Pharmaco-technological research and stability of the drug for the treatment and prevention of vitamin and trace element deficiency in animals

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Abstract

The article presents the research results on the Development of manufacturing technology and the study of the stability of a solution for oral use based on carnitine hydrochloride, vitamins – E, B12, choline chloride, selenium, and zinc. “Devivit Carnitine” oral is used for the prevention of diseases of liver and muscle tissue metabolism in farm animals and poultry, nephrosis, myocarditis, prevention of stress (heat, transport, sound, food, post-vaccination, etc.), and prevention of vitamin and hypovitaminosis. When developing the drug technology, experimentally obtained pharmaco-technological properties of active substances and excipients were taken into account. The technological process of preparing an oral solution consists of four technological stages: preparation of canisters for filling, preparation of the solution, filling containers with medicine and packaging. The stability and shelf life of “Devivit Carnitine” (solution for oral use) in plastic canisters were determined. It was established that the drug sample in plastic canisters was stable, according to all quality control methods indicators, during the entire period of the study. The shelf life of the medicinal product with carnitine hydrochloride, vitamins E and B12, choline chloride, selenium, and zinc is 12 months. In the future, a study of preclinical data is planned to verify whether the drug does not reveal a particular hazard for animals based on conventional studies of safety pharmacology, repeated dose toxicity, genotoxicity, carcinogenicity, and developmental toxicity.

Keywords: manufacturing; quality; stability; carnitine hydrochloride; vitamins E and B12; choline chloride; selenium; zinc.

1. Introduction

An essential indicator of the quality of medicinal products for the treatment and prevention of vitamin and trace element deficiency in animals is pharmaco-technological research, stability, and shelf life, i.e., the time during which there are no adverse changes in the physicochemical, pharmacological, and consumer characteristics of the drug (Liapunov et al., 2011; Nemchenko et al., 2017; Sachuk et al., 2022; Sachuk et al., 2023). Technological indicators, the stability of medicines, and their quality are closely related. That is why the study of stability, depending on various factors of determining the expiration dates of finished drugs, is one of the most critical problems, the solution of which specialists from various pharmacy branches are solving (Kraidashenko et al., 2015).

When developing the composition of a new medicinal product, the expiration date is determined experimentally by periodic evaluation of all indicators included in quality control methods (QC) (Nemchenko et al., 2017; Sachuk et al., 2022; Sachuk et al., 2023). The shelf life of medicinal products means the period during which they must fully retain their therapeutic activity and harmlessness and, in terms of qualitative and quantitative characteristics, meet the requirements outlined in the regulatory documentation, according to which the finished drugs were released and stored (Kovalenko et al., 2020; Gutyj et al., 2022; Kushnir et al., 2022; Vlizlo et al., 2023).

The stability of the medicinal product largely depends on the chemical composition and properties of the packaging material (Panagopoulos & Georgarakis, 1990; Zarivna, 2023). From the moment of receipt to the patient’s reception, these substances are in contact, and various chemical interactions are possible. When investigating the possibility of using packaging material, it is necessary to conduct preliminary physical and chemical tests. Both the stability of the packaging material and its ability to protect the medicinal
product from the influence of temperature, light, and humidity of the environment are of great importance. Therefore, after studying the stability of the packaging material, the stability of samples of the medicinal product packed in such packaging is investigated (Rahman et al., 1990). Of all the types of packaging used for oral solutions in the veterinary pharmaceutical industry, canisters ensure tightness during long-term storage. Canisters are the most common type of container for storing vitamin oral preparations. The canisters are resistant to fats, do not allow moisture to pass through, and serve as a barrier to air oxygen and UV radiation. Glass containers have also lost their relevance. It is not permeable to liquids, steam, gases, and microorganisms, which favorably distinguishes glass from polymers and allows it to be used to store most medicines.

Microbiological control is an important quality indicator when researching the developed drug (Seyfarth, 1985; Walker et al., 2003; Shintani, 2015; Temrikar et al., 2023). Almost all medicinal products that have not undergone sterilization can be contaminated with microorganisms to varying degrees. Even though microbes in viscous environments develop much more slowly than in liquid ones, they can survive for a long time in the medicinal product itself and multiply in it. Sources of microbial contamination can be raw materials, water, air, packaging, equipment, etc. (Derzhava Farmakopeia Ukrainy, 2014; 2015).

**The purpose of the work** is to study the technological features of the manufacture and the stability of the solution for oral use with carnitine hydrochloride, vitamins E, B12, choline chloride, selenium, and zinc.

2. **Materials and methods**

The solution for oral use was produced using the following equipment: vibrating sieving machine Analysette 3, vibrating micromill Pulversette 0, cavitator, reactor-mixer, and mixer at the site for the production of solutions for oral use of “DEVIE” LLC.

