Stability Study of a Sulfadimidine-Containing Medicated Premix and Its Mixture with Farm Feed

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The objective of this paper was to study the stability of a medicated premix and a medicated farm feed containing sulfadimidine as the active ingredient. The medicated premix is supplied as powdered form and administered orally after mixing with animal feed. The sulfadimidine analytical method described in United States Pharmacopoeia 23 cannot be used to carry out the stability study because of its lack of specificity for different degradation products. Therefore, a high-performance liquid chromatography method was developed to assay sulfadimidine. This method was optimized and validated for the stability study. According to International Committee Harmonization (ICH) guidelines, the samples were stored under long-term testing and accelerated conditions for the stability study of the premix and the medicated farm feed. Sulfadimidine degradation was not detected in either form under any of the conditions studied.

Keywords: *Sulfadimidine; medicated farm feed; medicated premix; stability study; HPLC*

INTRODUCTION

In addition to other requirements, current regulations of the European Union (EEC 1981, 1986, 1990, 1992) regarding the commercialization of veterinary medicines require specific terms to be fulfilled:

(a) analytical assay using validated procedures that are in agreement with the most recent scientific findings, and

(b) conduct of a stability study.

Studies carried out to determine the shelf life of the product are required to be described and justified, as well as storage conditions and post shelf life specifications. The information regarding the shelf life of both the premix and medicated feed should be included in accord with the recommended instructions.

Sulfonamides may be mixed with animal feed in order to be administered to animals. The medicated premix, that is, the veterinary medicinal product prepared with the purpose of including it in the farm feed, is supplied as powdered form and administered orally after mixing it with animal feed.

The objective of this paper is to study the stability of veterinary products containing sulfadimidine as active ingredient. To achieve this objective, an analytical method employing high-performance liquid chromatography (HPLC) is optimized and validated.

Both the stability study and the validation of the analytical method were planned according to the current regulations of the Committee for Proprietary Medicinal Products of the International Committee for Harmonization (CPMP/ICH, 1995a,b).

This medicine is expected to be commercialized as such and as a mixture with feed, therefore this stability study was carried out for both the medicated premix and the medicated farm feed.

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EXPERIMENTAL PROCEDURES

Materials. Raw materials included sulfadimidine (purity, minimum 99%), calcium carbonate (purity, minimum 99%), and feed for pigs (ingredients, cereals and oily seeds; composition, protein (17%), fats (6%), cellulose (4.5%), ashes (6.2%), vitamin A (8000 I.U./kg), vitamin D_3 (1800 I.U./kg), vitamin E (15 mg/kg), and copper (90 mg/kg)) supplied by Andrés Pintaluba Corporation, Reus, Spain. The medicated premix consisted of sulfadimidine and calcium carbonate (25:75) and was mixed by Andrés Pintaluba Corporation, Reus, Spain. The medicated farm feed was formed by medicated premix and animal feed (1:10 000 of sulfadimidine). They were mixed by Andrés Pintaluba Corporation, Reus, Spain. Sachets lined internally with polyethylene and two sheets of opaque kraft paper (70 g) were used for packaging of the medicated feed and were also supplied by Andrés Pintaluba Corporation, Reus, Spain.

The apparatus for conditioning of the sample was a V blender (Turu S). Apparatus for assay consisted of a high-performance liquid chromatograph (Gilson, model 807), detector (Gilson 116 UV), integrator (SP 4270/4290), columns (C 18 reversed-phase microbondapack 300 mm × 3.9 mm i.d., particle size 10 μm and Spherisorb P 300 mm × 3.9 mm i.d.. particle size 10 μm), potentiometer (Crison Micro TT 2022), and pH meter (Beckman, model Tanssonic 460). Apparatus for the stability study included ovens (Radiber model Din 43700 and Salvis model Pt 100) and a thermohygrometer (Testoterm 6000/6010).

Potentiometry. The sulfadimidine analytical method described in United States Pharmacopoeia 23 (United States Pharmacopoeia, 1995a) is a potentiometric one. As the principal path of degradation of sulfonamides does not affect the primary amine group, it is supposed that sodium nitrite will react with both the unaltered sulfadimidine and any product of degradation.

With the aim of checking whether the proposed analytical method may be used for the stability study of sulfadimidine, its specificity was evaluated. The specificity, that is the capacity of the method to quantify only sulfadimidine without interference of the other compounds (products of degradation), was determined by studying the effect of aging on sulfadimidine dissolved in 1N NaOH (25 mg/mL) and 2N HCl

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(12.5 mg/mL). The solution was stored for 21 days at 90 °C and was analyzed later by the USP23 (1995a) potentiometric method.

