STABILITY OF EXTEMPORANEOUSLY COMPOUNDED PIROXICAM 10MG/ML SUSPENSION WITH ACETATE BUFFER

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INTRODUCTION

Piroxicam is a cyclooxygenase-1 and 2 (COX-1, COX-2) inhibiting, non-steroidal anti-inflammatory (NSAID) drug that is primarily used in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.[1] NSAID's specifically COX-2 inhibitors are currently under investigation for primary or adjunctive therapy in the treatment and prevention of cancer.[2] In veterinary medicine, piroxicam is commonly used as adjunctive therapy for bladder transitional cell carcinoma in canines and felines. It may also be beneficial in squamous cell carcinomas, mammary, adenocarcinoma and transmissible venereal tumor (TVT).[3]

Drugs have an ionized and unionized form, both of which influences its absorption in the body. Compared to its ionized form, the unionized form of drugs are readily absorbed due to its lipid solubility and lack of charge.[4] Piroxicam is an ampholytic drug, thus it has two pKa values, 1.86 assigned to the weakly basic pyridyl nitrogen, and 5.46 assigned to the weakly acidic 4-hydroxy proton. Being an ampholytic drug, its ionization increases at low and high pH values, and decreases in the middle pH range where the drug is in its unionized form.[5] One of the main factors affecting stability is pH. Knowing the absorption of piroxicam is pH dependent, the addition of a buffer to piroxicam suspension is favorable. Buffers are employed within pharmaceutical solutions to control the pH of formulated products. Acetates are normal components of the diet of humans and animals and are produced in molar quantities daily in the gastrointestinal tract where they are rapidly and completely metabolized.[6] Acetate buffers in suspensions should be relatively safe for canines and felines.

The accelerated testing conditions of 40°C ± 2°C/75% RH ± 5% is based on the Food and Drug Administration (FDA) stability testing of drug substances and drug products.[7] According to Arrhenius' equation and the accelerated aging time (AAT) equation, 28 days under the following test conditions of 40°C ± 2°C/75% RH ± 5% with an aging factor (Q_{10}) 2, is equivalent to 3 months real-time at ambient temperature (T_{RT}) 23°C.[8,9]

Following USP <795> standards, suspensions not less than (NLT) 90% and not more than (NMT) 110% of the original piroxicam concentration with no visible caking, lumping or aggregation were considered stable.

OBJECTIVE

According to USP <795>, in the absence of stability information the storage period for compounded water-containing oral formulations is 14 days at temperatures 2° to 8°C. Due to a lack of established stability studies, this study examines the stability of extemporaneously compounded piroxicam 10mg/mL suspension with and without acetate buffer in glass amber bottles and plastic amber syringes under room temperature (20° to 25°C), refrigerated temperature (2° to 8°C) and accelerated conditions (40°C ± 2°C/75% RH ± 5%) equivalent to 3 months real-time.

MATERIALS

PIROXICAM SUSPENSION:

Piroxicam USP powder (Medisca Pharmaceutique Inc, Montréal, Quebec; lot 118943/Q, expiry April 2017)

Oral Suspend - Suspending Vehicle (Medisca Pharmaceutique Inc, Montréal, Quebec; lot 0015IM/A, expiry February 2018)

ACETATE BUFFER:

Sodium acetate (PCCA, Houston, Texas; lot C167751; expiry September 2018)

Acetic acid USP (Medisca Pharmaceutique Inc, Montréal, Quebec; lot 126426/D, expiry June 2017)

PACKAGING:

Glass Amber Bottles 60mL (E.D. Luce Packaging, Cerritos, California; item no. BA02)

Medisca Adapter Caps - Blue B, 20mm (Medisca Pharmaceutique Inc, Montréal, Quebec; lot 128320/D)

PreciseDose Dispenser Amber Syringes 60mL (Medisca Pharmaceutique Inc, Montréal, Quebec; lot 123799/J)

METHOD

COMPOUNDING

All compounds were done at Santa Clara Drug – The Compounding Shop in San Jose, CA. Piroxicam 10mg/mL suspension control (without acetate buffer) was prepared by adding 0.720 grams of Piroxicam USP powder in Oral Suspend quantity sufficient to make 720mL. Physical appearance was noted to be opaque yellowish-white with a pH of 4.30. VWR sympHony B10P pH meter was calibrated before use. Acetate buffer 5.33pH was compounded following USP35-NF30. 5.98 grams of sodium acetate was added to 3mL of acetic acid, pH was adjusted to 5.33, and water was added quantity sufficient to make 1000mL. Piroxicam 10mg/mL suspension with acetate buffer was prepared by adding 0.720 grams of Piroxicam USP powder in 144mL (20%) acetate buffer and Oral Suspend quantity sufficient to make 720mL. Physical appearance was noted to be opaque yellowish-white with a pH of 5.20. Each batch of 720mL was then divided into six 60mL glass amber bottles sealed with an adapter cap and six 60mL plastic amber syringes. Each group had two samples of 60mL. Each container was then labeled according to the following groups:

