Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

Scienze degli alimenti, nutrizione animale e sicurezza alimentare

Ciclo XXV

Settore Concorsuale di afferenza: 07/H4

Settore Scientifico Disciplinare: VET/07

VETERINARY DRUGS IN DRINKING WATER USED FOR PHARMACEUTICAL TREATMENTS IN BREEDING FARMS

Presentata da:
Salvatore Scandurra

Coordinatore Dottorato

Prof. Roberto Rosmini

Relatore

Prof.ssa Paola Roncada

Correlatore

Dott. Giorgio Fedrizzi

Esame finale anno 2013

A Lia e Laura

“There cannot be good living where is not good drinking”

Benjamin Franklin

Index

Abstract

Water is one of the simplest chemical substances present in nature, nevertheless it is the essential element for the life and all living organisms are mainly constituted of water. Water is susceptible to be used for numerous purposes, including edible, both for humans and animals.

In the food animal production, drinking water is frequently used as a way to carry out the most common pharmacological treatments. In these cases, there are many variables which could degrade drugs dissolved in this mean, even when properly arranged pharmaceutical formulations are used. In fact, although a product obtains a Marketing Authorization through appropriate laboratory studies both drug stability and solubility, on the other hand the solubility of the same drug in natural water used as a drinking water is not documented. In the present study has been evaluated the dissolution kinetics (at 0 hours and 24 hours) of products, having oxytetracycline and tylosin as active ingredient, used in drinking water samples in order to see how the different physical and chemical factors that characterize the drinking water may affect therapeutic efficacy. In fact, multiple factors, also of little relevance if individually considered, are able to adversely affect the pharmacological treatment carried out in drinking water.

For all based-oxytetracycline products considered, it was possible to observe a tendency to decrease in solubility between 0h and 24 h. In fact, for the different products, at 24h, active ingredient percentages between 20 and 62.8% (compared to that ones detected at 0h) were observed. About the based-tylosin products, the results were different. In fact, for some products was possible to observe a trend of solubility increase between 0h and 24h results. At 24h, for all based-tylosn different products, active ingredient percentages between 80% and 164% (compared to that ones detected at 0h) were detected. Therefore, the intrinsic characteristics of the water such as pH, hardness, conductivity and calcium may affect the dissolution of drugs tested in water. The results suggest that it would be appropriate to test the products in water samples under challenging conditions dissolution, in order to identify in advance possible problems.

1. Background

Water is one of the simplest chemical substances present in nature, nevertheless it is the essential element for the life and all living organisms are mainly constituted of water. Water is susceptible to be used for numerous purposes, including edible, both for humans and animals.

Therefore, by law, water must meet specific chemical, physical and microbiological characteristics. In the food animal production, drinking water is frequently used as a way to carry out the most common pharmacological treatments (Zaghini A., 2005). The oral medication by drinking water offers different advantages. In situations of disease, feed intake decreases. Water intake is also affected, although to a lesser extent. In fact, water consumption decreases 30% in situations of disease, whilst feed consumption decreases 40%. Water consumption also returns to normal levels much more quickly than feed consumption (Pijpers A. *et al*., 1991).

Furthermore, in contrast to medicated feed in feed bins, there is no dilution effect with drinking water. This makes dosage periods shorter and yields considerable economic and safety benefits, as withdrawal periods can be adjust more precisely. While with medicated feed, the product must first be ordered, then mixed and finally delivered to the farm, therapy by drinking water can start more quickly. Medicated

drinking water allows quick action when there is a disease outbreak and prevents loss of animals and deterioration of zootechnical parameters (Kunesch *et al*., 1986).

Studies have shown that a drug's bioavailability is greater when it is supplied in water than in medicated feed. The maximum absorption of drugs takes place in the small intestine. With gastric digestion, the stomach empties liquid contents faster than solids. Consequently, water-dissolved medicines are absorbed more quickly than medicines that have to be extracted from the food and dissolved by the digestive juices before they can absorbed (Reeve-Johnson L., 1998).

In the oral medication by the drinking water, there are many variables (for instance hardness, temperature, pH of the water and characteristics of mixing and distribution of the drugs) which could degrade drugs dissolved in this mean, even when properly arranged pharmaceutical formulations are used. The drugs solubility in the drinking water is a critical and challenging factor for the therapy. In fact, if the medication does not dissolve, its active ingredients will not be completely released and the animals will not receive an effective dose (Zaghini A., 2005).

The present study evaluates the dissolution kinetics of some veterinary drugs commonly used in drinking water for food animals bred in zootechnical farms. The aim of the study was to assess how the therapeutic efficacy might be affected by different factors that

characterize drinking water. In fact, there are multiple factors, even with little relevance when individually considered, which are responsible to make the pharmacological treatments virtually useless. These factors combine to modify the stability and/or solubility of individual active principles, or the formulation used, as well as excipients present in drinking water (Almond G. and Monahan K, 2011).

Furthermore, it is extremely important to consider the correct modality of mixing the veterinary drugs in water by the operators and the functionality both of the blending plant and the water distribution system in the farm, especially when the veterinary drugs used are poorly hydro soluble (Dorr P.M. *et al.*, 2009).

Finally, after the administration of the medicated water, it is absolutely critical to clean carefully the whole water distribution system in order to avoid that residues of the administered drugs could remain adherent to the internal walls of the plant tubes. In fact, these residues can be released again reaching animals in sub therapeutic doses or interfering in any way (chemical, dynamic, kinetic etc.) with following pharmacological treatments implemented by drinking water (Croubels S. *et al*., 2001).

2. Water and its characteristics

The water also known as Dihydrogen monoxide, H_2O, is defined as: "clear, colorless, odorless and tasteless liquid "(USGS, 2013). Water has a molecular weight of 18 Daltons and is constituted by one oxygen atom and two hydrogen atoms. Water is totally neutral, however, due the presence of oxygen, this molecule is polar and has a strong tendency to form hydrogen bonds (each water molecule can form hydrogen bonds with other four molecules of water) (A. F. I., 1991). Water is the essential element of life, all living organisms are mostly made up of water and life on Earth, in all its forms known, uses the physical and chemical properties of the water for its own existence. Flowing above and below the earth's surface, water make both its chemical function of soluble salts or minerals dissolution and its physical function of insoluble materials entrainment; moreover, water is able to incorporate gas, such as oxygen and carbon dioxide, allows that the animal life can take place in rivers, lakes and seas as well. The primary source of water is atmosphere because its precipitation (Figure 1). When the water is collected on the soil, it is a solution with a composition that could be extremely variable depending with both of the function of the substances which take contact with it and the time and physical modalities of the contact when it occurs. The primary sources of water can be classified as follows:

1. Meteoric water (rain, snow, hail);

2. Surface water (rivers, lakes, seas, oceans, etc.).

3. Groundwater (phreatic layer, aquifer layer).

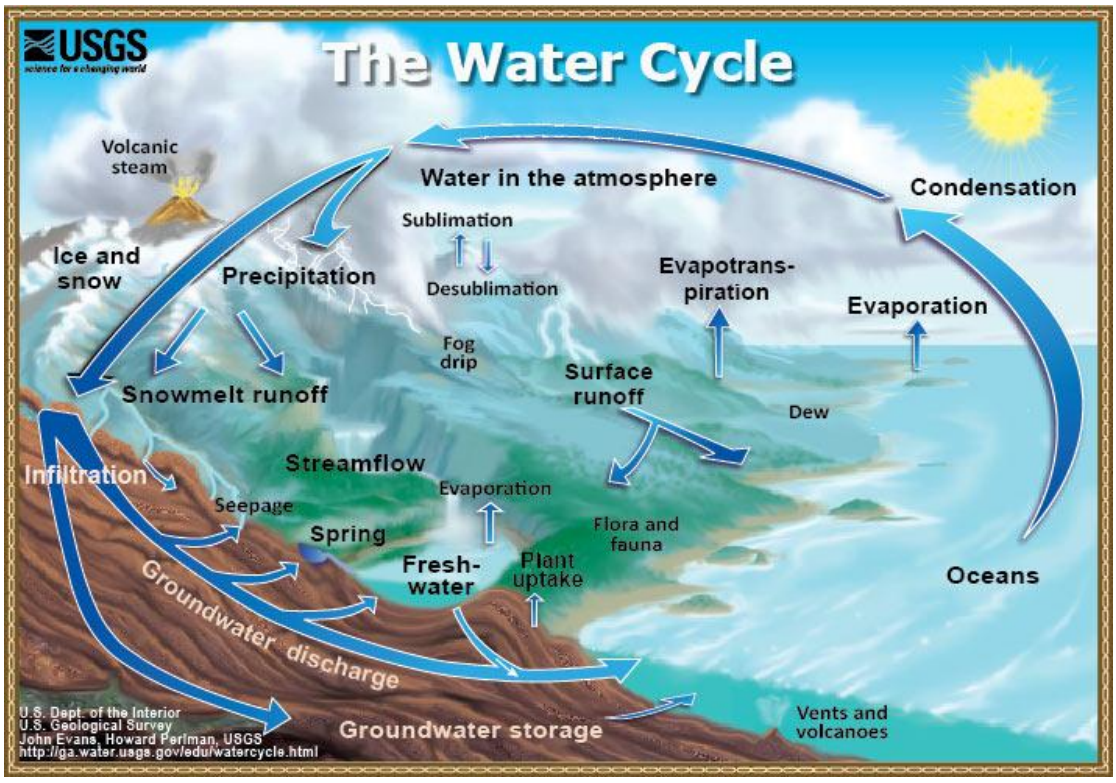


Figure 1. Water cycle (United States Geological Survey, 2013).

Some chemical and physical characteristics of the pure water (http://www.62.co.za/water_properties.html).

- Melting point with normal pressure……0°C
- Boiling point with normal pressure…...……..100°C
- Specific weight at 0°C (water)…..…….0.9998 g/cmc
- Specific weight at 3.98°C…….……..…….....1 g/cmc
- Specific weight at 20°C……..0.9982 g/cmc
- Specific weight at 0°C (ice)…….....…….……0.9168 g/cmc
- Specific heat at 15°C (water)……...…….1 cal/g
- Specific heat at 0°C (ice)…….....……..…0.4870 cal/g
- Specific heat at 100°C (vapor)…....……....……...0.4620 cal/g
- Dipole moment….....……..…...1.84x10-18
- Viscosity at 0°C……0.0179 poise
- Viscosity at 20°C……..…0.01 poise
- Viscosity at 100°C……...0.0028 poise
- Coeff. of compressibility (T and P normal).……4x10-5 cmq/kg
- Constant dielectric at 18°C……....81.07 Farad/m
- Eletric conductivity at 18°C……3.8x10-8 S/cm

2.1 Quality of drinking water

The groundwater can be:

1) **Phreatic**. This kind of water is less valuable because can be easily reached by infiltrant pollutants;

2) **Deep.** This kind of water offers the best requirements of quality of drinking water because passing trough underground layers of soil, is deeply filtered and properly purified.

Drinking water must meet precise organoleptic, bacteriological, physical and chemical requirements. The Presidential Decree n. 236 of May 24, 1988 (and subsequent updates) has enacted EEC Directive 80/778 concerning the quality of water intended for human consumption:
"*Water for human consumption are intended all waters, irrespectively of the origin, in which state they are or after processing, supplied for consumption; furthermore water for human consumption are all water used by food businesses by acquisition or contact for the manufacture, treatment , storage, placing on the market of products and substances intended for human consumption and which may have consequences for*

the health of the final food product. Are excluded from this decree, the mineral and thermal waters".

This is the broadest definition of drinking water because it includes all the different possibilities of use of water for edible or for multiple purposes. This Presidential Decree, as well as to extend the concept of drinking water according to its use, it gives also a precise definition of drinking water. Infact, there are over than 60 parameters to classify waters destined for human consumption, and most of these parameters regards physical characteristics.

Legislative Decree n. 31 of 2 February 2001, implementing Directive 98/83/EC, regulates the quality and ensure healthiness of water intended for human consumption in order to protect human health from the adverse effects of any contamination of water":

1) the treated or not treated waters, intended for drinking use, for the preparation of foods and beverages, or other domestic purposes, regardless of their origin, even if distributed through an acqueduct, from a tanker, from bottles or containers;

2) waters used in any food-production to preparing, processing, preservating or marketing products or substances intended for human

consumption (excluding those identified under Article 11, paragraph 1, letter s), which quality not affects the foodstuff healthyness.

In the past the risk was standed mainly by contaminated water, and sometimes exclusively by infections, today due the increasing of pollution sources caused by technological evolution, changing needs and living conditions, there is a considerable spread of chemicals in the environment with the consequent change of the water pollution type (calcium, magnesium, iron, manganese, silica, carbon dioxide, hydrogen sulfide, phosphate, copper, aluminum, arsenic, lead, cadmium, nitrates, algae, protozoa, etc.).

The following Table 1 and Table 2 summarize the possible substances present in water and the parameters useful as indicators of its quality.

In short, the main characteristics of water that must be kept under control are essentially: hardness, pH (acidity and alkalinity) and nitrites.

PHYSICAL STATE	SOURCE		
	Inorganic	***Organic***	***Biologic system***
Solution	Inorganic salts of Na^{+}, Ca^{2+} , NH^{4+}, NO^{2-}, NO^{3-}, Cl^{-} , Mg^{2+}, etc. Radioactive ions Gases: O_2, N_2, CO_2, NH_3, H_2S, etc.	Quaternary ammonium-based Artificial corrective systems: pesticides, fertilizers, herbicides, etc. Organic salts	Organic decomposition
Dispersion *suspension emulsion, colloid*	Erosion and/or corrosion products: sand, insoluble salts, etc. Treatment residues Micelles or large molecules (S, SiO_2, Fe_2O_3, Al_2O_3, etc.)	Immiscibles liquids Insoluble products Treatment residues Macromolecules Surfactants Humic acids	Microorganisms Algae Macromolecules

Table 1. Possible substances in the water (2).

PARAMETER	QUALITY INFORMATIONS
Cations and anions Specific conductivity Dissolved solids Hardness (Ca and Mg)	Geochmica degli strati attraversati Water salinity Organic variability of water Geochemistry of crossed layers
NO_2, NO_3, NH_3 total Nytrogen (Nytrogen cycle) PO_4 totale phosphorus (Phosphorus cycle)	Inorganic and biologic pollution
Dissolved oxygen	Oxidation Well-being of acquatic life
pH	Acidity and corrosivity Well-being of acquatic life
Radioactivity	Radiochemistry
Total carbon	Presence of organic substances
Pesticides Organic solvents	Presence of organic substances Ecological status
Turbidity Temperature Color, odor	Physical state
B.O.D.(Biochemical oxygen demand)	Organic pollution
Microorganisms	Human and animal pollution

Table 2. Water quality parameters (A.F.I., 1991).

2.1.1 Hardness

In nature, usually, the water hardness is determined usually from the substrate where it flows. Chemically, the water hardness occurs by measuring the presence of metal ions such as calcium and magnesium salts dissolved in the water itself.

The hardness is distinguished in:

- **temporary or carbonic hardness,** due by Ca and Mg soluble bicarbonates that, at boiling water temperature, pass to insoluble carbonates:

$$Ca(HCO_3)_2 \rightarrow CaCO_3 \downarrow + H_2O + CO_2 ;$$

- **permanent or non-carbonic hardness**, due by other salts of Ca and Mg (sulfates and chlorides mainly) that remain in solution even after a prolonged boiling of water;

- **Total hardness** is the sum of temporary hardness and permanent.

Hardness is measured in hydrotimetric degrees: French degrees (°F) and German degrees (°DH), where 1°F = 0.56 °DH (see Table 3):

CLASSES	HARDNESS			
	(°F)	**(°DH)**	**(°e)**	**(°a)**
1°)	1 8	0,56 4,47	0,7 5,6	0,58 4,60
2°)	12 20	6,71 11,18	8,4 14,0	7,00 11,60
3°)	24 32	13,42 17,89	16,8 22,4	13,90 18,50
4°)	36 40	20,13 22,36	25,2 28,0	20,90 25,20

Table 3. Natural water classification (A.F.I., 1991).

(°e) = English degree

(°a) = American degree

2.1.2 pH (acidity and alcalinity)

In freshwater, the pH ranges between 6.5 and 7.5 usually. The pH scale is logarithmic, thus the increase by one unit represents 10-fold the acidity or alkalinity increase.

2.1.3 Nitrites

The nitrites are derived from the ammonia conversion during the nitrogen cycle (Figure 2). Nitrites are transformed into less harmful nitrates. Nitrites and ammonia are signs of water pollution.

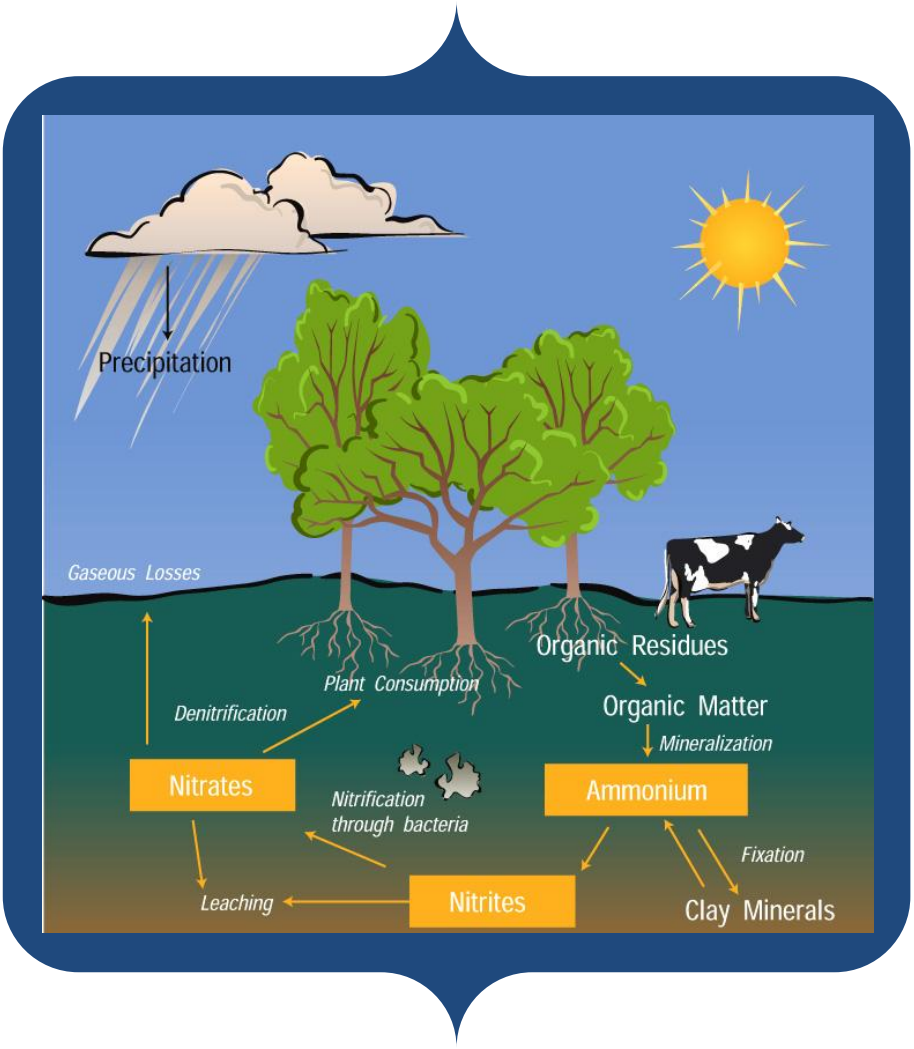


Figure 2. Nytrogen cicle (www.eo.ucar.edu, 2013)

2.2 Water quality in the livestock breeding

Water should be considered as an outright food since it is necessary and irreplaceable to the animals' survival. The water intake must even be done regularly. In fact, while for other nutrients (eg fat), animals are able to accumulate own reserves, water instead must be ingested daily. Typically an animal ingests a double water quantity compared to the consumed food (Canadian Water Quality Guidelines for Livestock, 1987).

The water used for livestock breeding has different origin: aqueduct, well, lake, river etc.

In the poultry, rabbit and swine breeding as well as other livestock species, several drugs, especially antibacterial, with different therapeutics purposes, are administered. Usually the most important administration route is the oral one, via food, but most often via drinking water (Adams H.R, 1999).

The most commonly drugs used via drinking water are the following:

1) antibacterial:

- Gentamicin;
- Spiramycin;
- Tylosin;
- Tiamulin;

- Colistin;
- Amoxicillin;
- Sulphadimethoxine-Trimethoprim;
- Chlortetracyclin;
- Oxytetracyclin;
- Doxycyclin;

2) Acetyl-salicylic acid;
3) Levamisole;
4) Vitamin K and B;
5) Acidifying agents: acetic acid, copper, etc;
6) Disinfectants: chlorine, hydrogen peroxide, etc;
7) Yeast.

Particularly the antibacterial use may depend on the need to obtain a therapeutic, prophylactic and methaphylactic action (Lawhorn B., 1998).
In the livestock breeding the drug administration with drinking water is an excellent and inexpensive way to carry out a drug treatment. In fact, the water "medication" allows to act very quickly and to obtain therapeutical effective levels with limited cost and with a little animal's "handling". In addition, in case of diseases (especially diarrhea and pneumonia), the animals might eat less, or stop eating completely, while they will always continue to drink water.

Finally, in case of some drugs (eg sulphanomides), the product powder is characterized by a remarkable electrostaticity, then these type of product tend to remain on the feeders surfaces, on the structures floors and in the air. Consequently, there is a very high risk of cross-contamination and carryover (Schneider P., 2003).

Other antibacterials (eg tetracyclines) because their strong chelating activity towards ions like calcium and iron, when added to the feed frequently give rise to chelates inactive and nonadsorbable at intestinal level (Kunesh J. *et al*., 1986). Accordingly, a highest quality of the drinking water is a very critical factor for the livestock production efficiency and health.

There are multiple and different factors that can determine water quality:

- **Biological factors:** presence of bacteria, protozoa (coccidia), intestinal helminths eggs, etc. For example, a high level of coliforms may indicate faecal contamination of the water (in general, the drinking water used for livestock should contain fewer than 100 total bacteria / ml and less than 50 coliforms / ml). Low levels of contamination can be managed using disinfectants (usually chlorine-based).

- **Physical factors**: color, taste, odor, clarity; water should be clear and odorless.

- **Chemical factors**: total dissolved solids (TDS), pH, iron, hardness, and nitrates/nitrites.

In detail, Total Dissolved Solids (TDS) represent a measure of the inorganic substance total concentration present and dissolved in water. Usually constitutes the "salinity" because water is determined from calcium, magnesium and sodium, in form of bicarbonates, chlorides or sulphates, and traces of iron, manganese and other molecules (Table 4) (Van Heugten E., 2011).

The consequences of lower water quality are:

- **Presence of bacteria**: diarrhoea, mastitis, metritis, abortion, etc.
- **pH, hardness**: cystitis, nephritis, metritis, locomotor disorders, etc.
- **Too high level of nitrates**: renal and reproductive problems, nervous disorders, low milk production, etc.
- **Too high level of iron**: obstruction of pipework, etc.
- **Too high level of sulphates**: laxative effect, etc.

Poor water quality will not always lead to problems in animals, but it might affect the efficiency of a water medication.

Inorganic substance concentration (mg/L)	Information
<1.000	No risk
1.000-2.999	Acceptable. Mild diarrhea in animals not used to this drinking water may occur.
3.000-4.999	Acceptable. Temporary denial of water and mild diarrhea may occur.
5.000-6.999	Sufficiently healthy, but to be avoided in pregnant and lactating animals.
7.000-10.000	Unsuitable for swine production. Pregnant and lactacting females and very young and stressed animals are particularly at risk.
>10.000	To be avoided.

Table 4. Water salinity (Van Heugten E., 2011).

2.2.1 pH

The range of acceptable pH values is from 6.5 to 8.5. PH values lower than the lowest (acidity) or above the highest (alkalinity) can cause pipes corrosion and consequently water could be contaminated by metals such as iron, copper, lead and cadmium. A high pH gets worse chlorination efficiency. By contrast, a low pH may cause precipitation of some antimicrobial agents administered through the drinking water. Sulfonamides lose their water solubility in acid pH values (Van Heugten E., 2011).

Water with pH lower than 5.5 can be harmful causing digestive and urinary tract problems, demineralization and skeletal fragility, materials corrosion. Often a low pH and a low hardness are associated and synergistically act on the mobilization of calcium from bones (Dorr P.M., 2005).

2.2.2 Hardness

As previously reported, water may be considered ***soft***, ***hard*** and ***very hard***. In general, the water hardness is a problem for the water distribution systems (build-up of deposits) especially for the inactivation of some antibacterials (for example: tetracyclines can form chelates, which may alter their effectiveness, as they cannot be absorbed in the presence of divalent ions such as calcium, magnesium and iron) (Kunesh

J. *et al.,* 1986) that could prejudice both animal health and the livestock performance. In this regard, the following considerations apply:

- **from15 to 50°F** water is considerated potable;

- **above 20 ° F** it is possible to have:

1. Oligoelements intestinal absorption decreased (reduction effect of the solvent power);
2. Reduction in the effectiveness of disinfectants used in solution;
3. Dissolved salts precipitation if water is heated to above 50 ° C;

- **Below 8 ° F** drinking water is not suitable for animals and is considered aggressive towards metals.

