ANALYTICAL CONTROL OF AMOXICILLIN AND ENROFLOXACIN IN VETERINARY DRUGS AND FEED SAMPLES FOR PIGS

Mrs. Esther Miralles-Cardiel [1], Dr. Isidre Casals-Ribes[1], Mr. Andrey Skryl[2], Dr. Sergey Shapovalov[2], Dr. Elena Kornilova[2], Dr. Andrey Potechin[3].

1 CCiTUB (Scientific and Technological Centers), University of Barcelona, Separative Techniques Unit
2 Limited Liability Company Research and Testing Center "Cherkizovo", Russia
3 Biokorm International S.L.

ANNOTATION

To assess the stability of feed drugs containing amoxicillin and enrofloxacin during the process of feed pelleting, the laboratories of the Russian Federation and Spain conducted studies of feed before and after granulation, as well as veterinary drugs under conditions simulating granulation. The HPLC method was used to determine the concentration of amoxicillin and enrofloxacin in samples of the veterinary drugs "Amoxigran" and "Enrogran" by Biokorm International, as well as in samples of feed for pigs with the addition of these drugs. In studies carried out both in Spain and in Russia, the average concentrations of amoxicillin in the feed with Amoxigran and enrofloxacin in the feed with Enrogran before and after granulation were above 90 and 94% of the theoretical content, respectively, which confirms the satisfactory stability of the active ingredient. The innovative development of micro-granulation of the drugs "Amoxigran" and "Enrogran" allows them to be effectively used in the production of granulated feed.

INTRODUCTION

Despite progress in the development of specific prophylaxis against bacterial diseases, pigs are often not protected due to the insufficient duration of hyperimmunity as a result of vaccination carried out after weaning. So the use of antibacterial agents in livestock in order to prevent economic losses due to bacterial infections seems to be justified. The use of drugs with feed is the most acceptable option, since large groups of animals can be treated without exposure to stress factors and with minimal labor costs.

In pig breeding, antibiotics of the group of penicillins and fluoroquinolones, which include amoxicillin and erofloxacin, which have a wide spectrum of bactericidal action, are among the most frequently used antibacterial drugs with feed. At the same time, the drugs used must meet all the requirements of feed production, namely, have the following properties: thermal stability (resistance to high temperatures during feed granulation), compatibility with feed components due to carefully selected auxiliary substances, protection from environmental factors (temperature, humidity, natural oxygen content of air and UV rays), as well as a high degree of flowability, absence of electrostatic properties and optimal granule size (0.3 - 0.7 mm).

Several studies have shown that amoxicillin is effective in treating streptoccosis and hemophilic polyserositis (Higgins and Gottschalk 2006; Rapp-Gabrielson et al. 2006). Most strains of Streptococcus suis and Haemophilusparasuis isolated from pigs are highly susceptible to the drug (Marie et al. 2002; Pejsak et al. 2005; Schwarz et al. 2007, 2008). When administered orally, amoxicillin is rapidly absorbed in the gastrointestinal tract and penetrates into most organs and tissues of the animal.

However, amoxicillin, like other penicillins, is known to be unstable to fluctuations in pH, temperature, pressure and humidity that occur during feed granulation and storage. This disadvantage of the drug is the main obstacle to its effective use in the composition of granulated feed. In addition to the inactivation of amoxicillin, the composition of the feed has a great influence on the stability of the drug during the granulation process. Thus, in the manufacture of a medicinal feed, agro-technicians and nutritionists are faced with problems of the active

ingredient stability. In this regard, various methods have been developed to protect amoxicillin (coating, encapsulation) from the influence of external influences (Derrieu et al. 2000; Bousquet et al. 2006).

Also, a number of works shows that enrofloxacin belongs to the fluoroquinolone series drugs, it is widely used to treat various respiratory, gastrointestinal and urogenital diseases of pigs caused by gram-negative and gram-positive bacteria, as well as mycoplasmas. Enrofloxacinis partially metabolized in the liver to form ciprofloxacin, which also has antibacterial activity, while pigs can be slaughtered for meat no earlier than 5 days later (Trouchonet al. 2016).

For the qualitative and quantitative analysis of amoxicillin and enrofloxacin in dosage forms and feed, the HPLC method with various detection methods is currently used (Peddi et al. 2016; Zemanova et al. 2008).