High-Performance Liquid Chromatography. *Optimization of the Chromatographic Method.* Initially, a chromatographic system proposed by Sharma et al. (Sharma, 1976) was tried. A Microbondapack C18 reversed-phase column (300 mm × 3.9 mm i.d.) with a particle size of 10 μm was used. The mobile phase was methanol and the detection wavelength was 254 nm. However, the method was optimized by changing different variables until a constant retention time was obtained.

The chromatographic system used to determine sulfadimidine operated with a column (C18 reversed-phase Microbondpack 300 mm × 3.9 mm i.d.; particle size, 10 mm) and a mobile phase of methanol and 66 mM phosphate buffer (pH 7.2), at a ratio of 30:70. The wavelength for ultraviolet (UV) detection was 254 nm; the flow rate was 0.8 mL/min, and the injection volume was 20 ml.

The assay of sulfadimidine in the medicated farm feed was carried out without any interference by using two columns simultaneously (C18 reversed-phase Microbondpack 300 mm × 3.9 mm i.d., 10 μm, and Spherisorb p 300 mm × 3.9 mm i.d., 10 μm).

Preparation of Standard Samples. Sulfadimidine (100 mg) was dissolved in 1N HCl and the volume was made up to 10 mL. The solution was diluted 100 times with distilled water. Different amounts of this solution were transferred to a 10-mL volumetric flask and the volume was adjusted up to the mark to obtain the desired concentration. By determining the linearity of the chromatographic method, 6 μg/mL of the solution was considered as 100%. The pH values of the resulting solution were in the range of 3 to 4.

Preparation of Test Samples. The medicated premix (400 mg, corresponding to 100 mg sulfadimidine) was treated in the same manner as the standard samples. The theoretical concentration of the final solution was 6 μg/mL.

For the medicated farm feed, 1000 mL of 1N HCl was added to 100 g of the medicated farm feed and the solution was agitated continuously for 15 min. The mixture was centrifuged and the supernatant was filtered. Sodium hydroxide solution (2N) was added to 10 mL of the filtrate to obtain a pH value between 3 and 4. The volume of the solution was made up to 20 mL by adding citrate buffer of pH 3.5. The theoretical concentration of sulfadimidine in the final solution was 5 μg/mL.

The amount of sulfadimidine in mg contained in the samples (Q_p) was calculated from

$$Q_p = C_s \frac{V_s A_p}{A_s} f(>)$$

where C_s = concentration of the standard solution injected, V_s = volume of the last dilution of the standard solution, A_s and A_p = peak area corresponding to standard and test solution, respectively, and f = dilution factor.

Validation of the Analytical Method. The method was validated according to the validation protocol described by Camacho et al. (1993) and taking into account the current guidelines of the analytical procedures provided by the International Committee for Harmonization (ICH).

The parameters determined were linearity, sensitivity, accuracy, precision, and specificity. For the linearity study standard samples were used. For this reason, a only linearity study is useful for both premix and farm feed. The concentration range was 2−10 μg/mL. The curve parameters, slope (b) and intercept (a), were calculated by ordinary least squares. The regression was statistically analyzed by the coefficient of correlation (r), the coefficient of determination (r^2), and an analysis of variance (ANOVA) for the null hypothesis of the slope ($b = 0$). The goodness of the linear fitting was determined by another ANOVA. Finally, the proportionality of the method or null hypothesis of the intercept ($a = 0$) was studied by a Student's t-test.

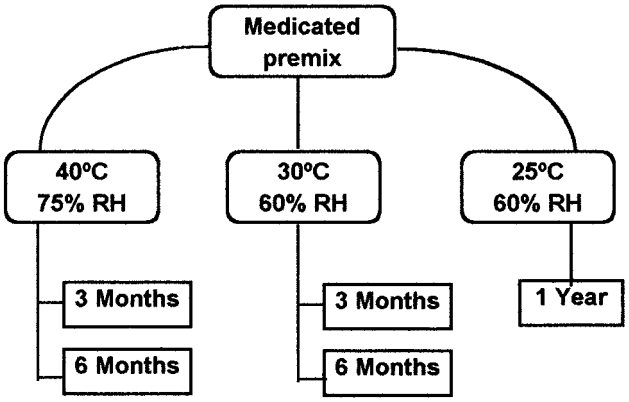

Figure 1. Schematic diagram of the stability study for the medicated premix (RH, relative humidity).