2.2.3 Chlorides

If chloride are present in quantities greater than 250-500 ppm can give to the water an unpleasant taste and then make it unpleasant to animals (Van Heugten E., 2011); often chlorides accompany the organic matter of animal origin and therefore may indicate pollution. It can not exclude the possibility that high concentrations may interfere on rumen fermentation. Avian species are also very sensitive to sodium chloride that is nephrotoxic.

2.2.4 Iron

The presence of iron in water can enhance the growyh of certain bacteria, which can result in the precipitation of ferric derivates. Although this does not affect animal health, however, iron quantities of 2-3 ppm may block drinking nozzles. At concentrations of 5 ppm or more, oxytetracycline and its derivates form chelates, which prevent its absortion. Finally concentrations higher than 10 ppm lead animals to turn down the water (Van Heugten E., 2011). Apramycin should not be used in water with a high ferric content, as it may form chelates, which will limit its therapeutic effectiveness (Manual of water medication, 2011).

2.2.5 Sulfates

Any activity laxative/purgative. Sulfates can cause diarrhoea in weaning piglets especially, but in adults as well; sulfates may be associated with bacterial contamination(Van Heugten E., 2011).

2.2.6 Nitrates and nitrites

Have a dual origin: organic matter degradation, leaching of soil after animal manure spreading and fertilizers. Beyond certain concentrations, nitrates have an own toxicity (growth retardation, digestive problems, decrease in the eggs deposition), but their danger remain mainly in the possibility to be converted into nitrites (especially in the cattle rumen),

very toxic for their action to formate methaemoglobin which makes it impossible to transport oxygen to the tissues. The maximum allowable concentration for nitrates is only 0.1 mg/l (Arduin M., 2004).

2.2.7 Ammonia

Ammonia is within the standards for drinking water but coming from organic matter can oxidize to nitrates (Arduin M., 2004).

2.2.8 Manganese

Beyond to abnormal flavors, a very high pH, can cause damage to the equipments (Arduin M., 2004).

2.2.9 Phosphorus

Phosphorus may have a chemical (e.g. fertilizers) or organic (e.g. manure) origin. Phosphates associated with bacterial pollution makes the water undrinkable (Arduin M., 2004).

Parameters	Maximum Allowable Concentration
Nitrates (mg/l NO_3)	50
Nitrites (mg/l NO_2)	0,5
Ammonia (mg/l NH_4)	0,5
Iron (pg/l Fe)	200
Manganese (µg/l Mn)	50
Copper (µg/l Cu)	1000
Phosphorus (µg/l P_20_5)	5000

Table 5. Legal parameters for certain undesirable substances (Arduin M., 2004).

Evidently, when the drinking water features does not meet the desired ones, it is not possible to carry out multiple and different therapeutic treatments.

The major obstacles encountered in using drinking water as "vehicle of drugs" are represented by water solubility and drug stability in water. To facilitate a perfect solubility of the active ingredients, the commercial formulations contain particular excipients and/or salified forms soluble in water.

Excipients most frequently added to the formulations to be used in water, beyond to facilitate dissolution of the active ingredient, can play a role in protection it as well.

In general excipients comprise:

- Antioxidants
- Buffer sistems
- Chelating
- Colorants
- Glidants
- Flavoring
- Preservatives
- Disperdants.

In any case, beyond any consideration of the complete and rapid formulation dissolution, must also be evaluated with particular attention the characteristics of the water distribution system where the product dissolution takes place. In fact, the system might influence the dissolution kinetics of the drug, with a possible incomplete dissolution that would lead to a reduced oral bioavailability and consequently sub-optimal hematic concentrations (Allen L.V. Jr, 2001). Therefore, it is very important to constantly verify the concentration both in the water in which the drug is dissolved and in the water at the level of the drinking troughs, in order to avoid an under-dosed drug and/or a subsequent solubilization of the precipitates with the possibility of a following cross-

contamination. This last case would involve, consequently, the presence of undesirable active substances in animal products (EMA, 2002).

3. Solubility of veterinary drugs

The solubility of a medication will depend on the product (active substance and excipients) and the water quality. As described by Marco E. (2009) the product's active substance must be able of ionizing in order to be water-soluble; otherwise it would precipitate out to form sediments. An example of a compound able of ionizing upon contact with water would be a salt, and this is the most common presentation of soluble medications. A salt will separate into two kinds of radicals: acid (positive) and base (negative). Not all compounds used will separate and give the same amount of acid and base radicals. This characteristic is expressed by means of the pKa constant (Manual of water medication, 2011).

The smaller this constant, the more acidic the compound. So, a compound with a pKa of 2.7 (the pKa of phenoxymethylpenicillin), will be considered as an acid, while a compound with a pKa of 7.6 (the pKa of lincomycin) will be considered as a base. When the pH of the medium in which it is dissolved coincides with its pKa value, the compound will be 50% ionized. For a correct solution, there should be full ionization. Thus, a compound that has a weakly basic character will be ionized better in water with a acidic pH, for example water from granite soils, while a

compound with a weakly acidic character will ionize better in a basic medium, for example water from calcareous soils (Marco E., 2009)

In practice, slightly acidifying or neutralizing drinking water may be of great value to achieve better solubility of thr products used. Thus, for example, to avoid problems with weakly basic compounds, such as tetracyclines, acidification of drinking water can be recommended.

To know the substances solubility is crucial, but it is not always easy to understand why a certain substance is less or more soluble in a specific solvent, since there are multiple factors that affect solubility and that often act contrasting each other. When we use the term "solubility" tacitly assume that it is the solubility equilibrium (Marco E., 2009).

In other words it is assumed that a solid (solute) is put in contact with a liquid (solvent), that the system is mantained under agitation until a state of equilibrium is reached. This equilibrium state is characterized by the fact that the solute concentration has reached a constant level.

In general there are several equilibrium situations:

- the solid phase is a pure compound and there is only one liquid phase;
- the solid phase is a pure compound and is present more than one liquid phase;

- two components form a solid solution so that there is an unlimited solubility in the solid phase;
- there are two solid solutions that are formed, in this case the solubility in the solid phase is limited.

Among the above possible situations, the first one is most common. The solubility of a solid is determined putting in contact a considered excessive quantity of this solid with a solvent, in an airtight container, at a predetermined and constant temperature, up to the achievement of an equilibrium state.

Conventionally 72 hours are considered necessary to achieve this state.

Using less time, the solubility can be determined by extrapolation, it means by constructing a curve taking samples from the medium every 12 hours and testing the concentration of the solute. If the compound is not very stable in aqueous solution it is necessary to use a different method that provides for faster results, such as not to allow the product to deteriorate (sampling every 20 minutes), and finally calculate results by the Nogami method.

The solubility is commonly expressed as molality, or as weight of solute per gram of solvent.

3.1 Temperature effect

The heat associated to the solution of a drug substance in solid form in a solvent (usually water) shows that the solubility is conditioned by the temperature. If during the dissolution heat will develop, the compound solubility decreases with increasing temperature, and in general, in simple cases, the opposite will occur. It is well known that when KOH is dissolved in water develops a lot of heat develops; but this doesn't mean that the solubility of the compound decreases with temperature. Indeed, the energy that is initially developed as heat is positive, but overall at the end when it reaches the saturation point it becomes negative, therefore actually, an increase in temperature leads to a further increase of the solubility.

3.2 Electrolytes effect

In aqueous solution the present electrolytes can greatly affect the solubility of a compound, particularly if the compound itself is an electrolyte.

3.3 Use of mixed solvents

The solvent used has considerable influence on the solubility and it must always to be specified. Often in pharmacology it is necessary to use mixed solvents to solubilize poorly soluble substances in water. The co-

solvents most commonly used are ethanol, propylene glycol, glycerin, polyoxyethylene glycol.

3.4 Dielectric costant effect

Frequently, the solubility is a function of the medium dielectric constant, and the relationship is described generally by the Jaffe equation:

$\ln [S] = (A / \varepsilon) + B$

Very often the solubility of hydrophobic substances decreases with the medium dielectric constant increase.

3.5 Solubility multiple peaks (Chameleonic effect)

For certain substances in certain mixtures of solvents is possible to observe multiple peaks in the graph that represents the solubility as a function of the solubility parameters of solvents.

3.6 Complexes formation

Substances can form complexes with complexing agents. In general, one of the two components of the system is defined substrate, while the other component is defined ligand.

This phenomenon is often associated with problems of solubility in the pharmaceutical field, but can be useful in the production process as well.

3.7 Cyclodextrins

These compounds (α, β and γ in relation to their size ring) form compounds with certain substances; cycledextrins hold the whole molecule, or some hydrophobic portions of it within their "cavity." This affects some physico-chemical properties of the complexed substance without affecting its pharmacological properties.

3.8 pH

As discussed before, the solvent pH influences the solubility of a substance which has in its structure acidic or basic groups, shifting the balance in one direction or in the opposite one, depending on wheter the reaction products or the reagents are incremented. So, use oh pH modifiers might affect the solubility of water-soluble antibiotics.

3.9 Mixture of soluble drugs

As suggested by Dorr P.M. *et al.* (2009), restrictions on mixing medications that may compromise the health and performance of the pigs should also be considered. Formation of precipitates when 20-mL aliquots of commonly used water-soluble medications prepared according to label directions were combined in pairs and observed for 24 hours (Table 6). Medications included in precipitation reactions were aspirin (ASA), sodium salicylate (Na sal), amoxicillin (amox), sulfamethoxazole-

trimethoprim (Smz-Tmp), potassium pencillin G (PotPen), neomycin (Neo1 and Neo2), tetracycline (tet), oxytetracycline (oxytet), chlortetracycline (chlortet), chlortetracycline-sulfamethazine (chlor-S), sulfamethazine (sulfa), and tiamulin (tiam). Gentamicin, lincomycin, and tylosin were also tested with other products with no observation of precipitate formation.

	Tet	Oxytet	Chlortet	Chlor-S	Sulfa	Tiam
ASA						
Na Sal						
Amox						
Smz-Tmp						
PotPen						
Neo 1						
Neo 2						
Tet						
Oxytet						
Chlortet						
Chlor-S						

Table 6: Red precipitate; green no precipitate (Dorr P.M. *et al.*, 2009)

4. Stability of veterinary drugs

When a medicated solution is stored for a longer time period than necessary, it loses quality (palatability and contamination). The optimal storage period should correspond to the maximum time during which the concentrate remains effective and safe. Poor water quality causes unstable products, which leads to low bioavailability, incapacity to optimize active substances (affecting both efficacy and safety), failure to respond proportionally to the dose, suboptimal dosing and undesirable following medication (Manual of water medication, 2011).

According to the Italian Official Pharmacopoeia, "A medicament is considered stable when, in a specific period of time, its essential properties do not change or change within tolerable limits, if stored in a suitable vessel, under defined conditions of temperature, humidity and exposure to light".

4.1 Stability period

Is the period between the preparation of the medication and the time in which the medication no longer meets the Official Pharmacopoeia requirements. Evidently, in this context, is considered the only chemical and physical stability, because the microbiological stability is assured by preservative and/or sterilizing treatments.

4.2 The time limit t10

It is defined as the required time so that the drug initial title undergoes a reduction of 10%. The values are calculated by the stability tests, possibly accelerated (http://www.galenotech.org/stabilit.htm; FDA 2013).

The main causes of instability of medicines are represented by **temperature, light and humidity.**

- **Temperature**: in general, a temperature increase of 10 degrees centigrade increases the rate of chemical reactions of 2-4 times. This means that it doubles or even quadruples the drug decomposition speed. However, it should be paid great attention to the extreme cold, for instance, insulin should be stored in a refrigerator, but does not tolerate freezing. In general, it is good practice to comply with the storage conditions indicated on the packaging and in absence of indications to keep drugs at room temperature, between 8 and 25°C.

- **Light**: some active ingredients are light sensitive; the packaging must be able to protect them from light (eg brown bottles, aluminum blisters or opaque) and the packs shall be kept strictly closed. Even in the absence of light sensitivity, it is not advisable to

prepare in advance the drug dose by pulling it out from the container.

- **Umidity**: may further a rapid deterioration of medicinal products, in general, they should be stored in a dry place. For example, the β-lactam antibiotics distinctly exhibit this problem and they are easily degradable in aqueous environment (fragility and β-lactam ring opening, with loss of antibacterial activity) ((http://www.galenotech.org/stabilit.htm; FDA 2013).

Below are reported some examples of molecules having pharmacological action and commonly administered via drinking water, for which some expedients are necessaries to avoid affecting the treatment:

- ***Acetyl salicil Acid.*** This non-steroidal anti-inflammatory, commonly used in swine production, is particularly sensitive to sodium carbonate which rapidly hydrolyzes it to salicylic acid with complete loss of the pharmacological activity (Moisescu S. *et al.*, 1975). Must be paid particular attention to the water hardness.

- ***Vitamins K and B1***. Both vitamins are characterized by a high sensitivity to light and air, respectively, which determine a very rapid degradation with loss of activity. Both vitamin K that vitamin B1 are inactivated very rapidly by alkaline solutions, while they are very stable in acid aqueous solutions (The Merck Index Centennials, 1989). It is recommended to prepare solutions administered to animals immediately before using; it should be also paid particular attention to the integrity of the metal pipes of water distribution systems (Carr J., 2002).

- ***Gentamicin sulfate*.** This aminoglycoside is characterized by a high stability in aqueous solutions from strongly basic ones to slightly acidic ones, at room temperature provided in tightly closed containers (Osborn E., 1976). Once dissolved in water, the gentamicin should not be stored in rusted containers as it is very quickly degraded; furthermore, it is good practice to prepare daily the aqueous solution to be used (FDA, 2013).

- ***Bacitracin methylene disalicylate***. This antibacterial agent is characterized by a good general stability and is not particularly

sensitive to changes of pH and temperature. However, the aqueous solution that contains it must be prepared daily (FDA, 2013).

- ***Tylosin***. This molecule is characterized by a fairly good solubility in water and its solutions are stable in a pH range between 4 and 9; for pH values lower than 4 it could occur the formation of a derivative (desmicosine) which nevertheless retains unaltered the antibacterial activity (The Merck Index Centennials, 1989).

- ***Oxytetracycline and chlortetracycline***. Similarly to most of the tetracyclines, are characterized by a good stability at different values both temperature and pH, although they are characterized by maximum stability at very acids pH (The Merck Index Centennials, 1989). Likewise to other molecules belonging to this antibacterials group, Oxytetracycline and chlortetracycline have propensity to form epimers; chlortetracycline, particularly, form epimers reversibly at the 4-diethylamino group level. This occurs slowly in water, but it is strongly accelerated in the range of pH between 2 and 6. The antibacterial activity of the epimers is virtually zero (Doerschuk A. *et al.*, 1955; McCormack J. *et al.*, 1957). To avoid possible changes in the pH of the water, which could be due to

tetracycline epimerization, should be paid particular attention to the water distribution systems metal pipes integrity.

- ***Doxycycline hyclate***. In an experimental study conducted in field, Vervaet C. *et al.*, (2003) evaluated the influence that the early addition of citric acid 0.1% (w / v) in water, could have on hydrosolubility of doxycycline hyclate. The water samples both from the mixer and the troughs were conducted at the following points: 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after the addition of drug. The doxycycline concentrations in the water samples were measured with HPLC method. The results obtained showed that the tetracycline concentration in water without citric acid addition was very low (30 mg/L versus a theoretical concentration of 200 mg/L), despite the literature reports as this salt is characterized by a good hydrosolubility. The citric acid addition of 0.1% has increased the doxycycline concentration in water up to a maximum of 125 mg/L. The authors believe that the suboptimal doxycycline concentration, even after the addition of citric acid, is caused by the mixer structure, probably insufficient for very high water volumes, as well as to the dispensing water system because of cohesion of the drug on the tubes walls. This study demonstrated, beyond the drug

hydrosolubility, the critical importance of all water distribution system and how it is always very important to control the drug concentrations at various points of the system itself.

- ***Ceftazidime*.** Although the β-lactam antibiotics are characterized by a high instability in aqueous vehicles, because of the β -lactam ring opening, this cephalosporin is characterized by a good stability, even in a wide range of concentrations, in aqueous solution at temperatures up to 25°C and at pH below 10 (Servais H. and Tulkens M.P., 2001).

- ***Sulfadiazine (sodium) and trimethoprim*.** With a sulfadiazine and trimethoprim powder soluble in water, Vervaet C. *et al*., (2003) evaluated samples of water concentrations of the two antibacterial taken both from the mixer and troughs. The water sampling was conducted at the following points: 0, 0.5, 1, 2, 3, 4, 7 and 9 hours after addition of the two drugs. The concentrations were measured using HPLC method. The results obtained have shown that the sulfonamide is soluted in water immediately after its addition, without requiring particular mixing. The sulfonamide concentration remains constant throughout the water distribution system and

throughout the experimental period. Conversely trimethoprim concentrations appear highly variable and always below the theoretical concentration. In fact, much of the drug remains in suspension because of its poor solubility in the conditions of use (temperature 8°C, pH 8.62). Therefore it would be required an optimal mixing system to ensure the best solubility and the best possible dispersion of trimethoprim throughout the system.

4.3 Stability of active substance in biofilm

Water quality and poor cleanliness will have an influence both on the stability of the medication and on the formation of biofilm. Biofilms are complex communities of microorganisms resistant to antibiotics, coated with an extracellular polymer that helps them to retain food and to protect themselves from toxic agents (Figure 3). The presence of a biofilm in water pipework is frequent due to the deficient application of cleaning methods. Normally, the purer the product (the less excipients, sugar, etc), the lower the production of biofilm, as fewer nutrients are available in the pipework. It may be advisable to perform a sensitivity culture of the microorganisms present in the biofilm using a sample of the product intended for use. An expected result is the maximum halo of growth inhibition, while an unexpected result is the existence of massive microbial growth (no inhibition).

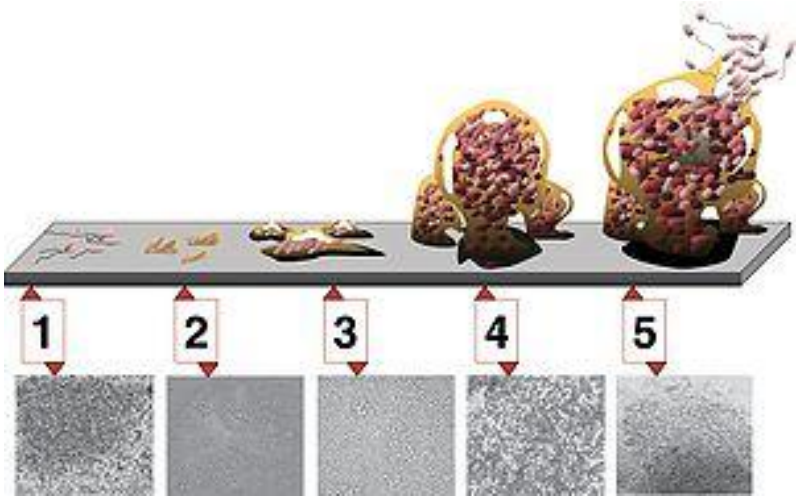


Figure 3. Five stages of biofilm development: (1) Initial attachment, (2) Irreversible attachment, (3) Maturation I, (4) Maturation II, and (5) Dispersion. Each stage of development in the diagram is paired with a photomicrograph of a developing *P. aeruginosa* biofilm. All photomicrographs are shown to same scale.

5. European legislation on veterinary drugs administered with drinking water.

The EMA guideline 540/03 (CVMP) (24), consider that preparations as emulsions, granules, powders, concentrated solutions and suspensions may be administered to animals with drinking water, with regard to the following points:

- possible excipients influence on the pH of the medicated water with consequent changes in solubility or stability of the active ingredient whose oral bioavailability would be altered;
- thinness level of the powders and solubility of all ingredients regardless of the type of water distribution system;
- the water solubility of the active ingredient at different temperatures and at different pH values;
- the solubility of the active ingredient in water when are necessary pre-dilutions;
- in case of powders, the solubilization have to take place in a reasonable time and that does not cause an excessive increase of the solution density;

- for emulsions must be evaluated the product dispersion at different pH values;
- stability to the temperature;
- duration of the product stability in water;
- stability at different types of water (pH, temperature, hardness, salts, metals, etc.) and light.

Therefore, the company that plans to market these pharmaceutical preparations will be required to perform various stability tests in relation to the aspects mentioned above. The results of these tests shall be printed on the label, so that who use the product can have clear indications.

6. Aim of the study

In the food animal production, drinking water is frequently used as a way to carry out the most common pharmacological treatments. In these cases, there are many variables (for instance hardness, temperature, pH of the water and characteristics of mixing and distribution of the drugs) which could degrade drugs dissolved in this mean, even when properly arranged pharmaceutical formulations are used. In fact, although a product obtains a Marketing Authorization through appropriate laboratory studies both drug stability and solubility, on the other hand the solubility of the same drug in natural water used as a drinking water is not documented. This solution ability remains strictly linked to the variables inherent the drinking water characteristics. In the present study has been evaluated the dissolution kinetics (at 0 hours and 24 hours) of some common veterinary drugs used in drinking water samples in order to see how the different physical and chemical factors that characterize the drinking water may affect therapeutic efficacy. In fact, multiple factors, also of little relevance if individually considered, are able to adversely affect the pharmacological treatment carried out in drinking water. These factors combine to modify the active ingredients stability and/or solubility in drinking water, the formulation used and the excipients present as well. Moreover, being quite common the unaware use of expired products by

operators, some expired products (from 1 to 4 years) have been included in the experimental test. In this study were considered products having oxytetracycline and tylosin as active ingredient. Such molecules have pharmacological activity susceptible to degradation and/or reduction in their therapeutic efficacy if particular aspects of the drinking water are not considered or not known.

7. Materials and methods

In the present study were randomly collected 9 drinking water samples in 9 breeding farms distributed in 9 provinces of 5 Italian regions. Furthermore, to these 9 samples, were added other 2 samples: 1 sample of distilled water and 1 sample resulting from the mixing of distilled water with water sampled from the Bologna acqueduct in a ratio 1:1 (Table 7). All 11 water samples were analyzed in order to measure all the zoothecnical potability parameters at the Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna "G. Ubertini" (IZSLER) Chemical Laboratories of Bologna.The technical tests related to the determination of zoothecnical potability parameters are shown in Tables 10, 11 and 12. Following this analysis, the samples 2, 4, 9, 10 and 11 (renamed respectively 1, 2, 3, 4 and 5) were selected as water samples to be analyzed to the solubility test of some veterinary products having as active ingredient oxytetracycline, and tylosin (a macrolide for veterinary purposes only).

Samples	Origin	Farm	Region/Province
01	Acqueduct	Swine	Veneto (TV)
02	Acqueduct	Rabbits	Emilia Romagna (FC)
03	Acqueduct	Poultry	Emilia Romagna (BO)
04	Well	Swine	Puglia (FG)
05	Well	Rabbits	Campania (CE)
06	Well	Swine	Lombardia (BS)
07	Well	Rabbits	Lombardia (MN)
08	Well	Swine	Piemonte (CN)
09	Lake	Poultry	Emilia Romagna (RN)
10	Distilled	///	///
11	Acqueduct:distilled (50:50)	///	Emilia Romagna (BO)

Table 7. Water samples origin.

The 6 products oxytetracycline based were identified respectively A, B, C, D, E, F and showed the characteristics indicated in Table 8.

ID	A. I.	Pharm. form	Expired
OTC-A	OTC 20%	LIQUID	4 YEARS
OTC-B	OTC 20%	LIQUID	2 YEARS
OTC-C	OTC 20%	POWDER	NO
OTC-D	OTC 20%	LIQUID	3 YEARS
OTC-E	OTC 20%	POWDER	NO
OTC-F	OTC 20%	LIQUID	1 YEAR

Table 8. Characteristics of based-oxytetracycline products.

The 6 products based on tylosin were identified respectively A, B, C, D, E, F and showed the characteristics indicated in Table 9.

ID	A. I.	Pharm. form	Expired
TYL-A	TYL 100%	POWDER	NO
TYL-B	TYL 100%	POWDER	NO
TYL-C	TYL 20%	LIQUID	1 YEAR
TYL-D	TYL 20%	LIQUID	1 YEAR
TYL-E	TYL 20%	LIQUID	NO
TYL-F	TYL 20%	LIQUID	NO

Table 9. Characteristics of based-tylosin products**.**

In the case of products based on 20% of oxytetracycline, assuming that the expected dosage for avian species is 50 mg of A.I. per kg of body weight and calculating that for a broiler weighting about 3 Kg the water consumption is about 100ml/Kg of B.W./day, were dissolved 50 mg of A.I. (corresponding to 250 mg of product) of the C product in 5 Becker (identified from C1 to C5) containing 100 ml of each water sample selected. Simultaneously, the same method was followed for the other 5 products remaining (A, B, D, E and F).

Each Becker was then stirred manually until complete dissolution. Then, from each Becker 1 mL of solution was taken and repeatedly diluted until to obtain a concentration of 100 ng/ml. Then, the tubes containing the obtained solutions were capped and vortexed for about 1 minute. From each tube 2 mL of solution were collected and placed in vials for the LC-MS/MS detection.

The standard was dissolved in methanol and then diluted in distilled water until obtaining a 100 ng/ml concentration following the same process used for products samples. After 24 hours, 1 mL of solution was collected from each Becker and repeatedly diluted until to obtain a concentration of 100 ng/ml.

