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Quality evaluation of solid pharmaceutical preparations for veterinary use containing cephalexin

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The analysis of pharmaceuticals is fundamental in both human and veterinary medicine. In this context, quality control must accompany the process from the acquisition of raw material to the transformation into a finished product, since its goal is to ensure safe and effective drugs for human or animal consumption. Considering the significant increase in veterinary prescriptions containing antimicrobial drugs, the present work carried out a search for information about the qualification of veterinary preparations available in the national market, highlighting, through legislation, the quality parameters applied to pharmaceutical products for veterinary use. The quality of solid pharmaceutical preparations containing cephalexin, acquired in the national veterinary pharmaceutical market, was also evaluated. The six different brands of cephalexin tablets and coated tablets analyzed in this study were approved with regard to identification, average weight, determination of mechanical strength in tablets, disintegration test, and content. The results of the determination of the content varied between 94.9% and 103%, being within the limits indicated for preparations for human use, as recommended by USP 44 and the Brazilian Pharmacopeia 6th edition. Even so, it is important to emphasize the need for improvement in the legislation that regulates the quality control of medicines for veterinary use, since the norms are not very specific, with an absence of parameters, specifications, and release procedures that ensure which tests should be performed. This study is a perspective that can bring subsidies for investigations of other therapeutic classes, considering the large number of formulations available in the Brazilian market of veterinary medicines.

Keywords: veterinary drugs; cephalexin; quality control.

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Introduction

Over the last decade, the animal health market has had a growth rate of over 9% per year. With the coronavirus pandemic, the Pet Commission points to a 30% increase in the acquisition of pets, a trend that will continue to grow. This link shows an increase in the care and demand for veterinary services, as well as an average increase of 45% in clinical care during the pandemic. The Market Information Commission points out that antimicrobials rank third in representativeness by therapeutic class (13). In Brazil, among the first-generation cephalosporins, cephalexin (CEP) appears prominently in veterinary dermatological medicine and as the first choice in the treatment of skin infections (1-9). The supervision of veterinary medicines is the responsibility of the Ministry of Agriculture, Livestock and Supply (MAPA) (2). Few bibliographic references report lack of conformity of veterinary products, added to the fact that many of these deviations are not notified (12). The use of medications must guarantee that the patient receives the concentration of the active ingredient correctly for the purpose for which it was intended: whether for therapeutic, prophylactic, or diagnostic purposes (10). The lack regarding the evaluation of the conformity of these drugs, as well as the expressive increase in veterinary prescriptions leads to the search for information about the qualification of pharmaceutical preparations available in the national market. This study aimed to evaluate the quality of solid pharmaceutical preparations, using CEP as a representative of the class of antimicrobials in the national veterinary pharmaceutical market.

Experimental section

Six different brands of cephalexin tablets and coated tablets, available in the Brazilian market of veterinary drugs, were evaluated. The parameters evaluated were identification, average weight, determination of mechanical resistance in tablets, disintegration test and determination of the content. The pharmaceutical forms were purchased in the local market and named using specific letters, as shown in Table 1.

The determinations of mean weight, tablet mechanical strength of tablets and disintegration tests were evaluated according to the Brazilian Pharmacopeia 6th edition (6).
The American Pharmacopeia (USP 44, 2021) was used for identification and content determination of commercial samples of CEP for veterinary use, tablets and coated tablets (14). The experimental conditions of the analytical method are set out in Table 2. The analytical balance

model Sartorius/TE214S was used for weighing the reference standard and commercial samples. The hardness was determined using a bench-top hardness tester, brand Nova Ética model 298-AT. For the friability test, the friability tester, brand Nova Ética model 300/1 was used. Disintegration analysis was evaluated using the disintegrator, brand New Ethics model 301AC.

Table 1. Samples of six veterinary drugs containing cephalexin available in the national market.

Sample	Dosage Form	Drug content
A	Coated Tablet	500mg
B	Tablet	300mg
C	Tablet	150mg
D	Tablet	75mg
E	Tablet	500mg
F	Tablet	75mg

Chromatographic analysis was performed in Agilent high-performance liquid chromatography equipment, model 1220 Infinity LC, coupled to a DAD detector. The column used was a Shimadzu C18 (250 mm x 4.6 m, 5 µm) Shim-pack VP-ODS model. The mobile phase was prepared by adding 0.985 g of sodium 1-heptanesulfate to the mixture of water, acetonitrile(ACN), methanol(MeOH) and triethylamine (170:20:10:3), adjusting to pH 3.0 with the addition of phosphoric acid. The mobile phase was filtered using a 0.45 µm nylon membrane (USP 44, 2021). Characterization was performed from the analysis of the retention time and UV spectrum obtained by HPLC.

Table2 2. Chromatographic conditions used in the HPLC method for cephalexin determination.

