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Study of Stability of $C_{19}H_{23}N_3$ (AMITRAZ) through Analysis FT-IR Spectroscopy

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Abstract

The work aims to study the stability of C19H23N23 in veterinary products through the technique of analysis by infrared spectroscopy and Fourier Transform with Transmission and Reflection (FT-IR).The potential and limitations of this method were investigated by analyzing the spectroscopic changes occurring inside and on the surface of the material. This work will present the sensitivity levels of the active principle as well as the small structural changes that may occur in the material.

Keywords: *ftir, amitraz, spectroscopy*

1 INTRODUCTION

The chemical and the pharmaceutical industry have over the time ensuring a key role in the process of products development. There are techniques that assist in directing the way forward, and methodologies for the analysis of a product are essential tools facing a market as crowded and narrow [1]. The spectroscopic techniques can be considered methodologies that assist in the product development process, characterized by studies of highly oriented principles and procedures.

In the pharmaceutical industry, when a mixture of chemical elements for a drug production is performed, we have noticed that each element has their structural properties altered significantly. That change may user loss of activity as the appearance of toxicity in the mixture, that is why is necessary to study all the consequences of it. The concentration of a solution is conditional on factors like the type of degradation (hydrolysis, oxidation or photolysis) and even the reaction speed.

In drugs samples most of the processes of degradation in solution is directly proportional to the concentration of active ingredient. To decrease the effects of degradation on drugs is also relevant the solution pH. Drugs are stable in pH between 4 and 8, however drugs formulated with extreme pH values lead to rapid decomposition.

Drugs may have their stability affected by destructive processes both in the hydrolysis, in which they interact with water molecules to form products with different chemical composition relative a oxidation, which destroys many types of drug molecules, occurring change in its color, organoleptic properties and precipitation.

The hydrolysis process is probably the main cause of decomposition of the drug, since a large number of it active ingredients comprises esters where are susceptible to the hydrolytic process.

When considering external factors which may affect the drug, must be taken into account since the formation of packaging containers even the effectof sun light exposure.

For the drug approval regardless for human or veterinary use, several tests are needed for stability. However, these medications may change if not properly stored. Thus, the objective of this work is to analyze the degradation of a drug in a product for veterinary utilization, whose active ingredient is known commercially as Armitraz C19H23N3, by spectroscopic and demonstrate that the degradation is directly involved with the improper handling or storage of the drug.

Thus, qualitative studies and spectroscopic analysis as well as simulations of physical weathering actions (temperature variations and light effects) will be carried out in three different commercial brands of drugs.

ASPECTS OF THE DEVELOPMENT OF VETERINARY PRODUCTS

It is understood by veterinary medicine, every chemical, biological, biotechnological substance manufactured, whose administration is applied individually or collectively, directly or mixed with food, for the prevention, diagnosis, cure or treatment of animal diseases. It can Include additives, supplies, promoters, enhancers of animal production, antiseptics, disinfectants for in their environmental use or equipments, pesticides and all products used in animals and / or its habitat, protect, restore or modify their physiological functions and physiological, as well as products for the beautification of the animals [2].

As veterinary medicines may leave toxic residues in food, they must follow quality and safety standards, not only for animal health, but especially for public health and the minimization of the environmental impacts..

The potential risks to human health arising from the use of these drugs in food-producing animals may be associated with residues of these levels above the maximum limits recommended by the legislation. Whereas the veterinary medicines in Brazil, the maximum residue limits (MRLs) are set by the National Plan for Control of Residues in Animal Products (Instruction / MAA No. 42, December 20, 1999), which considers it as a reference levels defined by international references, among which are MERCOSUR, Codex Alimentarius, FDA / USA and the European Union [3].

Among the veterinary medicines the tick pesticide noteworthy. It's applied against cattle tick (Rhipicephalus, Boophilus microplus) and the horn fly (Haematobia irritans). Ticks are bloodsucking parasites found in most countries worldwide, with higher incidence in tropical and subtropical areas.

Since it became an economic problem for the livestock, causing weight loss by the animal, lower milk production, losses to the leather, weakening that leads to widespread predisposition to disease and others problems, [4], the chemical pesticides have been a major instrument of effective control.