The study of the stability of the drug “Devivit Carnitine” (solution for oral use) was carried out in the laboratory for quality control, safety, and registration of veterinary medicinal products and feed additives of LLC “DEVIE”.

The method of researching the quality of the drug involved the use of the following equipment, dishes, and reagents: analytical scales with a weighing accuracy of 0.0001 g; ultrasonic bath UZU-01 or similar; sodium hydroxide, n.d.a.; phosphoric acid, n.d.a.; silver nitrate, n.d.a.; potassium iodide, n.d.a.; sodium thiosulfite, n.d.a.; membrane filters with a pore size of 0.2–0.5 μm; argentum nitrate, Hc; sodium eosin, chda; acetonitrile for chromatography; vitamin B12 (cobalamin), certified standard sample (CAS Number 68-19-9); water is purified by P, according to DFU 2.0, and laboratory dishes according to DSTU ISO 4787 (Berest, 2021).

3. **Results and discussion**

The following general approaches are essential when developing, establishing specifications, and evaluating the quality of substances and finished veterinary medicinal products submitted for registration.

**Characteristics of the medicinal product.**

Description: red solution (opalescence is allowed).

Composition: 1 ml of the drug contains active substances: carnitine hydrochloride – 50 mg, vitamin E – 30 mg, B12 – 0.01 mg, choline chloride – 10 mg, selenium – 0.05 mg, zinc – 1 mg, filler – up to 1 Jr.

**Pharmacological properties.** Devivit Carnitine oral is a metabolism stimulator, hepatoprotector, and anti-stress drug. This combination of active substances is most effective when additional energy is needed and to reduce the adverse effects of stress factors.

Carnitine hydrochloride participates in the breakdown of excess fatty acids, increases the use of cellular energy sources, and affects animal energy metabolism. It also improves appetite and stimulates cells of the immune system, which helps in the recovery period.

Choline chloride has several advantages: it has a methionine-preserving effect, improves appetite, stimulates live weight gain, increases egg production in poultry, increases chicken hatchability, prevents perosis, and prevents fatty infiltration of the liver.

Selenium prevents indigestion, increases resistance, and contributes to the normalization of metabolism, which allows for high growth rates, high egg production, preservation of young animals, and high productivity of farm animals and poultry.

Vitamins E and B12 are catalysts in the body and participate in protein synthesis.

**Application.** Devivit Carnitine oral is used for the prevention of diseases of liver and muscle tissue metabolism in farm animals and poultry, nephrosis, myocardosis, prevention of stress (heat, transport, sound, food, post-vaccination, etc.) and prevention of vitamin and hypovitaminosis.

**Dosage.** The drug is used in a dose of 0.9–1.0 ml/L of water for 3–5 days.

**Contraindication.** Individual sensitivity to the components of the drug.

Withdrawal period (withdrawal period). Livestock products are used without restrictions.

**Release form.** Canisters of 5 l.

**Storage.** Store the drug in a dry, dark place inaccessible to children at 5 to 25 °C. Store the drug separately from food and drinks, including animal feed.

**Expiration date.** One year from the date of manufacture.

**Production technology.** When developing the drug production technology, experimentally obtained pharmacotechnological properties of active substances and excipients were considered.

The technological process of preparing an oral solution consists of four technological stages (Fig. 1): 1. Preparation of canisters for filling. 2. Preparation of the solution. 3. Fill containers with medicine. 4. Packaging.

**VR 1.** Preparation of canisters for filling includes three technological operations.

**VR 1.1.** Unpacking. From the composition, canisters in polyethylene packaging are submitted for preview and treatment of the outer surface. The packaging is placed in a GF-1 bath and rinsed with hot drinking water until the washing water is clean.

Canisters without packaging are loaded into the GF-2 bath. Currently, the canisters are visually inspected, and the chips are rejected.

**VR 1.2.** Washing canisters. Soaking canisters in a bath is carried out in a 0.5 % detergent solution at a temperature of (55 ± 5) °C for two hours. Clean processing of canisters is carried out on the “Fortune” GF-3 machine with drinking water for 3–5 days.
water that has passed through the F-4 filter (in compartment I) and then in compartments II and III with water for injections. Water for injection into the machine comes from the production, storage, and distribution system of purified water SOV 19-18 LLC “THERMODISTILYATSIA RV”. In the II compartment, there is a rough rinsing with circulating water for injections, which comes through the F-6 filter from the III compartment. Water for injections enters the II and III compartments through nozzles. The water pressure from the nozzles should be at least (0.15 ± 0.1) MPa or (1.5 ± 0.1) kgf/cm².