The aim of this study was to determine the concentration of amoxicillin and enrolfoxacin by HPLC in commercial feed batches with the addition of the commercial drug Amoxigran and a number of other drugs containing amoxicillin, as well as the drug Enrogran, before and after granulation at various temperatures. In addition, the concentration of amoxicillin in the commercial drug Amoxigran and a number of other drugs containing amoxicillin was monitored during a laboratory experiment simulating granulation conditions.

MATERIALS AND METHODS

AMOXICILLIN

Standard samples. A certified standard of amoxicillin trihydrate (CAS: 61336-70-7) obtained from Sigma-Aldrich was used for the studies.

Standard solutions. A basic standard solution of amoxicillin (1 mg / cm^3) was prepared by dissolving a sample in a water / methanol 80/20 v / v mixture. When calculating the concentration, the purity of the standard and the content of amoxicillin base in the amoxicillin trihydrate molecule were taken into account. 5 calibration solutions with amoxicillin concentration from 10 to 200 μg / cm^3 were prepared fresh by appropriate dilutions of aliquots of the stock solution with a buffer solution (pH = 6.0).

Extraction.

Feeds. Before analysis, all feed samples were ground using a centrifugal mill (Retsch ZM 200) with a 1 mm sieve (Cherkizovo Lab) and a SPEX cryogenic mill model 6850 (University of Barcelona). The analysis was carried out in duplicate. A 25 g sample of milled feed was weighed in a conical flask with a volume of 250 cm^3. Then 50 cm^3 of chloroform was added thereto, the suspension was stirred, and then the flasks were transferred to an ultrasonic bath for extraction for 15 minutes. Thereafter, 100 cm^3 of buffer solution was added to the mixture, and extraction was continued with a horizontal shaker for 30 min at 200 rpm. Then the entire volume of the resulting mixture was transferred into centrifuge tubes with a volume of 50 cm^3 and centrifuged for 15 min at 15-20 ° C, 3000 rpm. The top layers from each respective tube were pooled in glass jars, and about 1 ml of the extract was filtered through a 0.45 μm nylon syringe filter.

Veterinary drugs. A commercial drug "Amoxigran" (Biokorm International) was used in the work in the form of a premix (A) containing 15% of amoxicillin trihydrate in the form of a microencapsulated powder and an excipient (feed base). Commercial drugs in the form of premixes (B) and (C) contained 10% and 20% of native amoxicillin trihydrate respectively. A 2 g sample was placed in a 200 cm^3 volumetric flask. About 190 cm^3 of methanol was added and extracted for 30 min using an ultrasonic bath. When the extract was cooled to room temperature, the volume was adjusted to the mark with methanol. An aliquot of 1 cm^3 was transferred into a 10 cm^3 volumetric flask and the volume was adjusted to the mark with a buffer solution. Thereafter, about 1 cm^3 of the diluted extract was filtered through a 0.45 μm nylon syringe filter.

HPLC-UV analysis. A system consisting of an S 5300 autosampler (SYKAM, Germany), an S 1130 pump (SYKAM, Germany) and an S 3350 diode-array detector (SYKAM, Germany) was used for research at Cherkizovo Lab, and a Wathers Alliance 2695 liquid chromatograph, a DAD detector Waters 2996 were used at the University of Barcelona. The wavelengths for the analysis of drugs and feed were 228 and 240 nm respectively. Isocratic elution was carried out for the drugs, and gradient elution for feed on a Mediterranea Sea 18.5 μm, 25x0.46 cm chromatographic column, Teknokroma (Cherkizovo Lab) and Ultrabase 100 (AkadyCromatografia; 4.6 × 100 mm; particle size 3.0 μm (University of Barcelona).In the isocratic mode, the mobile phase consisted of a mixture of a 0.15% solution of phosphoric acid in water, adjusted to pH = 2.6 with a solution of sodium hydroxide 0.1 M and methanol in a ratio of 80/20 v / v.In a gradient elution mode, the above solutions were separated into two banks (A - phosphoric acid solution) and (B - methanol). The gradient program is presented in Table 1.

Table 1 - Gradient program of solutions of phosphoric acid and methanol

Time, minutes	A, %	B, %
0	80	20
12	80	20
14	10	90
19	10	90
20	80	20
26	80	20

The analysis was carried out at a flow of 0.8 ml / min; the injection volume was 20 μL.

