

Stability of Methimazole in Poloxamer Lecithin Organogel to Determine Beyond-Use Date

Alyssa Pignato, BS
Marvin Pankaskie, PhD
Christine Birnie, RPh, PhD
Wegmans School of Pharmacy
St. John Fisher College
Rochester, New York

INTRODUCTION

A prevalent endocrine disorder for middle-aged and older cats is hyperthyroidism. This disease is characterized by the overproduction of thyroxine (T4) and triiodothyronine (T3), two hormones that are heavily involved in the body's metabolism. Commonly a cat is diagnosed as having hyperthyroidism when the T4 levels exceed the normal range of 1.0 ng/dL to 4 ng/dL. Although the T4 is the more frequently monitored, tests can be done to confirm if the T3 hormone is also in excess.¹ The cause of hyperthyroidism in cats is still debatable, but some supposed environmental agents include iodine, soybean, phthalates, and polyphenols found in canned foods.² Although surgical options are available for permanent treatment, a less invasive pharmacological therapy is the most popular choice.²

The thioimidazole antithyroid agent methimazole is used as a long-term solution for managing hyperthyroidism. Oral methimazole was found to regulate the production of thyroxine to normal levels within 7 to 14 days.² Side effects of the drug include vomiting, anorexia, and lethargy. The main source of vomiting and anorexia can be attributed to the bitter taste of the drug. Aside from the gastrointestinal (GI) side effects it can also be difficult to give an animal an oral

ABSTRACT

The purpose of this stability study was to determine the beyond-use date of the active ingredient methimazole extemporaneously prepared for transdermal absorption to treat feline hyperthyroidism. The methimazole was prepared in poloxamer lecithin organogel and subjected to a three-month stability study. It was determined that under room temperature conditions (25°C ± 2°/60% RH ± 5%) the methimazole concentration remained above 90% after 62 days. An accelerated condition of 35°C and refrigerated condition of 5°C were also employed to see how temperature affected the stability of the drug. Under all three conditions the methimazole concentration dropped below 90% after day 62. It was observed that the refrigerated conditions appeared to be least stable both physically and chemically. A control sample was also prepared, stored, and packaged similarly to the sample preparations. As a result, the beyond-use date was determined to be 60 days at room temperature. The drug preparation should not be stored in a refrigerated condition.

dose, such as a tablet. To avoid the challenge of administering the drug orally, a compounded transdermal formulation has become a more feasible alternative.³ The medication has been effectively compounded in a poloxamer lecithin organogel (PLO) that is rubbed on the inner pinna of the cat's ear. A recent study tested the efficacy of the transdermal formulation in PLO. The study concluded that the transdermal formulation resulted in a significant decrease in T4 levels for all 10 cats tested.² It was also reported that no GI side effects were seen in any of the participating cats. A separate study found that the oral route did produce better results after two weeks. But once the blood samples were compared to a reference after four weeks, no significant difference was observed between the oral or transdermal methods. This study also noted that only the cats treated with the

medication orally showed visible GI side effects.⁴ These studies support that the topical methimazole is not only effective in decreasing the T4 hormone but also successful in reducing some of the major side effects encountered with the oral route.

In addition to the PLO formulation, other compounded formulas of topical methimazole are currently being used. Lipoderm and anhydrous PLO formulas have also been used for what appears to be an effective base for methimazole transdermal (Professional Compounding Centers of America [PCCA], Houston, Texas, personal communication on September 24, 2008).⁵ Despite some potential advantages to these other base formulas, the majority of therapeutic studies incorporate methimazole in the original PLO formulation, and, for this reason, the study was limited to that same formulation.

Currently there is inadequate stability data available for compounded methimazole in PLO. In accordance with the *United States Pharmacopeia (USP)* guidelines for compounding, this formulation should be assigned a 14-day beyond-use date (BUD), stored at refrigerated conditions (4°C to 8°C) due to the presence of water in the formulation.⁶ The *USP* has recently expanded its standard setting to include compounded veterinary formulations. The monograph for methimazole transdermal gel was reported to be “in development” due to needed stability testing.⁷ Therefore, it is of great interest to understand the stability of the drug over a long period of time, not only to determine a BUD, but to further aid in other aspects of methimazole research. Based on this rationale, the aim of this study was to determine a BUD for methimazole in PLO gel by a three-month stability study under standard conditions (25°C), accelerated conditions (35°C), and refrigerated (5°C) conditions.

MATERIALS AND METHODS

Materials

Methimazole (Lot C127616), lecithin soya fine powder (Lot C127601), Poloxamer 407 NF (Lot C132541), Isopropyl Palmitate NF (Lot# C127510), and sorbic acid (Lot C12815) were obtained from PCCA (Houston, Texas). High performance liquid chromatographic (HPLC)-grade methanol (Lot 096964) and potassium phosphate monobasic (Lot 064592) were supplied by Fisher Scientific (Pittsburgh, Pennsylvania).