Among the groups of chemicals components that act as pesticides stand out: arsenic, chlorine, carbamates, organophosphates, pyrethroids, formamidine, fluazuron, fipronil and avermectin.

The main stages of testing and validation related with veterinary products development can be summarized as follows:

Step 1 - Analysis of product content and residue tolerance, where activities are conducted for the development and validation of analytical method for the determination of content and residues of the active ingredients of the product.

Step 2 - Analysis of similarity of plots and stability, aims to prove that the amount of the active content remains close in different production batches. In this study, 03 sequential batches are produced, and performed chromatographic analysis of content of active principle by applying the validated method, maintaining values close to each other. One of the lots analyzed is incubated in a climatic chamber and

conduct will be the accelerated stability study of 6 months (40 º C and 75% RH) and the study of long-term stability 24 months (30 º C and 65% RH).

Step 3 - Safety, where clinical trials are conducted to verify that the drug product does not intoxicate the target species. Are involved in this study testing oral LD50, LD50 dermal, eye irritation / corrosion oral, dermal, ocular, dermal sensitivity maximized; acute inhalation toxicity.

Step 4 – Period Validity, involves the study of determining the qualifying period of active products in the matrices to be evaluated, defining analytical methods that meet the MRLs (Maximum Residue Limit) established for each matrix studied.

Step 5 - Effectiveness of the products, which selected strains of ticks are used to artificially infest cattle of the target species to see if the veterinary product has at least 95% efficiency within 14 days after application.

Given the prominent position of Brazil as the largest exporter of beef, the market for veterinary medicines is growing, with an increasing demand for new products, mainly for acquired resistance by parasites to products in use.

In this process, analytical methods need to be developed and validated to meet the demands of national and international regulatory agencies regarding registration of these products not only for adequate quality control of veterinary products, as well as the guarantee of food security conditions consumers.

2 MATERIALS AND METHODS

For the work development it was used the spectroscopy technique and Fourier transform infrared-FT-IR with a Perkin Elmer FT-IR 3600. The technique of transmission FTIR-MIR (casting film) and FTIR-microscopy shows more clearly the structural changes, because it does not have the reflection characteristic, and also shows spectroscopic changes, more subtle, and may also be regarded as an attractive methodology to analyze materials, polyamides and others [5] [6]. The potential and limitations of these methods will be investigated by analyzing the spectroscopic changes that occur inside and on the surface of materials as well as changes related to NH-CN-C = O, exposing the material to ultraviolet (UV) through a 1200W lamp.

The samples used were randomly chosen so that all three drugs are for veterinary use, and having C19H23N3 as the active principle, known as Amitraz. The samples were classified as A1, A2 and A3 in order to preserve the trademark. Initially, spectra were acquired in FT-IR spectra taking these as initial.

The samples were divided in two batches. One them was wrapped in the commercial packaging and maintained in an environment away from the light, the other are was maintained in an environment with to ultraviolet light exposure in order to a exposure.

After a period of 30 days FT-IR measurements of different brands we obtained the first measurement acquired was of the pucket spectrum (background) followed by the measurements of samples spectra. It is noted that each spectrum is composed of an average of 25 scans.

As shown in Figures 1, 2 and 3 it's noticed that the active principle (which has a vibrational energy in the wavelength range of approximately 1330cm-1) [7].

Both samples showed a decrease in their concentration as seen through transmittance intensity. This decrease originated storage as suggested by the manufacturer as well of by in storage with exposure to UV light.

It's also noted in Figure 4 the sample 3, which has the highest concentration, showed the largest decrease, with the relevant fact given that all samples should be diluted for use.