Validation of the method. In order to perform the validation in University if Barcelona the next was done: 1) Aliquots of blank mash feed, 25g, were spiked with amoxicillin solution at 100 mg/kg and 300 mg/kg. The repeatabilitiy for n=6 calculated as RSD was 1.0% at both concentrations. 2) Aliquots of blank mash feed, 25g, were spiked with amoxicillin raw material, at 300 mg/Kg. The repeatabilitiy for n=6 calculated as RSD was 2.5%. 3) A pellet feed sample medicated at 225 mg/kg of amoxicillin was analysed under repeatability conditions and the precision found for n=6 was RSD=2.0%. 4) Pellet feed samples medicated at 150 mg/Kg and 200 mg/Kg of amoxicillin were analysed under reproducibility conditions and the RSD found was 10%. The intra-day and inter-day precision are suitable for the analysis of medicated feed samples. The recovery of the method must be established for every laboratory. The recovery found with samples added with amoxicillin solution or amoxicillin raw material was about 75%. The recovery found in samples medicated with Amoxigran, was in the range 70-80%.

ENROFLOXACIN

Standard samples. A certified standard for enrofloxacin (CAS: 93106-60-6) obtained from Sigma-Aldrich was used for the studies.

Standard solutions. A stock standard solution of enrofloxacin (100 mg / cm^3) was prepared by dissolving a weighed portion in an extraction solution (0.1 M sodium phosphate buffer pH 7.4 / ethanol 60/40 v / v). When calculating the concentration, the purity of the standard was taken into account. Five calibration solutions with enrofloxacin concentration from 0.5 to 10 μg / cm^3 were prepared ex tempore by appropriate dilutions of aliquots of the stock solution with the extraction solution.

Extraction. Before analysis, all feed samples were milled using a centrifugal mill (Retsch ZM 200) with a 1 mm sieve. The analysis was carried out in duplicate. A 5 g sample was weighed in a borosilicate glass jar with a volume of 100 cm^3. Then 50 cm^3 of the extraction solution was added to it and the sample was extracted with a horizontal shaker for 60 min at 350 rpm. Then 2 cm^3 of the obtained extract was transferred into microcentrifuge tubes with a

volume of 2 cm^3 and centrifuged for 5 min at 14500 rpm. The supernatant was filtered through a 0.2 μm polyethersulfone syringe filter.

HPLC-UV analysis. A system consisting of an S 5300 autosampler (SYKAM, Germany), an S 1130 pump (SYKAM, Germany) and an RF-20A Prominence fluorescence detector (Shimadzu) was used. The excitation and emission wavelengths were 280 and 465 nm, respectively. Gradient elution was performed on a Reprosi-Pur Basic C18, 5 μm, 15x0.46 cm, Dr. Maisch chromatographic column with a ReproSil-Pur Basic-C18, 5 μm 10x4.6 mm guard column.

Solutions of mobile phases A (phosphoric acid 2.9 g / L (1.75 ml / L) adjusted to pH 2.3 with triethylamine) and B (acetonitrile) were divided into two banks. The gradient program is presented in Table 2.

Table 2 - Gradient program of solutions of phosphoric acid and acetonitrile

Time, minutes	A, %	B, %
0	90	10
5	65	35
6	65	35
6.1	90	10
8.5	90	10

The analysis was carried out at a flow of 1.5 ml / min; the injection volume was 5 μL.

All tests were carried out according to the above methods.

RESULTS

The results of determining the antibiotic content in premix samples exposed to different temperatures are presented in Table 3.

Table 3 - Stability of amoxicillin concentration in premix samples when exposed to different temperatures

Product	Temperature, °C (exposure 1 min)	Expected content of amoxicillin, mg / g	Research results		% of losses of AI
			Actual AI concentration, mg / g	error, ±	
(A)	without heating	150.0	149.5	7.5	-
	75	150.0	150.5	7.5	0
	85	150.0	149.5	7.5	0
	90	150.0	147.0	7.3	**1.7**
(B)	without heating	100.0	98.4	4.9	-
	75	100.0	85.7	4.3	13.0
	85	100.0	83.9	4.2	**14.7**
	90	100.0	86.9	4.3	11.7
(C)	without heating	200.0	167.9	8.4	-
	75	200.0	162.0	8.1	-3.5
	85	200.0	159.4	8.0	-5.1
	90	200.0	148.9	7.4	**-11.3**

The results presented in table 1 indicate that heat treatment of the premixes led to a decrease in the concentration of the active ingredient. And the greatest degree of inactivation of amoxicillin was observed when the samples were processed at the maximum temperature.