Característica	Descrição
Mobile phase	H_2O:ACN:MeOH: triethylamine (170:20:10:3) sodium 1-heptanosulfonate pH 3.0
Column	Shimpak C18 (250 mm x 4.6 mm)
Flow	1.5 mL.min^{-1}
Wavelenght	254 nm
injection	20 µL
Diluent	Mobile phase

(USP 44, 2021)

The Kruskal-Wallis test was used to test the difference in the content of the six presentations, using SPSS statistical software. The Kruskal-Wallis test is a non-parametric option to the analysis of variance test, used on this occasion because we had a limited sample size for parametric analysis (4).

Results and Discussion

In the last decades, the veterinary drug industry presented an expressive expansion of its activities due to the need for therapeutic support to domestic animals. The insertion of different therapeutic classes, associated with the availability of new treatments, allowed not only to prolong life, but also to qualify their health condition. However, while there is an expansion in the offer of alternative medicines, data on the quality of veterinary preparations available are still quite precarious (11). When searching for information, it was verified the lack of data regarding the quality in the industry of veterinary drugs, even considering that these have been widely used for the prevention, treatment, and promotion of animal health. The growth of the veterinary drug industry brings with it a crucial issue regarding the qualification of these products in order to ensure compliance, safety, and efficacy of these drugs. When veterinary medicines are not produced in accordance with good manufacturing practices for products for veterinary use, these products may be ineffective for their intended purpose.

The precariousness of the quality of medicines can determine the spread of diseases among animals, risks of illness as a result of the lack of adequate content, stability of the formulation, presence of impurities from degradation products or traces of synthesis route reagents (10).

In a study carried out in Sub-Saharan Africa, a market highly affected by counterfeit veterinary drugs, the research identified cases of adverse reactions and therapeutic ineffectiveness. The results found showed a total of 62% of the evaluated drugs were suspected of adverse reactions or lack of efficacy, pointing to the need for a national system of continuous and effective veterinary pharmacovigilance (11). Another research conducted in South Korea evaluated 1,650 drugs and the average noncompliance rate was close to 2%.

The main cause of noncompliance of the products was related to an insufficient or excess amount of the drugs in the veterinary preparations (7). In another study conducted in Vietnam, analysis of veterinary antimicrobials indicated that among the 144 samples analyzed, about 91% met the national quality standard. Ten antimicrobials (6.9%) contained less than half the concentrations of the labeled content(8). With regard to the inspection of veterinary drugs in Brazil, there is an important gap in MAPA's inspection bodies regarding the conformity assessment of products being marketed in the domestic market. MAPA has 6 official analytical laboratories to contemplate the different demands of the veterinary area in Brazil.

In this sense, the creation of the Guide for Validation and Analytical Quality Control - Drugs in Products for Animal Feed and Veterinary Drugs can be considered an important contribution to the quality control of medicines produced in the country. Nationally, the production of veterinary drugs has grown significantly in recent years. However, the inspection of these drugs needs to be more rigorous.

A study that performs the quantification of beta-lactam antibiotics in veterinary drugs shows that the three drugs evaluated showed concentration of the drug different from that indicated in the package insert, ranging from -12% to +21% (10).

The present study was conducted using CEP as the antimicrobial of choice due to its high application in veterinary medicine, especially in pets, and due to the variety of preparations available in the national market. Due to the lack of normative reference guides for veterinary pharmaceutical specialties, the formulations containing cephalexin were evaluated according to the American and Brazilian pharmacopeias. The identification of the samples in the tablet pharmaceutical form was performed by comparing the retention times of the solution prepared with cephalexin reference standard (RS). The chromatograms obtained from the analysis of the RS and tablets and coated tablets samples are presented in Figure 1.

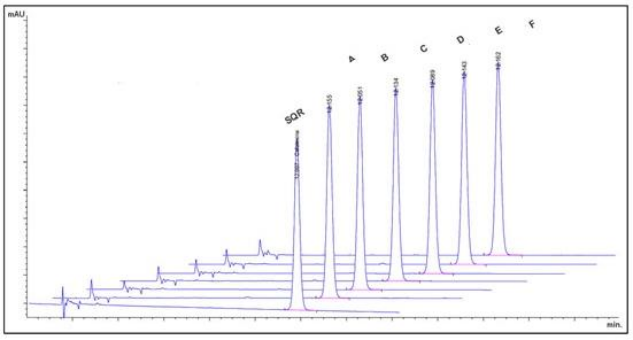

Figura 1: Chromatograms obtained by HPLC analysis of cephalexin SQR and cephalexin tablet solutions, brands A B, C, D and F (0.4 µg.mL-1) Chromatographic conditions: Shimadzu C18 column (250 mm x 4.6 mm, 5 µm); mobile phase H2O:ACN:MeOH:triethylamine (170:20:10:3); flow of 1.5 mL.min-1; UV detection at 254 nm.