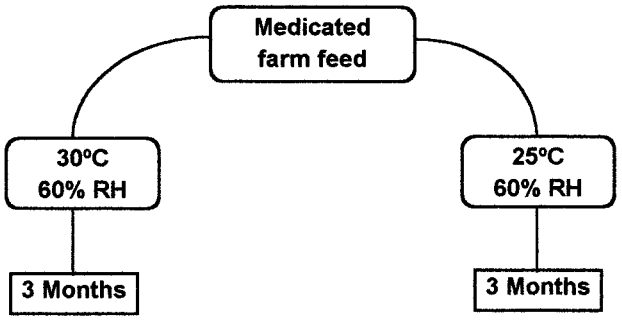

Figure 2. Schematic diagram of the stability study for the medicated farm feed.

Table 1. Linearity: Regression Line Parameters, Regression Variance Analysis

total number of samples	14
linear range (μg/mL)	2−10
correlation coefficient, r	0.9974
determination coefficient, r^2	0.9950
slope b (A^b × mL/μg)	40226.02
standard deviation of b (A × mL/μg)	1072.60
intercept a (A)	−511.81
standard deviation of a (A)	6821.33
CV residual (%)	2.81
ANOVA test F regression	1406.48[a]
F linear model	1.83
t-test of proportionality	0.08

[a] $P<0.01$. [b] A, peak area. [c] CV, coefficient of variation.

For the sensitivity study, the experimental results of the linearity study were used. It was calculated by the calibration sensitivity (b), the analytical sensitivity (b/Sy), and the discriminator capacity ($(b/Sy)t$, where t is the Student's t-test value for a 0.05 significance level).

For the precision study, test samples were analyzed. Repeatability and intermediate precision were determined. The number of the sample replication for a precise assay was calculated by the coefficient of variation obtained in the repeatability study (Camacho, 1993). Time as different day of analysis was considered in the intermediate precision study. The coefficient of variation obtained in the intermediate precision study was compared with that obtained in the repeatability study to determined the possible influence of the time factor in the analytical results.

For the accuracy study, test samples were analyzed. The mean recovery in percentage was calculated and compared with 100% (theoretical recovery) by a Student's t-test.

The specificity of the analytical method was determined according to the objective of the work, which was a stability study. Thus, chromatograms of the samples were compared: standard sample, freshly prepared test sample, and test samples after aging.

Table 2. Sensitivity of the Analytical Method

calibration sensitivity ($A \times$ mL/μg)	40 226.02
mean analytical sensitivity (mL/μg)	6.66
discriminatory capacity (μg/mL)	0.32

Table 3. Precision and Accuracy: Statistical Parameters

	premix (6 μg/mL)	farm feed (5 μg/mL)
average peak value (A)	233 028.50	103 482
CV repeatability (%) ($n = 6$)	2.71	3.61
mean recovery percentage, R (%) ($n = 6$)	98.52	103.22
Student's t test	0.80	2.19
CV intermediate precision (%) ($n = 30$)	1.98	

Stability. *Sample Preparation.* Samples of both medicated premix and farm feed were packed in sachets made of the materials used for the final product packaging.

Storage Conditions. The designs of the stability studies for premix and its mixture with animal feed are shown schematically in Figures 1 and 2, respectively. The storage time for the stability study of the medicated farm feed (Figure 2) was three months, because it was considered time enough for the feed to be consumed.

Analysis of the Samples. The quantity of the active ingredient present in each of the samples, both medicated premix and farm feed, was determined by the analytical method described previously.

Statistical Treatment of the Results. Statistical treatment was used to analyze the results obtained from the stability study. First, the mean of the active ingredient present in 100 mg of the product was calculated, and then the corresponding percentage of the active ingredient was also calculated by comparing with the theoretical value and in the same way respective deviation was calculated. The coefficient of variation was calculated, and from the value corresponding to the Student's t-test, the mean of the experimental content was compared with the theoretical value. In this way, it was determined whether a significant difference exists between the calculated and theoretical values, and then a conclusion was drawn regarding whether the samples contained the correct dose.

Considering all the initially assayed samples as reference, a comparative study of the samples assayed in different times and conditions was carried out. By means of a Student's t-test it was determined if there was significant difference between the mean of the two populations for a certain degree of acceptation. 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 as 100%. On the other hand, in the event of significant difference, it was considered that degradation occurred and the degradation was quantified by calculating the unaltered percentage of active ingredients.