Premix (A) had the highest thermal stability, while the concentration loss of the active ingredient was 1.7%. At the same time, for premixes (B) and (C), a decrease in the same parameter was observed by 14.7% and 11.3% respectively.

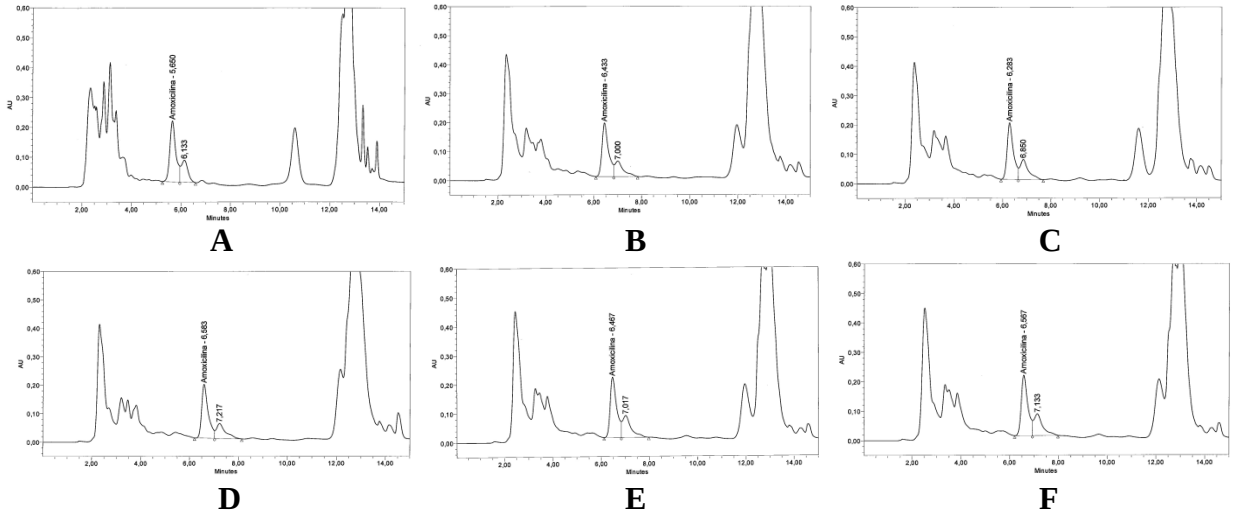
In addition to the results showing how stable each drug is when heated, it is important to pay attention to the obtained amoxicillin content in the samples before heating. In drugs A, B and C, the declared content is 15, 10 and 20% or (150, 100 and 200 mg / g) respectively. As can be seen from the table, the content of amoxicillin in drugs A and B corresponds to the declared (149.5 and 98.4 mg / g respectively). However, the content of the active substance in drug C differs significantly from that declared by the manufacturer (167.9 mg / g against the declared 200 mg / g). All three formulations contain amoxicillin trihydrate. Amoxicillin trihydrate (molecular weight 419.5 g / mol, (PubChem)) contains 87.1% of amoxicillin base (molecular mass 365.4 g / mol, (PubChem)). The therapeutic dose of the drug is calculated from the amount of pure amoxicillin, not amoxicillin trihydrate. Taking this into account, manufacturers of drugs A and B put in their formulas such an amount of amoxicillin trihydrate that the amount of amoxicillin in them corresponded to the declared amount (150 and 100 mg / g respectively). If we recalculate the content of amoxicillin in drug C (167.9 mg / g) for amoxicillin trihydrate, we get 192.8 mg / g, which is much closer to the declared 200 mg / g. According to the prescribing information for drug C, it should contain 200 mg / g of amoxicillin trihydrate, while calculating the dosage it is mentioned that the dose is calculated from the content of amcosicillin. Naturally, this conversion is not included in the prescribing information. Moreover, the personnel who formulate the feed and are responsible for the amount of the drug added to the feed are also not aware of such details, since they do not have a chemical-pharmaceutical degree. As a result, despite the fact that the drug is dosed in accordance with the prescribing information, the amount of active ingredient in the feed is underestimated, which significantly affects the production of the final product. Thus, when choosing a drug, special attention should be paid to how much amoxicillin base the product contains.

