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RESEARCH ARTICLE

Effect of Different Conservation Conditions on the Active Compounds and Pharmacovigilance Screening of Different Florfenicol Preparations [1]

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Summary

This study was aimed to evaluate the state of utilization of florfenicol, along with the investigation of adverse drug reactions and to evaluate the active substance content in different storage conditions. In this study, three different preparations were kept in different storage conditions and active substance content along with physical composition was determined at the beginning, 3rd, 6th and 9th months. No physical difference was observed for different condition storage preparations. In pharmacovigilance surveys; adverse drug declarations in which veterinarian reported were mostly from the second drug, following the third drug and the first drug lastly. Overall, active substance content lost was seen for the first drug groups. No significant difference of florfenicol levels (P>0.05) was observed for the second and the third drug groups stored both in room temperature and +4°C for the initial, 3rd, 6th and 9th month periods.

Keywords: *Florfenicol, Pharmacovigilance, Stability, Poultry, Veterinary drugs*

Florfenikol İhtiva Eden Preparatlara Farklı Saklama Şartlarının Etkileri İle Farmakovijilans Taramaları

Özet

Bu çalışma, Türkiye'deki florfenikol kullanım durumu ve florfenikolden kaynaklanan ters ilaç tepkimelerinin olup olmadığını araştırmak; florfenikolün farklı saklama koşullarında tutulması ve bu koşullardaki etkin madde miktarlarındaki değişimi belirlemek amacı ile yapıldı. Çalışmada, 3 farklı müstahzar farklı saklama koşullarında muhafaza edildi, başlangıçta, 3, 6 ve 9. aylarda etkin madde miktarları yönüyle analizleri yapıldı ve fiziki yönden de değerlendirildi. Farklı depolama şartlarında saklanan preparatlarda hiçbir fiziksel değişiklik gözlenmedi. Farmakovijilans araştırmalarında; veteriner hekimler tarafından ters ilaç etkisi bildirimi en fazla ikinci ilaç için bildirildi, bunu üçüncü ilaç ve son olarak ilk ilaç takip etti. Genel olarak, etken madde kaybı birinci ilaç grubu için görüldü. İkinci ve üçüncü ilaç gruplarının hem oda ısısında hem de +4°C'de muhafaza edilenlerinde başlangıç, 3., 6. ve 9. aylarda hesaplanan değerler arasında (P>0.05) önemli bir fark görülmedi.

Anahtar sözcükler: *Florfenikol, Farmakovijilans, Dayanıklılık, Kanatlı hayvan, Veteriner ilaçları*

INTRODUCTION

The antibacterial drugs used in veterinary medicine are often used for the treatment of the illnesses, the protection from the illnesses and for increasing the productivity of animals. For this reason, it is very important to follow the production, storage and market control conditions for the benefit from these drugs at the most level. Likewise the stability and pharmacovigilance works for drugs are done [1,2].

The Affects of Different Storage Conditions

Like the human drugs, the veterinary drugs and growing products are also the products made through the scientific studies and technological works. Consequently, the great usage of economical resources at the all stages of delivering and producing of these drugs are always relevant to the public

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care, the knowledge-ability experience at the most level, the practices on veterinary medicine and the contemporary growing principles [3,4].

Stability means the ability of an active substance or a pharmaceutical product to protect its specifications within definite limitations thorough the shelf life. Stability tests are the tests made for determining the shelf life and usage period of an active substance or a pharmaceutical product in its packing and on its storage conditions. Which is essential to stability is that the active substance be stable in specialties prepared. In the course of time, as a result of internal and external effects degradation products begin to form because the active substance changes. Besides, stability is not only valid for the active substance. The stability of helping substances used in formulations at the same time is also important [2,5].

Pharmacovigilance

Drugs may cause toxic effects even in normal usage conditions. Drug safety is researched at the all levels of developing preclinical and clinic drugs in priority and detail. However, doing these things perfectly and presenting great safety proofs often can not even prevent the adverse effects that may happen by being used by great majority after drug marketing. Consequently, drugs must be followed for safety during the period after marketing and there must be awareness about their potential toxicity [6-9].

Veterinary pharmacovigilance is a study of discipline which searches whether it is about taking drugs as a result of the adverse affects of the usage of veterinary medical drugs or not and also searches the degree of this. Following the veterinary medical drugs after marketing includes evaluating and preventing the probable adverse affects on animals which are treated and the effects on human health and environment at the same time [1,7,10].