Group A (glass amber bottles, accelerated conditions 40°C ± 2°C/75% RH ± 5%):
- AI. control (piroxicam in Oral Suspend)
- AII. buffer (piroxicam + acetate buffer in Oral Suspend)

Group B (plastic amber syringes, accelerated conditions 40°C ± 2°C/75% RH ± 5%):
- BI. control
- BII. buffer

Group C (glass amber bottles, room temperature 20° to 25°C):
- CI. control
- CII. buffer

Group D (plastic amber syringes, room temperature 20° to 25°C):
- DI. control
- DII. buffer

Group E (glass amber bottles, refrigerated 2° to 8°C):
- EI. control
- EII. buffer

Group F (plastic amber syringes, refrigerated 2° to 8°C):
- FI. control
- FII. buffer

A stability-indicating High Performance Thin Layer Chromatography (HPTLC) method for piroxicam[10] was utilized to indicate piroxicam suspension concentrations on study days 0, 7, 14, 21 and 28, with continuous observation of ease of resuspension, physical appearance and pH. All samples were shipped overnight to KB Analytical, LLC in Oakdale, CT where all the HPTLC analyses were performed.

HPTLC

Piroxicam in Oral Suspend assay by High Performance Thin Layer Chromatography:

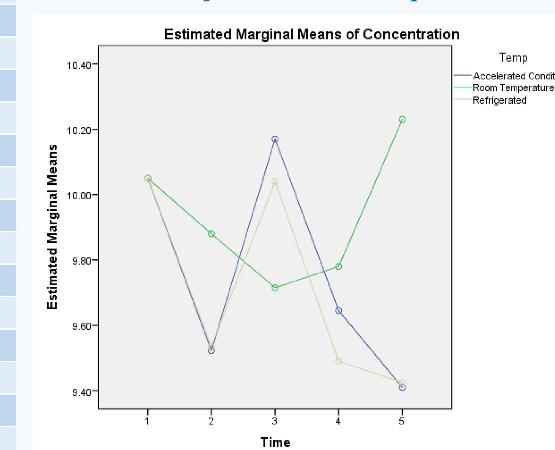
1. Shake supplied sample bottle or syringe 25 times
2. Pour out sample aliquot into a 15mL test tube (remainder used for pH test)
3. Quantitatively transfer 1.0mL sample to a 15mL glass centrifuge tube
4. Add 100uL 0.1N HCl to each centrifuge tube
5. Quantitatively add 2.0mL chloroform to each centrifuge tube
6. Cap tightly
7. Sonicate tubes for 15 minutes followed by shaking tubes in a shaker for an additional 15 minutes
8. Centrifuge tubes for 5 minutes
9. Transfer 1 – 1.5mL of the chloroform layer to a 2.0mL autosampler vial and seal
10. Apply standards to HPTLC plate (5 point standard curve) 0.5uL – 2.5uL
11. Apply samples to HPTLC plate 1.0uL
12. Develop plate in vertical tank with mobile phase (petroleum ether: methyl ethyl ketone: acetic acid)
13. Allow to dry
14. Visualize at 254nm
15. Quantitative scan at 360nm

RESULTS & DISCUSSION

All study groups retained NLT 90 and NMT 110% of the original concentration with no observable physical changes across all time points. All statistical analyses were performed with IBM SPSS Version 24. Three mixed-design ANOVA ($p<0.05$) were used to analyze the data. The within-subjects variables were the 5 time points (Day 0, 7, 14, 21 and 28) and the between-subject factors were temperature, with buffer and storage container, respectively. Significant concentration degradation across the five time points was verified with statistically significant differences in all three mixed-design ANOVA performed, $F(4,36)=4.776$, $p=0.003$; $\epsilon=0.544$, $F(2.177,21.769)=3.708$, $p=0.038$; $F(4,40)=3.405$, $p=0.017$. There is no statistical significant differences in concentrations between the three temperatures, $F(2,9)=3.523$, $p=0.074$; between the control and buffer group, $F(1,10)=0.057$, $p=0.816$; and between storage containers, $F(1,10)=4.138$, $p=0.069$, respectively. There was a significant interaction between time and temperature, $F(8,36)=2.755$, $p=0.018$. Following-up this interaction there was statistical difference of the accelerated conditions group from Day 0 (baseline) to Day 28, $p=0.016$ and refrigerated temperature group from Day 0 (baseline) to Day 28, $p=0.019$. On Day 28, Group F's control and buffer concentrations were 9.07mg/mL (90.7%) and 9.03mg/mL (90.3%), respectively.