Finally, the tubes containing the obtained solutions were capped and vortexed for about 1 minute. From each tube 2 mL of solution were collected and placed in vials for the LC-MS/MS detection.

Similarly, for products based on 20% of tylosin, assuming that the expected dosage for avian species is 25 mg of A.I. per kg of body weight and calculating that for a broiler weighting about 3 Kg the water consumption is about 100ml/Kg of B.W./day, were diluted 25 mg of A.I. (corresponding to 125 mg of product) mg of the product C in 5 Becker (identified from C1 to C5) containing 100 ml of each water sample selected. Simultaneously, the same method was followed for the other 5 products (D, E and F). About the remaining products A and B, based on 100% of tylosin, assuming that the expected dosage for avian species is 25 mg of A.I. per kg of body weight and calculating that for a broiler weighting about 3 Kg the water consumption is about 100ml/Kg of B.W./day, were diluted 25 mg of A.I. (corresponding to 25 mg of product) mg of the product A in 5 Becker (identified from A1 to A5) containing 100 ml of each water sample selected. Simultaneously, the same method was followed for the other product remaining (B).

Each Becker was then stirred manually until complete dissolution. Then, from each Becker 1 mL of solution was taken and repeatedly diluted until to obtain a concentration of 100 ng/ml. Then, the tubes containing the

obtained solutions were capped and vortexed for about 1 minute. From each tube 2 mL of solution were collected and placed in vials for the LC-MS/MS detection.

The standard was dissolved in methanol and then diluted in distilled water until obtaining a 100 ng/ml concentration following the same process used for products samples. After 24 hours, 1 mL of solution was collected from each Becker and repeatedly diluted until to obtain a concentration of 100 ng/ml.

Finally, the tubes containing the obtained solutions were capped and vortexed for about 1 minute. From each tube 2 mL of solution were collected and placed in vials for the LC-MS/MS detection.

All the samples derived by the based-oxytetracycline and based-tylosin products solubilization in 5 samples of water selected after the determination of the zootechnical potability parameters, were analyzed in a Waters Alliance 2795 HPLC system (Milford MA USA) with Synergy Polar column (2.0mmx150mm; 4 micron) (Phenomenex USA), coupled to a mass spectrometer Four Last Platinum (Micromass Manchester UK).

8. Results

In determining the zootechnical potability parameters relevant differences were not found between the nine water samples taken from different farms but consequently this analysis, the samples 2, 4, 9, 10 and 11 (renamed respectively 1, 2, 3, 4 and 5) were selected and they were used to carry out the solubility test of the products. The water samples characteristics are described in tables 10, 11 and 12.

DRINKING WATER SAMPLES				
TEST	**1**	**2**	**3**	**4**
pH	7,7	7,9	7,3	7,9
Chlorine residual free	<0,01 mg/l	<0,01 mg/l	<0,01 mg/l	<0,01 mg/l
Oxydability	<0,02 mg/l di O_2	0,48 mg/l di O_2	<0,02 mg/l di O_2	0,24 mg/l di O_2
Hardness	18 FD	21,5 FD	17,5 FD	14,5 FD
Total dissolved Solids at 180° C	160 mg/l	221 mg/l	387 mg/l	150 mg/l
Nitrites	<0,05 mg/l	<0,05 mg/l	<0,05 mg/l	<0,05 mg/l
Nitrates	3 mg/l	4,5 mg/l	4,6 mg/l	3,2 mg/l
Colour	0 Hazen	0 Hazen	0 Hazen	0 Hazen
Conductivity	385 µS/cm	444 µS/cm	596 µS/cm	371 µS/cm
Chlorides	3,2 mg/l	16,9 mg/l	19,2 mg/l	6,1 mg/l
Sulphites	31,8 mg/l	22,2 mg/l	56,5 mg/l	2,9 mg/l
Ammonia	<0,1 mg/l	<0,1 mg/l	<0,1 mg/l	<0,1 mg/l
Phosphorus	<70 µg P_2O_5/l	<70 µg P_2O_5/l	<70 µg P_2O_5/l	<70 µg P_2O_5/l
Iron	<0,01 mg/l	<0,01 mg/l	<0,01 mg/l	<0,01 mg/l
Calcium	83,6 mg/l	86,1 mg/l	136,4 mg/l	80,4 mg/l

Table 10. Zootchenical potability parameters analysis results for samples 1-4.
In red, results of samples 2 and 4 selected for the dissolution test. FD: French degrees.

DRINKING WATER SAMPLES				
TEST	**5**	**6**	**7**	**8**
pH	7,2	8,0	7,9	7,1
Chlorine residual free	<0,01 mg/l	<0,01 mg/l	<0,01 mg/l	<0,01 mg/l
Oxydability	<0,02 mg/l di O_2	1,36 mg/l di O_2	0,28 mg/l di O_2	<0,02 mg/l di O_2
Hardness	16,5 FD	23 FD	22 FD	20,5 FD
Total dissolved Solids at 180° C	220 mg/l	174 mg/l	211 mg/l	406 mg/l
Nitrites	<0,05 mg/l	0,12 mg/l	<0,05 mg/l	<0,05 mg/l
Nitrates	5,2 mg/l	<1 mg/l	1,3 mg/l	30,9 mg/l
Colour	0 Hazen	20 Hazen	0 Hazen	0 Hazen
Conductivity	409 µS/cm	392 µS/cm	427 µS/cm	589 µS/cm
Chlorides	14,8 mg/l	2 mg/l	2,4 mg/l	7 mg/l
Sulphites	1,5 mg/l	<1 mg/l	<1 mg/l	43,7 mg/l
Ammonia	<0,1 mg/l	2 mg/l	0,1 mg/l	<0,1 mg/l
Phosphorus	<70 µg P_2O_5/l	<70 µg P_2O_5/l	<70 µg P_2O_5/l	<70 µg P_2O_5/l
Iron	<0,01 mg/l	0,5 mg/l	0,28 mg/l	<0,01 mg/l
Calcium	68,1 mg/l	88,4 mg/l	103 mg/l	154,4 mg/l

Table 11. Zootchenical potability parameters analysis results for samples 5-8. FD: French degrees.

DRINKING WATER SAMPLES			
TEST	**9**	**10**	**11**
pH	8,4	7,2	7,3
Chlorine residual free	<0,01 mg/l	<0,01 mg/l	<0,01 mg/l
Oxydability	4,88 mg/l di O_2	<0,02 mg/l di O_2	<0,02 mg/l di O_2
Hardness	30 FD	<0,5 FD	20 FD
Total dissolved Solids at 180° C	649 mg/l	0 mg/l	166 mg/l
Nitrites	0,06 mg/l	<0,05 mg/l	<0,05 mg/l
Nitrates	3,5 mg/l	<1 mg/l	2,7 mg/l
Colour	20 Hazen	0 Hazen	0 Hazen
Conductivity	768 µS/cm	5,69 µS/cm	370 µS/cm
Chlorides	37,9 mg/l	1,5 mg/l	10,5 mg/l
Sulphites	180,5 mg/l	<1 mg/l	28,9 mg/l
Ammonia	0,2 mg/l	<0,1 mg/l	<0,1 mg/l
Phosphorus	<70 µg P_2O_5/l	<70 µg P_2O_5/l	<70 µg P_2O_5/l
Iron	<0,03 mg/l	<0,01 mg/l	<0,01 mg/l
Calcium	81,4 mg/l	<0,1 mg/l	69,7 mg/l

Table 12. Zootchenical potability parameters analysis results for samples 9-11. In red, results of samples 9, 10 and 11 selected for the dissolution test. FD: French degrees.

The oxytetracycline concentrations obtained both at 0 h and after 24 h are described in tables 13-14 and in graphics 1-12.

	1	**2**	**3**	**4**	**5**
	pH 7.96 FD 21.5	**pH 7.94 FD 14.5**	**pH 8.40 FD 30.0**	**pH 7.22 FD <0.5**	**pH 7.31 FD 20.0**
OTC A	35	37	35	36	23
OTC B	66	64	61	53	61
OTC C	135	145	139	142	132
OTC D	30	41	40	27	14
OTC E	124	127	80	118	83
OTC F	88	55	87	84	77

Table 13. Oxytetracycline concentrations in ng/ml at 0h. FD: French degrees.

	1	**2**	**3**	**4**	**5**
	pH 7.96 FD 21.5	**pH 7.94 FD 14.5**	**pH 8.40 FD 30.0**	**pH 7.22 FD <0.5**	**pH 7.31 FD 20.0**
OTC A	7	6	6	20	9
OTC B	16	8	11	16	9
OTC C	40	84	89	37	95
OTC D	6	9	8	15	11
OTC E	53	88	51	49	80
OTC F	33	49	33	23	32

Table 14. Oxytetracycline concentrations in ng/ml at 24h. FD: French degrees.

The solubility differences of based-oxytetraycline products registered at 0h and 24h, expressed as percentage changes of solubility at 0h and 24h (product concentration at 24h/product concentration at 0h * 100), are described both in table 15 and in graphics 25-30.

	1	**2**	**3**	**4**	**5**
	pH 7.96 **FD 21.5**	**pH 7.94** **FD 14.5**	**pH 8.40** **FD 30.0**	**pH 7.22** **FD <0.5**	**pH 7.31** **FD 20.0**
OTC A	20	16	17	56	39
OTC B	24	13	18	30	15
OTC C	30	58	64	26	72
OTC D	20	22	20	56	79
OTC E	43	69	64	42	96
OTC F	38	89	38	27	42

Table 15. Solubility differences of based-oxytetraycline products registered at 0h and 24h (expressed in percentage). FD: French degrees.

The tylosin concentrations obtained both at 0 h and after 24 h are described in tables 16-17 and in graphics 13-24.

	1	2	3	4	5
	pH 7.96 **FD 21.5**	**pH 7.94** **FD 14.5**	**pH 8.40** **FD 30.0**	**pH 7.22** **FD <0.5**	**pH 7.31** **FD 20.0**
TYL A	71	70	73	67	75
TYL B	65	51	60	68	59
TYL C	36	22	18	21	33
TYL D	19	18	23	34	20
TYL E	16	22	38	22	19
TYL F	21	34	19	18	22

Table 16. Tylosin concentrations in ng/ml at 0h. FD: French degrees.

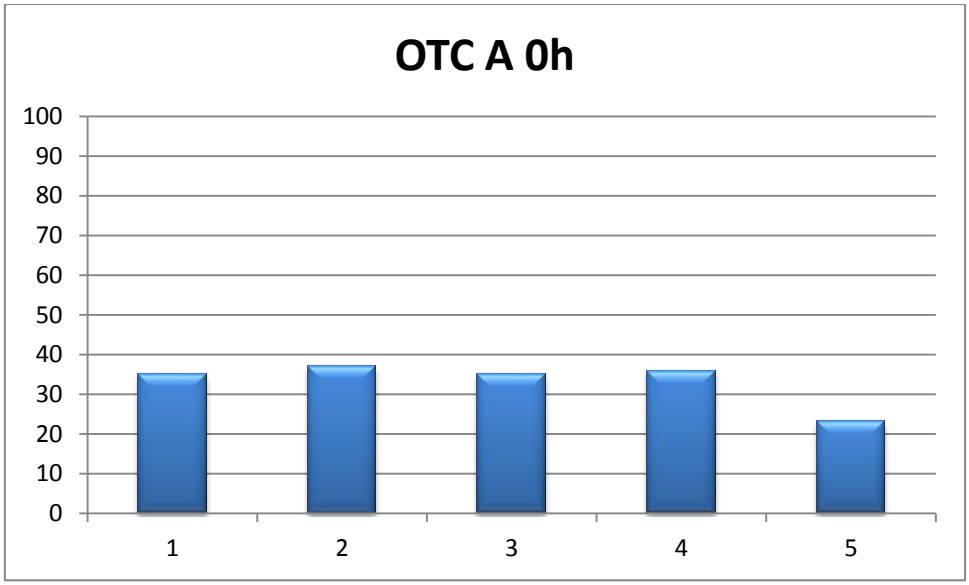
	1	2	3	4	5
	pH 7.96 **FD 21.5**	**pH 7.94** **FD 14.5**	**pH 8.40** **FD 30.0**	**pH 7.22** **FD <0.5**	**pH 7.31** **FD 20.0**
TYL A	59	56	53	53	65
TYL B	51	42	48	60	44
TYL C	40	37	43	39	38
TYL D	32	25	24	26	25
TYL E	20	24	20	25	22
TYL F	36	28	23	24	25

Table 17. Tylosin concentrations in ng/ml at 24h. FD: French degrees.

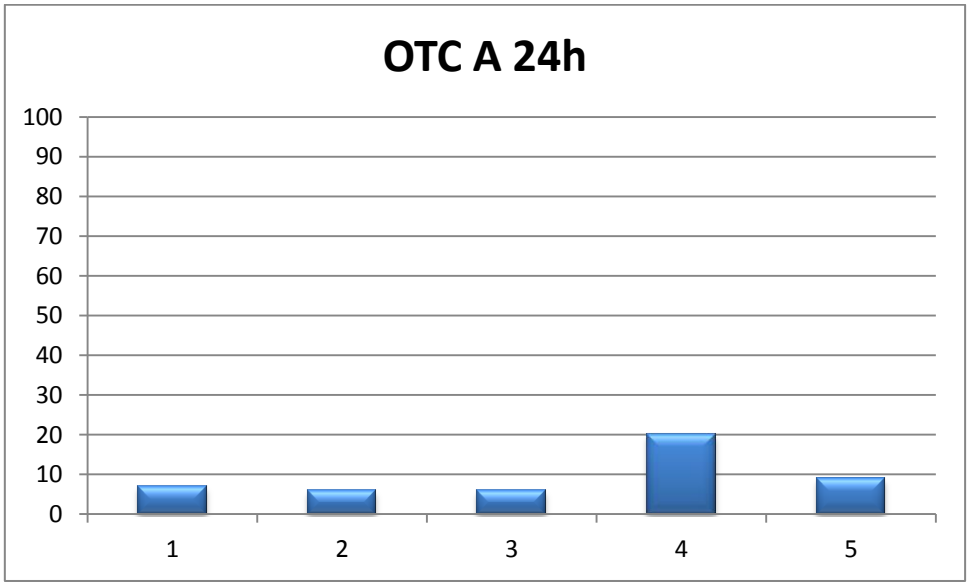
The solubility differences of based-tylosin products registered at 0h and 24h, expressed as percentage changes of solubility at 0h and 24h (product concentration at 24h/product concentration at 0h * 100), are described both in table 18 and in graphics 31-36.

	1	**2**	**3**	**4**	**5**
	pH 7.96 **FD 21.5**	**pH 7.94** **FD 14.5**	**pH 8.40** **FD 30.0**	**pH 7.22** **FD <0.5**	**pH 7.31** **FD 20.0**
TYL A	83	80	73	79	87
TYL B	78	82	80	88	75
TYL C	111	168	239	186	115
TYL D	168	139	104	76	125
TYL E	125	109	53	114	116
TYL F	171	82	121	133	114

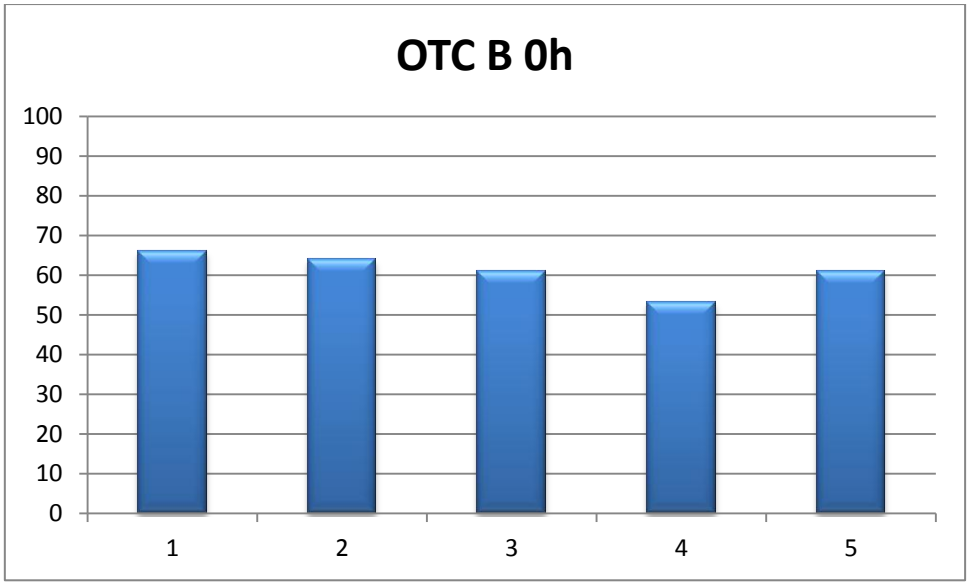
Table 18. Solubility differences of based-tylosin products registered at 0h and 24h (expressed in percentage). FD: French degrees.



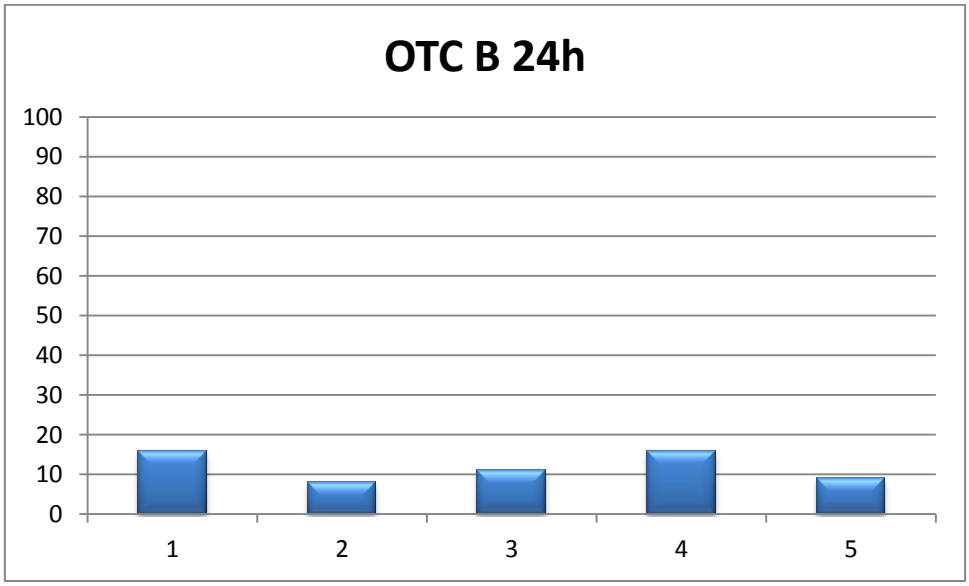
Graphic 1. OTC A concentrations at 0h (ng/ml).



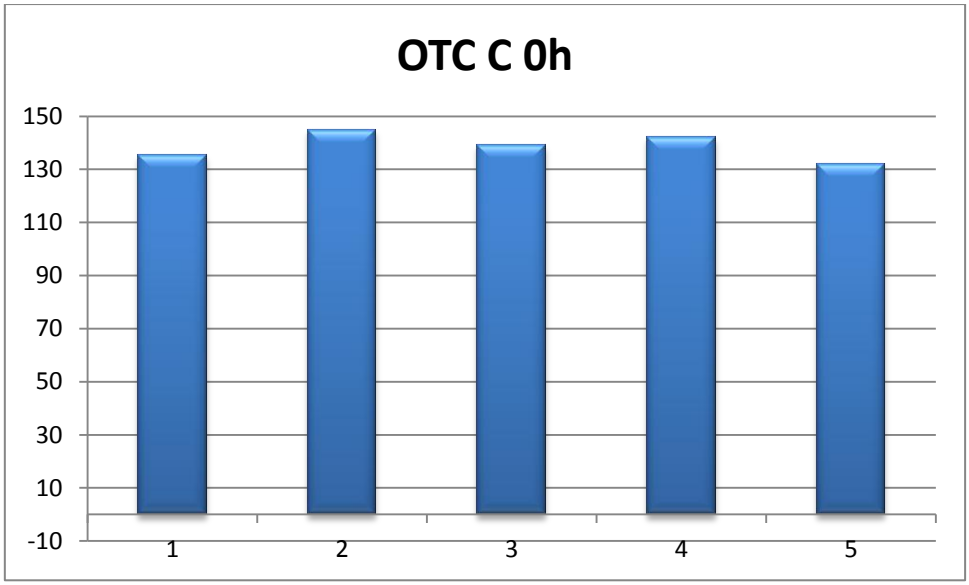
Graphic 2. OTC A concentrations at 24h (ng/ml).



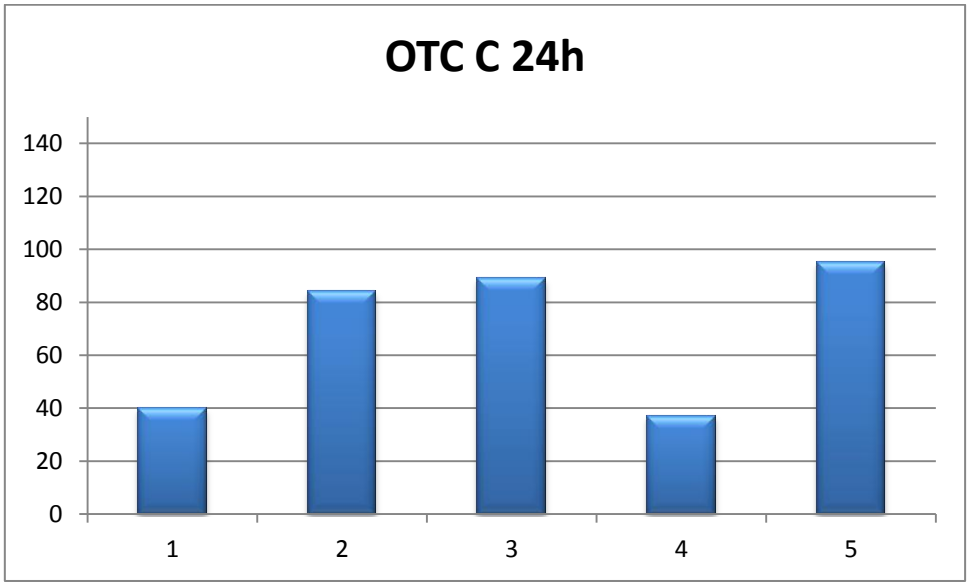
Graphic 3. OTC B concentrations at 0h (ng/ml).



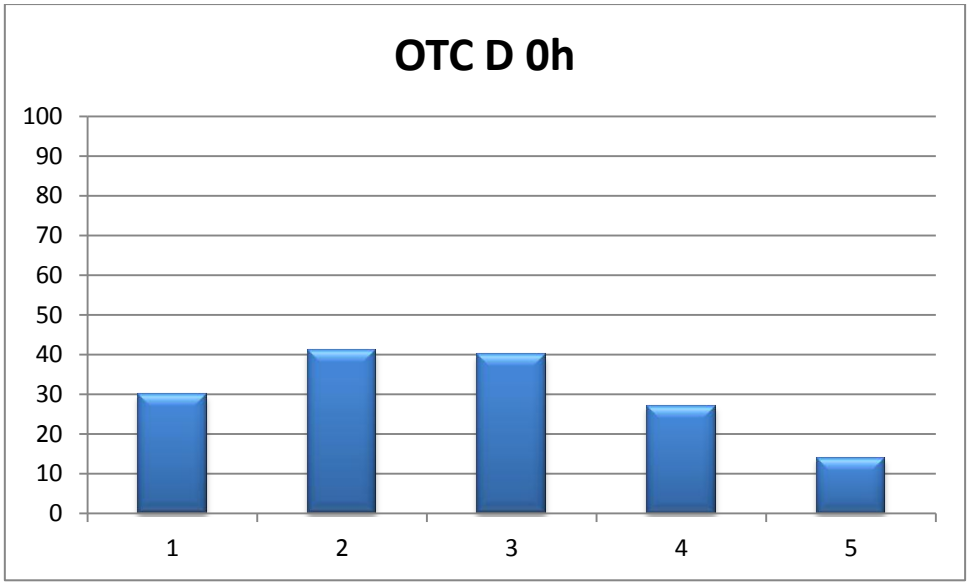
Graphic 4. OTC B concentrations at 24h (ng/ml).



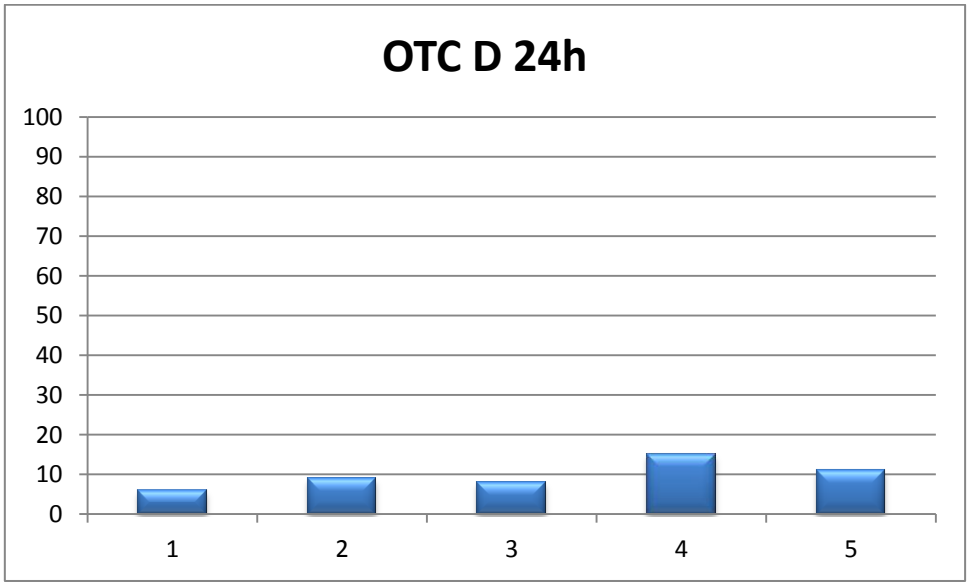
Graphic 5. OTC C concentrations at 0h (ng/ml).