The HPLC analysis used in this study was developed by a laboratory at the University of Barcelona based on the reference method for the analysis of amoxicillin (European Pharmacopoeia, 2005), modified by an original extraction procedure adapted to feed samples. The results of determining the content of amoxicillin in 6 feed samples before granulation and after granulation at temperatures from 70 ° C to 90 ° C for 5 minutes, carried out at the University of Barcelona, are presented in table 4.

Table 4- Stability of amoxicillin concentration in feed samples with the drug "Amoxigran" when exposed to different temperatures

Sample No	Feed type	The temperature at granulation, °C	The amount of amoxicillin, mg / kg	Losses, %
1	granules	70	304.7	3.94
2	granules	75	290.7	8.36
3	granules	80	300.7	5.21
4	granules	85	314.9	0.73
5	granules	90	306.5	3.38
6	mixture	-	317.2	-

At the same time, the revealed differences in the concentration of the antibiotic in the granulated feed and flour were significantly lower than the standard deviations of concentrations within each type of feed ($P > 0.05$).



Pic 1 Chromatograms obtained when analyzing feed samples for amoxicillin content: A - feed before granulation, B - feed after granulation at 70ºC, C - feed after granulation at 75 ° C, D - feed after granulation at 80 ° C, E - feed after granulation at 85 ° C, F - feed after granulation at 90 ° C

As part of the control of therapeutic doses of veterinary drugs at the Cherkizovo Lab, a study of the stability of two commercial drugs containing amoxicillin as an active ingredient was carried out, one of which was Amoxigran. The experiment was built in a similar way to what was previously carried out at the University of Barcelona, as described above. Both drugs were added to the feed at the same dosage to obtain 200 ppm of active ingredient, and then the feed was tested for amoxicillin content before and after granulation. The feed granulation parameters were as follows: temperature 62.5 ± 2.5 ° C, holding time 45 sec, steam pressure 1-1.5 bar. The research results are presented in table 5.

Table 5 - Stability of amoxicillin concentration in feed samples containing drug A ("Amoxigran") and drug B

Drug A ("Amoxigran")					**Drug B**			
Sample type	Amoxicillin content, mg / kg	Expected content of amoxicillin, mg / kg	The actual content of amoxicillin relative to the expected, %	Loss of analyte as a result of granulation, %	Amoxicillin content, mg / kg	Expected content of amoxicillin, mg / kg	The actual content of amoxicillin relative to the expected, %	Loss of analyte as a result of granulation, %
Placer	200.1	200.0	100.1	4.6	186.1	200.0	93.1	22.4
Granule	190.8	200.0	95.4		144.4	200.0	72.2	
Placer	194.5	200.0	97.3	0.6	194.5	200.0	97.3	50.6
Granule	193.3	200.0	96.7		96.1	200.0	48.1	
Placer	215.7	200.0	107.9	12.0	189.5	200.0	94.8	60.5
Granule	190.0	200.0	95.0		74.9	200.0	37.5	

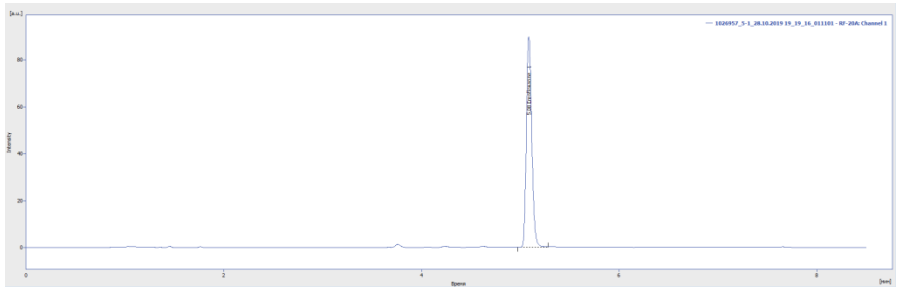
The results shown in Table 5 indicate the importance of testing the feed for active ingredient content before and after granulation when using thermolabile substances. Amoxicillin content in feed samples with drug A (Amoxigran) before granulation ranged from 97 to 108% of the expected value. At the same time, the loss of amoxicillin as a result of feed granulation was