VR. 1 Preparation of canisters for filling

VR. 1.1. Preparation of containers
VR. 1.2. Washing canisters
VR. 1.3. Canister drying

TP. 2 Preparation of the solution
TP. 2.1. Weighing
TP. 2.2. Dissolution
TP. 2.3. Standardization
TP. 2.4. Filtration
TP. 3.1. Filling canisters
TP. 3.2. Closing canisters
UMO 4.1. Preparation of containers
UMO 4.2. Marking
UMO 4.3. Packaging
UMO 4.4. Label

VR. 1 Preparation of the canister for packaging

Ready product

Fig. 1. Block diagram of the manufacturing process of the drug "Devivit Carnitine"

The productivity of the washing machine is 2500–3000 canisters per hour.

The washing machine is cleaned from fighting at least twice per shift. The washer controls the machine's operation. Average water consumption is statistical (1.75 ± 0.50) m³/hour. The washed vials are placed in sheets (according to the GF-5 scheme) in the “neck down” position and installed on an inclined stand (according to the GF-4 scheme). Then they are placed in clean boxes without mechanical inclinations, GF-6 boxes, and transferred to position GF-7 is a dry heat sterilizer. Sterilized vials are sent to the rinsing table according to the GF-10 scheme.

Control of canisters for mechanical inclusions.

5–10 canisters are selected before bottling in a specially prepared stainless steel cassette with a lid. Selected canisters in the “clean” control room are filled to half their volume with water, washing the inner surface with a jet. Each canister is viewed for 10 minutes at a distance of 25 to 30 cm from the eye on black-and-white screens illuminated by a 60 W electric lamp.

There should be no visible mechanical inclusions in the washes from the inner surface of the vials.

Control of the cleanliness of the outer surface of the canister.

1–2 canisters are taken from the cassette, the outer surface of which is successively rinsed with water, in a chemical beaker with a capacity of up to 500 ml. The washing water is poured into tested vials and viewed in the inspection area for 10 minutes. Washing water should not contain visible mechanical inclusions (TV 42-51-20-93).

The washing department cleaners carry out control at least once per shift, the shift master selectively, and the results are recorded in the work log.

VR 1.3. Canister drying. Canisters are checked for cleanliness and free from water.

TP 2. The preparation of an oral solution includes the following technological operations:

TP 2.1. Weighing substances and measuring water. They are weighed on manual scales (Table 1).

Table 1
Quantitative composition of substances and auxiliary substances

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>carnitine hydrochloride</td>
<td>0.5 kg</td>
</tr>
<tr>
<td>vitamin E</td>
<td>0.3 kg</td>
</tr>
<tr>
<td>vitamin B₂</td>
<td>0.1 g</td>
</tr>
<tr>
<td>choline chloride</td>
<td>1.0 kg</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Water</td>
<td>up to 100 liters</td>
</tr>
</tbody>
</table>
Water for injections is measured.

TP 2.2. Dissolution. In a stainless steel (AISI type) reactor equipped with electric heating and an electric stirrer, vitamins are dissolved in a portion of water during stirring. Then, bring the water up to the mark. Stir.

TP 2.3. Standardization. The quantitative content of vitamins in the injection solution was determined by liquid chromatography. In case of increased concentration, water was added to the solution for injection, considering its volume. In the case of a decrease in concentration - the calculated amount of vitamins.

TP 2.4. Filtering. The injection solution is filtered three times through a pleated filter made of filter paper.

TP 3. Filling canisters with medicine. Includes two technological operations:

TP 3.1. Filling canisters. The filtered solution in a sterile chemical reactor is poured into canisters with a volume of 5.0 liters.

TP 3.2. Clogging of canisters. To ensure the tightness of the package and fix the rubber stoppers, the canisters are screwed with a cork.

4. Packaging. Includes the following technological operations:

4.1. Preparation of containers.

4.2. Marking. On the canister, with the help of a label, indicate the name of the drug in Ukrainian and Latin, the concentration of the solution, and the volume of the solution in ml.

4.3. Packaging. 5 canisters each are placed in contoured cell packaging made of polyvinyl chloride film, printing aluminum foil, and varnished. Contour packages of 5 pieces are placed in a cardboard package. Each package includes five instructions for use. The boxes are pasted over with a wrapping made of printing paper. The ends of the package are sealed with a label made of printing or label paper. Boxes of 10 pieces are packed in a stack with wrapping paper. Feet are placed in plywood boxes, lined in advance with wrapping paper. The free space in the boxes is filled with waste paper. A packing sheet is placed in the box.

4.4. Labeling. The box, contour packaging, and label indicate the name of the manufacturing plant and its trademark; the name of the drug in Latin and Ukrainian; the concentration of the solution; the volume of the solution in the vial; the number of ampoules in the package; the indication “sterile”; “intramuscular”; storage conditions; registration certificate number; serial number; and expiration date. The foot label and group box additionally indicate the number of packages without indicating the price.