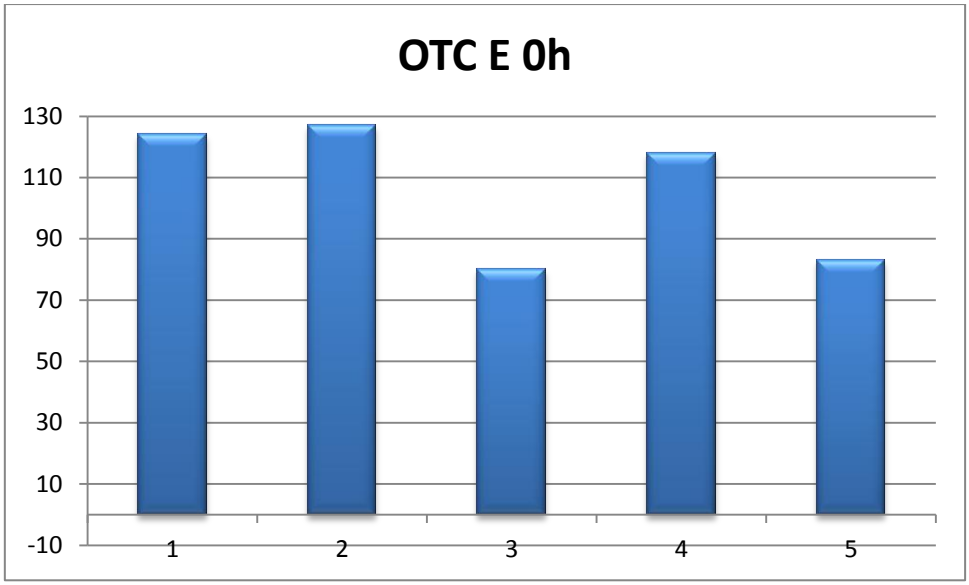
Graphic 6. OTC C concentrations at 24h (ng/ml).



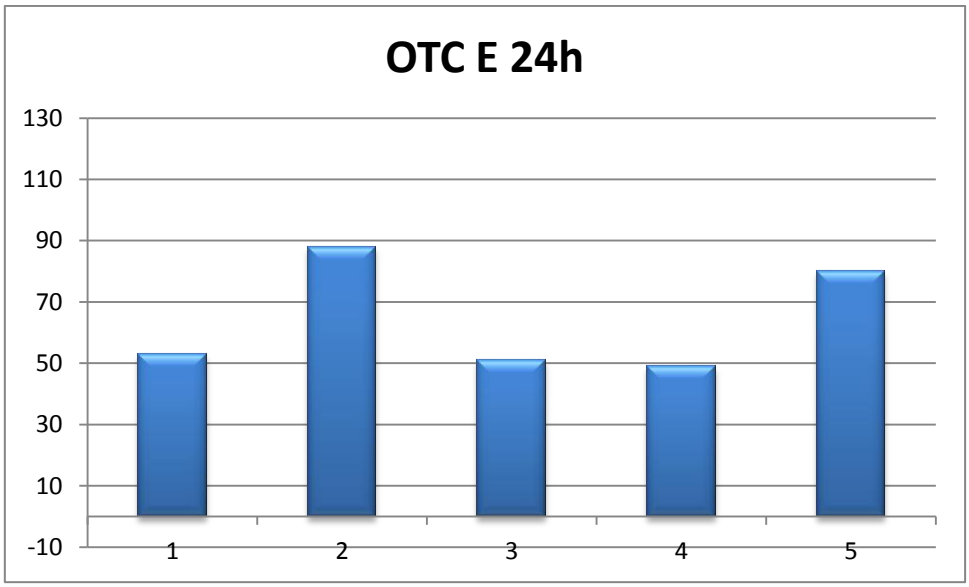
Graphic 7. OTC D concentrations at 0h (ng/ml).



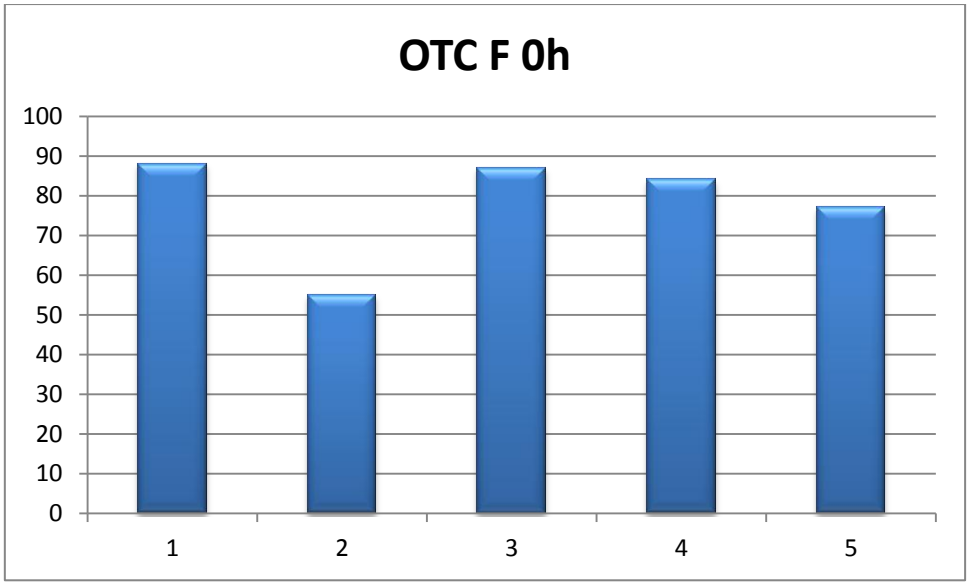
Graphic 8. OTC D concentrations at 24h (ng/ml).



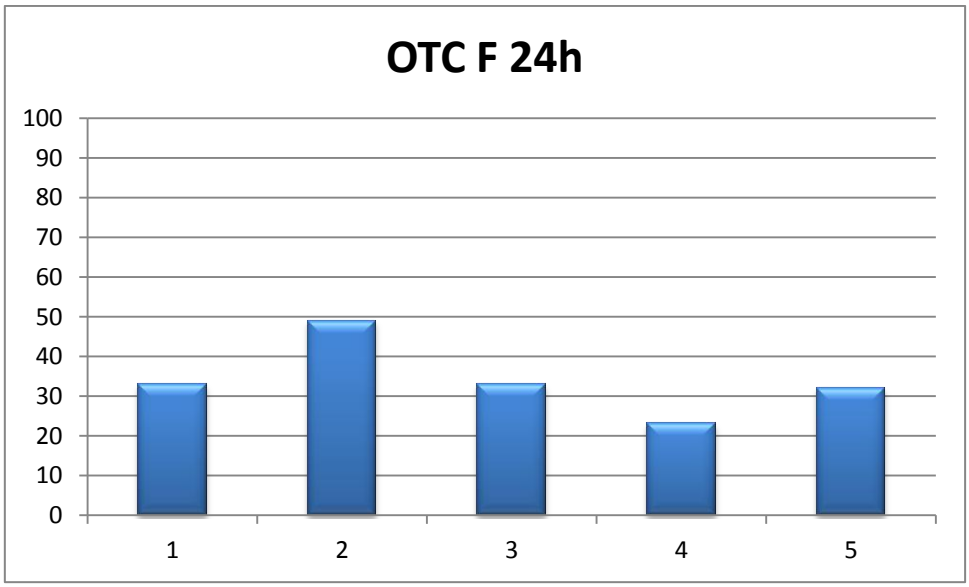
Graphic 9. OTC E concentrations at 0h (ng/ml).



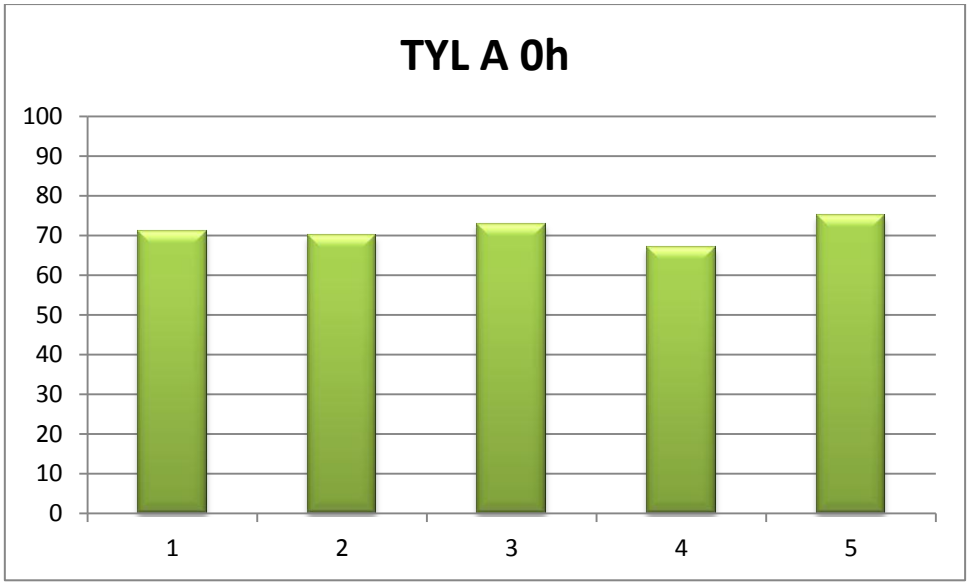
Graphic 10. OTC E concentrations at 24h (ng/ml).



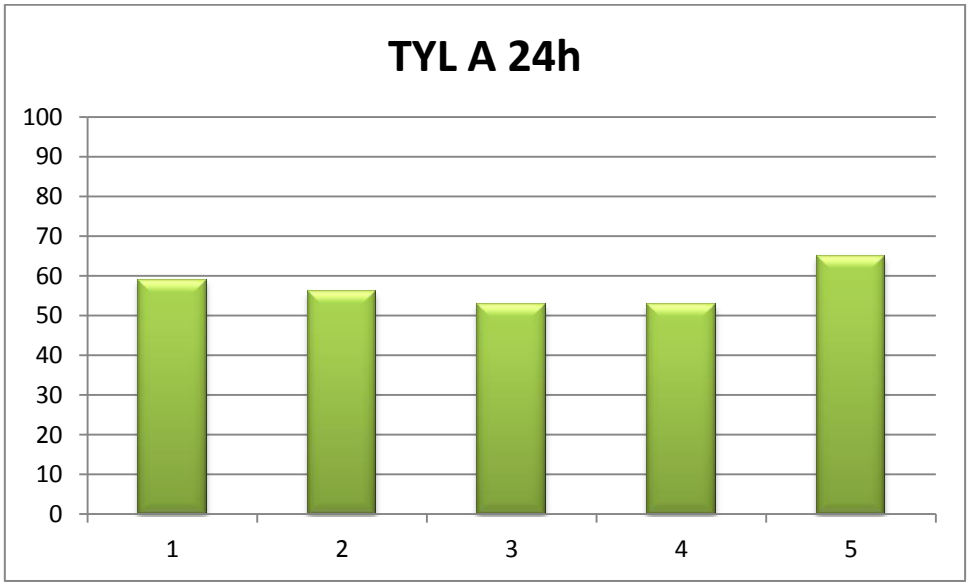
Graphic 11. OTC F concentrations at 0h (ng/ml).



Graphic 12. OTC F concentrations at 24h (ng/ml).



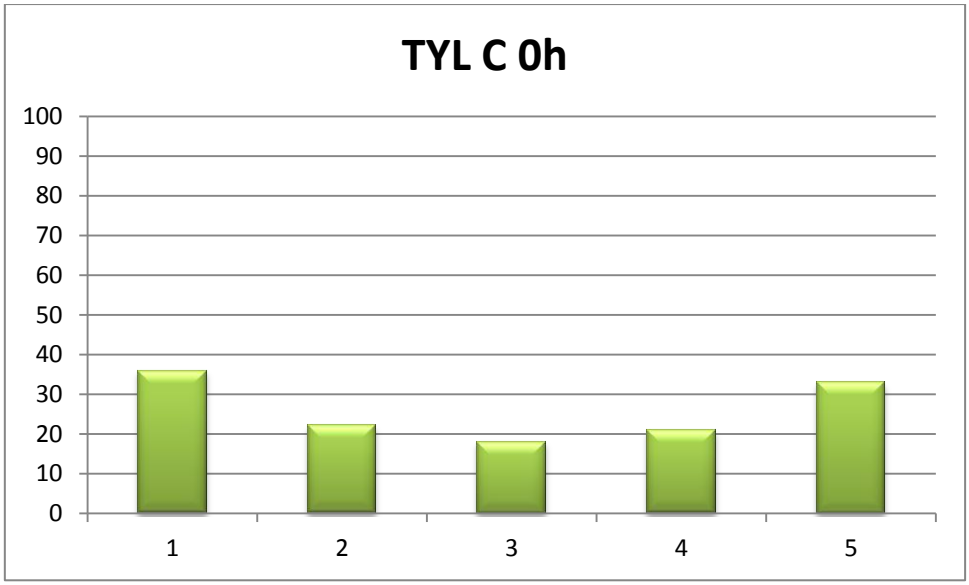
Graphic 13. TYL A concentrations at 0h (ng/ml).



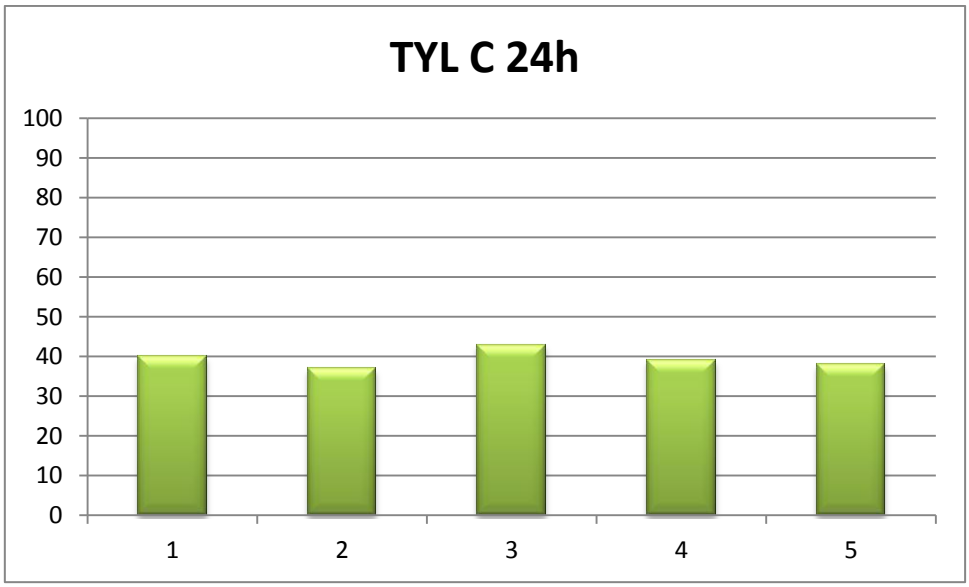
Graphic 14. TYL A concentrations at 24h (ng/ml).

Graphic 15. TYL B concentrations at 0h (ng/ml).

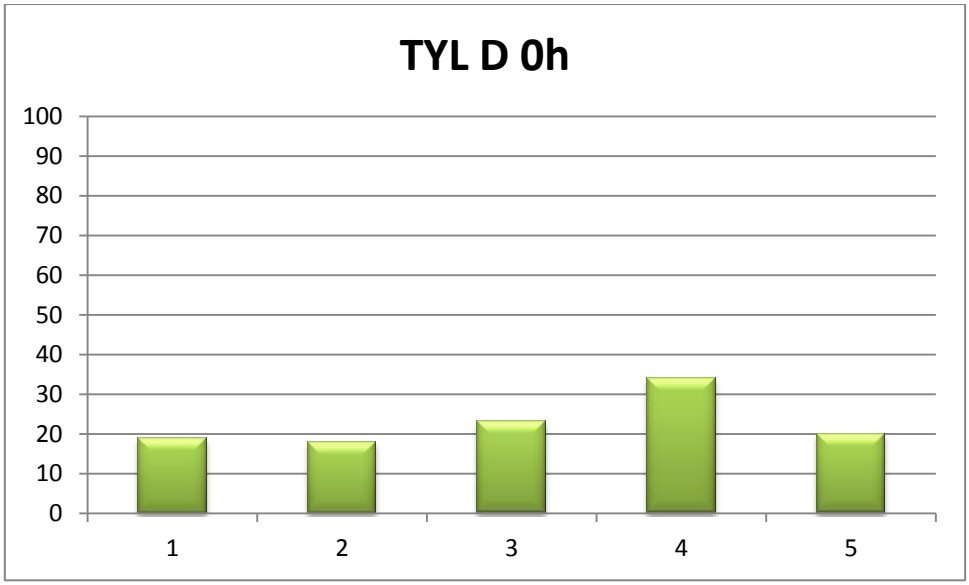
Graphic 16. TYL B concentrations at 24h (ng/ml).



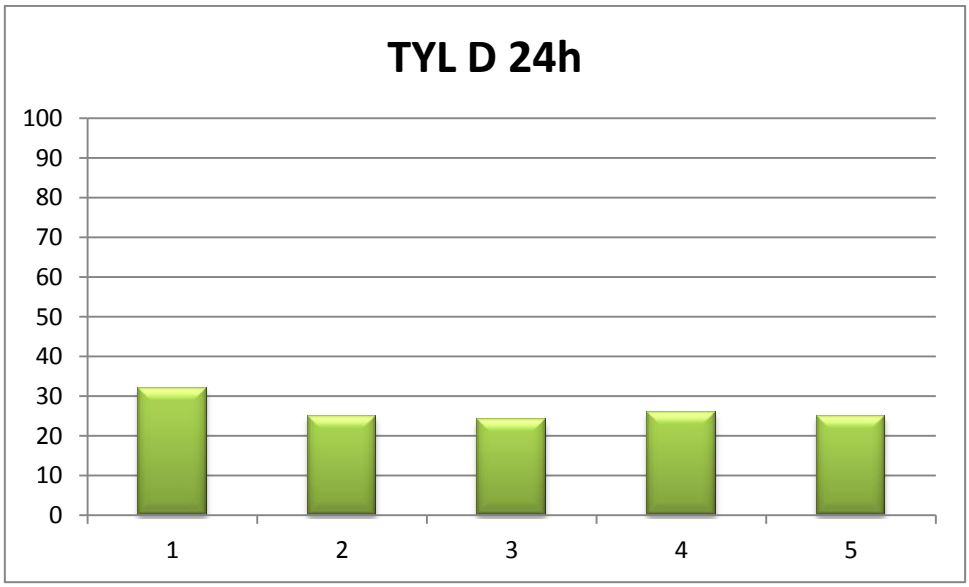
Graphic 17. TYL C concentrations at 0h (ng/ml).



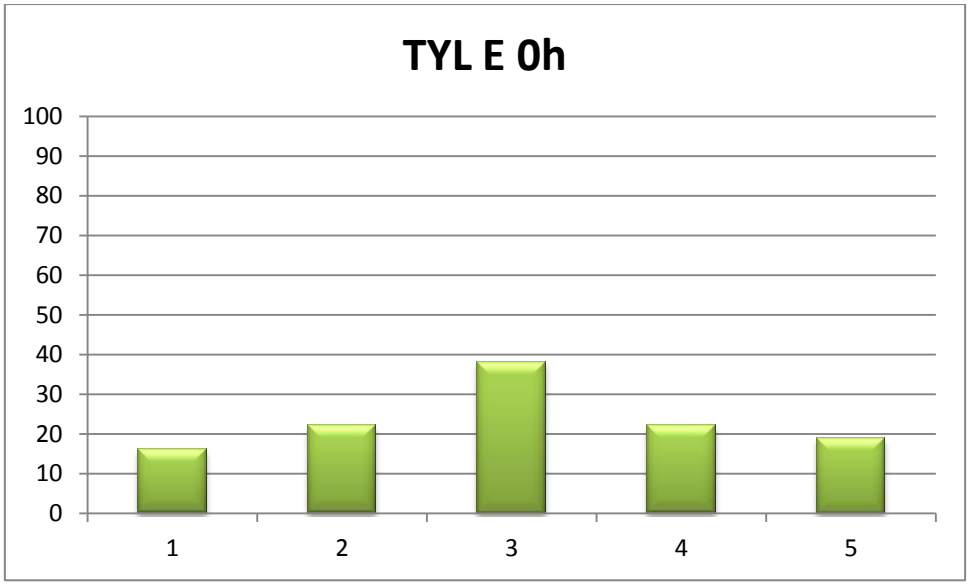
Graphic 18. TYL C concentrations at 24h (ng/ml).



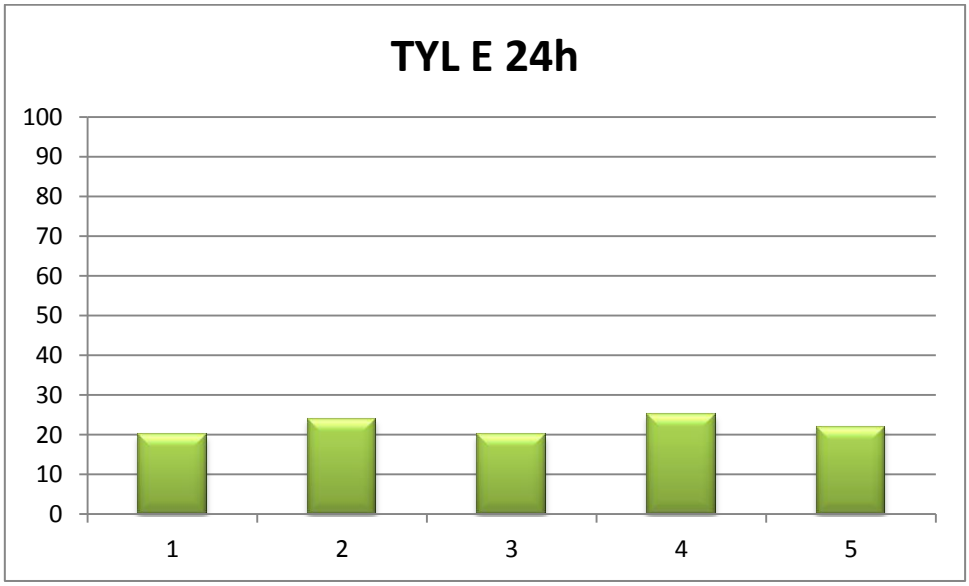
Graphic 19. TYL D concentrations at 0h (ng/ml).



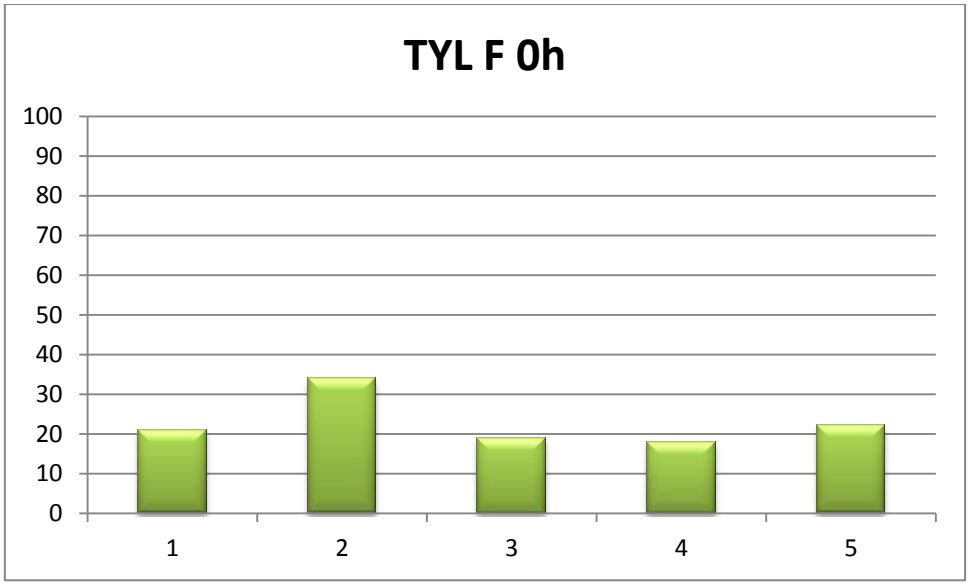
Graphic 20. TYL D concentrations at 24h (ng/ml).