0.6-12%, which, in addition to the granulation itself, can partially be caused by an error in the research method. Samples of feed before granulation, with veterinary drug B in the formulation, contained from 93 to 97% of amoxicillin from the expected value. After granulation, the loss of the analyte was 22 - 60%, which indicates the instability of the amoxicillin molecule in this veterinary drug.

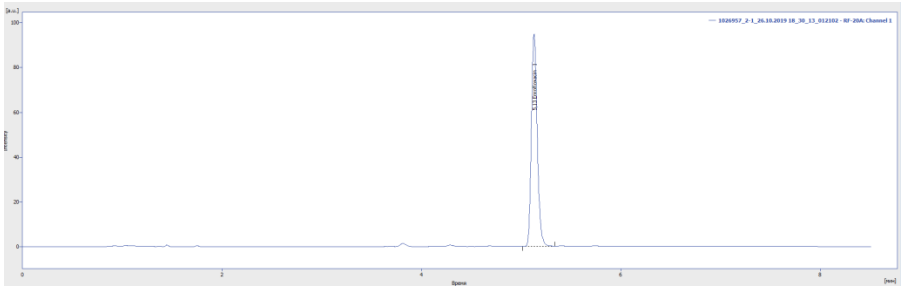
In order to determine the stability of the drug "Enrogran" when used at the feed mills of one of the largest enterprises in Russia, monthly samples of feed were taken before and after granulation from 3 different plants of the enterprise. The analysis was carried out at Cherkizovo Lab. The feed granulation parameters were as follows: temperature 82.5 ± 2.5 ° C, holding time 45 sec, steam pressure 1-1.5 bar. The results obtained for 6 months of using the drug are presented in table 6.

Table 6 - Concentration stability of enrofloxacin in feed samples containing the drug "Enrogran"

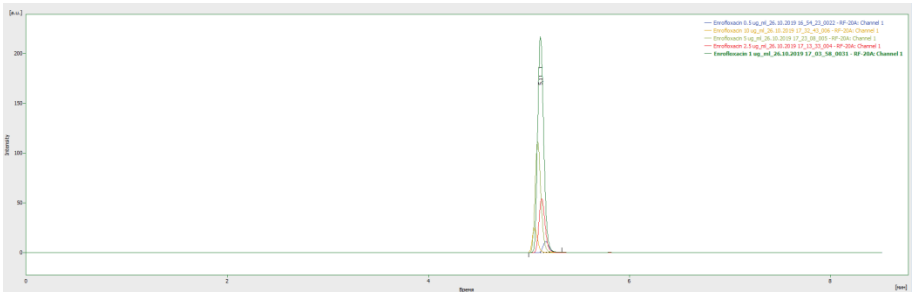
Feed production date	Sample type	Enrofloxacin content, mg / kg	Expected content of enrofloxacin, mg / kg	The actual content of enrofloxacin relative to the expected, %	Loss of analyte as a result of granulation, %
29.03.2019	Placer	42.8	50.0	85.6	-
	Granule	45.0	50.0	90.0	
15.04.2019	Placer	52.8	50.0	105.6	1.7
	Granule	51.9	50.0	103.8	
22.04.2019	Placer	49.6	50.0	99.2	-
	Granule	49.8	50.0	99.6	
14.05.2019	Placer	47.3	50.0	94.6	-
	Granule	48.9	50.0	97.8	
15.05.2019	Placer	47.7	50.0	95.4	4.0
	Granule	45.8	50.0	91.6	
17.05.2019	Placer	40.0	50.0	80.0	-
	Granule	51.1	50.0	102.2	
11.09.2019	Placer	54.5	50.0	109.0	6.8
	Granule	50.8	50.0	101.6	
24.09.2019	Placer	55.5	50.0	111.0	1.4
	Granule	54.7	50.0	109.4	
17.09.2019	Placer	52.6	50.0	105.2	3.4
	Granule	50.8	50.0	101.6	
18.10.2019	Placer	51.8	50.0	103.6	-
	Granule	54.5	50.0	109.0	
Average for the entire period		49.9	50.0	80.0-111.0	1.4-6.8



