Elanco Rumensin®

Tech Report

SCIENTIFIC UPDATE FROM ELANCO ANIMAL HEALTH

Assessment of Dissolution Characteristics of Rumensin® and Monovet®[1,2]

Mike Brown, PhD, PAS, Technical Consultant
Laura Foust, Statistical Advisor
Beverly Krabel, Research Advisor
Gary Fuller, Marketing Consultant

STUDY OVERVIEW

- Laboratory conditions were used to evaluate the dissolution of monensin from Type A medicated articles of the pioneer (Rumensin 90) and a generic monensin approved in the U.S. (Monovet 90). This dissolution test measures the rate of dissolution in two synthetic rumen fluids
- Two lots of Rumensin 90 (P1 and P2) and two lots of Monovet (A1 and A2) were evaluated. Expiration dates for all products were between May and August 2021
- Monensin potencies were 200.0 (P1), 198.3 (P2), 212.0 (A1) and 203.7 (A2) mg/g
- Twelve replicates of each lot were used to generate the dissolution profile. Dissolution was performed by adding the products into a vessel with simulated rumen fluid at 39° C with constant stirring. In Experiment 1, the simulated rumen fluid mimicked the ruminal conditions of cattle fed a high-forage ration. In Experiment 2, the simulated rumen fluid mimicked the ruminal conditions of cattle fed a high-concentrate ration. Sample aliquots were collected from each vessel at 15, 30, 45, 60, 90, 120, 240, 360 and 480 min
- Within-lot variation was below a relative standard deviation (RSD) of 10 (i.e., less than 10% per time point) at all time points for Rumensin lots and after 45 min for Monovet lots
- The standard f_1 and f_2 computations described in FDA guidance for use when variation is acceptably low were utilized to interpret the data from both experiments. Thus, the alternative method of determining similarity of dissolution profiles when the RSD>10 (i.e., greater within-lot variation) using tolerance limits as was used in the Monovet 90 approval was not needed.[3] Analysis of variance techniques were not used, as they are not part of the FDA assessment of such data
- In both experiments, the Rumensin lot (P2), which had the lower dissolution profile, was used as the reference. Monovet lots A1 and A2 were compared to the reference lot (P2). Both Monovet lots had lower dissolution profiles than the reference
- Evaluation of the f_1 and f_2 test statistics indicated that neither Monovet lot (A1 and A2) was similar to the Rumensin reference number (P2) in either Experiment 1 or 2. Data suggest that the active ingredient would be released more slowly and/or to a lesser extent from Monovet than from Rumensin under the conditions tested

INTRODUCTION

Rumensin has a long history of use in the cattle feeding and dairy industries across the globe. There are several generic monensin products commercially available outside the U.S., and Monovet 90 was recently approved for use in the U.S. Final product characteristics are influenced by manufacturing processes from fermentation through final formulation. Assessment of the attributes of generic products is needed to better understand potential production implications for customers.

A large dissolution project involved several international monensin sources in addition to the specific Rumensin 90 and Monovet 90 lots evaluated in this report. The analyses reported here only include the in-date lots of Rumensin 90 and Monovet 90 involved in the large project.

STUDY OBJECTIVE

The objective of this study was to determine in vitro monensin dissolution characteristics of Rumensin 90 and Monovet 90 Type A medicated articles using industry-standard techniques.

MATERIALS AND METHODS

Dissolution Procedure

The products evaluated included Rumensin 90 (Elanco, Greenfield, IN) and Monovet 90 (Huvepharma, Sofia, Bulgaria). Rumensin lot numbers were D148565 (P1, manufacture date 18SEP2019) and D148566 (P2, manufacture date 18SEP2019). Monovet lot numbers were 19051514010 (A1, manufacture date MAY2019) and 19061514034 (A2, manufacture date JUN2019).

The assay conditions and media preparations used were according to United States Pharmacopeia and National Formulary (USP-NF), 2019,[4] and monensin was assayed by high-performance liquid chromatography (HPLC) according to USP-NF, 2017.[5] Briefly, 75 mg +/-10 mg of Type A medicated article was added to a 1,000-mL dissolution vessel containing simulated rumen fluid at 39°C with constant stirring (250 rpm). Sample aliquots were collected from the dissolution vessel at 15, 30, 45, 60, 90, 120, 240, 360 and 480 min and assayed for monensin content. The media used in Experiment 1 was simulated rumen fluid of cattle fed a high-forage ration (pH = 6.8) and in Experiment 2 simulated rumen fluid of cattle fed a high-concentrate ration with a pH of 5.5 (referred to as high-grain in USP).