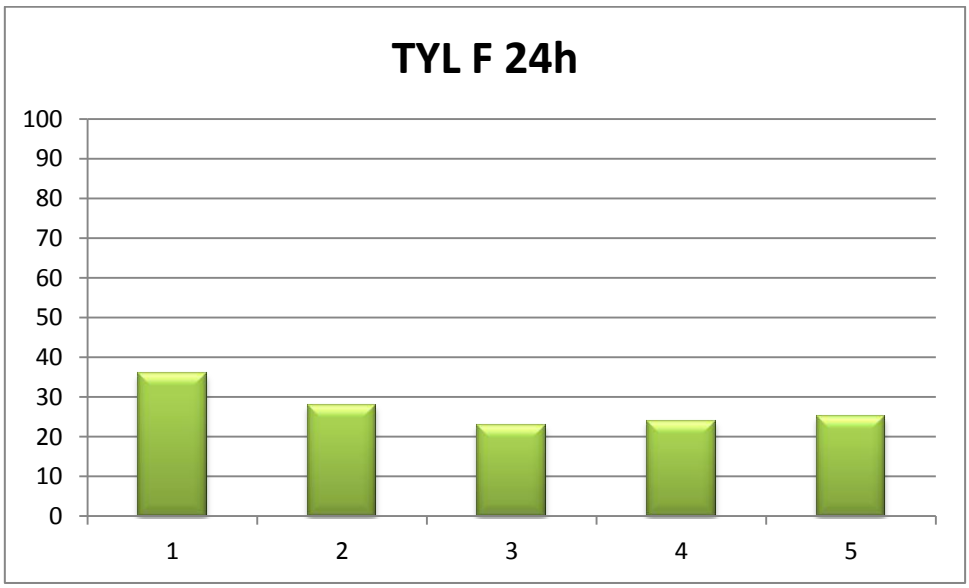
Graphic 21. TYL E concentrations at 0h (ng/ml).



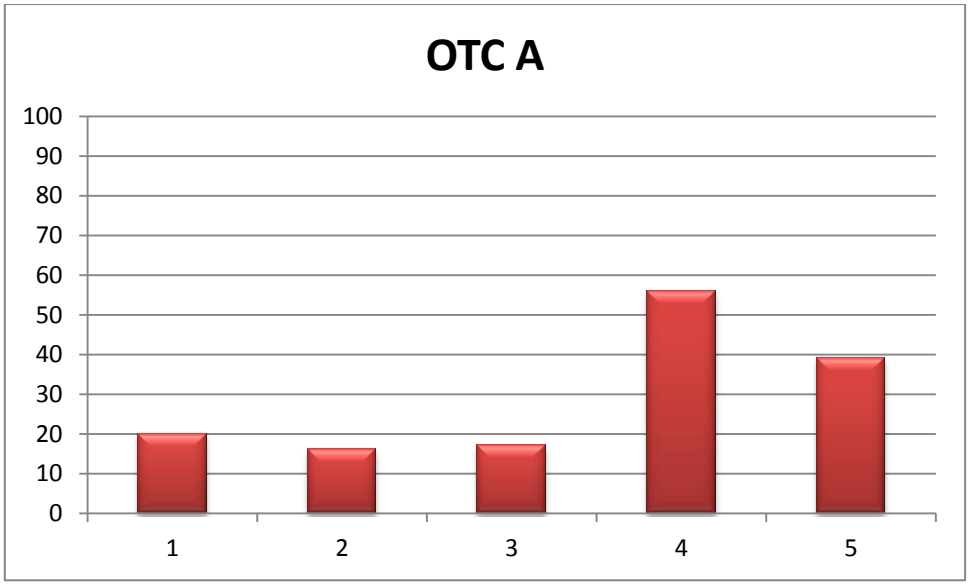
Graphic 22. TYL E concentrations at 24h (ng/ml).



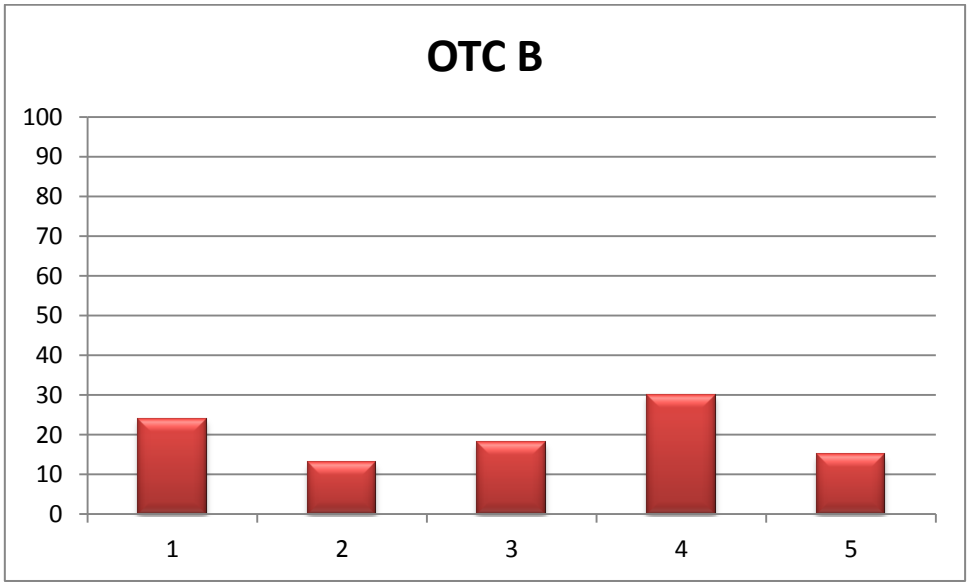
Graphic 23. TYL F concentrations at 0h (ng/ml).



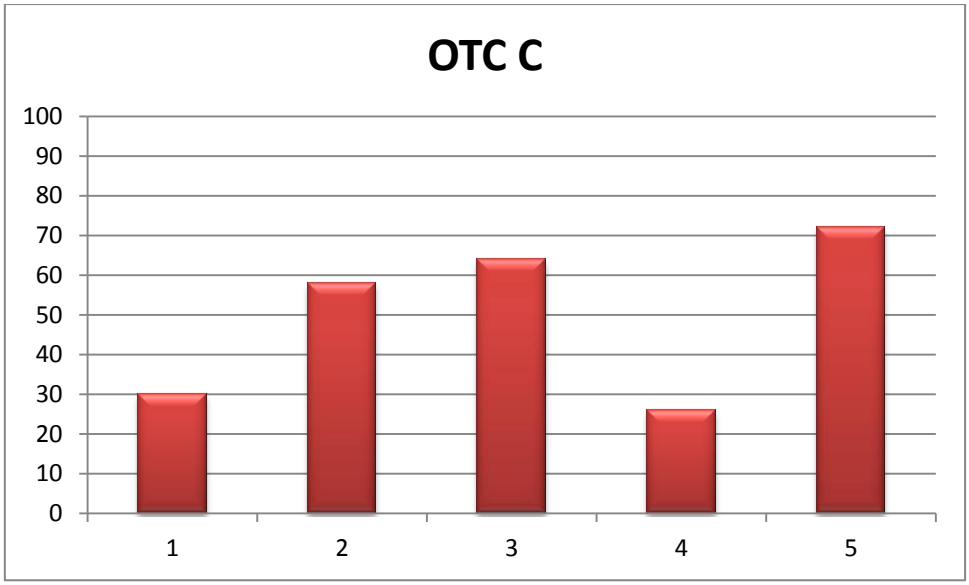
Graphic 24. TYL F concentrations at 24h (ng/ml).



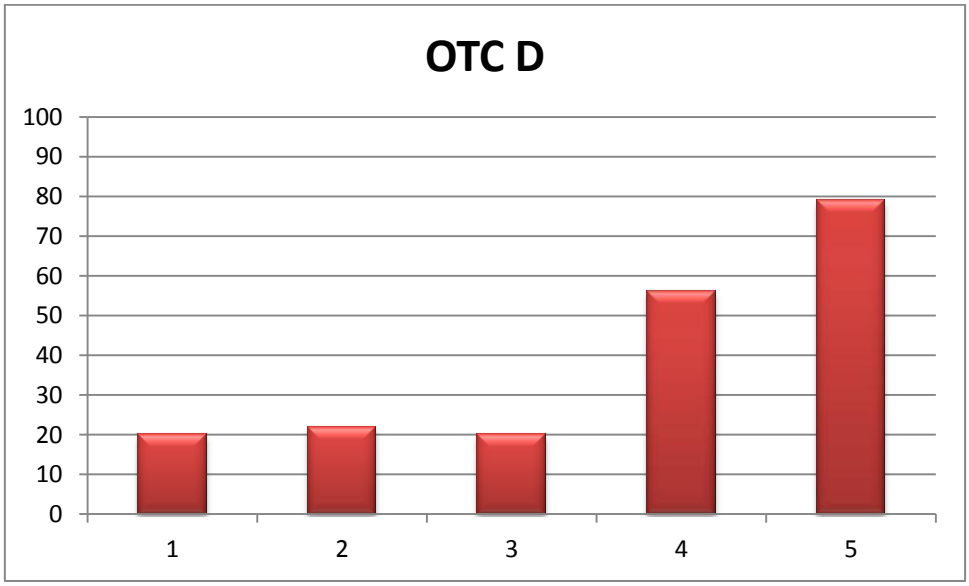
Graphic 25. OTC A product solubility differences at 0h/24h (in percentage).



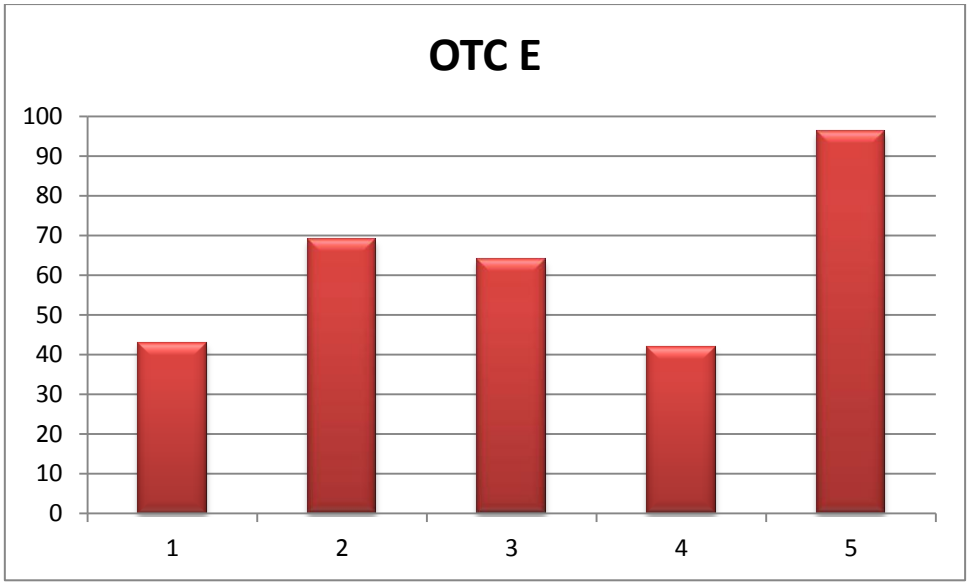
Graphic 26. OTC B product solubility differences at 0h/24h (in percentage).



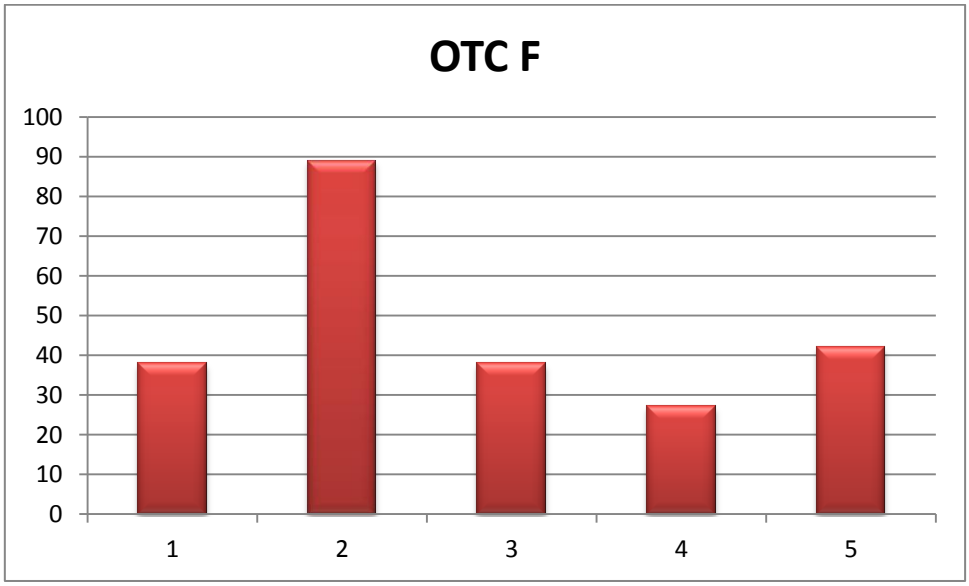
Graphic 27. OTC C product solubility differences at 0h/24h (in percentage).



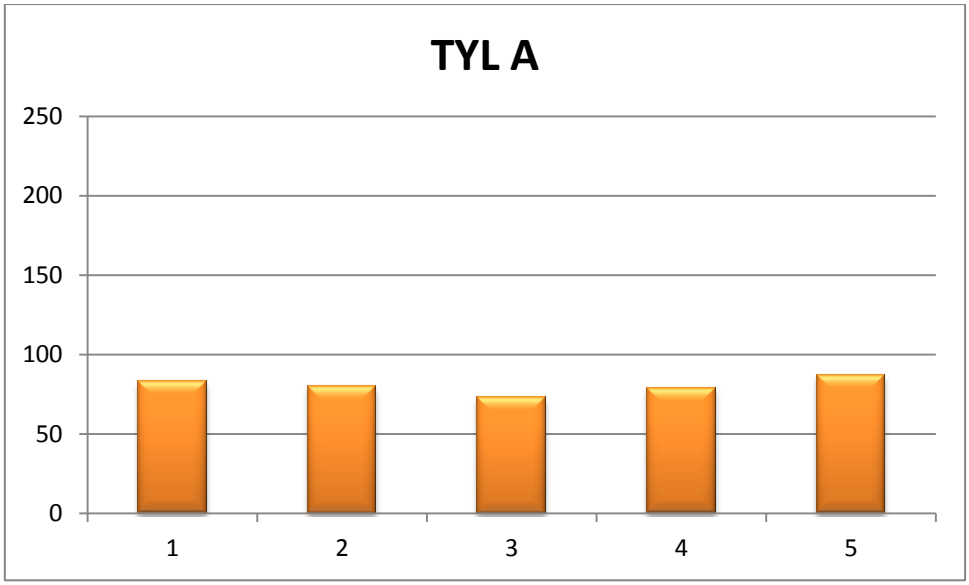
Graphic 28. OTC D product solubility differences at 0h/24h (in percentage).



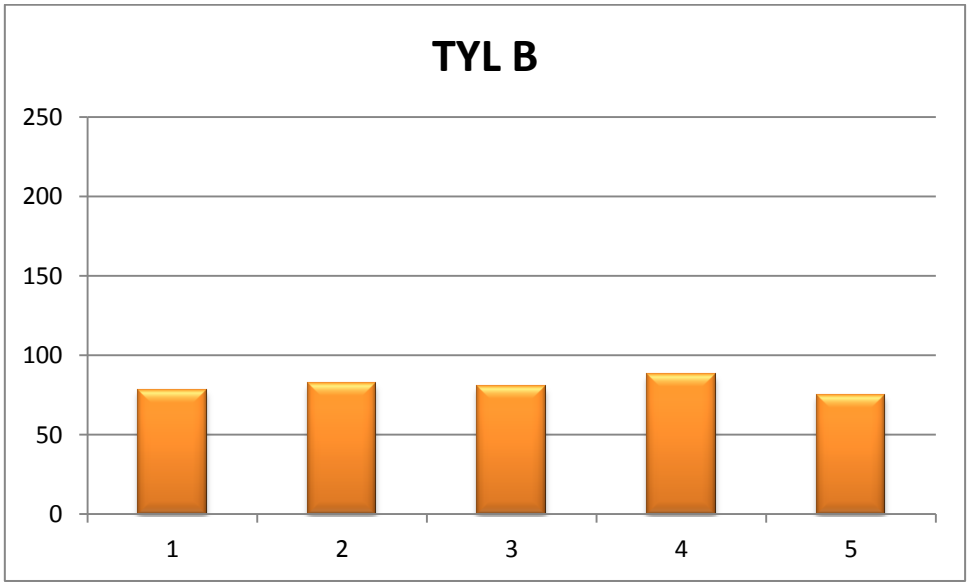
Graphic 29. OTC E product solubility differences at 0h/24h (in percentage).



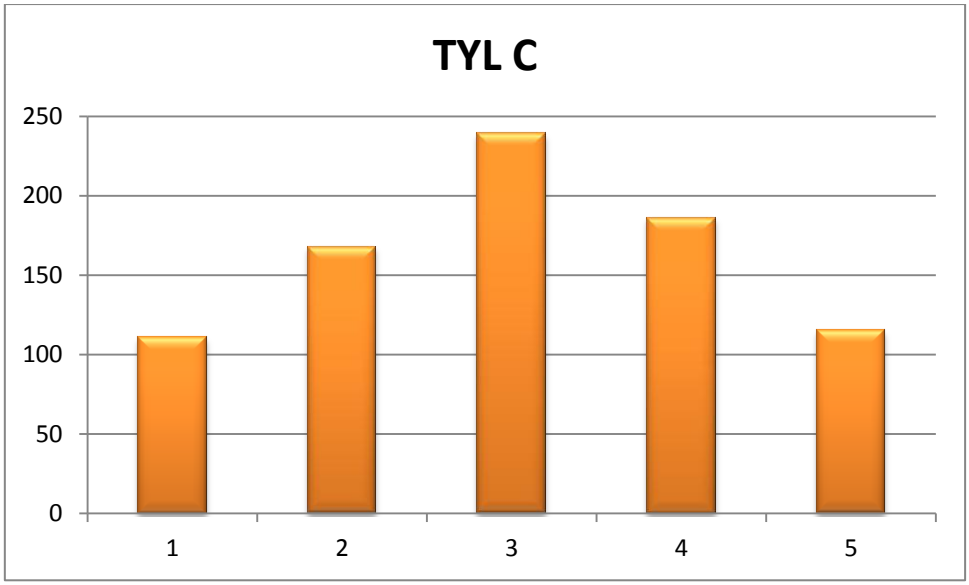
Graphic 30. OTC F product solubility differences at 0h/24h (in percentage).



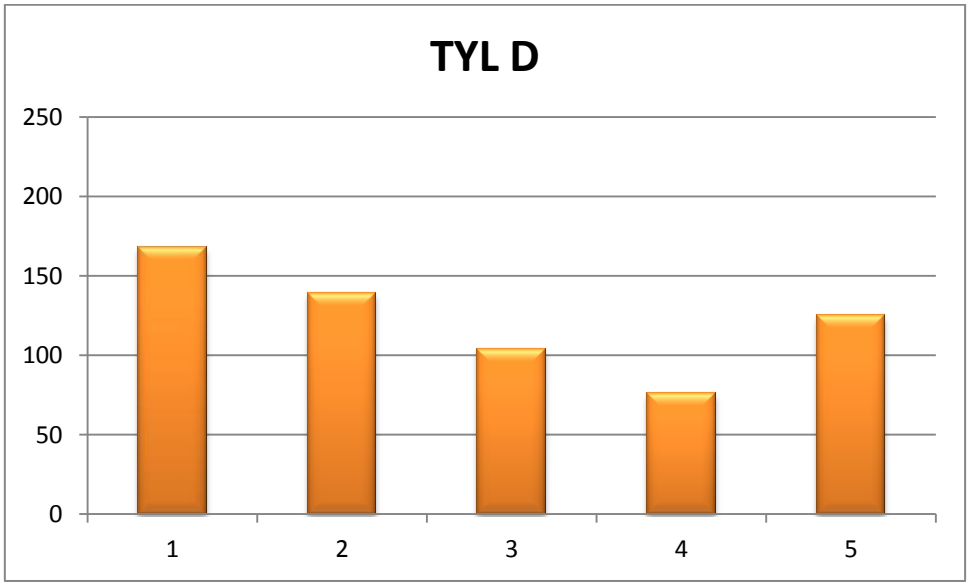
Graphic 31. TYL A product solubility differences at 0h/24h (in percentage).



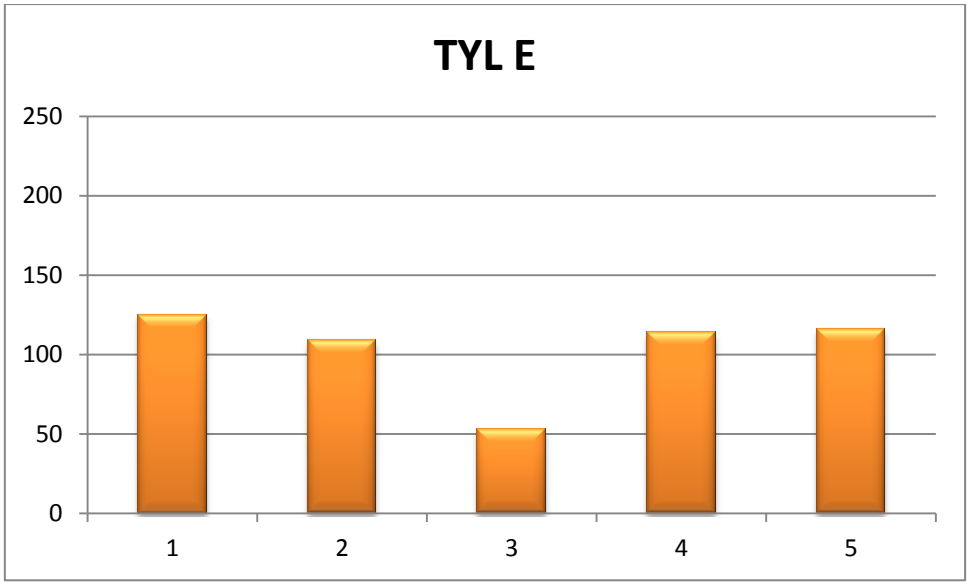
Graphic 32. TYL B product solubility differences at 0h/24h (in percentage).



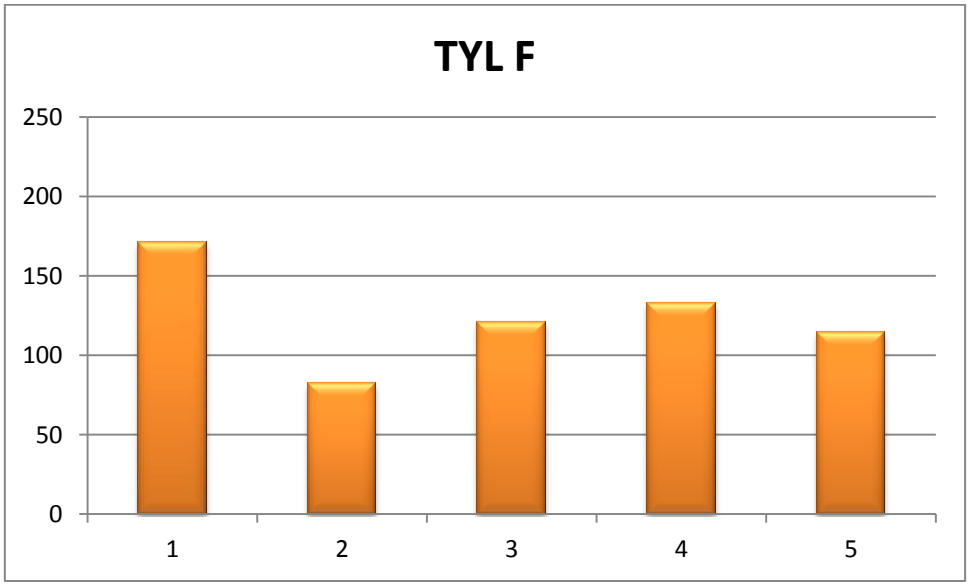
Graphic 33. TYL C product solubility differences at 0h/24h (in percentage).