Table 2
Results of stability analysis of samples of solution for oral use during storage at a temperature of 5–25 °C

<table>
<thead>
<tr>
<th>Name of indicators</th>
<th>Storage temperature 5–25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Norm</td>
</tr>
<tr>
<td>description</td>
<td></td>
</tr>
<tr>
<td>Carnitine hydrochloride content</td>
<td>50.0 ± 10 %</td>
</tr>
<tr>
<td>Vitamin E content, mg</td>
<td>30.0 ± 10 %</td>
</tr>
<tr>
<td>Vitamin B12 content, mg</td>
<td>0.01 ± 10 %</td>
</tr>
<tr>
<td>Content of choline chloride, mg</td>
<td>10.0 ± 10 %</td>
</tr>
<tr>
<td>Selenium content, mg</td>
<td>0.05 ± 10 %</td>
</tr>
<tr>
<td>Zinc content, mg</td>
<td>1.0 ± 10 %</td>
</tr>
</tbody>
</table>

Stability of the drug.

According to the Decree of November 21, 2007, N 1349 “On the approval of the provisions on the state registration of veterinary drugs, feed additives, premixes and ready-made feeds”, the manufacturer, in the case of initial registration of the drug, has the opportunity to provide data on stability for experimental and industrial series with a deadline observation, at least 12 months for normal conditions (temperature 25 ± 2 °C, relative humidity 60 ± 5 %) and conditions of accelerated testing (temperature 40 ± 2 °C, relative humidity 75 ± 5 %), predicting product quality compliance for two years. This approach makes it possible to carry out registration by considering this forecast in a shorter time frame while considering all the risks for the animal.

The investigated veterinary medicinal product was stored at a controlled room temperature (5–25 °C), and its stability was determined in real-time.

In the process of storing the solution for oral use “Devivit Carnitine”, the following indicators were determined: appearance and amount of carnitine hydrochloride, vitamin E, B12, choline chloride, selenium, and zinc.

Table 2 shows the results of an experimental study of the drug’s stability under different storage conditions.

The results of studying the stability of the drug in other studied series were identical. The analysis of the obtained data shows that the samples of the solution, which were stored under conditions of 5–25 °C, withstood the tests according to all indicators of the MQ of the medicinal product, and the samples, which were stored under conditions of temperature above 25 °C, after 12 months of storage, acquired the smell of bitter of oil, which indicates the presence of oxidation processes in the dispersed phase of the solution. The acid number values confirm this.

To determine the thermal stability, 5–6 glass tubes with a diameter of 15 mm and a height of 150 mm were taken. The test tubes were filled with 8–10 ml of the studied samples and placed in a TS-80M-2 thermostat with a temperature of (42.5 ± 2.5) °C for seven days. After that, the samples were transferred for seven days to a refrigerator with a temperature of (6 ± 2) °C, and then, for three days, kept at room temperature. Stability was determined visually: if no delamination was observed in any test tube, the sample was considered stable.

To establish the causes of potential impurities’ formation, stress studies of the active substance and the drug “Devivit Carnitine” were carried out under the influence of various factors: high temperature, acids, alkalis, strong oxidants, and metal ions (especially iron) that can enter the medicinal substance or the finished product dosage form from the surface of the equipment.
The research data confirmed the high stability of the product, even under the influence of harsh factors - only a strong oxidizer leads to the formation of small amounts of impurities. Considering the study of stability, stress stability, and the results of accelerated storage, it has been proven that the formation of impurities in the preparation of “Devivit Carnitine” does not occur. The content of impurities is kept at the level of the content of impurities at the zero point of the study, and their only source can be the raw materials used to obtain the solution for oral use.

Therefore, based on the above studies, it was established that the solution for oral use, which was stored in canisters during the entire study period, met all the indicators of the methods of quality control of the medicinal product.

4. Conclusions

The results of research on the Development of the technology of an oral use solution based on carnitine hydrochloride, vitamins E, B12, choline chloride, selenium, and zinc are presented.

The stability and shelf life of “Devivit Carnitine” (solution for oral use) in plastic canisters were determined. It was established that the drug experimental samples were stable, according to all indicators of MKY, during the entire period of the study in plastic canisters.

The shelf life of the medicinal product with carnitine hydrochloride, vitamins E and B12, choline chloride, selenium, and zinc is 12 months.

In the future, a study of preclinical data is planned to verify whether the drug does not reveal a particular hazard to animals based on conventional studies of safety pharmacology, repeated dose toxicity, genotoxicity, carcinogenicity, and developmental toxicity.

Conflict of interest

The authors claim no conflicts of interest.

References


