Procedure To Evaluate the Stability during Processing and Storage of a Medicated Premix and Medicated Farm Feed: Erythromycin Thiocyanate

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In this paper, a stability study of a medicated premix and medicated farm feed containing erythromycin thiocyanate was planned. No drug degradation was detected during the medicated farm feed processing. In the medicated premix stability study, significant drug degradation was detected only at 40 °C and 75% relative humidity. Because after 2 years of storage at 25 °C and 60% relative humidity no degradation of erythromycin thiocyanate was detected, this period of time is proposed as the premix shelf life. In the medicated farm feed stability study, drug degradation was detected under accelerated conditions, but it was not detected under long-term storage conditions for 3 months. Therefore, the proposed shelf life of the medicated farm feed is 3 months, as this is time enough to be consumed. The planned stability study—storage conditions, testing frequency, and proposed data evaluation—allowed an easy and reliable evaluation of veterinary medicine stability.

Keywords: *Erythromycin thiocyanate; shelf life; stability; veterinary drugs; feeds processing*

INTRODUCTION

In the European Union, current regulations referring to the commercialization of veterinary medicines (*1*−*7*) require stability studies to be fulfilled. The studies carried out to determine the shelf life of the product are required to be described and justified, along with storage conditions and post shelf life specifications.

When a medicated premix is concerned, that is, when a veterinary medicinal product is prepared with a view to include it in farm feed, the stability information should include data about the stability of the medicinal product (premix) during the manufacture of the medicated farm feed. In addition to this information, stability data of the medicated premix over the storage and shelf life of the medicated farm feed prepared with the premix should be included.

The stability studies should be carried out on, preferably, three batches of medicated feed, which should be prepared from at least two different batches of the medicated premix.

The objective of this paper was to propose a procedure to evaluate the stability of a medicated premix and medicated farm feed. Erythromycin thiocyanate was used as active ingredient.

The stability study designed includes the suggestion of the shelf life for a medicated premix and farm feed and the stability of the medicinal product during the processing of the medicated feed.

Erythromycin is a macrolide for oral administration. The empirical formula is $C_{37}H_{67}NO_{13}$. It occurs as white or yellowish, odorless or almost odorless, slightly hygroscopic crystals or powder. It is slightly soluble in water but less soluble at higher temperatures; soluble to freely soluble in alcohol; and soluble in chloroform, in ether, and in methyl alcohol. With the aim of masking the intense bitter taste of the base, increasing its gastric resistance, or increasing its water solubility, different derivatives (acid salts and esters) are used (*8*, *9*).

Erythromycin thiocyanate is used in veterinary medicine for treating birds, specifically chickens and turkeys (*10*, *11*). One hundred grams of erythromycin thiocyanate (93 g of base) per ton of feed is administered orally as an aid in the prevention of chronic respiratory disease during periods of stress in chickens and turkeys and as an aid in the prevention of infectious coryza in chickens. For the former use, chickens and turkeys must be given the medicated feed as the only ration from 2 days before stress until 3−6 days after stress. For prophylaxis of infectious coryza, chickens must be given the medicated feed as the only ration for 7−14 days. In both cases, the treatment must be withdrawn 24 h before slaughter. When erythromycin thiocyanate is used as an aid in the prevention and reduction of lesions and in lowering the severity of chronic respiratory disease, 185 g (171 g of base) per ton of feed is administered orally to chickens and turkeys for 5−8 days. The treatment must be withdrawn 48 h before slaughter and must not be used in birds producing eggs for food purposes.

MATERIALS AND METHODS

Materials. The following materials were used in this work.

Raw materials included erythromycin thiocyanate (purity, minimum 99%) and dextrose (purity, minimum 99%).

Feed included cereal seeds, oily seeds, vitamins, and minerals. The composition was as follows: crude protein, 15%; crude fat, 6%; crude fiber, 4%; arginine, 0.83%; glycine and serine, 0.58%; histidine, 0.22%; isoleucine, 0.50%; leucine, 0.83%; lysine, 0.60%; methionine + cystine, 0.50%; methionine, 0.25%; phenylalanine + tyrosine, 0.83%; phenylalanine, 0.45%; threonine, 0.57%; tryptophan, 0.14%; valine, 0.52%; linoleic acid,

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1%; calcium, 0.7%; phosphorus (available), 0.35%; potassium, 0.30%; sodium, 0.15%; chlorine, 0.12%; magnesium, 500 mg/kg; manganese, 30 mg/kg; zinc, 35 mg/kg; iron, 60 mg/kg; copper, 6 mg/kg; iodine, 0.35 mg/kg; selenium, 0.10 mg/kg; vitamin A, 1500 IU/kg; vitamin D, 200 IU/kg; vitamin E, 5 IU/kg; vitamin K, 0.50 mg/kg; riboflavin, 10 mg/kg; pantothenic acid, 10 mg/kg; niacin, 11 mg/kg; vitamin B_{12}, 0.003 mg/kg; choline, 900 mg/kg; biotin, 0.10 mg/kg; folacin, 0.25 mg/kg; thiamin, 1.3 mg/kg; pyridoxine, 3 mg/kg.