For the calculations of the Student's t-test, it was considered if the variances were homogeneous or not, and this was determined by the Snedecor F-test.

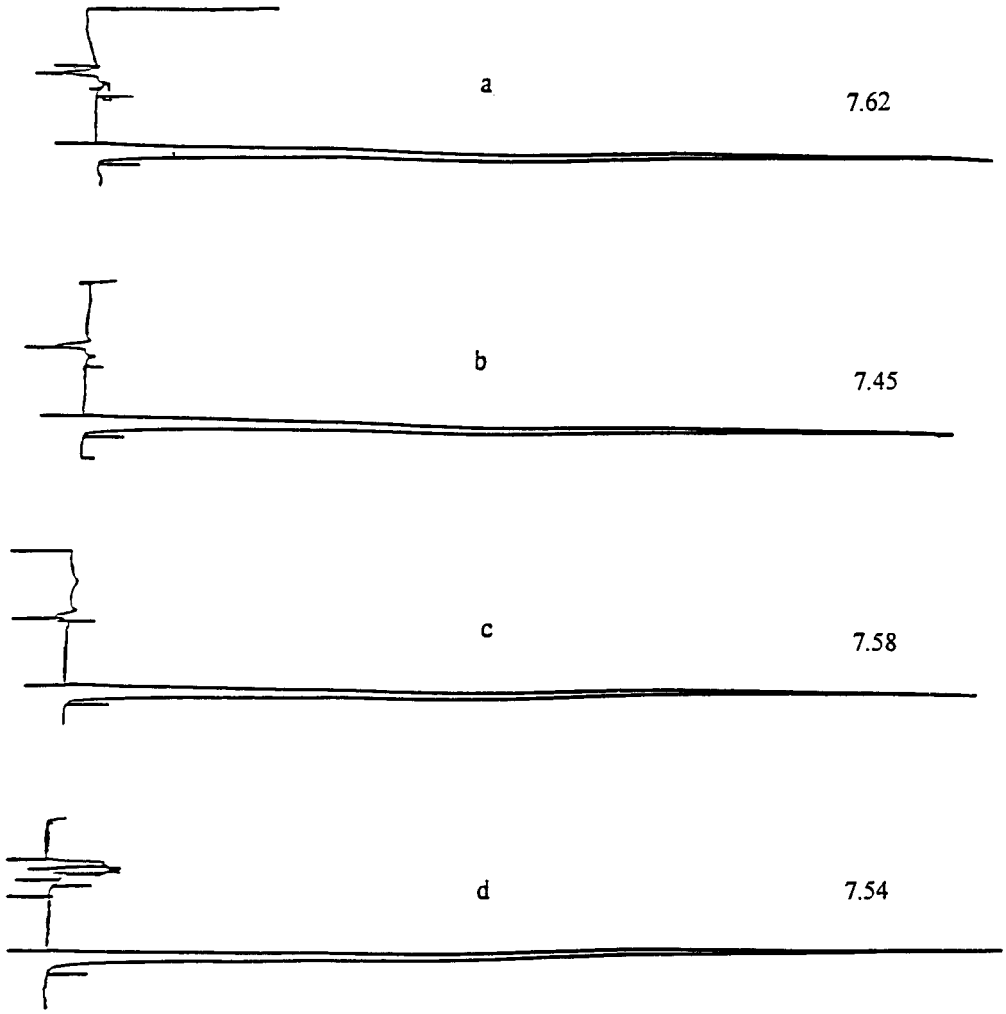

Figure 3. Specificity of the chromatographic method for the assay of sulfadimidine in the medicated premix: (a) standard sample; (b) freshly prepared sample; (c) sample stored for 6 months under accelerated conditions (30 °C, 60% RH); (d) sample stored for 12 months under long-term testing conditions (25 °C, 60% RH).