Veterinary pharmacovigilance involves great scope. It has a priority to clinical safety. It includes the adverse affects of the product on animals treated in normal conditions or also human beings; because the person using it is in close contact with the animals treated [1,11-13].

It is aimed to search the usage of florfenicol in Turkey and the adverse affects of drugs, to keep florfenicol in different storage conditions and to determine the changes in quantity of the active substance in these conditions.

MATERIAL and METHODS

Reagents and Instruments

All reagents and solvents were analytical grade unless otherwise specified. Florfenicol standard (Fluka, Sigma-Aldrich, St. Louis, MO), Ethyl acetate (Sigma-Aldrich, St. Louis, MO), Acetonitrile (Merck, Darmstadt, Germany), Polyethylene glycol (PEG, Sigma-Aldrich, St. Louis, MO), Ultra-pure water was obtained from a Millipore system (Millipore, Molfheim, France). First drug: 300 mg/ml florfenicol; oral solution, 200 ml/bottle, second drug: 300 mg/ml florfenicol; oral solution, 1 L/bottle, third drug: 300 mg/ml florfenicol; oral solution, 1 L/bottle.

Shimadzu LC-20A, the high pressured liquid chromatography (HPLC, Shimadzu, Tokyo, Japan) system has a foto diode-array dedector and inertsil ODS-3 column (GL Sciences, Eindhoven, The Netherlands). The column heat 30°C, mobile phase; acetonitrile:water (27:73; v/v), wavelenght 223 nm and flow speed 1 ml/min.

Storage Conditions

The changes in quantity of the active substance of the preparation found in different storage conditions (room temperature and +4°C fridge) were followed. For this reason, 3 preparations including florfenicol as an active substance are determined. 5 drugs having the same serial number for each preparation were preserved in room temperature and +4°C, at the beginning these preparations were analyzed with their quantities of the active substance in the 3rd, 6th and 9th months and determined physically (turbidity, particulate substance, precipitation and color change).

The active substance in preparation was analyzed by means of HPLC parameters in the methods explained by Shen et al.[14].

Pharmacoviligance

Adeverse effects of the preparations including florfenicol were determined. The forms prepared for this reason were directly caused to reach the veterinarians working in the sector of animals having poultry. The information which was useful for veterinarians and presenting the adverse drug reactions was included in the forms.

Statistics

"SPSS 15.0 for Windows" statistic packet programme was used for the statistical calculations. The preparation including florfenicol for stability works were applied to HPLC and their quantities were determined with the peak areas obtained. The data were explained by arithmetic mean ± standard deviation at the most and least values. The variance analysis with the one direction (ANOVA) for the beginning, 3rd, 6th and 9th months of the drugs were applied and the importance of the differences between the groups was determined with the Duncan test. T- test was applied for the data of each drug in room temperature and +4°C in the beginning, 3rd, 6th and 9th months.

RESULTS

The Findings of Storage Conditions

Before first drugs, second drugs and third drugs (5 of each) were stored in room temperature and +4°C, each drug were

determined with the turbidity, particulate substance, precipitation, color change and the results were recorded. The same process was repeated in the following 3rd, 6th and 9th months. According to this, turbidity, particulate substance, precipitation, color change were not determined in each drug. However, white sediments were seen in the shell of the first drugs after they were taken for the analysis from the 3rd months. This was not seen in the second and third drugs.

Twenty µl polyethylene glycol (PEG) was applied to the HPLC before the determination of the quantities of the active substance of the preparation including florfenicol. In this way, PEG was controlled about whether it caused peak or not. Florfenicol standard was placed in autosampler after preparing the first, second and third drugs with the definite intensity (0.5 mg/ml and 2 mg/ml); the autosampler was ensured to use 20 µl from this mixture and the times of the peak were determined by controlling whether it causes proper peak or not *(Fig. 1)*. The results obtained are presented in *Table 1*.

Then in order to determine the quantity of the active substance of the first, second and third drugs at the beginning, 2 mg/ml of the density for each of them was diluted by considering the quantities stated in their prospectuses and they were placed in the autosampler by being put in vials. These samples enabled the autosampler to use 20 µl. All these works were repeated in the drugs stored in +4°C and in the room heat from the first month to the 3rd, 6th, and 9th months. The peak areas obtained were recorded.