Preparation of Methimazole Gel

The compounded preparation followed the previously published formulation.⁸ The lecithin:isopropyl palmitate solution (LIPS) and poloxamer 20% gel were previously prepared. These methods accompany this manuscript. The formulation was adjusted to a 35-mL volume to ensure an adequate sample size over the course of the stability study. Three batches labeled A, B, and C of the methimazole gel were

mixed in 35-mL plastic syringes (Luer-Lok Monoject syringes). A blank was also prepared containing only poloxamer (20%) and LIPS. Each of the four 35-mL syringes were then subdivided and distributed into three 10-mL syringes. These 10-mL syringes were each placed in plastic storage bags and stored in their respective stability chambers; 25°C ± 2°C chamber with 60% ± 5% relative humidity, 35°C ± 2°C chamber and refrigerator at 5°C.

Chromatographic Conditions

The initial chromatographic conditions used were previously reported and modified as described below.⁹ The HPLC instrument was a Shimadzu LC-2010A HT Liquid Chromatograph (Fairlawn, New Jersey) consisting of a serial dual plunger pump, autosampler, and a ultraviolet (UV) detector set at 254 nm. Separation was achieved on a Hypersil GOLD aq C18 column (Thermo Scientific, West Palm Beach, Florida) with a mobile phase consisting of 5% methanol/95% potassium phosphate (0.02 M, pH 4.8) at a flow rate of 1 mL/min. Sample injection volume was 20 µL and methimazole eluted with a retention time of 3.47 minutes. Quantitative analysis was performed using a standard curve as described below.

Stability Indicating Assay

To confirm that this assay was stability-indicating, aqueous solutions of methimazole were subjected to accelerated degradation, using four separate conditions: 1N HCl, 1N NaOH, 3% hydrogen peroxide, and air. For each condition, ~0.5 g of pure methimazole was dissolved in 10 mL of each solution, stored at 40°C in glass vials for seven days. Oxidation was achieved by merely dissolving the methimazole in water and leaving the vial open to air at 70°C for 24 hours. Samples were analyzed by HPLC to determine if any degradation products co-eluted with the methimazole peak. With the exception of a small peak eluting at a retention time of 3.5 minutes, no other degradation peaks were observed, although the solutions did

change color over the seven-day incubation period. These results were consistent with previous reports suggesting that degradation of methimazole occurs via hydrolysis of the imidazole ring forming non-UV active preparations.

Preparation of Calibration Curve

A stock solution of methimazole was made containing 204.3 mcg/mL using purified water. From this stock solution, calibration standards were prepared ranging in concentration values from 4.1 µg/mL - 36.8 µg/mL. Each standard was analyzed in duplicate, and the average peak area plotted against concentration to obtain a working calibration curve.

Sample Preparation

The samples were assayed on days 0, 1, 5, 7, 14, 21, 28, 49, 62, and 91. Each sample was rehomogenized using a two-syringe mixing method. A 0.5-g sample of gel was dissolved in methanol in a 100-mL volumetric flask. A 5.0-mL aliquot of this first solution was transferred to a second 100-mL volumetric flask which was then diluted with water. This sample was then placed in glass vials and analyzed. The samples were visually inspected for any color change or gel separation each day of analysis. The pH was recorded at the start and conclusion of the study to monitor any major fluctuations. The sorbic acid was added to the vehicle to act as a preservative and, therefore, no microbial testing was performed.

RESULTS

Calibration Curve

The retention time for the methimazole was determined to be 3.7 minutes. The calibration curve over the range 4.1 mcg/mL to 36.8 mcg/mL yielded an equation $y = 161053x + 2097.14$ with a correlation coefficient $r^2 = 0.9999$.

Stability Study

The stability study was designed using the guidance of previously published stability study recommendations.¹⁰ The methimazole concentration in the samples

under the following conditions, $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with $60\% \pm 5\%$ R.H., $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$, and 5°C can be found in Table 1 and visualized in Figure 1. For the purpose of this study the drug preparation was considered to have reached its BUD once the original concentration of methimazole fell below 90%. It was determined at day 62 that the percent of methimazole remaining in the samples stored at 5°C was 92.2 ± 6.4 , at 25°C was 100.9 ± 5.3 , and 35°C was 96.9 ± 9 . As seen in Figure 1, the methimazole concentration of all three conditions fell below 90% after 62 days. The refrigerated condition showed a significant change from a milky yellow to an almost white milky color. Under the 25°C condition, the color remained a milky yellow, and for the 35°C , a darker yellow hue

TABLE 1. Average Concentration (% Relative to Day 0) of Methimazole.