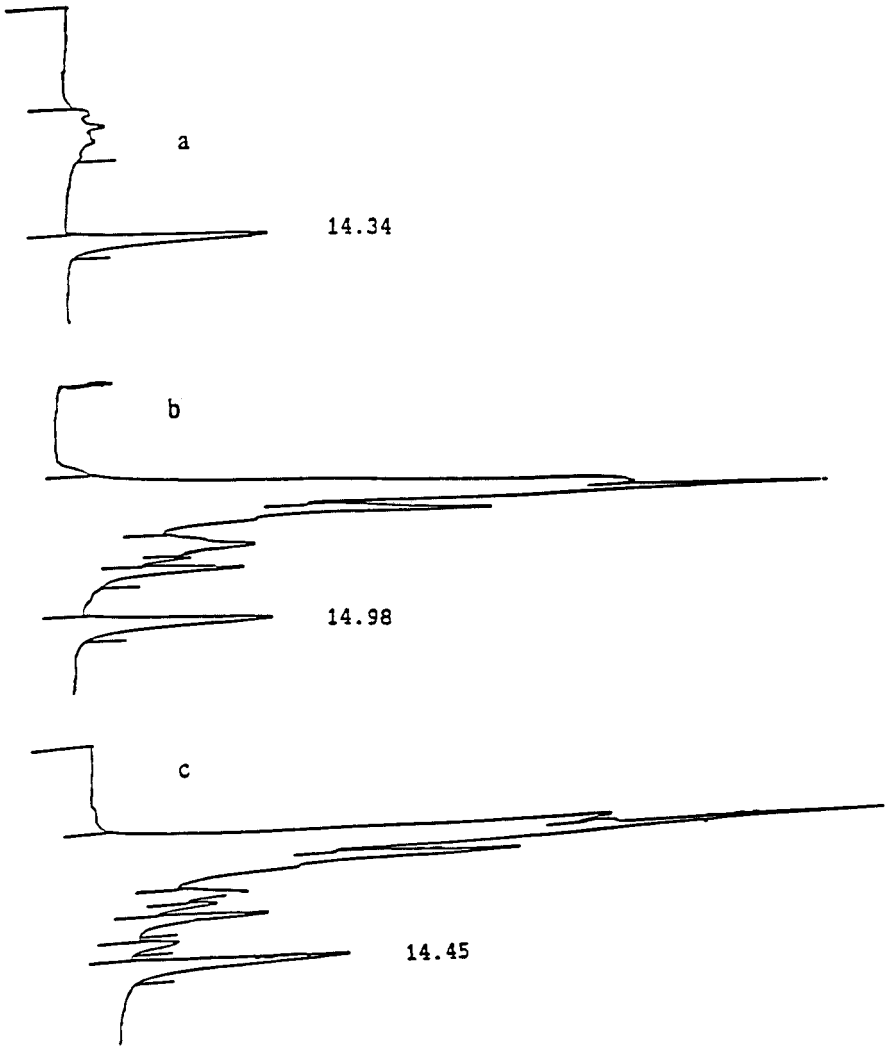

Figure 4. Specificity of chromatographic method for the sulfadimidine assay in the medicated farm feed: (a) standard sample; (b) freshly prepared sample; (c) sample stored for three months under accelerated conditions (30 °C, 60% RH).

RESULTS

Assay Methods. In the specificity study of the potentiometric method, all the samples with sulfadimidine degradation products presented a quantity of unaltered drug higher than that before aging of the sample. It was confirmed that sodium nitrite reacts with both the unaltered sulfadimidine and any product of degradation. Thus, the potentiometric method was determined to be not valid for the objective of the present work, which is a stability study. Then, the high-performance liquid chromatographic method described above was used.

Validation of the Chromatographic Analytical Method. Table 1 contains the parameters of linear regression calculated for the calibration curve. The curve parameters, slope, and intercept are shown. The regression may be statistically understood through the values of the coefficients of correlation ($r = 0.9974$) and determination ($r^2 = 0.9950$), as well as through the F value from the ANOVA of regression (1406.48). The F value from the ANOVA of the linear model (1.83) shows the goodness of the linear fitting. The proportionality of the method may be studied by the value of the t-test (0.08) from comparing the intercept with 0.

Table 2 contains the sensitivity parameters of the chromatographic method in the range of concentration studied (2−10 μg/mL).

As far as the precision and accuracy studies for the analysis of both premix and farm feed are concerned, the statistical treatment of the data are presented in Table 3.

The small values of the repeatability coefficients of variation of both premix and farm feed determined the precision of the analytical method. The value of the intermediate precision coefficient of variation of premix was even smaller than that of the repeatability coefficient.

For the accuracy study, through the t-test value obtained, it can be said that there was no significant difference between the mean recovery (in percentage) and the theoretical value (100%) for both premix and farm feed.

The chromatograms of the specificity study of the method are shown in Figures 3 and 4 for premix and farm feed, respectively.

Stability. Table 4 contains the statistical parameters for the concentration of sulfadimidine in the medicated premix at the initial time of storage and during the accelerated storage and long-term storage conditions.