By considering the peak area as 100% which was obtained from 2 mg/ml of the florfenicol active substance; the peak areas in the first, second, third drugs at all times and in different storage conditions were transferred into mg/ml according to this value. The comparison of the arithmetic mean±standard

Table 1. *The florfenicol active substance, first, second and third drugs HPLC output times*

Tablo1. *Florfenikol etkin maddesi, ilk, ikinci ve üçüncü ilaçların YBSK'da çıkış zamanları*

Effective Substance	Mean Peak Time (Minute)
Florfenicol Standard	13.25
First drug	13.35
Second drug	13.50
Third drug	13.40

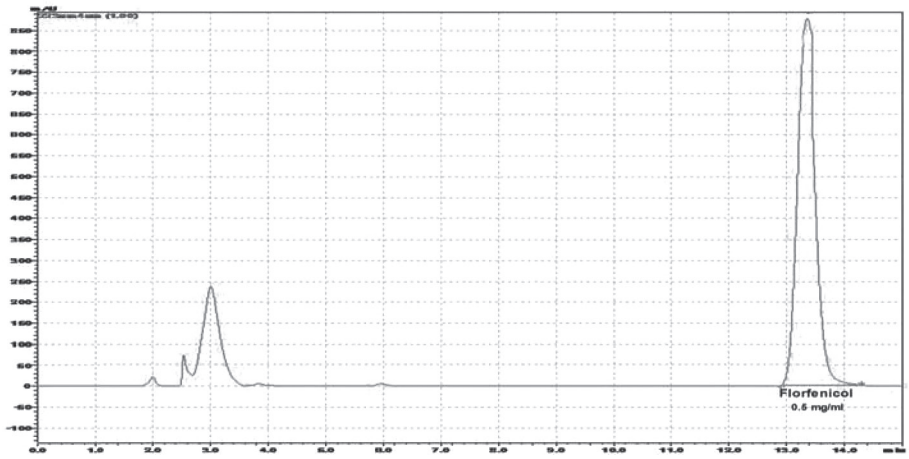

Fig 1. The chromatogram of the 0.5 mg/ ml density of the first drug in HPLC

Şekil 1. İlk ilacın 0.5 mg/ml'lik yoğunluğunun YBSK'deki kromatogramı

Table 2. *The comparison of the quantities of the preparation including florfenicol in point of the room temperature and +4°C in the beginning, 3rd, 6th, and 9th Months (arithmetic mean±standard deviation, the lowest-the highest values)*

Tablo 2. *Florfenikol ihtiva eden preparatların başlangıçta, 3., 6. ve 9. aylarda oda sıcaklığında ve +4°C'deki etkin madde miktarlarının karşılaştırılması (aritmetik ortalama±standart sapma, en alt-en üst değerleri)*

Drugs	Storage Conditions	Beginning (mg/ml)	3rd Months (mg/ml)	6th Months (mg/ml)	9th Months (mg/ml)
First drug	room temperature	1.917±0.081 [a] (1.784-1.991)	1.917±0.081 [a] (1.783-1.990)	1.804±0.017 [b] (1.778-1.827)	1.70±0.055 [c] (1.645-1.773)
	+4°C	1.731±0.161 [a] (1.585-1.998)	1.679±0.072 [a] (1.585-1.747)	1.576±0.070 [ab] (1.487-1.679)	1.483±0.135 [b] (1.270-1.615)
Second drug	room temperature	1.769±0.238 (1.435-2.00)	1.768±0.238 (1.434-1.998)	1.767±0.238 (1.433-1.996)	1.766±0.238 (1.432-1.994)
	+4°C	1.620±0.173 (1.448-1.897)	1.619±0.173 (1.448-1.896)	1.618±0.173 (1.447-1.895)	1.617±0.173 (1.446-1.893)
Third drug	room temperature	1.489±0.155 (1.272-1.643)	1.488±0.156 (1.270-1.642)	1.486±0.156 (1.268-1.642)	1.485±0.156 (1.265-1.641)
	+4°C	1.782±0.177 (1.589-2.00)	1.780±0.177 (1.587-1.998)	1.779±0.177 (1.584-1.996)	1.777±0.177 (1.581-1.994)

a,b,c, *Mean within in the same line with different letters are statistically significant (P<0.05)*

deviation, the lowest-the highest values, all drugs in the individual data in +4°C and room temperature are given in *Table 2*.

Pharmacovigilance Findings

80 questionnaire forms were used for the evaluation of the pharmacovigilance in 17 cities of Turkey. The questionnaires were filled by the veterinarians working in the service for poultry. According to the data obtained from all the questionnaires, it was observed that the preparation including florfenicol applied to poultry can be taken easily. The information about the areas where the preparations were stored is given in *Table 3*. The information given by veterinarians surveyed about adverse drug reactions are given in *Table 4*. By considering the notes about the problem of "the drugs not being dissolution completely" explained by the veterinarians in the choice of "the other "of the "WHAT ARE YOU GOING TO GIVE INFORMATION ABOUT?" part of the questionnaire; it was observed that the first and second drugs could not dissolution completely in normal water used for drinking but they could dissolution completely in warm water. It was stated that the third drugs could not dissolution completely in both normal water for drinking and warm water and the drug particles floated in the water.