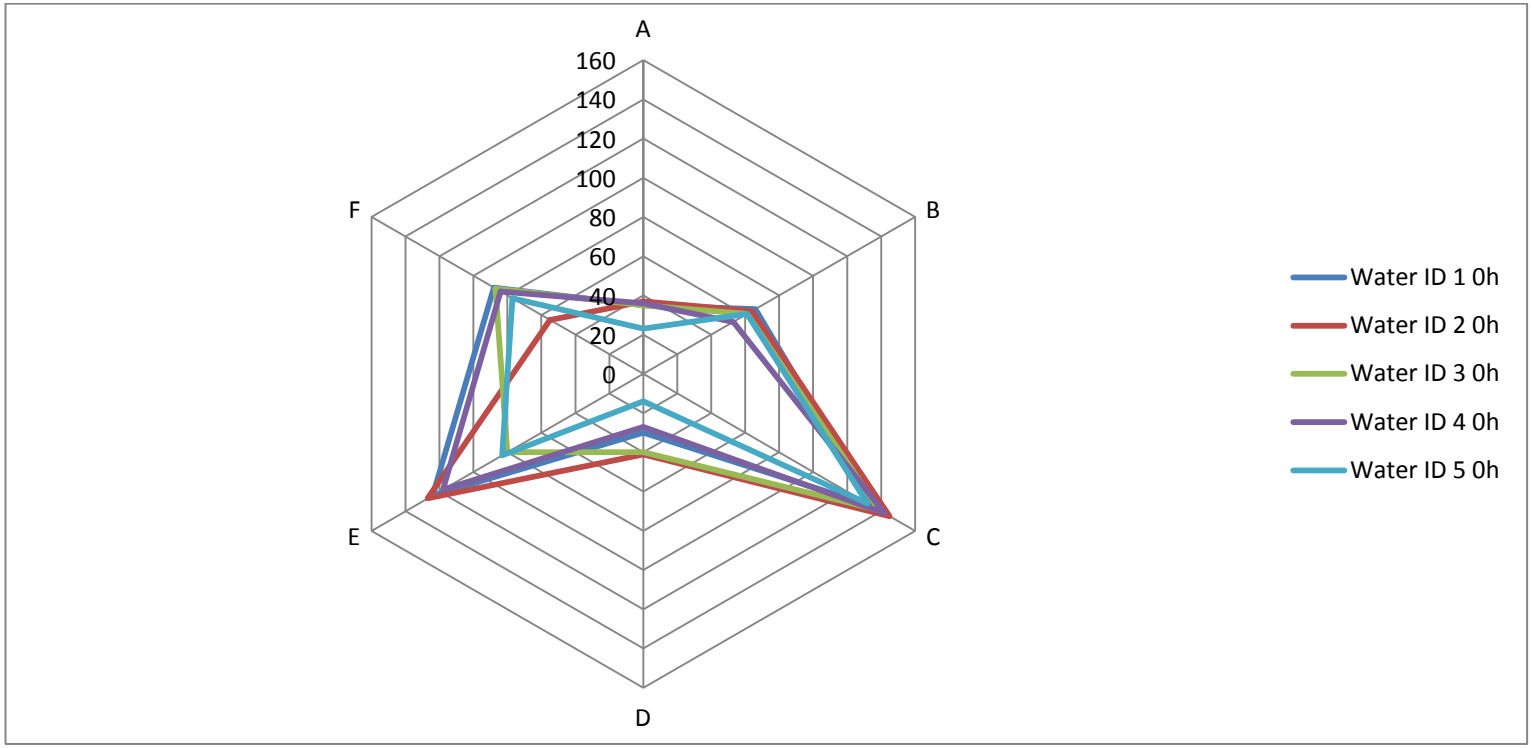
Graphic 34. TYL D product solubility differences at 0h/24h (in percentage).



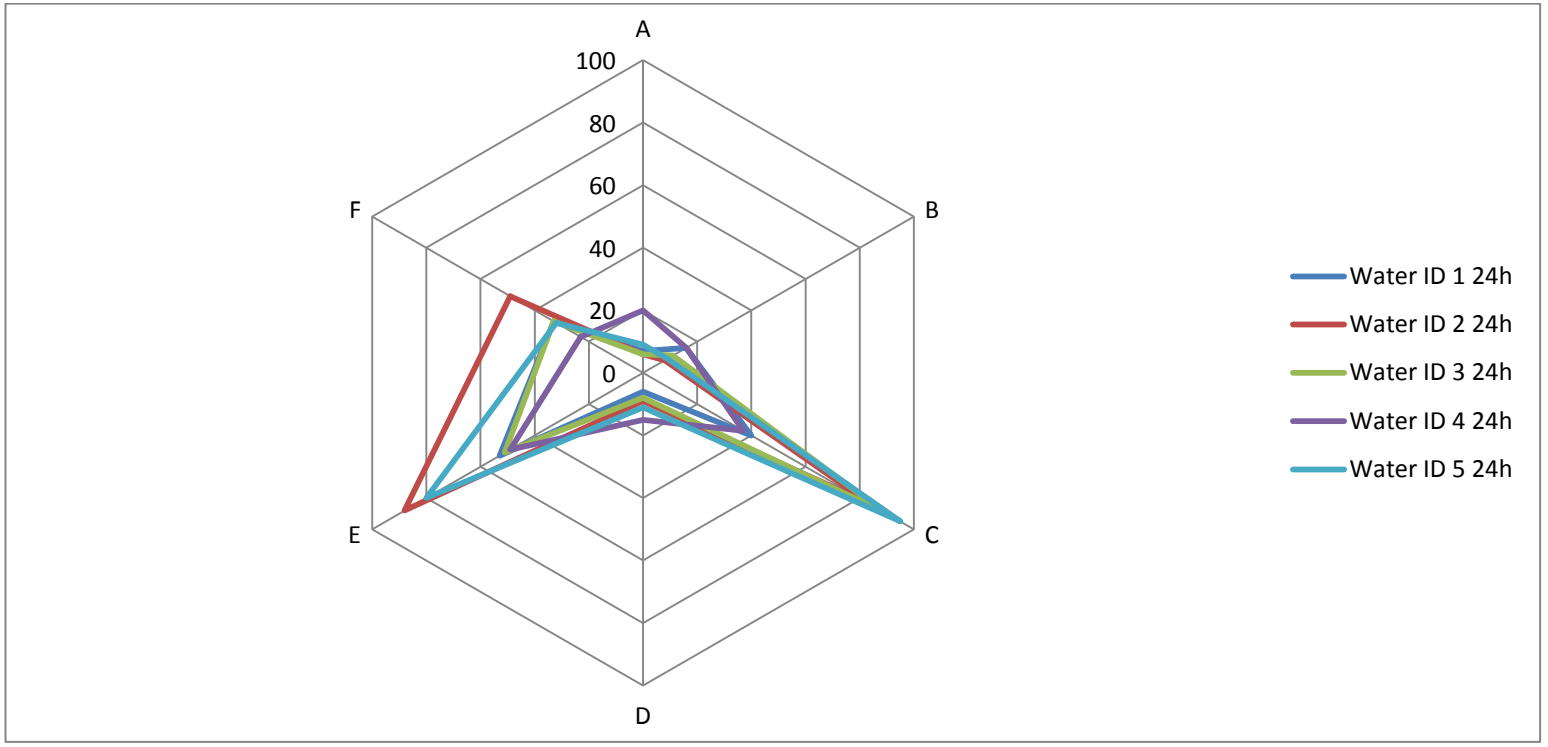
Graphic 35. TYL E product solubility differences at 0h/24h (in percentage).



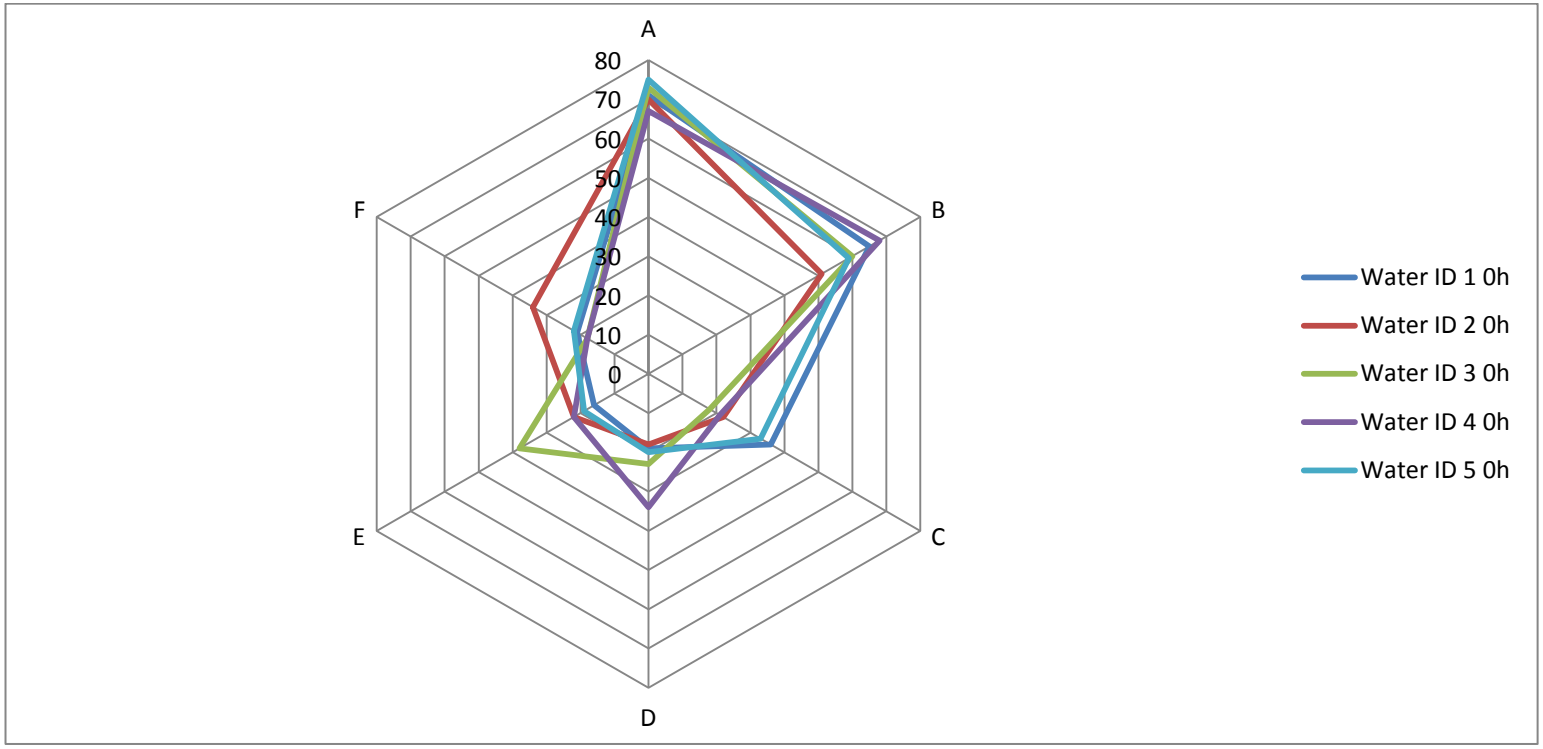
Graphic 36. TYL F product solubility differences at 0h/24h (in percentage).



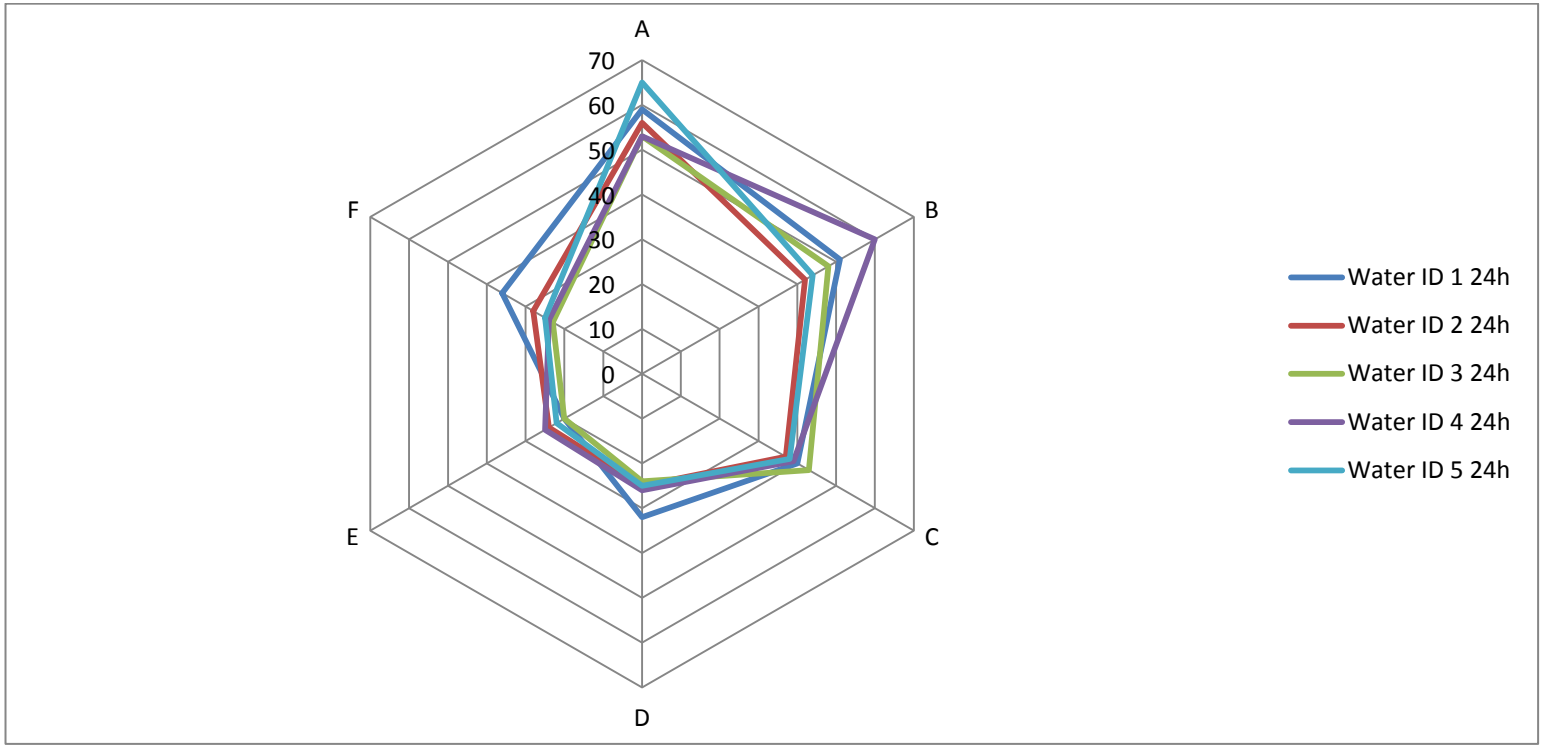
Graphic 37. Oxytetracycline products concentrations at 0h (ng/ml). Characteristics of water samples identified from 1 to 5: Water ID 1 pH 7.96 FD 21.5; Water ID 2 pH 7.94 FD 14.5; Water ID 3 pH 8.40 FD 30.0; Water ID 4 pH 7.22 FD <0.5; Water ID 5 pH 7.31 FD 20.0.



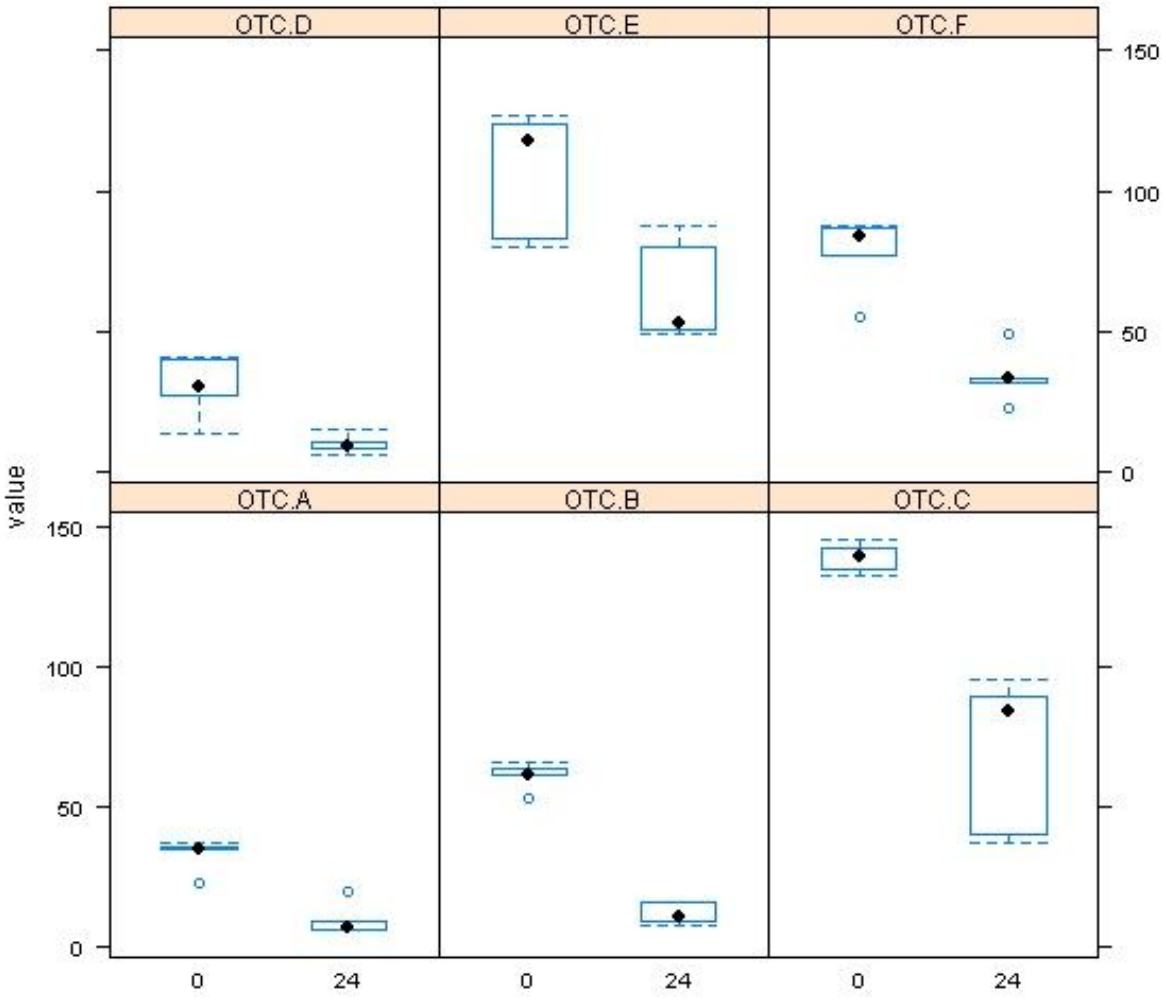
Graphic 38. Oxytetracycline products concentrations at 24h (ng/ml). Characteristics of water samples identified from 1 to 5: Water ID 1 pH 7.96 FD 21.5; Water ID 2 pH 7.94 FD 14.5; Water ID 3 pH 8.40 FD 30.0; Water ID 4 pH 7.22 FD <0.5; Water ID 5 pH 7.31 FD 20.0.



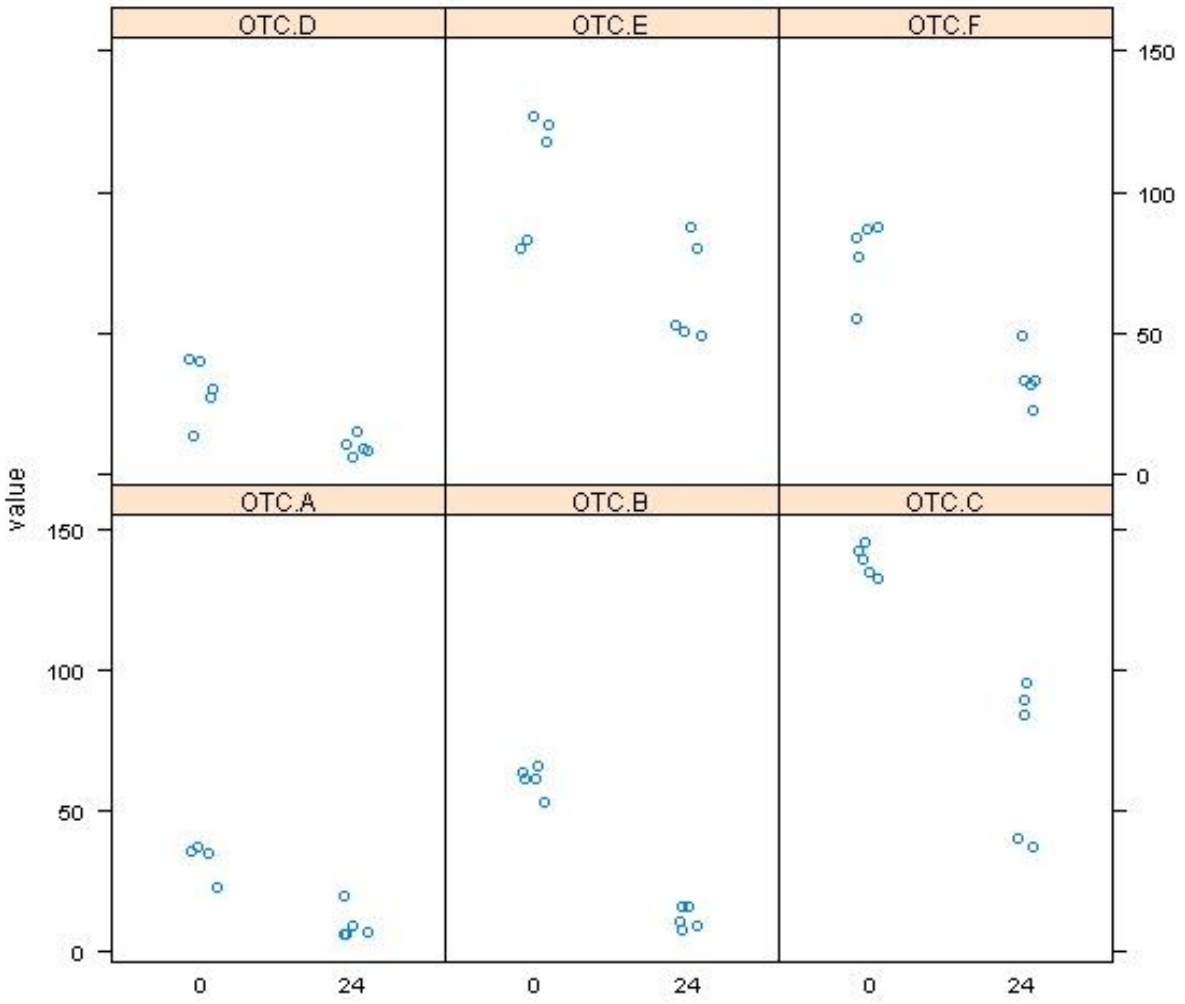
Graphic 39. Tylosin products concentrations at 0h (ng/ml). Characteristics of water samples identified from 1 to 5: Water ID 1 pH 7.96 FD 21.5; Water ID 2 pH 7.94 FD 14.5; Water ID 3 pH 8.40 FD 30.0; Water ID 4 pH 7.22 FD <0.5; Water ID 5 pH 7.31 FD 20.0.



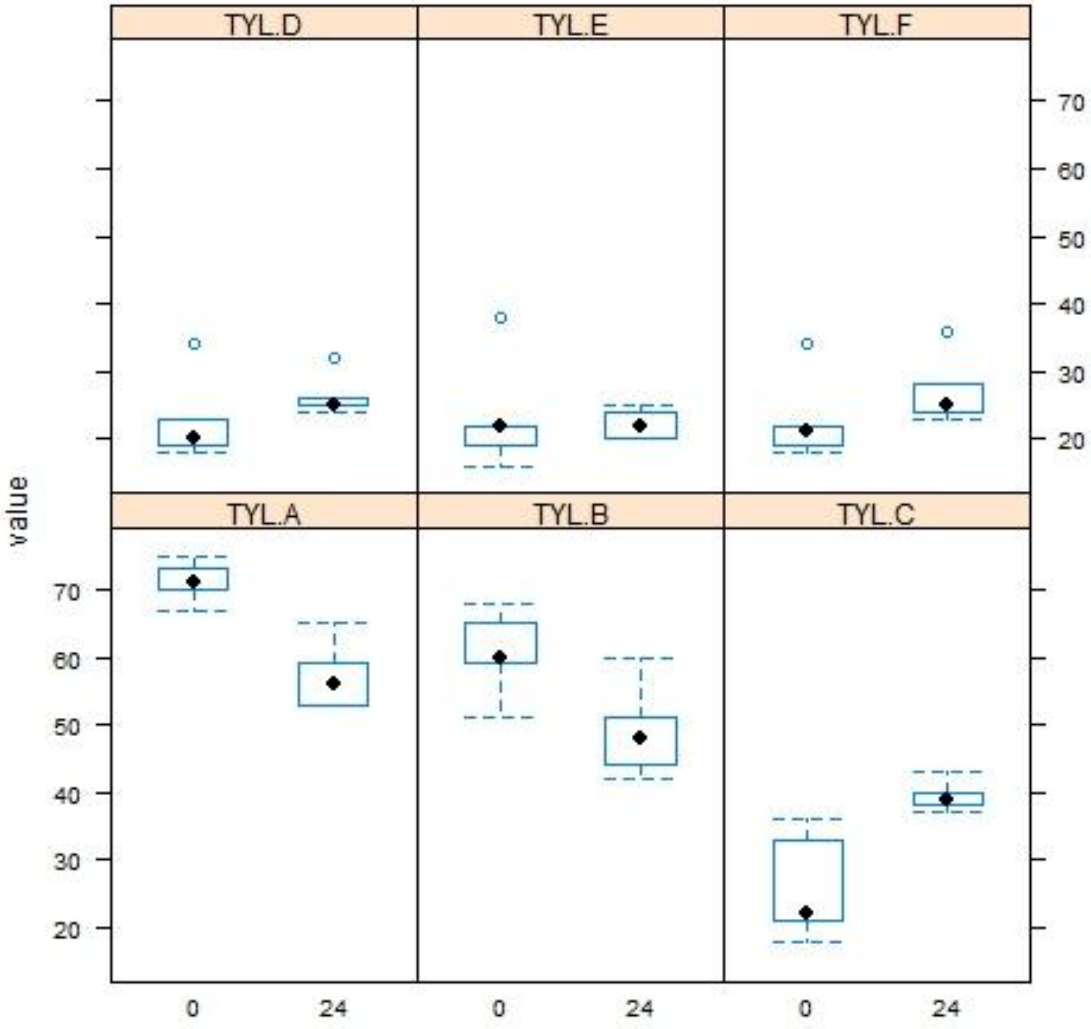
Graphic 40. Tylosin products concentrations at 24h (ng/ml). Characteristics of water samples identified from 1 to 5: Water ID 1 pH 7.96 FD 21.5; Water ID 2 pH 7.94 FD 14.5; Water ID 3 pH 8.40 FD 30.0; Water ID 4 pH 7.22 FD <0.5; Water ID 5 pH 7.31 FD 20.0.



