulations at high temperatures has not been reported.

Storage at room temperature (25°C) also resulted in excessive degradation in the present study, although this occurred at a slower rate than when samples were stored at 37°C, with the formulation expected to be stable when stored at this temperature (without exposure to light) for an average of 33 or 29 days, respectively, on the basis of results of linear regression analysis and Monte Carlo simulations. Storage at –20°C did not result in excessive degradation during the study period, and linear regression analysis suggested that the formulation would be stable for 41 days when stored at this temperature, although Monte Carlo simulations predicted that the formulation would be stable for a mean of only 32 days.

Storage of the pergolide formulation at 8°C (ie, refrigerated) resulted in the least degradation in the present study. Linear regression analysis suggested that the formulation would be stable when refrigerated for 59 days, and Monte Carlo simulations predicted that the formulation would be stable when refrigerated for a mean of 39 days. Thus, we recommend that compounded pergolide formulations be stored in a refrigerator at temperatures between 2° and 8°C.

An interesting aspect of the present study was the difficulty encountered in compounding the original formulation. Use of a mortar and pestle technique resulted in a formulation with a pergolide concentration much higher than expected. The most likely cause of this high concentration was the loss of suspending vehicle on the surface of the mortar and pestle during mixing, as the final volume of the suspension was less than the intended 20 mL. Additionally, a target concentration of only 1 mg/mL is more difficult to achieve than are higher concentrations. Importantly, starting with commercially available tablets, even if they were still available, would have increased the level of difficulty because although individual tablets contain between 0.9 and 1.1 mg of pergolide, the exact potency of each tablet is unknown. Additionally, use of commercially available tablets to formulate oral suspensions contributes excipients and binders important for tablet compression that also make precise formulation difficult. All of these factors further illustrate the variability that can be obtained while

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compounding preparations and emphasize the need for standardized formulas and methods for compounding drug preparations used in animals.

Finally, it must be stressed that the exemption allowing compounding of pergolide from bulk substance is special and, hopefully, temporary. These formulations may still be subject to regulatory actions by the FDA if they prove to be harmful to patients or their human caregivers, and the exemption will be rescinded if a commercially available pergolide formulation is marketed. Other regulations for compounding pharmacies still apply, including regulations prohibiting pharmacies from manufacturing and distributing these products in mass quantities outside of a valid veterinarian-client-patient relationship.[12] For any other drug intended for use in animals that has not received a similar exemption, it is still considered illegal to compound from bulk substances as well as to produce products from bulk substance that mimic FDA-approved products.[12] The US Pharmacopeial Convention has recently developed veterinary monographs for drugs such as pergolide and potassium bromide that are not available for treatment of animals through any source other than compounding pharmacists.[13] Veterinarians should seek out compounding pharmacists that use this officially recognized formula.

a. Professional Compounding Centers of America, lot No. C119829, Houston, Tex.
b. Fisher Scientific, Fair Lawn, NJ.
c. B Braun Medical Inc, Bethlehem, Pa.
d. Ora-Plus Vehicle for Oral Suspension-NF, Paddock Laboratories Inc, Minneapolis, Minn.
e. Ora-Sweet Vehicle for Oral Solution-NF, Paddock Laboratories Inc, Minneapolis, Minn.
f. Cryule polypropylene vials, Wheaton Science Products, Millville, NJ.
g. Agilent series 1100, Agilent Technologies, Wilmington, Del.
h. Agilent series 1050 variable wavelength detector, Agilent Technologies, Wilmington, Del.
i. HPChem, Agilent series 1100 Chemstation software, Agilent Technologies, Wilmington, Del.
j. Zorbax RX-C18 4.6 × 150-mm reverse-phase column and Zorbax RX-C18 guard column, Agilent Technologies, Wilmington, Del.
k. United States Pharmacopeia, Rockville, Md.
l. Fig.P, version 2.98, Fig.P Software Corp, Durham, NC.
m. Crystal Ball, version 7.0, Oracle, Denver, Colo.

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7. United States Pharmacopeia (USP-NF). USP30–NF25. Chapter 1191: stability considerations in dispensing practice. In: *The United States Pharmacopeia*. Rockville, Md: US Pharmacopeial Convention, 2007;655–658.
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12. US FDA Center for Veterinary Medicine. Chapter 6, Subchapter 600, Sec. 608.400—compounding of drugs for use in animals, July 2003. In: *Compliance policy guide: compliance policy guidance for FDA staff and industry*. Rockville, Md: FDA, 2003.
13. Pergolide oral suspension, veterinary. In: *The pharmacists' pharmacopeia*. 2nd ed. Rockville, Md: US Pharmacopeial Convention, 2008;461.

Appendix 1

Composition of an aqueous vehicle for oral suspension[d] used for compounding.

Ingredient	Amount
Cellulose, microcrystalline	800 mg
Xanthan gum	200 mg
Carrageenan	150 mg
Carboxymethylcellulose sodium (high viscosity)	25 mg
Citric acid	250 mg
Sodium phosphate, dibasic	120 mg
Simethicone	0.1 mL
Potassium sorbate	100 mg
Methylparaben	100 mg
Purified water (sufficient quantity to make 100 mL)	NA

NA = Not applicable.

Appendix 2

Composition of an aqueous vehicle for oral solution[e] used for compounding.

Ingredient	Amount
Sucrose	80 mg
Glycerin	5 g
Sorbitol	5 g
Sodium phosphate, dibasic	120 mg
Citric acid	200 mg
Potassium sorbate	100 mg
Methylparaben	100 mg
Purified water (sufficient quantity to make 100 mL)	NA

NA = Not applicable.

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