Day	Condition 25°C	5°C	35°C
0	100	100	100
1	95.9 ± 3.0	97.8 ± 3.5	97.5 ± 1.5
5	95.4 ± 2.9	98.8 ± 2.0	94.6 ± 2.1
7	98.7 ± 2.3	99.6 ± 3.3	99.4 ± 2.1
14	93.3 ± 2.2	95.0 ± 3.0	94.6 ± 3.1
21	102.8 ± 10.8	98.0 ± 5.5	97.8 ± 3.0
28	98.6 ± 2.6	99.0 ± 1.2	97.5 ± 4.1
49	101.5 ± 2.8	100.3 ± 1.3	101.8 ± 0.9
62	100.9 ± 5.3	92.2 ± 6.4	96.9 ± 9.7
91	84.4 ± 2.2	83.3 ± 1.9	86.4 ± 8.6

was recorded. Visible separation was recorded under the refrigerated condition. Two distinct layers were noted when the samples were initially removed from the refrigerator before sampling. One layer appeared to be a clear gel, while the second layer was an opaque light-yellow gel. This separation was not observed in the 25°C or 35°C conditions. The consistency of the gels was also observed. The refrigerated condition appeared to be thin and was very easy to mix. Under the 35°C condition, the gel had a high thickness and proved very difficult to mix. No significant change from the initial mean \pm standard deviation pH (5.60 ± 0.049) occurred between any of the samples and no visible microbial growth was observed.

DISCUSSION

Although the gel showed acceptable stability for all three storage conditions, it was surprising to observe slightly increased degradation of the preparation in the refrigerated sample. This may be due to the reverse thermal property of the poloxamer.^{11,12} The samples exposed to the refrigerated condition showed visible separation between the aqueous poloxamer and LIPS. There is a significant decrease in the concentration of the refrigerated samples when compared to the 25°C and 35°C samples. This separation may cause a disruption of the gel emulsion and, therefore, the drug is no longer evenly dispersed throughout the gel. Even the rehomogenizing process may not remedy the broken emulsion, resulting in the lower concentration value. The color change in the refrigerated and accelerated samples may be due to degradation. The 25°C and 35°C also decreased significantly at the conclusion of the study, falling below 90% by day 91, but no visible separation was seen among these samples.

From the results of this study, it is recommended that the BUD for this product be 60 days. After day 62, the concentrations among all three conditions drops below 90% and, therefore, not recommended for patient use. Also, it should be noted that the medication should not be refrigerated due to the phase separation that occurs in the gel.

Rx

METHIMAZOLE 5 MG/0.1 ML IN
PLURONIC LECITHIN ORGANOGEL

For ? mL

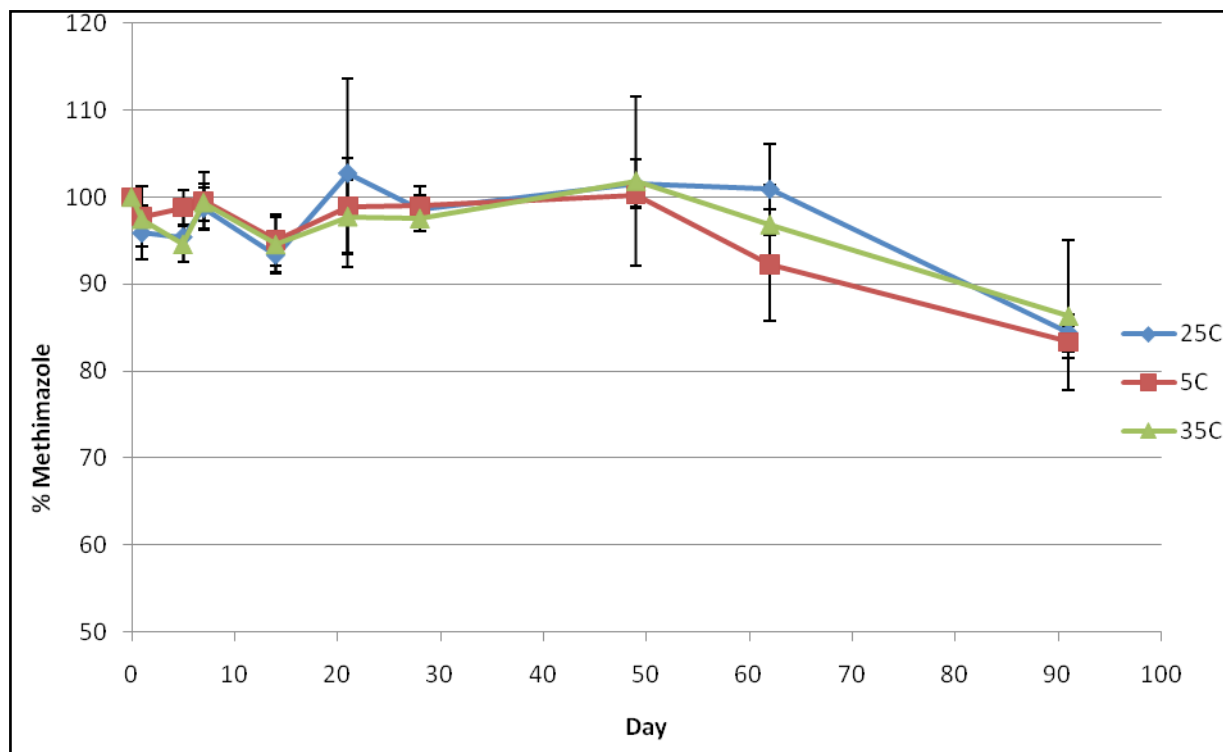
Methimazole	150 mg
Lecithin:isopropyl palmitate solution	0.66 mL
Poloxamer 20% gel	qs 3 mL