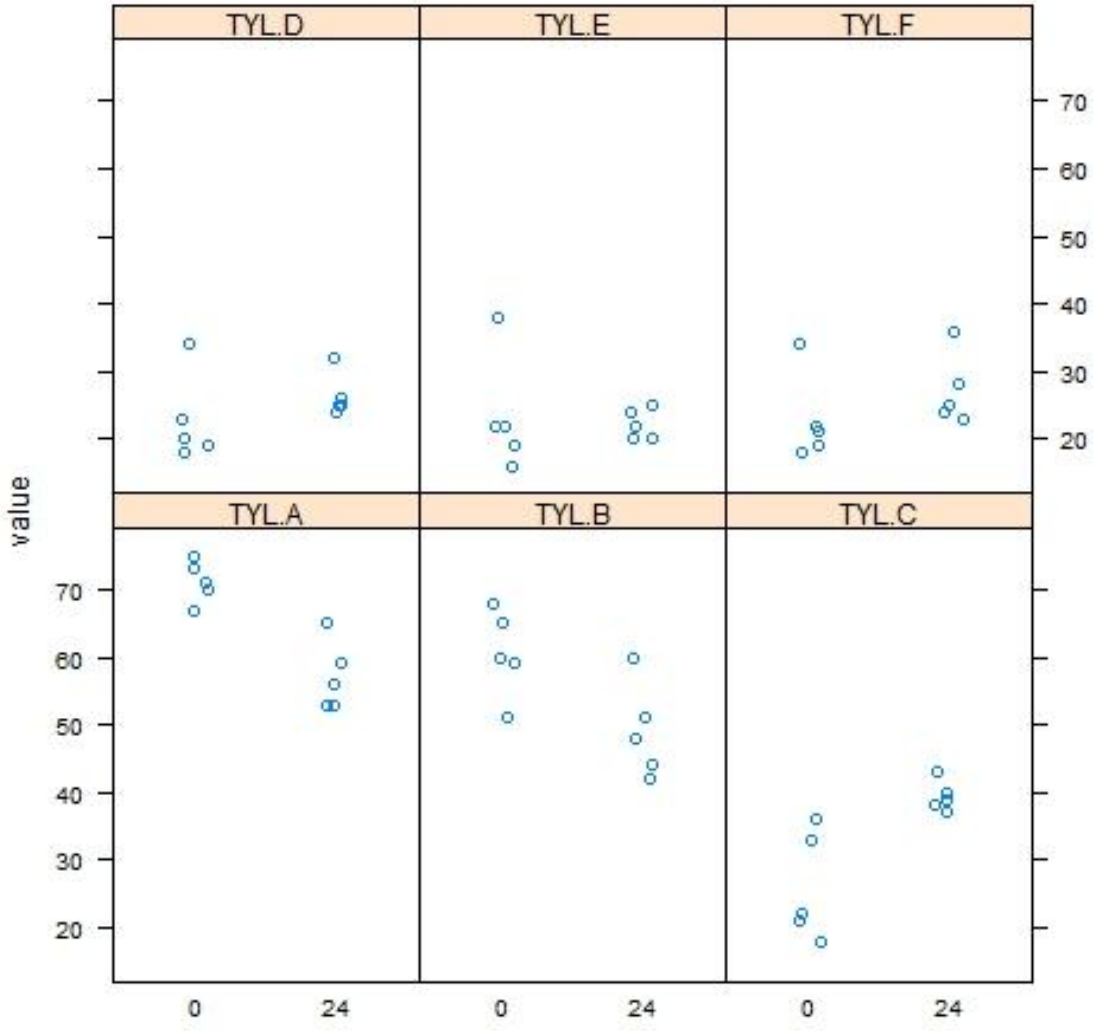
Graphic 41. Box plot displaying the based-oxytetracycline products distribution.



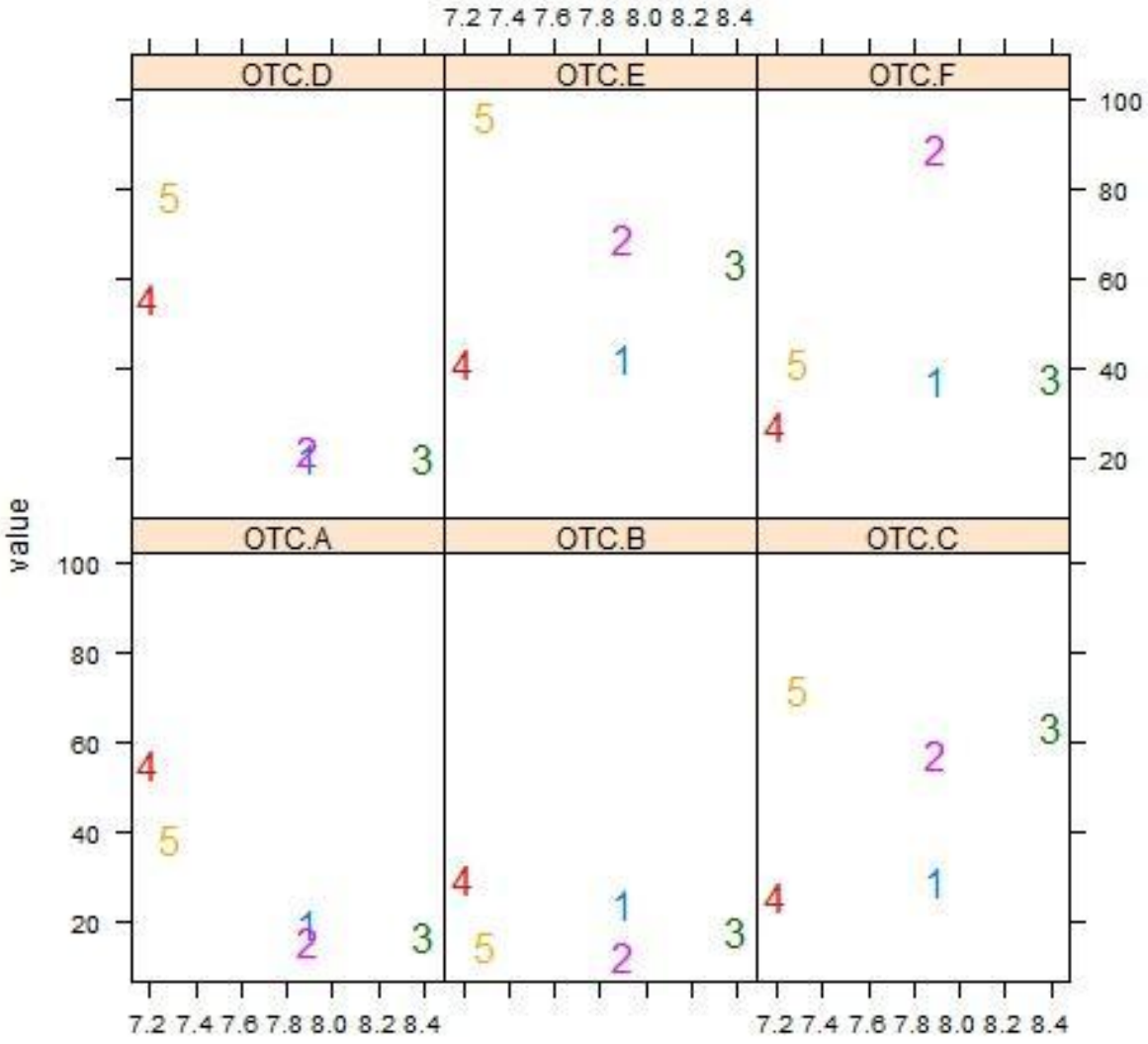
Graphic 42. Strip plot displaying every observation point of based-oxytetracycline products.



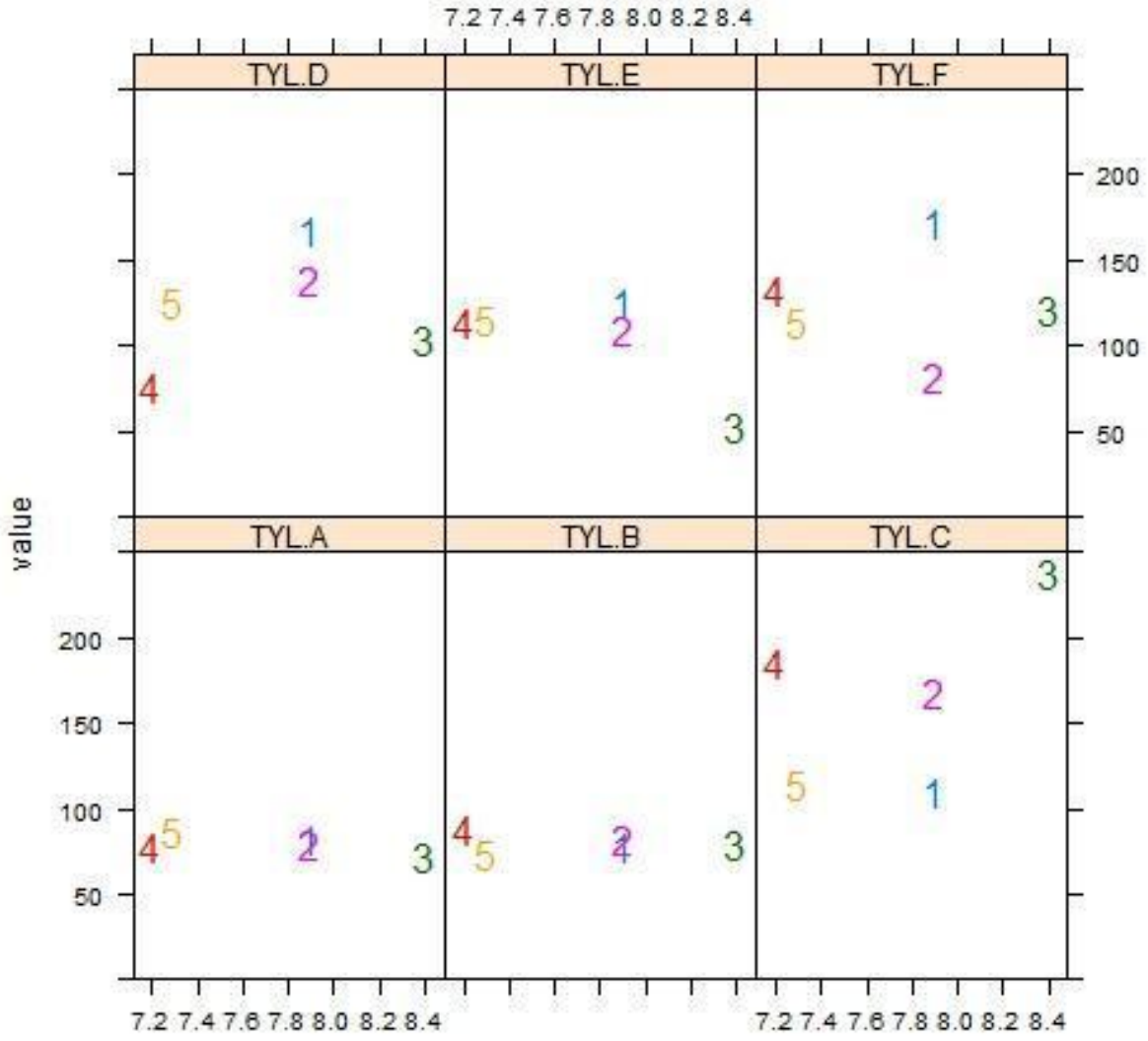
Graphic 43. Box plot displaying the based-tylosin products distribution.



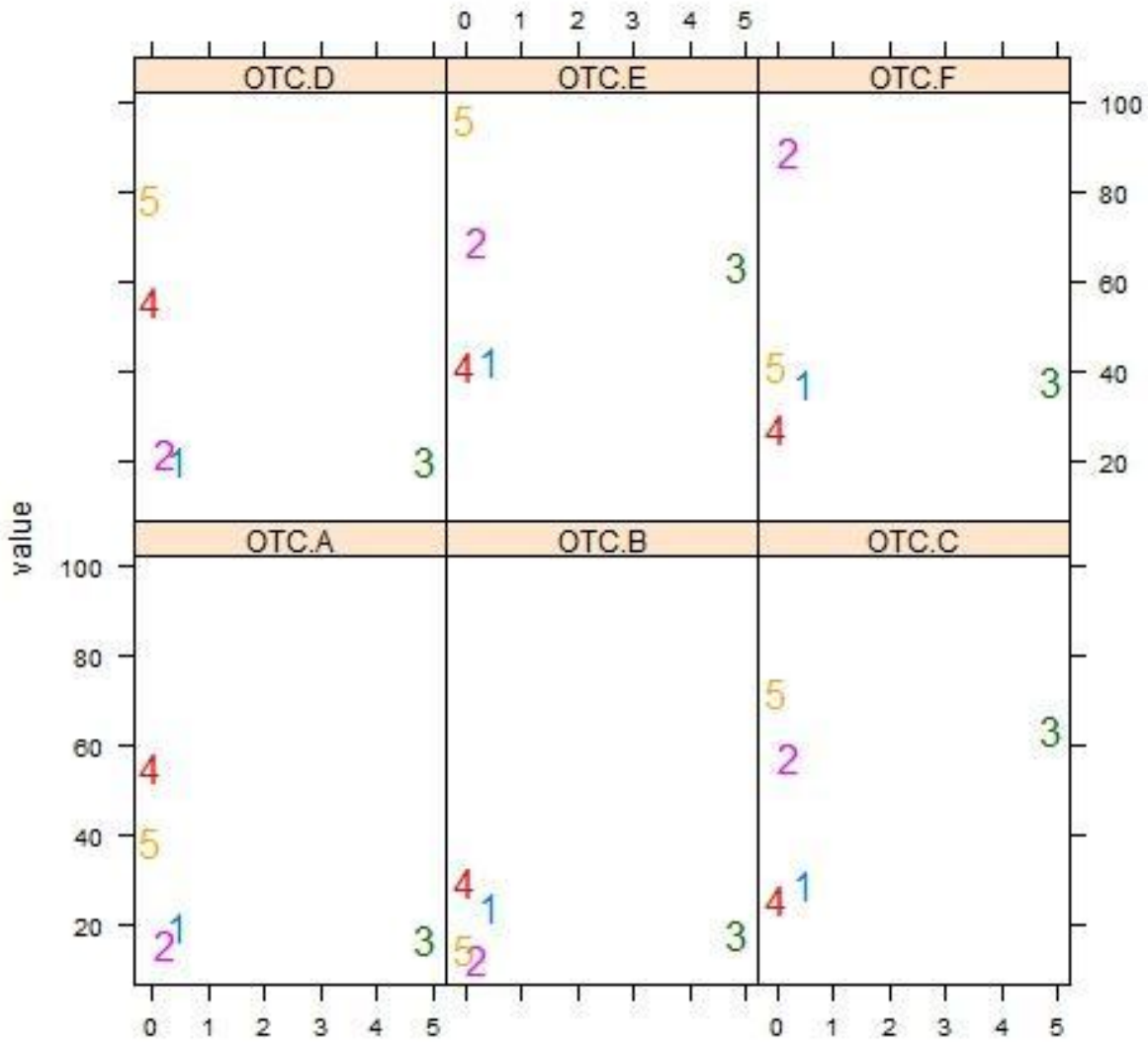
Graphic 44. Strip plot displaying every observation point of based-tylosin products.



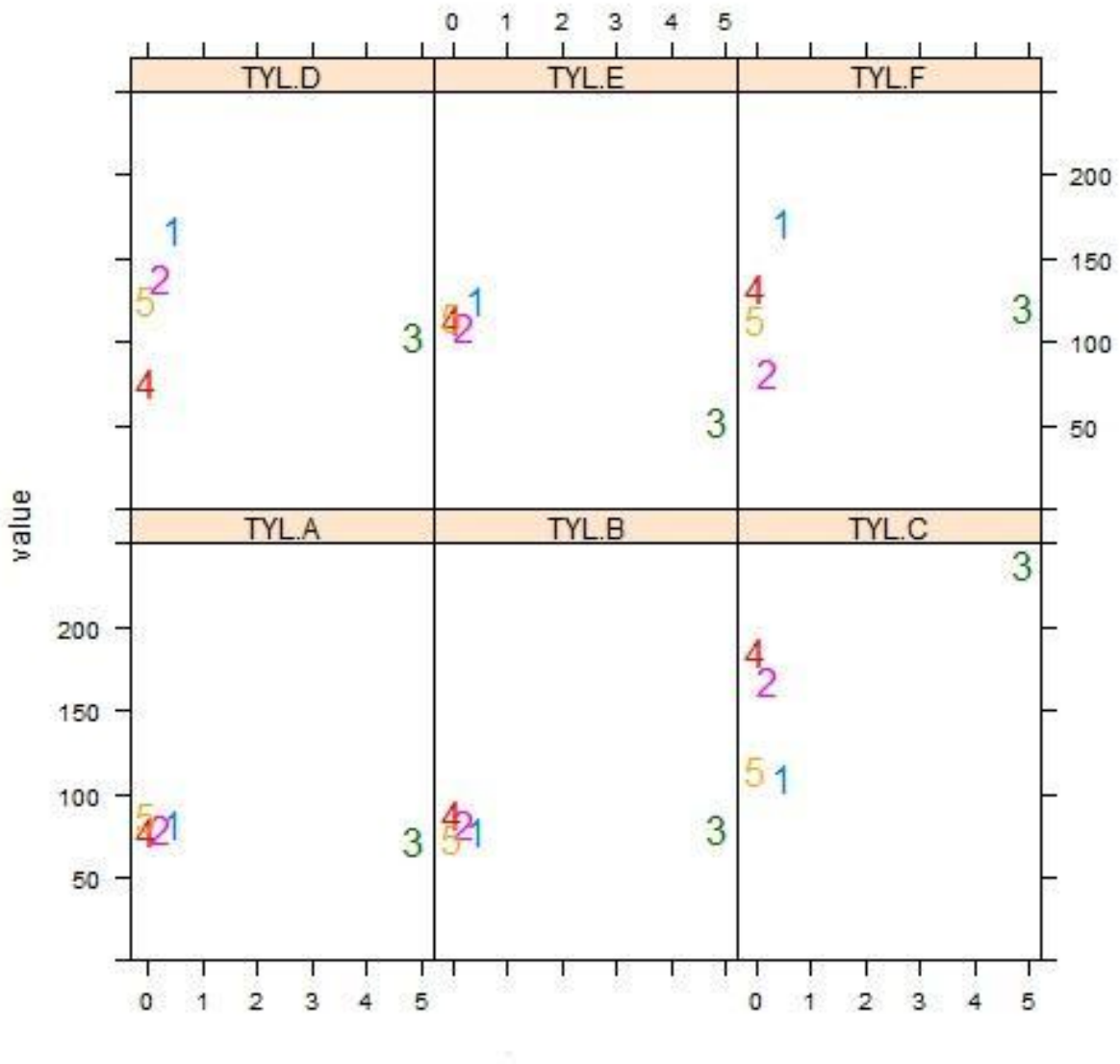
Graphic 45. pH and based-oxytetracycline products.



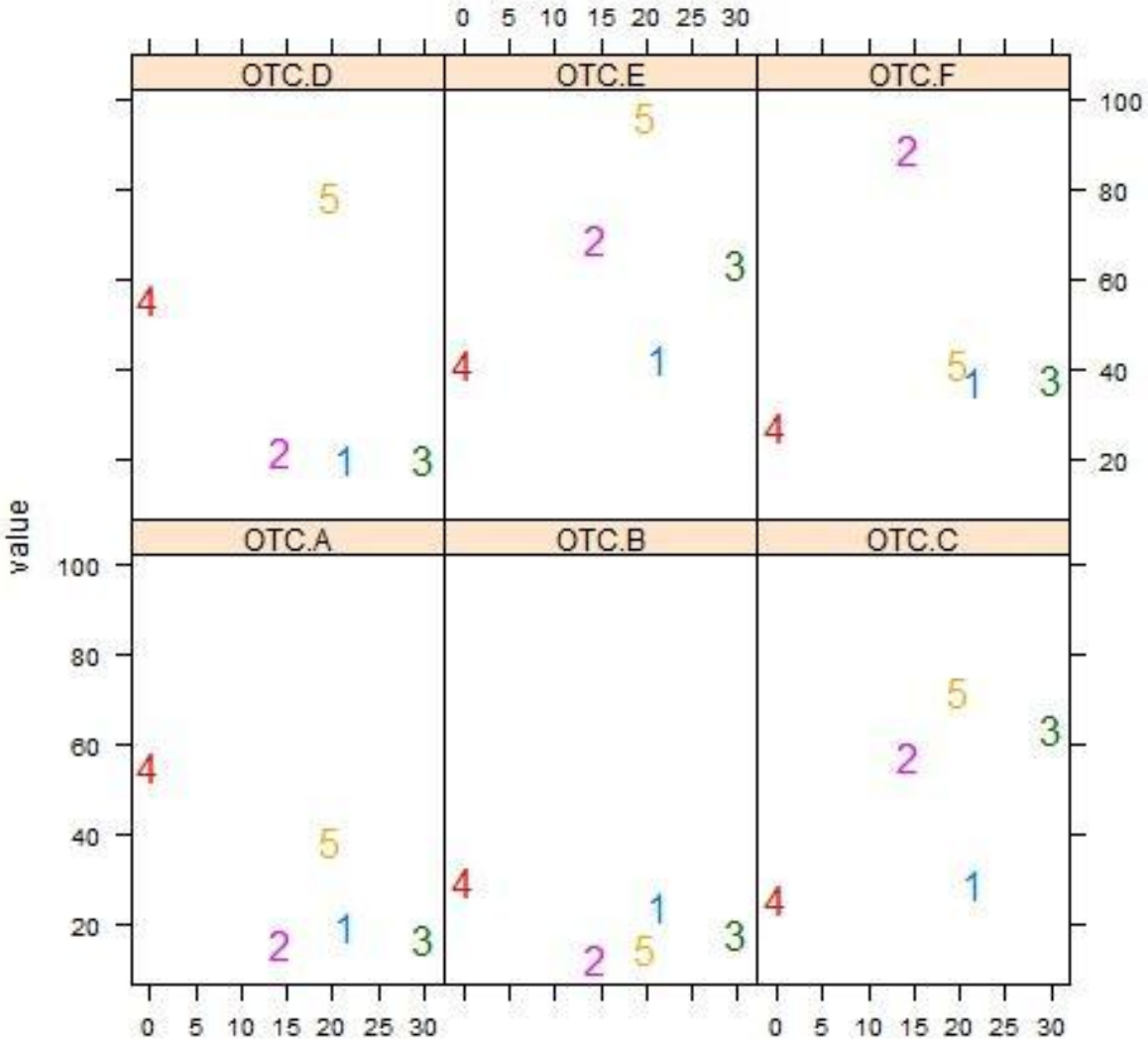
Graphic 46. pH and based-tylosin products.