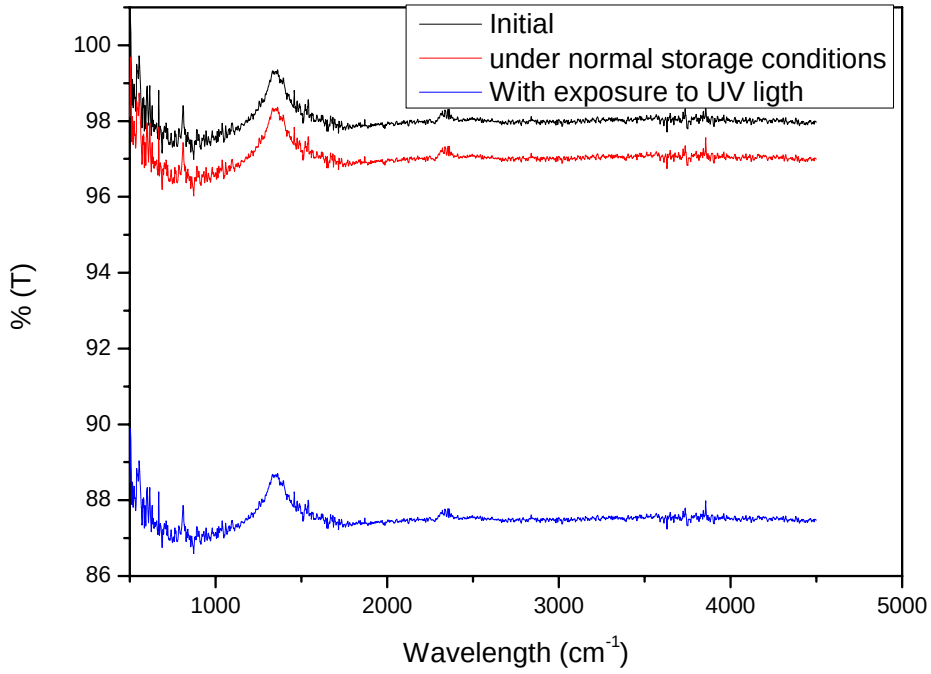

Figure 1- FT-IR spectra for sample A1

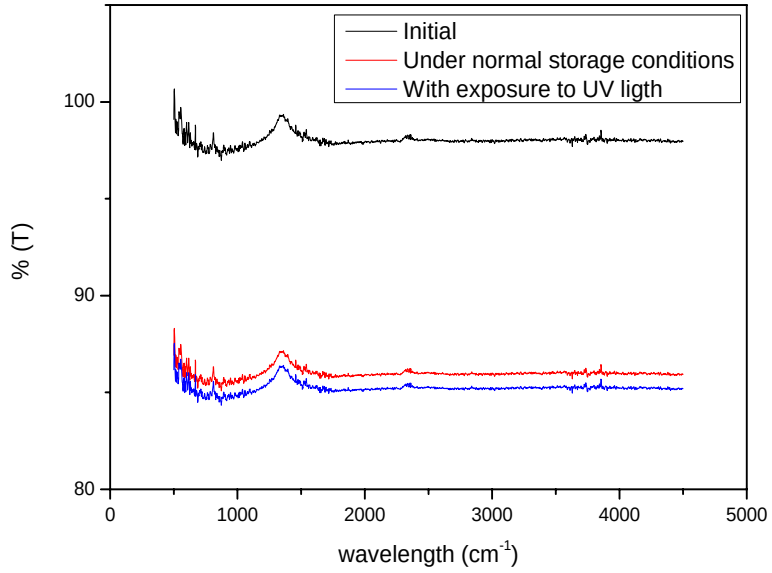

Figure 2- FT-IR spectra for sample A2

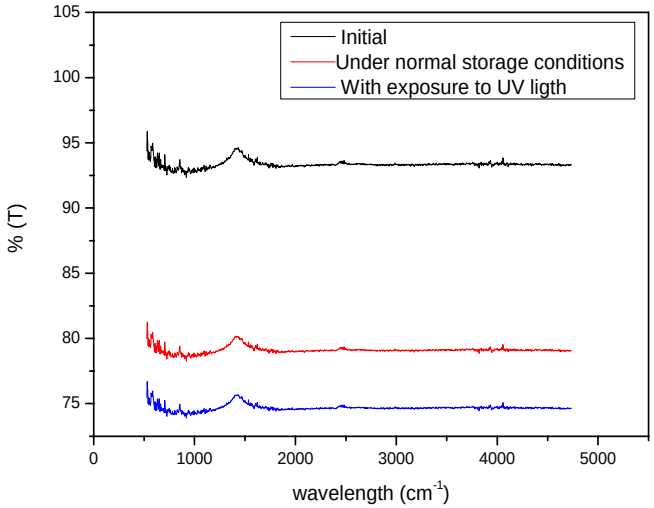

Figure 3- FT-IR spectra for sample A3

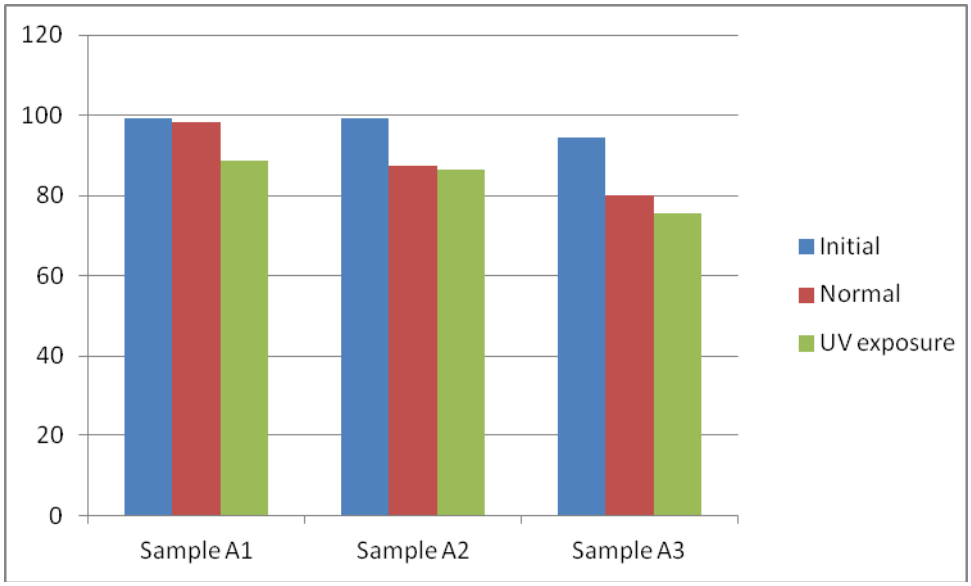

Figure 4- Comparison of the relative amount of the active principle and initial storage of 30 days after

4 CONCLUSION

The technique was sensitive to detect small structural changes occurring in the material that may suggest the formation of ¾ OH, HOC = O-and-C = O, and structural changes related to NH and CN-C = O groups.

In the case of packaging, it's noted that the plastic packages are formed by organic molecular structures, not inert, resulting in processes related to permeability, removal, and absorption or adsorption. The passage of the volatile constituents of certain drug molecules to the outside. Causes significant loss of its volume. In other cases the reverse may occur, the contact of drug with oxygen and other molecules that pass from outside to inside can cause oxidation. PVC for example allowed the passage of molecules in the form of steam of N2, O2, CO2 and

H2O, thus causing a decrease in the concentration of the active principle of the samples.

The dispersion analysis in the environment was important since it is reported that Equine sprayed with aqueous solutions of sulfur 0.2%, showed marked neurologic signs, similar to that received orally Amitraz [8]. But there is no description of experiments that have used this concentration solutions (0.2%). Studies show that Amitraz is also harmful to pets like dogs and cats, in general, presenting the same clinical signs of poisoning ranging from transient hyperglycemia to sedation, hypothermia, and bradycardia, bradyarrhythmia of different intensities.

Thus, its use in food should be done cautiously. According to the results obtained, the application of Amitraz in crops of apples, 20 days before harvest, does not guarantee its elimination in sufficient quantities to avoid the intoxication of consumers, since almost 80% of product remains on the surface fruit, so even contradict the vigilance agencies, suggesting that foliar application has a security in an interval of 20 days with acceptable daily intake (ADI) of 0.01 mg / kg bw [9]. The dilution should also be taken into account, since the information has Amitraz has agricultural indications as acaricide advocating its use in water, although the literature data on ticks control guide diluting concentration of 10Kg/1.000L [] to stabilize the mixture pH 12 to prevent loss of action [10] [11].

It also may be noted that the higher the concentration of the active principle greater decline due to its storage and exposure to storms of nature.

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