Table 5 contains the statistical parameters for the concentration of sulfadimidine in the medicated farm feed at initial time and during the accelerated storage and long-term storage conditions.

Table 4. Stability Results: Statistical Parameters for the Concentration of Sulfadimidine in the Premix Both for Accelerated Storage Conditions and Long-Term Testing Conditions with Respect to the Initial Assay of the Premix

		3 months		6 months		12 months
	$t = 0$	30 °C, 60% RH[a]	40 °C, 75% RH	30 °C, 60% RH	40 °C, 75% RH	25 °C, 60% RH
mg of sulfadimidine/100 mg of premix, x	24.60	25.94	24.81	26.14	25.82	24.71
number of replicates, n	10	5	5	5	5	10
standard deviation, σ (mg/100 mg)	2.09	1.55	1.66	1.60	1.58	2.79
coefficient of variation, CV (%)	8.47	5.97	6.70	6.13	6.11	11.28
F_{exp}		1.8120	1.5728	1.6925	1.7457	1.7871
t_{exp}		1.2658	0.1974	1.5835	1.2707	0.0999
unaltered percentage, UP		100	100	100	100	100

[a] RH, relative humidity.

Table 5. Stability Results: Statistical Parameters for the Concentration of Sulfadimidine in the Farm Feed for Accelerated Storage Conditions and Long-term Testing Conditions after Three Months of Storage with Respect to the Initial Assay of the Farm Feed

	$t = 0$	30 °C, 60% RH	25 °C, 60% RH
x (mg/100 g)	9.58	8.73	8.55
n	6	6	6
σ (mg/100 g)	0.55	0.74	1.07
CV (%)	5.78	8.43	12.48
F_{exp}		1.7696	3.7141
t_{exp}		2.2149	2.0712
UP		100	100

(x, mg of sulfadimidine/100 g of farm feed)

DISCUSSION

Potentiometry. The analytical method for sulfadimidine described in United States Pharmacopoeia 23 (1995a),a potentiometric method, cannot be used to carry out the present stability study because of its lack of specificity for different products of degradation.

Validation of the analytical method. *Linearity*. The chromatographic method was linear and proportional in the range of concentration studied. A correlation coefficient value of 0.9974 indicates that 99.5% of the variation in peak area is due to the variation in the concentration.

Sensitivity. From the sensitivity parameters of the chromatographic method, it was concluded that the small difference of concentration detected by the method was 0.32 μg/mL.

Precision. As for the repeatability study, the value of the coefficient of variation indicates that the number of replications for precise assay of the active ingredient should be 2 in the case of medicated premix, and it should be 4 in the case of medicated farm feed, with an acceptation level of 0.05. In both cases, the required number was below the number of samples actually analyzed.

From the coefficient of variation for the intermediate precision, it can be said that the time factor does not affect the precision of the results.

Accuracy. From the value of the Student's t-test, it can be concluded that the analytical method was accurate for both the premix and the premix/animal feed mixture.

Specificity. As for the specificity study, from the qualitative point of view it was observed that the chromatograms corresponding to premix were very similar. In addition, all of them were clearly defined.

In the case of the medicated farm feed, the sulfadimidine retention time was greater (approximately twice) than that for the premix due to the fact that two columns were used simultaneously. Because of this fact, the peak corresponding to the active ingredient is easily detectable; however, because of the presence of the soluble components of the feed, the chromatograms from the mixture became less clear than those of the premix. To determine whether the presence of other components had any interference, the resolution between the peak for sulfadimidine and the nearest peak was calculated (chromatograms b and c in Figure 4) (USP 23, 1995b). For the chromatograms b and c, this factor was 2.69 and 1.18, respectively. As both of them were higher than unity, it was considered that the peak areas of the sulfadimidine in the samples corresponds exclusively to the active ingredient, without any interference.

The analytical method developed from the HPLC in this work is valid and applicable for both medicated premix and farm feed containing the antibiotic.

Stability. There was no significant degradation of sulfadimidine in the premix during the study period at the chosen conditions.

There was no statistically significant degradation of the active ingredient in the medicated farm feed.

Sulfadimidine degradation was not detected in the premix or its mixture with animal feed, under any of the conditions studied.

Abbreviations Used. CPMP, Committee for Proprietary Medicinal Products; ICH, International Committee of Harmonization; i.d., internal diameter; HPLC, high-performance liquid chromatography; USP 23, United States Pharmacopoeia; UV, ultraviolet.

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