A



B



C

Pic 2 Chromatograms obtained by analyzing feed samples for the content of enrofloxacin. A - feed before granulation, B - feed after granulation; C - calibration solutions

The table shows that the actual enrofloxacin content in the feed varied from 80 to 111% of the expected content, with most of the results (18/20) being in the 90-111% range. The loss of analyte during granulation, defined as the difference between the result before and after granulation, referred to the result before granulation, was no more than 6.8%. The research results show both the stability of "Enrogran" drug during the feed granulation process at different factories, and the homogeneity of the drug from batch to batch.

DISCUSSION

The obtained results of experiments on the stability of drugs from different manufacturers during the granulation process indicate the obvious importance of such experiments in deciding which drug will retain its activity in the final product, granulated feed. Each manufacturer has its own unique technology for protecting the molecule of the active substance in the drug, aimed at preventing the degradation of the active pharmaceutical substance due to factors such as oxidation, thermal and photodegradation, etc. However, in the granulation process, there are many factors, such as high temperature, high pressure, and as a result, possible chemical reactions between the active ingredient and other components of the feed or premix, which can negatively affect the content of the active ingredient in the final product. In addition, the conditions for granulation in different factories and for different brands of feed can differ significantly, which makes it impossible to say for sure whether the degradation of the active substance will occur or not. Such experiments are extremely important for thermolabile

molecules with known instability when exposed to high temperatures, such as amoxicillin. A similar type of analytical method has been previously described for the analysis of amoxicillin in medicinal premixes (Dousa and Hosmanová 2005). In addition, a combined HPLC / mass spectrometry method has been used successfully for the analysis of amoxicillin in feed (De Baere and De Backer 2007).

CONCLUSIONS

1. The concentration of feed amoxicillin tested in the experiment corresponds to the recommended level, equivalent to the dosage regimen of 10-15 mg / kg, which is equivalent to 0.67-1.0 g of "Amoxigran" per 10 kg of body weight per day. This therapeutic concentration is consistent with the pharmacodynamics of amoxicillin against Streptococcus suis and Haemophilusparasuis (Schwarz et al. 2008).

2. The difference between the average concentrations of amoxicillin in samples before and after granulation of flour and granulated feed ranged from 0.73% to 8.36%. In other studies using native amoxicillin in feed formulations, the difference was significantly higher, reaching 20–40% (Derrieu et al. 2000, Bousquet et al. 2006).

3. The results of experiments on the analysis of feed before and after granulation, carried out both at the University of Barcelona and at Cherkizovo Lab, showed a good stability of “Amoxigran” drug when exposed to various temperatures during the granulation process (maximum 90 ^{o}C), with losses not exceeding 12.0%. At the same time, the results obtained at Cherkizovo Lab on the content of amoxicillin in feed before and after granulation containing veterinary drug A, showed an unsatisfactory stability of the latter, with losses of up to 60.5% in the granulated feed.

4. The study of feeds of a Russian manufacturer, containing the veterinary drug "Enrogran", also showed good stability of enrofloxacin during the granulation process, as well as the uniformity of the drug itself from batch to batch, since the study was carried out within 5 months and samples were taken monthly from different feed factories.

5. The concentration of feed enrofloxacin tested in the experiment corresponds to the recommended level, equivalent to the dosage regimen of 2.5-5.0 mg / kg, which is equivalent to 0.25-0.5 g of "Enroran" per 10 kg of body weight per day. This therapeutic concentration is consistent with the pharmacodynamics of enrofloxacin against Actinobacilluspleuroneumoniae and Salmonella spp. (Zhixin Leiet al. 2017, HaihongHaoet al. 2015).

6. The stability of amoxicillin and enorofolsacin in the commercial drugs “Amoxigran” and “Enrogran” by Biokorm International, presented in the form of microencapsulated powders, is ensured by the presence of multilayer protective granule shells.

BIBLIOGRAPHY

Bousquet E, Derrieu G, Raynier B, Segot C, Lemaire F, Sanquer A, Casado P, CollMasvidal T, MorilloAlujas A, Cano Lopez G, Villalba Mata D, Goutalier J, Combeau S 2006: Comparative study of different amoxicillin premixes (homogeneity and stability in feed). In: 27th Anaporc Symposium. Badajoz, Spain, pp. 185-188.