Medicated premix is supplied in a powdered form and administered orally after mixing with animal feed. It was presented in sachets, lined internally with polyethylene and two sheets of opaque kraft paper, containing a mixture of 7.5 g of erythromycin thiocyanate (200 g kg^{-1}) and dextrose (800 g kg^{-1}).

Medicated farm feed was prepared by mixing medicated premix and pellets of bird feed (3% w/w of premix and 97% w/w of feed). One kilogram of feed includes 6 g of erythromycin thiocyanate. The samples for the stability study were packed in the same kind of sachets as the premix samples. Each sachet contained 600 g of the medicated farm feed.

All materials were supplied by Andrés Pintaluba Corp., Reus, Spain.

Apparatus. The instrument used for processing medicated farm feed was a V blender (Turu Corp., Tarrasa, Spain). For the stability study, ovens (Radiber model Din 43700 and Salvis model Pt 100, Barcelona, Spain) and a thermohygrometer (Testoterm 6000/6010, Kainos, Madrid, Spain) were used.

Storage Conditions. Both medicated premix and farm feed were tested under accelerated and long-term storage conditions.

Thus, storage conditions of medicated premix were established according to ICH recommendations for the stability testing of new drug products (*12*, *13*).

Initially the temperature and relative humidity (RH) for the accelerated conditions were 40 ± 2 °C and 75 ± 5%, respectively. The samples were tested after 6 months of storage. If the active ingredient undergoes a chemical degradation, the study at accelerated conditions is carried out at an intermediate condition: 30 ± 2 °C and 60 ± 5% RH.

The temperature and relative humidity for the study under long-term storage conditions were 25 ± 2 °C and 60 ± 5% RH, respectively. Samples were analyzed every 6 months. The storage conditions used for the medicated farm feed were the same as those used for the medicated premix, but the length of time of the stability study is shorter due to the prompt use of the product after its mixture with animal feed in veterinary practice.

Analysis of the Samples. The stability study was carried out for the samples of three batches of medicated premix and medicated farm feed. Every batch of the latter was elaborated from the corresponding batch of the medicated premix. The samples were assayed before (initial time) and after the storage under the different conditions.

The quantity of the active ingredient was determined by using a microbiological bioassay method. The method was previously validated to quantify the erythromycin thiocyanate included both in the medicated premix and in farm feed (*14*, *15*). The number of replicates (n) needed to obtain a valid result was <4 in all cases, for a probability level of 0.05.

The analytical conditions of the method were as follows: microorganism, *Bacillus subtilis* ATCC 6633; test agar, A; test agar pH, 9; diameter of the paper disks, 6 mm; range of erythromycin concentration, 0.3, 0.7, 1.0 (reference concentration), 1.5, and 2.5 μg/mL; incubation temperature of the dishes, 33.5 ± 1.5 °C.

Statistical Treatment of the Results. To evaluate the stability of the premix during the processing of the medicated farm feed, the data of the active ingredient content both for premix and for medicated samples before storage (initial time) were used. These data were expressed as percentage of declared amount: 20 mg of erythromycin thiocyanate/100 mg of product in the premix and 600 mg of erythromycin thiocyanate/100 g of product in the medicated farm feed.

Table 1. Results of the Erythromycin Thiocyanate Stability Study during the Processing of the Medicated Farm Feed

	batch 1		batch 2		batch 3	
	x^a (%)	σ^b (%)	x (%)	σ (%)	x (%)	σ (%)
premix	105.0	9.6	100.9	6.9	109.9	10.1
mixture	101.5	9.7	99.1	6.5	103.3	4.3
t^c	0.78		0.31		1.90	
P^d	0.44		0.76		0.08	

[a] x = erythromycin thiocyanate average percentage respect to the declared value. [b] σ = standard deviation. [c] t = Student's t test. [d] P = significance level.

The data of the premix were compared with those of the medicated feed elaborated from the same batch. Student's t test was performed.

To propose a shelf life for the premix and medicated farm feed, the data of the active ingredient content both for premix and for medicated farm feed samples before and after storage were used. These data were expressed as milligrams of erythromycin in 100 mg of product. In a first step, the homogeneity among samples coming from the three batches was tested by means of an ANOVA (analysis of variance). If the homogeneity was proved, the data of the three different batches were mathematically treated together, and if it was not shown, the treatment was applied to samples of each of the batches.