Monensin Analysis

At each time point, a 10-mL aliquot was collected from each dissolution vessel into a 16 mm x 100 mm test tube. The sample aliquot was diluted 1:10 with methanol (vol:vol), mixed, filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter and transferred to an HPLC vial. Monensin was analyzed by HPLC according to USP-NF procedures.[5] The HPLC was equipped with a Zorbax® RX C18 column (250 mm x 4.6 mm) that was maintained at 30° C. The monensin standard curve (0.025, 0.05, 0.1, 0.25, 0.5, 1, 5 and 25 µg/mL) was performed at the beginning and end of every HPLC run. The standard curve was generated using a log-log algorithm. A control sample (1 µg/mL), three solvent blanks and one media blank were also included in the HPLC run. On average, an HPLC run consisted of 54 unknowns. The assay limits of detection and quantitation for a sample were 1.3 and 3.3 mg/g monensin, respectively. A run was considered acceptable if the R^2 of the standard curve was >0.9990 and the control sample recovery was +/-10%. The average control sample recovery across all acceptable runs was 96.3% (a range of 90.0% to 98.5%) for the simulated high-forage rumen fluid and averaged 96.2% (a range of 93.4% to 98.6%) for the simulated high-concentrate rumen fluid.

Calculations

The Rumensin 90 lot (P2) with the lowest dissolution in each experiment was designated as the control or reference for subsequent calculations. Thus, A1 and A2 were evaluated relative to P2.

The formulas for determining the test statistics are provided below.

$$f_1 = \{[\Sigma_{t=1}^{n} \mid R_t - T_t \mid]/[\ \Sigma_{t=1}^{n} R_t]\} \cdot 100$$

$$f_2 = 50 \cdot \log \{[1+(1/n)\Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \cdot 100\}$$

where n = number of time points, R_t = the reference profile mean percentage of dissolved at time t, and T_t = the test profile mean percentage of dissolved at time t.

The fundamental data requirements according to established FDA guidance[6] to evaluate dissolution profiles include: 1) at least three time points are needed; 2) only one time point after a sample reaches 85% dissolution may be used; and 3) the RSD at early time points is <20% and subsequent time points are <10%. None of the materials evaluated in Experiment 1 or 2 reached a peak of 85% dissolution. As the profile means failed to achieve 85% dissolved at any time point, the test and reference profiles were normalized so the highest reference mean included in the f_1 (difference factor) and f_2 (similarity factor) was equal to 85%, as recommended by Martinez et al.[7]

The standard f_1 and f_2 calculations described in FDA guidance[6] for use when variation is acceptably low was utilized for time points from 45 min to 240 min (peak dissolution) for Experiment 1 (Table 1) and from 15 min to 240 min (peak dissolution) for Experiment 2 to interpret the data. Thus, the alternative method of assessing dissolution profiles when the RSD>10 (i.e., greater within-lot variation) using tolerance limits,[7] as was used in the Monovet 90 approval,[3] was not needed. Analysis of variance techniques for repeated measures or area-under-the-curve were not used because these techniques are not utilized by the FDA[6] for assessment of such data.

The f_1 statistic represents the difference factor, whereas the f_2 statistic represents the similarity factor (Table 2). Two sample dissolution profiles are considered to be different if f_1>15 and f_2<50.[6]

TABLE 1.

ACTUAL SAMPLE VARIATION (RSD) IN SIMULATED HIGH-FORAGE RUMEN FLUID (EXPERIMENT 1) AND SIMULATED HIGH-CONCENTRATE RUMEN FLUID (EXPERIMENT 2)

	INCUBATION TIME (MIN)								
	15	30	45	60	90	120	240	360	480
Experiment 1 treatment[a]									
P1	7	5	7	7	4	4	4	7	5
P2	7	6	6	4	4	3	5	5	3
A1	9	10	15	9	5	4	9	8	6
A2	6	25	5	5	7	5	5	5	3
Experiment 2 treatment[a]									
P1	8	8	7	6	5	5	4	5	4
P2	6	5	5	6	4	3	6	5	4
A1	16	8	6	5	5	5	5	4	6
A2	5	7	4	5	3	2	6	6	5

[a]P1 = Rumensin 90 lot number D148565, P2 = Rumensin 90 lot number D148566, A1 = 19051514010, A2 = 19061514034.