Note: The lecithin:isopropyl palmitate solution can be prepared by mixing 0.2 g sorbic acid, 50 g soy lecithin, and 50 g isopropyl palmitate.

Note: The poloxamer 20% solution can be prepared by mixing 0.2 g sorbic acid, 20 g poloxamer, and sufficient purified water to make 100 mL.

METHOD OF PREPARATION

1. Calculate the required quantity of each ingredient for the total amount to be prepared.
2. Weigh and/or measure each ingredient accurately.
3. Pour the methimazole powder carefully into the barrel of a Luer-Lok 3-mL syringe.
4. Add the lecithin:isopropyl palmitate solution and replace plunger.
5. Add 2 mL of the poloxamer gel, using a second 3-mL syringe.
6. Attach a Luer-Lok adapter to connect the two syringes, and thoroughly mix the contents back and forth.
7. Push all the preparation into one syringe and measure the volume.
8. Remove the other syringe and add sufficient poloxamer 20% gel to obtain the desired volume.
9. Reattach syringes and mix thoroughly back and forth.

FIGURE 1. Concentration of methimazole in Pluronic lecithin organogel (%).

CONCLUSION

Compounded methimazole in PLO gel should be assigned a BUD of 60 days, stored at 25°C in plastic syringes. Due to the reverse thermal property of the poloxamer 20% gel, the preparation should not be refrigerated.

REFERENCES

- Davidson G. Understanding feline hyperthyroidism. *IJPC* 2003; 7(5): 345–347.
- Lécuyer M, Prini S, Dunn ME et al. Clinical efficacy and safety of transdermal methimazole in the treatment of feline hyperthyroidism. *Can Vet J* 2006; 47(2):131–135.
- Hoffman SB, Yoder AR, Trepanier LA. Bioavailability of transdermal methimazole in a pluronic lecithin organogel (PLO) in healthy cats. *J Vet Pharmacol Ther* 2002; 25(3): 189–193.
- Sartor LL, Trepanier LA, Kroll MM et al. Efficacy and safety of transdermal methimazole in the treatment of cats with hyperthyroidism. *J Vet Intern Med* 2004; 18(5): 651–655.
- [No author listed.] Products. [Dallas Parade Compounding Pharmacy Website] 2010. Available at: www.compoundingpharmacy.com.au/home/. Accessed May 18, 2010.
- United States Pharmacopeial Convention, Inc. *United States Pharmacopeia 33–National Formulary 28*. Rockville, MD: US Pharmacopeial Convention, Inc.; 2010: 315.
- Neal AS, DeVeau I, Schnatz RG. Publication expansion of United States Pharmacopeial Convention's Veterinary Standards and Clinical Information. *IJPC* 2008; 12(5): 405–409.
- Allen LV Jr. *Allen's Compounding Formulations*. Washington DC: American Pharmaceutical Association; 2003: 376–377.
- Bowman BJ, Iacoban PM. *Chemical Stability of Methimazole and Progesterone Transdermal Ointments*. Poster presentation at American Pharmacists Association Meeting. Atlanta, GA. March 16–19, 2007.
- Williams LA, Hastings MB. Identifying the criteria of a valid stability study. *IJPC* 2009; 13(1): 32–36.
- Sinko PJ. *Martin's Physical Pharmacy and Pharmaceutical Sciences*. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006: 428–430.
- Raymond RC, Sheskey PJ, Weller PJ, eds. *Handbook of Pharmaceutical Excipients*. 4th ed. London, UK: Science and Practice; 2003: 314–315, 447–448.

Address correspondence to Christine Birnie, RPH, PhD, Associate Professor and Chair, Department of Pharmaceutical Sciences, Wegmans School of Pharmacy, St. John Fisher College, 3690 East Avenue, Rochester, NY 14618-3597. E-mail: cbirnie@sjfc.edu