In a second step, a comparative study of data from samples before and after storage is carried out by means of a Student's t test.

For all of the stability studies, a Student's t test was employed to determine if there was a significant difference between the mean of the two populations for a certain degree of acceptance. When the difference was not significant, it was concluded that there was no degradation of the active ingredient and the unaltered percentage of the active ingredient was considered to be 100%. On the other hand, in the event of significant difference, it was considered that the active ingredient underwent a chemical degradation that was quantified by calculating the unaltered percentage of the active ingredient in relation to the initial value.

The acceptance level for all of the statistical parameters was 0.05.

RESULTS AND DISCUSSION

The results of the stability study of the erythromycin thiocyanate during the manufacture of the three batches of medicated farm feed are shown in the Table 1. It is important to express the active ingredient content as a percentage with respect to the theoretical value in order to compare the medicated premix results with the ones obtained from the medicated farm feed. In the stability study of the erythromycin thiocyanate during the manufacture of the medicated farm feed, the three batches were compared with their respective batches of premix; therefore, the comparison is batch to batch. The difference between the average values expressed as the percentage of the declared value of the three batches was not statistically significant. For this reason it was concluded that there was no degradation of the drug during the preparation of the medicated farm feed.

Table 2 shows the quantity of erythromycin thiocyanate included in the three batches of the premix at initial time. At initial time, from the F value obtained by ANOVA test, it was concluded that the variance of the erythromycin thiocyanate content for the three batches of premix was not statistically significant and, therefore, they were considered to be homogeneous. For this reason, the average content of erythromycin thiocyanate was calculated from the three batches of the medicated premix altogether. The possibility of com-

Table 2. Initial Amount of Erythromycin Thiocyanate Present in the Three Batches of the Premix and Statistical Parameters

	batch 1	batch 2	batch 3
n	10	10	10
x^a (mg)	21.01	20.18	21.98
σ (mg)	1.92	1.37	2.03
F^b (P)		2.50 (0.10)	
X^c (mg)		21.05	
σ (mg)		1.89	

[a] x = mg of erythromycin/100 mg of product. [b] $F = F$-Snedecor. [c] X = average drug content.

Table 3. Initial Amount of Erythromycin Thiocyanate Present in the Three Batches of the Medicated Farm Feed and Statistical Parameters

	batch 1	batch 2	batch 3
n	10	10	10
x (mg)	609.00	594.60	619.80
σ (mg)	57.04	37.99	25.18
F (P)		0.83 (0.44)	
X (mg)		607.80	
σ (mg)		41.89	

bining the results coming from the three batches augments the statistical power for later comparisons because the data number at initial time increases from 20 to 30.

Table 3 shows the initial content of erythromycin thiocyanate of the three batches of the medicated farm feed. The F value obtained for the medicated farm feed at initial time indicated that the three batches were homogeneous and, then, the average erythromycin thiocyanate content was calculated.

Tables 4 and 5 contain the statistical parameters for the three batches of the medicated premix under accelerated (6 months) and long-term storage conditions (1 and 2 years), respectively. From the data obtained in the stability study of the medicated premix, it can be observed that the F value was statistically significant for the samples of premix stored for 6 months at 40 °C and 75% RH. Therefore, the initial content was compared with the final content for each batch separately. The Student's t test result proved that the erythromycin thiocyanate included in the samples of batches 1 and 2 of the premix stored at 40 °C and 75% RH underwent a chemical degradation, which was not observed in the samples of batch 3. The active ingredient degradation was, approximately, 10% in batch 1 and 16% in batch 2. The result of the batch that undergoes a higher drug degradation (batch 2) will be considered as final result of this study.

As a consequence of these results, the study was carried out under new accelerated storage conditions: 30 °C and 60% RH. The statistical parameters of the stability study at this condition are shown in Table 4. By ANOVA test, it was concluded that the variance of the three batches of the medicated premix was not statistically significant. In this case, the average drug contents before ($n = 30$) and after ($n = 15$) storage were compared. No significant degradation of the erythromycin thiocyanate contained in the samples of the three batches of the premix stored for 6 months at 30 °C and 60% RH was detected through the value of Student's t test. Then the unaltered percentage of drug was considered to be 100%. For this reason, the product will be handled at a temperature and relative humidity not higher than 30 °C and 60%, respectively.