Table 3. *The storage conditions of the preparation including florfenicol (n: 80)*
Tablo 3. *Florfenikol içeren preparatların saklama koşulları (n: 80)*

Storage Conditions	Number (n) and percent (%)
In Clinics	n: 17 - 21.25%
In refrigerator	n: 24 - 30%
In Room temperature	n: 19 - 23.75%
The dark warehouse	n: 20 - 25%

Table 4. *The pharmacovigilance notifications about the preparation including florfenicol (n: 80)*
Tablo 4. *Florfenikol içeren preparatlara ilişkin farmakovijilans bildirimleri (n: 80)*

Advers Drug Reactions	Number (n) and Percent (%)
There was no negativity in animals	n: 14-17.5%
The ineffectiveness of the drug/lack of sufficient activity	n: 16-20%
Drug did not dissolution in the drinking water of animals	n: 50-62.5%

DISCUSSION

Much quality control was applied to the preparation presented in the market during the period of their production. The stability tests done in both short and long duration are the most attractive ones. These tests are done to determine the shelf lives of the preparation. However, the works done to determine the quantity of the active substance after the presentation to the market are in limited numbers. Although the quantity of the active substance and the shelf life of the preparation are determined by means of the quality control and the stability tests during the period of their productions, it can be observed that the quantity of the active substance may diminish from the production date to the expiry date and then the products may be spoilt before the expiry date.

According to the study of Canefe et al.[15] on the stabilities of the drugs in the structure of liquid dispersion in Turkey's climate conditions; the results of the physical stability tests in long duration presented much more mutability than the results of the chemical stability tests and in spite of the fact that there was no chemical defeat in the active substance of the preparation during the period of observation, physical defeats came into being and some drugs could not be used.

By means of the study of Hismiogullari and Yarsan [2] on the contents of the active substance of the some authorized veterinary antibacterial drug specialities (aminoglycosides and sulfonamides) in Turkey and the effects of some storage conditions on these levels; it was revealed that the prior factors about the defeat in the speciality were the storage condition and the storage period and furthermore the chemical characteristics of the active substance in the speciality were important in this point of view. This study is similar to the one done by Hismiogullari and Yarsan [2] in this point of view.

The formulation of 11 including 5% PEG and the formulation of 12 including 5% PEG subjected to the stability tests for 3 months in the study of Bekmezci [16]. It was stated that the dissociation speed of the Formulation11 and Formulation 12 formulations decreased at the end of the 3rd month. According to the results of the study on the stability in long term of the chosen antibiotic standards done by German et al.[17]; it was seen that excessive defeat did not reveal during all the storage period and an important change didn't happen at the level of dirtiness.

It was understood that the drug was usually preferred for the respiration infections in poultry according to the pharmacovigilance questionnaires. The number of the studies on the concept "pharmacovigilance" is extremely limited in our country. There are extremely few studies on this concept in veterinary medicine. Doğan [1] determined that the vitamin-mineral group caused mostly the adverse drug reaction by means of pharmacovigilance scanning and he also stated that the local reactions in the anaphylaxis and injection area are the mostly stated adverse drug reactions.

4474 adverse drug reactions, 4250 of which are related to animals and 224 of which are related to human beings were stated in the report published by EMA [18]. 143.000 sick poultry were declared in the same report and it was emphasized that all these animals were affected and two urgent reports were published about all of them. In the same time in this report, more than 50.000 adverse drug reactions about veterinary pharmacovigilance were reported until 2010. In this report, it was stated that the usage of the injection and oral preparation including florfenicol for the eradication of

A. pleauropneumoniae off-label use caused the illnesses in productivity system and so this hadn't to be used in pregnant pigs in Denmark in 2009 and all the countries which are the members of the council were informed about this event.

The users must be informed about the fact that there is a close relationship between the effiency of the drugs they have bought and their storage conditions and that they should store the drugs in suitable conditions in order to get maximum benefit from the drug. The concept pharmacovigilance must be given more importance in Veterinary Medicine Education. Besides, following and controlling the veterinary adverse drug reaction made by authorized person in our country must be done by the State like the other countries for the sake of animal health and economic managership.

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