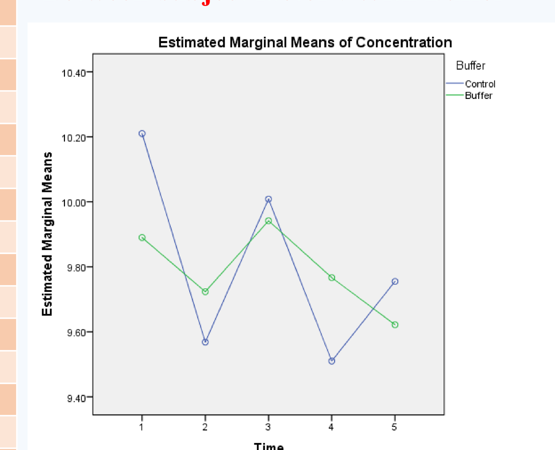
Descriptive Statistics of Concentration (mg/mL)				
	Temperature	Mean	Std. Dev.	N
Day 0	Accelerated Conditions	10.05	0.18	4
	Room Temperature	10.05	0.18	4
	Refrigerated	10.05	0.18	4
	Total	10.05	0.17	12
Day 7	Accelerated Conditions	9.52	0.33	4
	Room Temperature	9.88	0.29	4
	Refrigerated	9.54	0.13	4
	Total	9.65	0.30	12
Day 14	Accelerated Conditions	10.17	0.39	4
	Room Temperature	9.72	0.47	4
	Refrigerated	10.04	0.36	4
	Total	9.98	0.42	12
Day 21	Accelerated Conditions	9.65	0.30	4
	Room Temperature	9.78	0.37	4
	Refrigerated	9.49	0.36	4
	Total	9.64	0.33	12
Day 28	Accelerated Conditions	9.41	0.16	4
	Room Temperature	10.23	0.12	4
	Refrigerated	9.43	0.44	4
	Total	9.69	0.47	12

Mixed-design ANOVA ($p<0.05$)
Within-subjects variables: 5 Time Points
Between-subject factor : Temperature



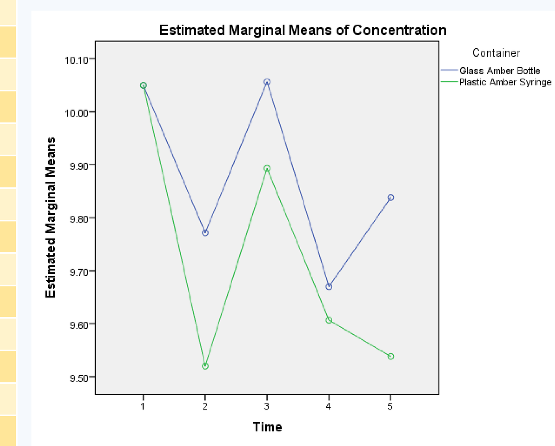
Descriptive Statistics of Concentration (mg/mL)				
	With Buffer	Mean	Std. Dev.	N
Day 0	Control	10.21	0.00	6
	Buffer	9.89	0.00	6
	Total	10.05	0.17	12
Day 7	Control	9.57	0.32	6
	Buffer	9.72	0.28	6
	Total	9.65	0.30	12
Day 14	Control	10.01	0.34	6
	Buffer	9.94	0.52	6
	Total	9.98	0.42	12
Day 21	Control	9.51	0.24	6
	Buffer	9.77	0.38	6
	Total	9.64	0.33	12
Day 28	Control	9.76	0.48	6
	Buffer	9.62	0.50	6
	Total	9.69	0.47	12

Mixed-design ANOVA ($p<0.05$)
Within-subjects variables: 5 Time Points
Between-subject factor : With Buffer