The similarity of the profiles of the RS solution and the samples, as well as the retention time, around 12 minutes, indicate that all samples contain the drug in their composition. Figure 2 shows the confirmation of the identity of cephalexin in the samples through the evaluation of the absorption spectrum profile in the UV region (200-400nm), obtained by the DAD detector, of the cephalexin RS solutions and tablet samples, which presented a spectral profile similar to each other.

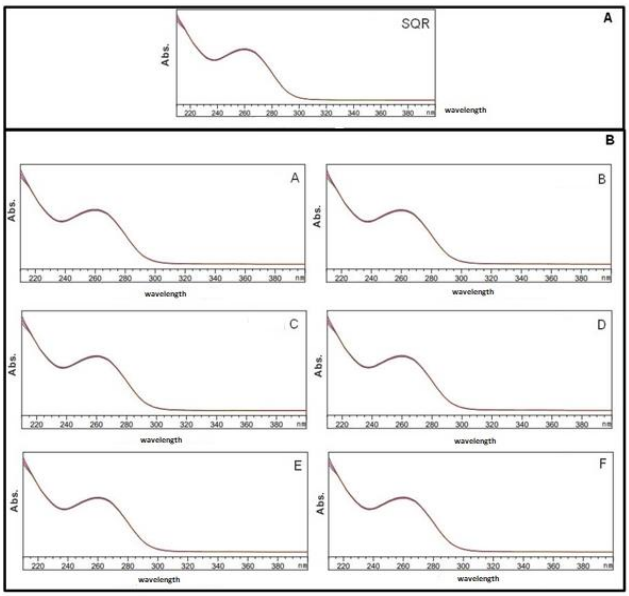

Figura 2: Absorption spectra in the UV region (200 – 400nm), obtained by the HPLC system, from SQR cephalexin solutions and from tablet samples.

The determination of the average weight of the commercial samples was obtained by individual analysis of each tablet or coated tablet, and the values expressed as mean, standard deviation, and relative standard deviation for each sample, according to Table 3.

Tabela 3: Average weight values, standard deviation, relative standard deviation, of different brands of veterinary solid dosage forms containing cephalexin

Sample	Average weight (g)	SD	RSD
A	1.0870	0.0077	0.71
B	0.5039	0.0112	2.22
C	0.2530	0.0044	1.76
D	0.2073	0.0031	1.49
E	0.6659	0.0056	0.84
F	0.1822	0.0020	1.11

Sample A (coated tablet); Sample B-F (tablets); SD (standard deviation); RSD (relative standard deviation)

The determination of average weight is indicative of a homogeneous therapeutic dose and, therefore, its efficacy in therapeutic treatment. This test is considered primordial in the QC of solid forms, and may indicate problems in the drug production process. It is noteworthy that the analysis of other parameters related to the control of solid pharmaceutical forms becomes unnecessary when the formulation fails in determining the average weight, corroborating the relevance of this parameter in the set of required analyses. According to the Brazilian Pharmacopeia (6), the weight determination test applies to solid pharmaceutical forms in unit dose, which includes, for example, coated and uncoated tablets, and capsules, among others. The six different brands evaluated are presented in tablets and coated tablets, which fit for the application of this test. The values obtained in the average weight determination test indicated that no value was outside the pharmacopeial limits. The allowed variation

limits are between 5% and 10%. The hardness and friability tests are official analyses within the Brazilian Pharmacopeia, considered useful elements in the evaluation of the integral quality of tablets, seeking to demonstrate the tablets' resistance to rupture caused by friction or falls (6). The results obtained in the determination of hardness, as well as the mean, standard deviation and relative standard deviation for each sample are presented in Table 4. The formulations present different average values that may reflect characteristics inherent to their composition and production technology.

Table 4. Results obtained, average, standard deviation and relative standard deviation, of different brands of tablets evaluated in the hardness test.

Test	A	B	C	D	E	F
T 1	191.5	58.1	102.7	58.1	58.7	36.8
T 2	250.1	47.9	125.6	48.6	67.3	31.9
T 3	221.3	61.4	114.8	50.9	69.9	29.2
T 4	179.7	60.7	119.0	66.6	52.2	33.5
T 5	203.3	71.5	117.4	68.2	71.5	31.9
T 6	181.0	84.3	112.2	73.2	74.5	32.5
T 7	207.2	65.3	122.3	57.1	75.5	36.8
T 8	197.0	69.9	127.2	79.4	78.4	37.8
T 9	188.8	58.1	96.4	58.7	60.4	36.8
T 10	205.9	71.5	127.9	87.6	62.3	27.6
média	202.6	64.9	116.6	64.8	67.1	33.5
SD	21.0	10.0	10.5	12.5	8.4	3.5
RSD	10.4	15.4	9.0	19.3	12.6	10.5

Sample A (coated tablet); Sample B-F (tablets); SD (standard deviation); RSD (relative standard deviation)

However, it is possible to observe high values of relative standard deviation among the analyzed brands. This fact may be related to the potential lack of homogeneity in the production and control process of these veterinary pharmaceutical preparations in solid form. In this context, variations may occur in the release process of the pharmaceutical active ingredient in the animal's body. The results obtained in the determination of friability, as well as the percentage of mass loss for each sample, are presented in Table 5.