From the F value obtained in the medicated premix stability study carried out under long-term storage conditions, it was concluded that there was no significant difference among the variances of the three batches of the premix stored under long-term storage conditions during 12 months. Therefore, the average drug content of the three batches was calculated and compared by a Student's t test with the average content obtained at initial time. No significant drug degradation after 12 months of storage was detected. Because of this, the stability study was prolonged for up to 24 months. After 2 years of storage at 25 °C and 60% RH, no degradation of the erythromycin thiocyanate was detected. Therefore, this period of time could be proposed as the shelf life of the product for this storage condition. The unaltered percentage of drug was 100%.

Table 6 contains the statistical parameters for accelerated storage conditions and long-term storage conditions of the three batches of the medicated farm feed. The stability study was carried out at 30 °C/60% RH because the active ingredient included in the medicated premix had undergone a chemical degradation at 40 °C and 75% RH.

In the stability study of the medicated farm feed it was observed, from the F value, that the variance of the three batches of the medicated farm feed, both for accelerated storage conditions and for long-term testing conditions, was not statistically significant. Therefore, by Student's t test, the average drug content of the stored samples was compared with that obtained at initial time.

The erythromycin thiocyanate of the samples of the three batches of the medicated farm feed stored during 3 months under accelerated storage conditions underwent a chemical degradation. The unaltered percentage was 84.84%. During this period of time there was no drug degradation at 25 °C and 60% RH. Because of that, the product must be stored and distributed at a temperature and relative humidity not higher than 25 °C

Table 4. Statistical Parameters for the Data Obtained from the Accelerated Stability Study of the Three Batches of the Premix after 6 Months of Storage

	40 °C/75% RH			30 °C/60% RH		
	batch 1	batch 2	batch 3	batch 1	batch 2	batch 3
n	5	5	5	5	5	5
x (mg)	18.93	16.93	20.91	19.88	20.18	21.58
σ (mg)	0.77	1.11	2.36	2.23	0.99	1.59
CV[a] (%)	4.05	6.56	11.29	11.21	4.90	7.37
F (P)		8.06 (0.01)			1.46 (0.27)	
X (mg)					20.55	
σ (mg)					1.74	
t (P)	4.34 (0.02)	4.71 (0.00)	0.15 (0.88)		0.86 (0.39)	
UP[b]	90.12	83.88	100		100	

[a] CV = coefficient of variation. [b] UP = unaltered percentage.

Table 5. Statistical Parameters for the Data Obtained from the Stability Study of the Three Batches of Medicated Premix after 12 and 24 Months of Storage under Long-Term Storage Conditions

	12 months			24 months		
	batch 1	batch 2	batch 3	batch 1	batch 2	batch 3
n	10	10	10	10	10	10
x (mg)	20.33	20.50	20.78	20.47	19.65	20.71
σ (mg)	1.77	1.33	1.58	1.46	1.49	2.10
CV (%)	8.71	6.49	7.60	7.13	7.58	10.13
F (*P*)		0.21 (0.81)			0.85 (0.44)	
X (mg)		20.54			20.21	
σ (mg)		1.53			1.77	
t (*P*)		1.15 (0.25)			1.76 (0.08)	
UP		100			100	

Table 6. Statistical Parameters for the Stability Study of the Three Batches of the Medicated Farm Feed after 3 Months of Storage under Accelerated and Long-Term Conditions

	30 °C/60% RH			long-term storage conditions		
	batch 1	batch 2	batch 3	batch 1	batch 2	batch 3
n	5	5	5	5	5	5
x (mg)	519.80	505.27	522.00	587.85	607.81	609.95
σ (mg)	26.90	46.30	32.07	55.65	73.08	32.02
CV (%)	5.17	9.16	6.14	9.46	12.02	5.26
F (*P*)		0.32 (0.73)			0.22 (0.80)	
X (mg)		515.69			601.87	
σ (mg)		35.09			53.58	
t (*P*)		7.21 (0.00)			0.43 (0.67)	
UP		84.84			100	

Table 7. Pattern of Stability Study Report: Storage and Distribution Recommendations and Proposed Shelf Life

storage and distribution recommendations
premix: ≤30 °C; <60% RH
farm feed: ≤25 °C, ≤60% RH
shelf life proposed
premix: 2 years
farm feed: 3 months

and 60%, respectively. Three months can be proposed as the shelf life of the samples of the medicated farm feed because this is time enough to be consumed.

Table 7 shows a pattern of the stability study report.

A review of the related literature reveals that there are not many works. Most previous studies have determined the stability of the medicated premix and medicated feed during the treatment period by analyzing the drug content the day dosing was started and the day of withdrawal, taking into account the limits of potency lost (*16*, *17*). No other stability study of macrolide antibiotics in medicated premixes or medicated feed has been found.

ABBREVIATIONS USED

ICH, International Committee of Harmonization; RH, relative humidity; *n*, number of replicates; ANOVA, analysis of variance.

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