De Baere S, De Backer P 2007: Quantitative determination of amoxicillin in animal feed using liquid chromatography with tandem mass spectrometric detection. Anal ChimActa 586: 319-325

Derrieu G, Raynier B, Ascher A, Gardey L 2000: Interest of a stabilized amoxicillin in feedstuffs manufacturing. Rev Med Vet 151: 109-117

Dousa M, Hosmanova R 2005: Rapid determination of amoxicillin in premixes by HPLC. J Pharm Biomed Anal 37: 373-377

European Pharmacopoeia 2005: Amoxicillin trihydrate. In: European Pharmacopoeia, supplement 5.3. 5th ed. European Directorate for the Quality of Medicines and Healthcare, Strasbourg, pp. 3440-3442

Higgins R, Gottschalk M 2006: Streptococcal diseases. In: Straw B, Zimmerman JJ, D'Allaire S, Taylor DJ (Eds): Diseases of Swine. Blackwell Publishing, pp. 767-783

Marie J, Morvan H, Berthelot-Herault F, Sanders P, Kempf I, Gautier-Bouchardon AV, Jouy E, Kobisch M 2002: Antimicrobial susceptibility of Streptococcus suis isolated from swine in France and from humans in different countries between 1996 and 2000. J AntimicrobChemother 50: 201-209

HaihongHao, Huafang Pan, Ijaz Ahmad, Guyue Cheng, Yulian Wang, Menghong Dai, Yanfei Tao, Dongmei Chen, Dapeng Peng, Zhenli Liu, Lingli Huang, Zonghui Yuan 2013 Susceptibility Breakpoint for Enrofloxacin against Swine *Salmonella* spp.Clinical Veterinary Microbiology 51(9): 3070-3072

Peddi P., Rajeswari T., Ganji R. 2016: Development and Validation of Stability Indicating RP-LC Method for Estimation of Related Substances of Enrofloxacin in Bulk and Its Pharmaceutical Formulations.Research Journal of Pharmaceutical, Biological and Chemical Sciences 7(1): 1440-1450

Pejsak Z, Jablonski A, Zmudzki J 2005: Drug sensitivity of pathogenic bacteria isolated from the respiratory system of swine. Med Wet 61: 664-668

Rapp-Gabrielson VJ, Oliveira SR, Pijoan C 2006: Haemophilusparasuis. In: Straw B, Zimmerman JJ, D'allaire S, Taylor DJ (Eds.): Diseases of swine. Blackwell Publishing, pp. 681-690

Schwarz S, AlEesik E, Grobbel M, Lubke-Becker A, Werckenthin C, Wieler LH, Wallmann J 2007: Antimicrobial susceptibility of streptococci from various indications of swine, horses, dogs and cats as determined in the BfT-GermVet monitoring program 2004-2006. Berl Munch TierarztlWochenschr 120: 380-390

Schwarz S, Bottner A, Goosens L, Hafez HM, Hartmann K, Kaske M, Kehrenberg C, Kietzmann M, Klarmann D, Klein G, Krabisch P, Luhofer G, Richter A, Schulz B, Sigge C, Waldmann KH, Wnllmann J, Werckenthin C 2008: A proposal of clinical breakpoints for amoxicillin applicable to porcine respiratory tract pathogens. Vet Microbiol 126: 178-188

Trouchon T., Lefebvre S. 2016 Review of Enrofloxacin for Veterinary Use, Open Journal of Veterinary Medicine, 6 (2).

Zemanova M., Maxa J., Hera A., Haas Z., Lemaire F., Sanquer A., Bousquet E. 2008 Analytical Control of a Starter Pig Feed Medicated with Amoxicillin.Acta Vet. Brno 77: 341–345

Zhixin Lei, Qianying Liu, Bing Yang, Jincheng Xiong, Kun Li, Saeed Ahmed, Liping Hong, Pin Chen, Qigai He and Jiyue Cao 2017 Clinical Efficacy and Residue Depletion of 10% Enrofloxacin Enteric-Coated Granules in Pigs. Frontiers in Pharmacology 8: 1-11