Table 5. Values obtained in the initial weighing, before the test and final after the friability test and percentage of loss of the different brands of tablets evaluated in this study.

Sample	Wi (g)	Wf (g)	D (g)	%
A	NA	NA	NA	*
B	10.0796	10.0543	0.0253	0.25
C	5.1130	5.1071	0.0059	0.12
D	4.2049	4.1930	0.0119	0.28
E	6.5819	6.5414	0.0405	0.62
F	3.6266	3.6224	0.0042	0.12

*NA: not applied; Wi: initial weight; Wf: final weight; D: weight difference; D%: percentual difference Sample A (coated tablet); Sample B-F (tablets).

According to the Brazilian Pharmacopeia 6th edition, the loss in mass of the tablets should be equal to or less than 1.5% of their original weight. The five brands evaluated were approved, demonstrating that the products are suitable for the process of eventual loss of mass by abrasion and do not impact the quality of the product. The disintegration test can reveal whether the technological characteristics of the solid formulation allow the release of the active pharmaceutical ingredient and, therefore, directly impacts its biopharmaceutical profile. Evaluation of the disintegration time of the samples tested for the disintegration test is presented in Table 6.

Table 6. Results obtained in the disintegration test of the different brands of tablets evaluated in this study.

Sample	Disintegration Time*
A	57 minutos e 17 segundos
B	45 segundos
C	41 segundos
D	23 segundos
E	57 segundos
F	2 minutos e 9 segundos

*(average of six tablets)

The samples of uncoated tablets (B-F) are in conformity with the limit specified by the Brazilian pharmacopeia, since they present disintegration in less than 30 minutes. The sample A (coated tablets) had a disintegration time of less than 60 minutes in accordance with the pharmacopeial regulations.

The determination of CEP content in the samples through the HPLC-UV is shown in Table 7. The values found in the analyses varied between 94.90% and 103.0% and are within the limits indicated for preparations for human use, as recommended by USP 44 (2021) and Brazilian Pharmacopeia 6th edition (2019), where CEP tablets should contain at least 90.0% and at most 120.0% of the declared value of CEP ($C_{16}H_{17}N_3O_4S$).

Table 7. Results of the drug content, relative standard deviation, of the samples A-F obtained by HPLC to determine the content of cephalexin tablets for veterinary use.

Sample	CEP1	CEP2(g)	(%)	RSD
A	500	491.41	98.30	0.66
B	300	293.73	97.90	0.36
C	150	148.41	98.90	0.55
D	75	74.72	99.60	0.74
E	500	478.35	94.90	0.55
F	75	77.25	103.00	0.49

Samples: A-F; CEP1: nominal CEP content; CEP2: experimental CEP content ; RSD: relative standard deviation

Statistical analysis did not identify any difference between the contents of the analyzed samples of the six different presentations (p=0.416), according to the variance analysis applied. The six formulations meet the criteria of the Brazilian Pharmacopeia 6th edition and USP-44, and can be safely used in animals (6, 14).

Conclusions

The review of the literature on the QC of pharmaceutical preparations for veterinary use showed a reduced number of publications on the subject, demonstrating the shortage of data related to the minimum parameters for qualification of these preparations and their impact on therapy. The data collected in the literature point to the need for improvement in the legislation that regulates the QC of veterinary drugs, since the regulations are not very specific, with a lack of well-defined parameters, specifications, and release procedures to ensure that the necessary and relevant tests are performed. The six different brands of CEP analyzed in this study were approved with regard to identification, average weight, determination of mechanical strength in tablets, disintegration test and content. The identification of the drug confirmed the authenticity of the substance with respect to its identity. The determination of the content of the six different preparations indicated that the drug content is in the pharmacopeial limit range established by the regulations, indicating its suitability for the intended antimicrobial treatment. Statistical analysis also showed no significant difference between the presentations in relation to drug content, demonstrating the reliability of currently available pharmaceutical products for veterinary use containing CEP. Considering the scarcity of data related to the Quality Control of veterinary drugs nationally, this study contributed to the investigation of other therapeutic classes, due to the high number of veterinary formulations available nowadays. Therefore, it makes relevant the deepening of research in the analytical area that ensures the qualification of pharmaceutical preparations intended for veterinary use.

Conflict of interest

The authors declare no conflicts of interest.

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