RESULTS

Average monensin potency for P1, P2, A1 and A2 were 200.0, 198.3, 212.0 and 203.7 mg/g of Type A medicated article, respectively. Thus, all treatments were within +/-6% of the target value of 200 mg/g or 90.7 mg of monensin/lb of Type A article. The lot mean potency was required to calculate the percent monensin dissolved for each sample, calculated by dividing the measured monensin potency result (mg/g) by the lot mean potency (mg/g), then multiplying by 100.

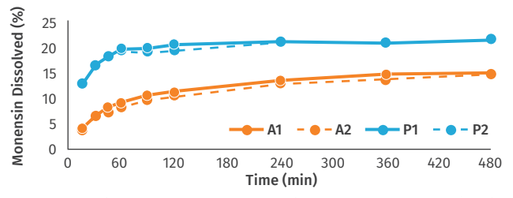
The raw dissolution profile in Experiment 1 (Figure 1) indicates that P1 and P2 reached a peak of approximately 20% dissolved, and A1 and A2 peaked at approximately 12% dissolved. The raw dissolution profile in Experiment 2 (Figure 2) suggests a similar pattern; however, the profile for A2 approached that of P1 and P2 after 6 to 8 hours. Nevertheless, the mean dissolution of the Monovet lots at 240 min (peak) was similar to that observed in Experiment 1 (approximately 13%).

Rumensin lot P2 was selected as the reference product because it displayed numerically lower dissolution across time than P1. In both experiments, monensin dissolution profiles of A1 and A2 were lower than that observed for the reference product (P2) (Table 2).

The profile assessment techniques used in these experiments[6] are different than those used in the Monovet 90 approval.[3] The Monovet FOI Summary indicated that an alternative technique to accommodate within-lot variation was utilized, and the results of those calculations indicated that Rumensin 90 and Monovet 90 displayed similar dissolution profiles.

FIGURE 1.

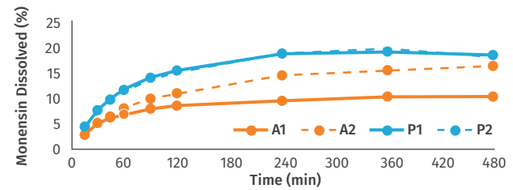
MEAN MONENSIN DISSOLUTION (% OF TOTAL) IN THE INCUBATION MEDIUM ACROSS TIME OF RUMENSIN 90 AND MONOVET 90 (12 REPLICATES/TIME) IN A SIMULATED HIGH-FORAGE RUMEN FLUID (EXPERIMENT 1)[a]



[a]A1 = Monovet 90 lot 19051514010, A2 = Monovet 90 lot 19061514034, P1 = Rumensin 90 lot D148565, P2 = Rumensin 90 lot D148566.

FIGURE 2.

MEAN MONENSIN DISSOLUTION (% OF TOTAL) IN THE INCUBATION MEDIUM ACROSS TIME OF RUMENSIN 90 AND MONOVET 90 (12 REPLICATES/TIME) IN A SIMULATED HIGH-CONCENTRATE RUMEN FLUID (EXPERIMENT 2)[a]



[a]A1 = Monovet 90 lot 19051514010, A2 = Monovet 90 lot 19061514034, P1 = Rumensin 90 lot D148565, P2 = Rumensin 90 lot D148566.

TABLE 2.

TEST STATISTICS FOR DETERMINING DISSOLUTION PROFILE SIMILARITY OF MONENSIN SOURCES INCUBATED IN A SIMULATED HIGH-FORAGE RUMEN FLUID (EXPERIMENT 1) OR A SIMULATED HIGH-CONCENTRATE RUMEN FLUID (EXPERIMENT 2)[a]

	Experiment 1		Experiment 2	
Comparison[b]	f_1	f_2	f_1	f_2
P2 and A1	45	23	41	31
P2 and A2	50	20	27	41

[a]P2 = Rumensin 90 lot number D148566, A1 = Monovet lot number 19051514010, A2 = Monovet lot number 19061514034. [b]Two sample dissolution profiles are considered to be different if f_1>15 and f_2<50.[5]

CONCLUSIONS

In this study, the within-lot variation was adequately low to utilize established FDA procedures to evaluate product dissolution. Data indicate that monensin from Rumensin 90 dissolves more rapidly and/or more extensively than Monovet 90 under these in vitro conditions simulating feeding a high-forage and a high-concentrate ration. This dissolution study showed that the products are not similar in these media.