Descriptive Statistics of Concentration (mg/mL)				
	Storage Container	Mean	Std. Dev.	N
Day 0	Glass Amber Bottle	10.05	0.18	6
	Plastic Amber Syringe	10.05	0.18	6
	Total	10.05	0.17	12
Day 7	Glass Amber Bottle	9.77	0.31	6
	Plastic Amber Syringe	9.52	0.24	6
	Total	9.65	0.30	12
Day 14	Glass Amber Bottle	10.06	0.49	6
	Plastic Amber Syringe	9.89	0.37	6
	Total	9.98	0.42	12
Day 21	Glass Amber Bottle	9.67	0.32	6
	Plastic Amber Syringe	9.61	0.37	6
	Total	9.64	0.33	12
Day 28	Glass Amber Bottle	9.84	0.41	6
	Plastic Amber Syringe	9.54	0.52	6
	Total	9.69	0.47	12

Mixed-design ANOVA ($p<0.05$)
Within-subjects variables: 5 Time Points
Between-subject factor : Storage Container



The pH of both the control and buffer groups remained consistent throughout the study with no statistical significant difference comparing Day 1 to Day 28. At the end of the study on Day 28, the control group mean pH and standard deviation was 4.28±0.04, and the buffer group mean pH and standard deviation was 5.13±0.08.

There were several limitations to the study. Due to limited time for the project, only 28 days of real-time stability was explored. Since HPTLC was performed offsite at KB Analytical, LLC in Oakdale, CT, researchers were not able to personally observe the HPTLC process and monitor storage conditions, pH and physical appearance. Due to the advancement of analytical chemistry, drug stability is more commonly associated with formation of low levels of degradants rather than loss of drug potency over time.[8] No degradation products of piroxicam, particularly 2-aminopyridine (2AP), were noted on any of the TLC plates, but was mentioned in the article referenced as the basis of the stability-indicating HPTLC determination of piroxicam.[10]

Future recommendations for this study is to explore a prolonged stability study with duplicate or triplicate samples. Explore method development for the stability-indicating method of piroxicam utilizing other methods such as High Performance Liquid Chromatography (HPLC). Potentially continue this study to a randomized control trial for efficacy and safety of extemporaneously compounded piroxicam 10mg/mL suspension with acetate buffer in canines and felines.

CONCLUSION

Piroxicam 10mg/mL suspensions in Oral Suspend with or without acetate buffer stored in glass amber bottles can be stable for up to 90 days at room temperature (20° to 25° C) and refrigerated temperature (2° to 8°C). When stored in plastic amber syringes, piroxicam suspension with or without buffer can be stable for up to 90 days at room temperature (20° to 25°C) and up to 28 days at refrigerated temperature (2° to 8°C).

ACKNOWLEDGMENT

I would like to thank my preceptor Dr. Vishal Purohit and Thiaba Fame, R&D Associate from Medisca, for guidance throughout the study period. I would also like to thank William Georgian, Director of KB Analytical LLC, for performing all the HPTLC analyses.

FUNDING: This study was funded by an unrestricted educational grant from Medisca Pharmaceutique Inc.

COMPETING INTEREST: Other than grant support, no competing interests were declared.

REFERENCES

1. Piroxicam. Lexi-Drugs. Lexicomp. Wolters Kluwer Health, Inc. Hudson, OH. Accessed October 2015.
2. Rayburn ER, Ezell SJ, Zhang R. "Anti-Inflammatory Agents for Cancer Therapy." *Mol Cell Pharmacol.* 2009;1(1): 29–43.
3. Plumb DC. *Plumb's Veterinary Drug Handbook Sixth Edition*. Stockholm, WI: PharmaVet Inc; 2008.
4. Shargel L, Wu-Pong S, and Yu ABC. *Applied Biopharmaceutics & Pharmacokinetics: 5th Edition.* New York: Appleton & Lange Reviews/McGraw-Hill, Medical Pub. Division; 2005.
5. Shohin, IE, Kulinich JI, Ramenskaya GV, et al. "Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Piroxicam." *J. Pharm. Sci.* 2013;103(2): 367-77.
6. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). "Scientific Opinion on the safety and efficacy of acetic acid, sodium diacetate and calcium acetate as preservatives for feed for all animal species." *EFSA Journal.* 2012;10(2): 2571.
7. International Conference of Harmonization Q1A(R2), 2003. Stability Testing of New Drug Substances and Products (Revision 2). Federal Register 68, 65717-65718.
8. Waterman KC, Adami RC. "Accelerated aging: Prediction of chemical stability of pharmaceuticals." *International Journal of Pharmaceutics.* 2005; 293(1-2):101–125.
9. Westpak. "Accelerated Aging Time (AAT) Calculator". *Reference ASTM F1980-07*. http://www.westpak.com/page/calculators.
10. Puthli SP, Vavia PR. "Stability indicating HPTLC determination of piroxicam." *Journal of Pharmaceutical and Biomedical Analysis.* 2000;22(4): 673-677.