The label contains complete use information, including cautions and warnings. Always read, understand and follow the label and use directions.

Caution: Consumption by unapproved species or feeding undiluted may be toxic or fatal. Do not feed to veal calves.

Growing beef steers and heifers fed in confinement for slaughter:

For improved feed efficiency: Feed 5 to 40 g/ton of monensin (90% DM basis) continuously in a complete feed to provide 50 to 480 mg/hd/day.

For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii*: Feed 10 to 40 g/ton of monensin (90% DM basis) continuously to provide 0.14 to 0.42 mg/lb of body weight/day monensin, depending upon severity of challenge, up to a maximum of 480 mg/hd/day.

Growing beef steers and heifers on pasture (stocker, feeder, and slaughter) or in a dry lot, and replacement beef and dairy heifers:

For increased rate of weight gain: Feed 50 to 200 mg/hd/day of monensin in at least 1.0 lb of Type C Medicated Feed. Or, after the 5th day, feed 400 mg/hd/day every other day in 2.0 lbs of Type C Medicated Feed. The Type C Medicated Feed must contain 15 to 400 g/ton of monensin (90% DM basis). Do not self feed.

For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii*: Feed at a rate to provide 0.14 to 0.42 mg/lb of body weight/day monensin, depending upon severity of challenge, up to a maximum of 200 mg/hd/day. The Type C Medicated Feed must contain 15 to 400 g/ton of monensin (90% DM basis).

Type C free-choice medicated feeds: All Type C free-choice medicated feeds containing Rumensin must be manufactured according to an FDA-approved formula/specification. When using a formula/specification published in the Code of Federal Regulations (CFR), a Medicated Feed Mill license is not required. Use of Rumensin in a proprietary formula/specification not published in the CFR requires prior FDA approval and a Medicated Feed Mill License.

Feeding Directions

Dairy cows: For increased milk production efficiency (production of marketable solids-corrected milk per unit of feed intake):

Total Mixed Rations ("complete feed"): Feed continuously to dry and lactating dairy cows a total mixed ration ("complete feed") containing 11 to 22 g/ton monensin on a 100% DM basis.

Component Feeding Systems (including top dress): Feed continuously to dry and lactating dairy cows a Type C medicated feed containing 11 to 400 g/ton monensin. The Type C medicated feed must be fed in a minimum of 1.0 lb of feed/cow/day to provide 185 to 660 mg/hd/day monensin to lactating cows or 115 to 410 mg/hd/day monensin to dry cows. This provides cows with similar amounts of monensin they would receive by consuming total mixed rations containing 11 to 22 g/ton monensin on a 100% DM basis.

For calves (excluding veal calves)

For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii*: Feed at a rate of 0.14 to 1.00 mg/lb of body weight/day, depending upon severity of challenge, up to a maximum of 200 mg of monensin/hd/day. The monensin concentration in Type C medicated feed must be between 10 and 200 g/ton.

Beef cows:

For improved feed efficiency when receiving supplemental feed: Feed continuously at a rate of 50 to 200 mg/hd/day of monensin. Cows on pasture or in dry lot must receive a minimum of 1.0 lb of Type C medicated feed per head per day. Do not self feed.

For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii*: Feed at a rate of 0.14 to 0.42 mg/lb of body weight/day, depending upon severity of challenge, up to a maximum of 200 mg/hd/day.

[1] Elanco Animal Health. Data on file.
[2] Elanco Animal Health. Data on file.
[3] Freedom of Information Summary, ANADA 200-639. 2019.
[4] United States Pharmacopeia and National Formulary. USP 42-NF 37: Update to Chapter <1236> Solubility Measurements. United States Pharmacopeia Convention, Inc. Rockville, MD. 2019; Supplement 2.
[5] United States Pharmacopeia and National Formulary. USP 42-NF 37: Monensin Type A Medicated Article. United States Pharmacopeia Convention, Inc. Rockville, MD. 2017.
[6] FDA. Guidance for Industry, Center for Drug Evaluation and Research (CDER): Dissolution testing of immediate release solid dosage forms. 1997.
[7] Martinez M, Zhao XA. A simple approach for comparing the in vitro dissolution profiles of highly variable drug products: a proposal. AAPS J. 2018;20(4):78.

PM-US-20-0520(2)