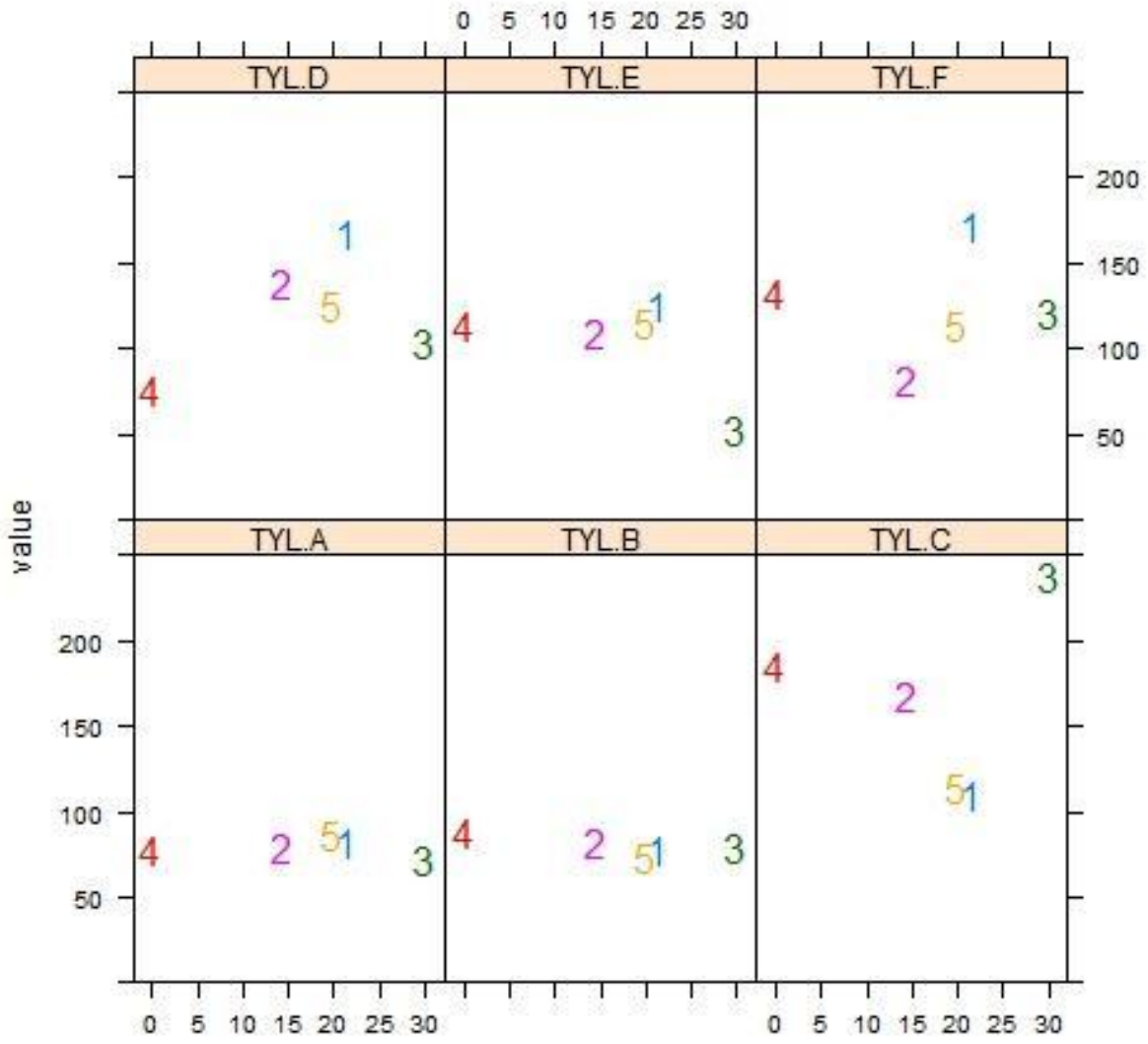
Graphic 47. Oxydability and based-oxytetracycline products.



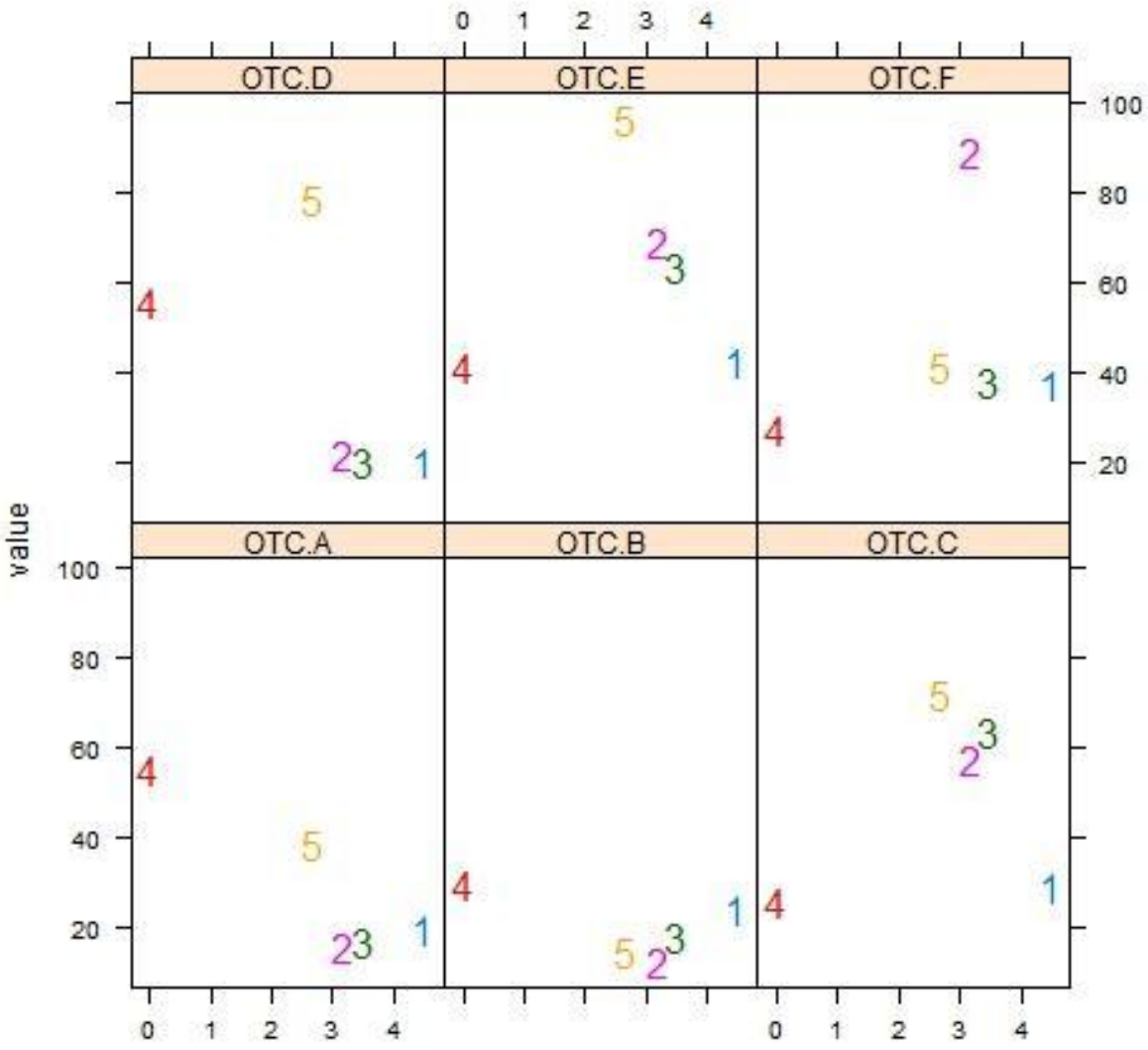
Graphic 48. Oxydability and based-tylosin products.



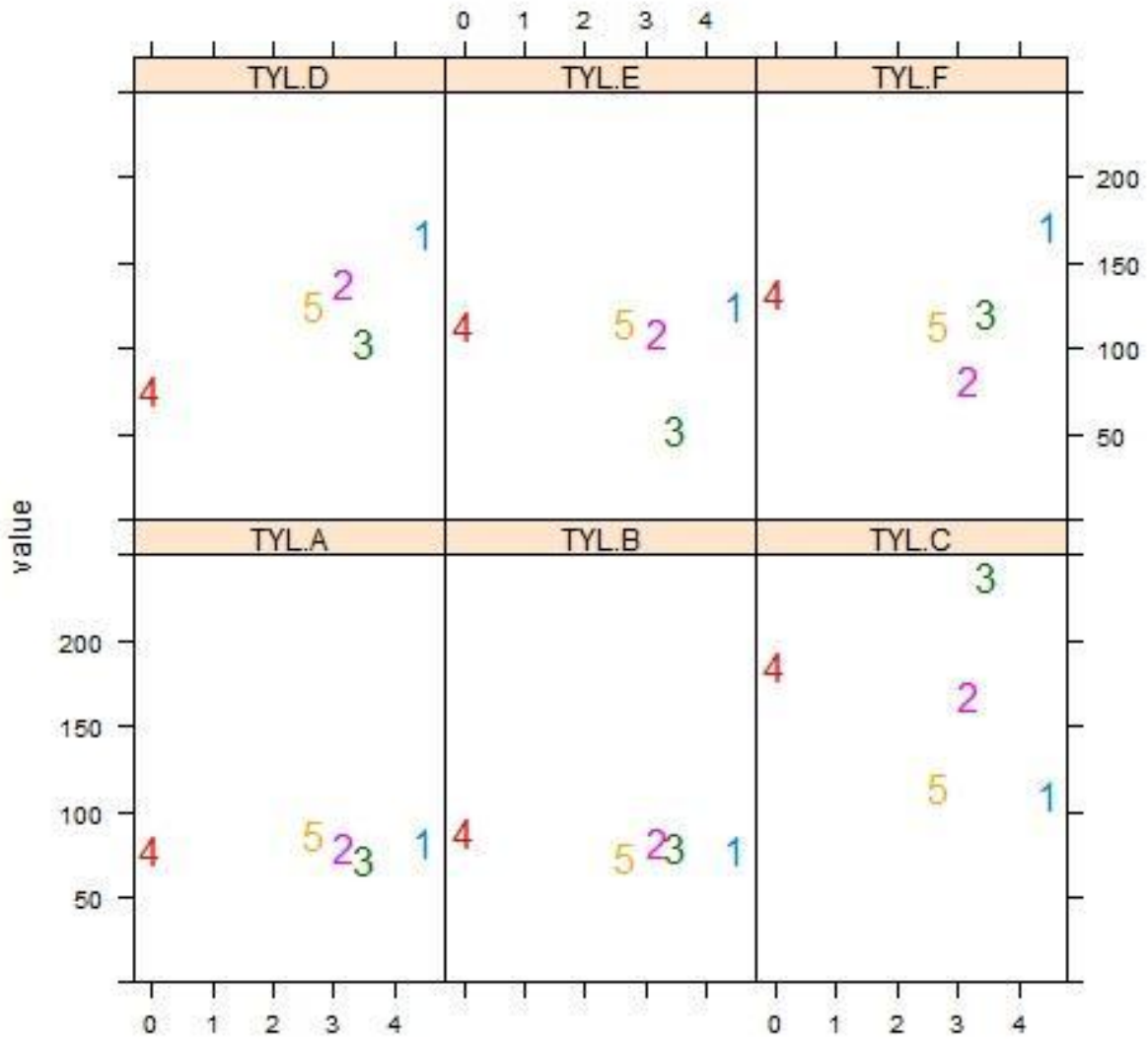
Graphic 49. Hardness and based-oxytetracycline products.



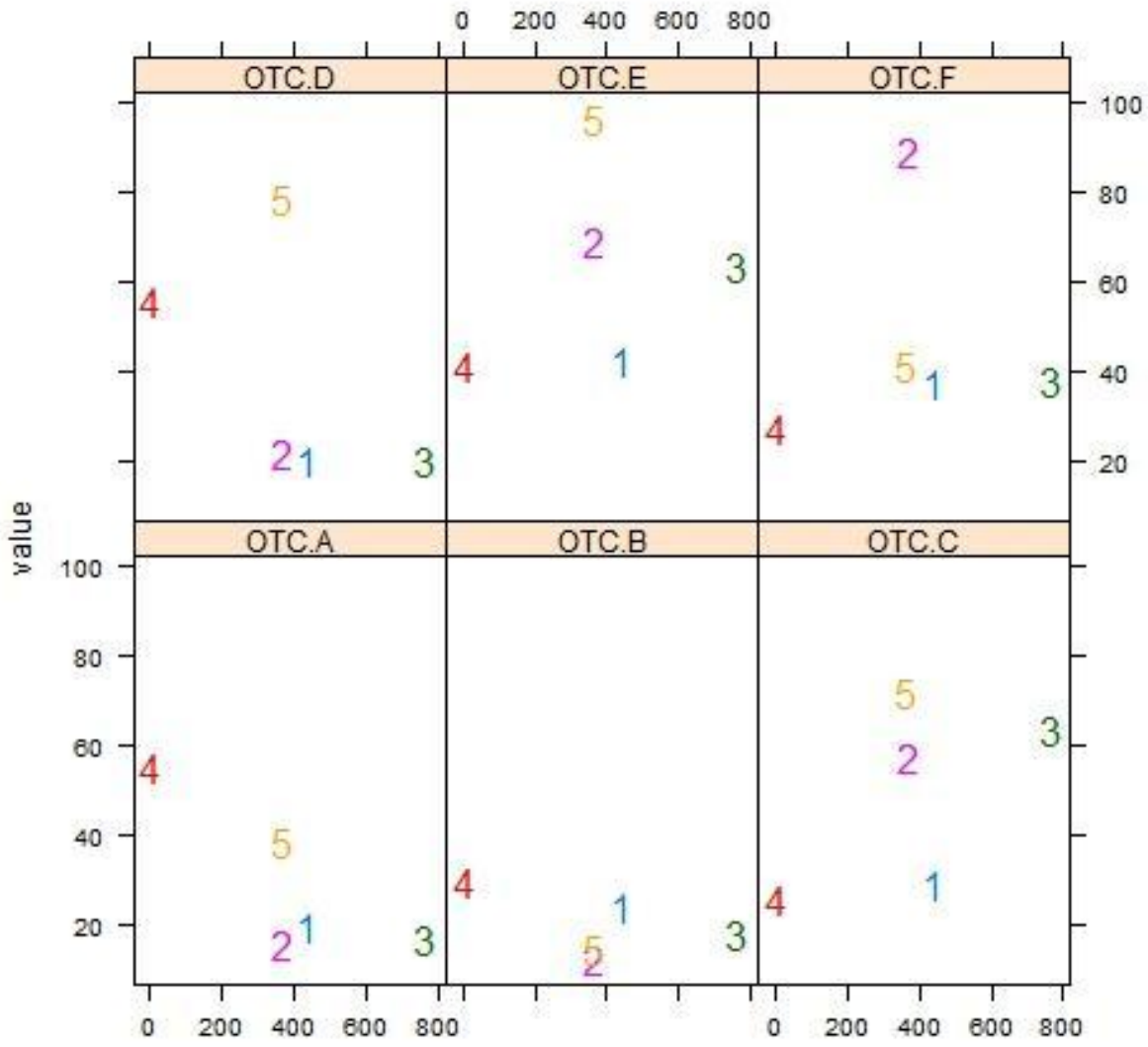
Graphic 50. Hardness and based-tylosin products.



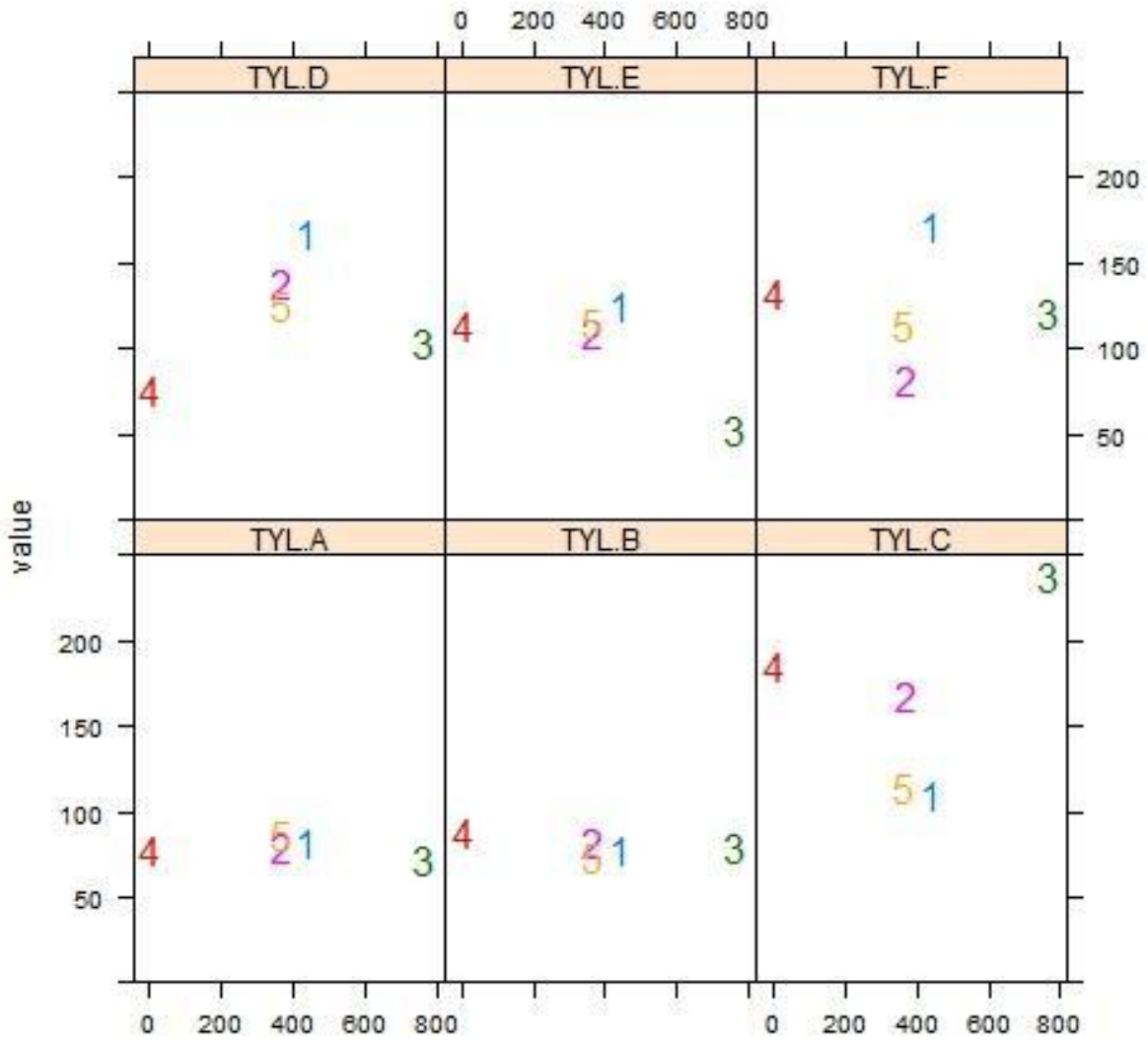
Graphic 51. Nitrates and based-oxytetracycline products.



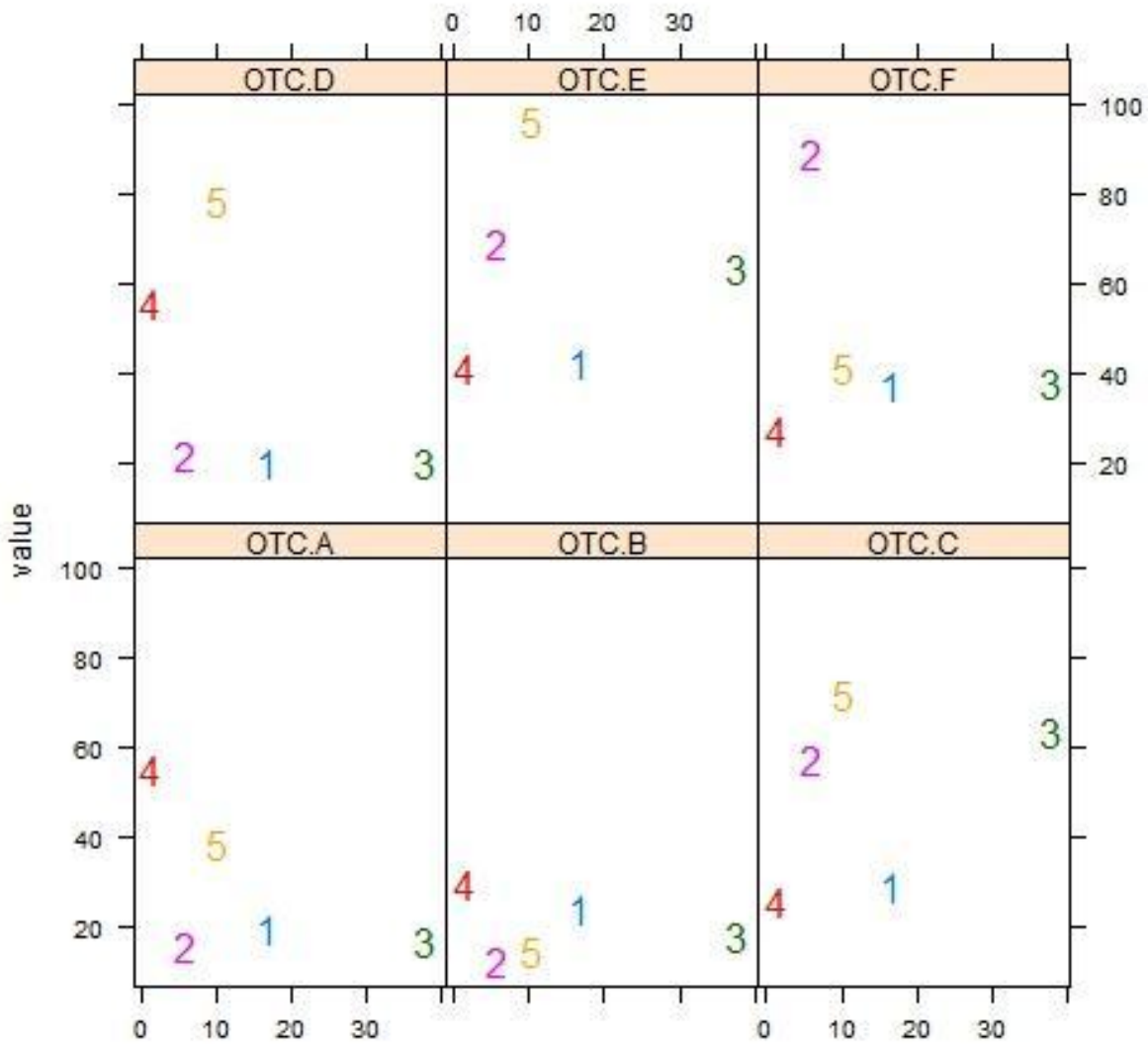
Graphic 52. Nitrates and based-tylosin products.



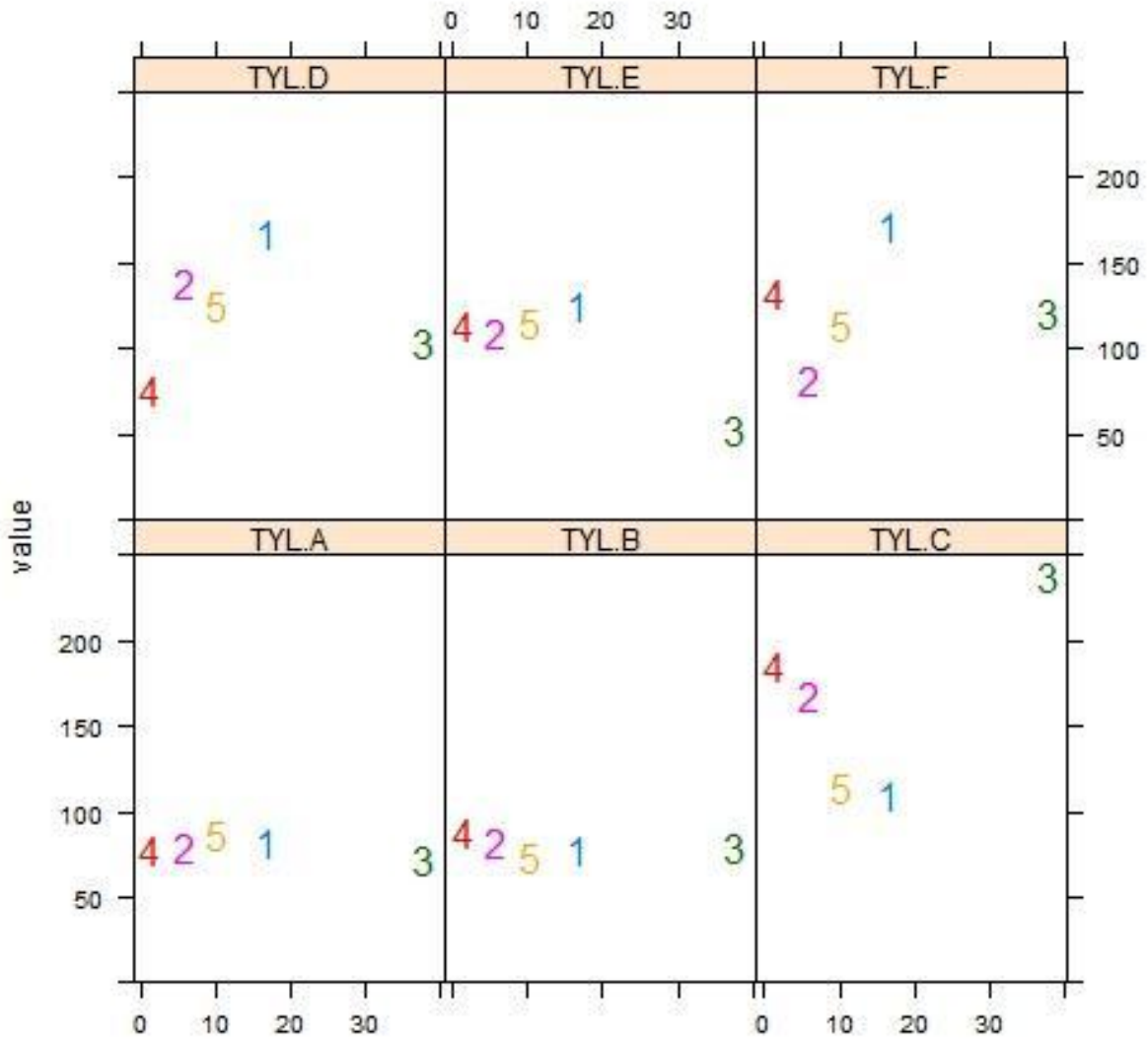
Graphic 53. Conductivity and based-oxytetracycline products.



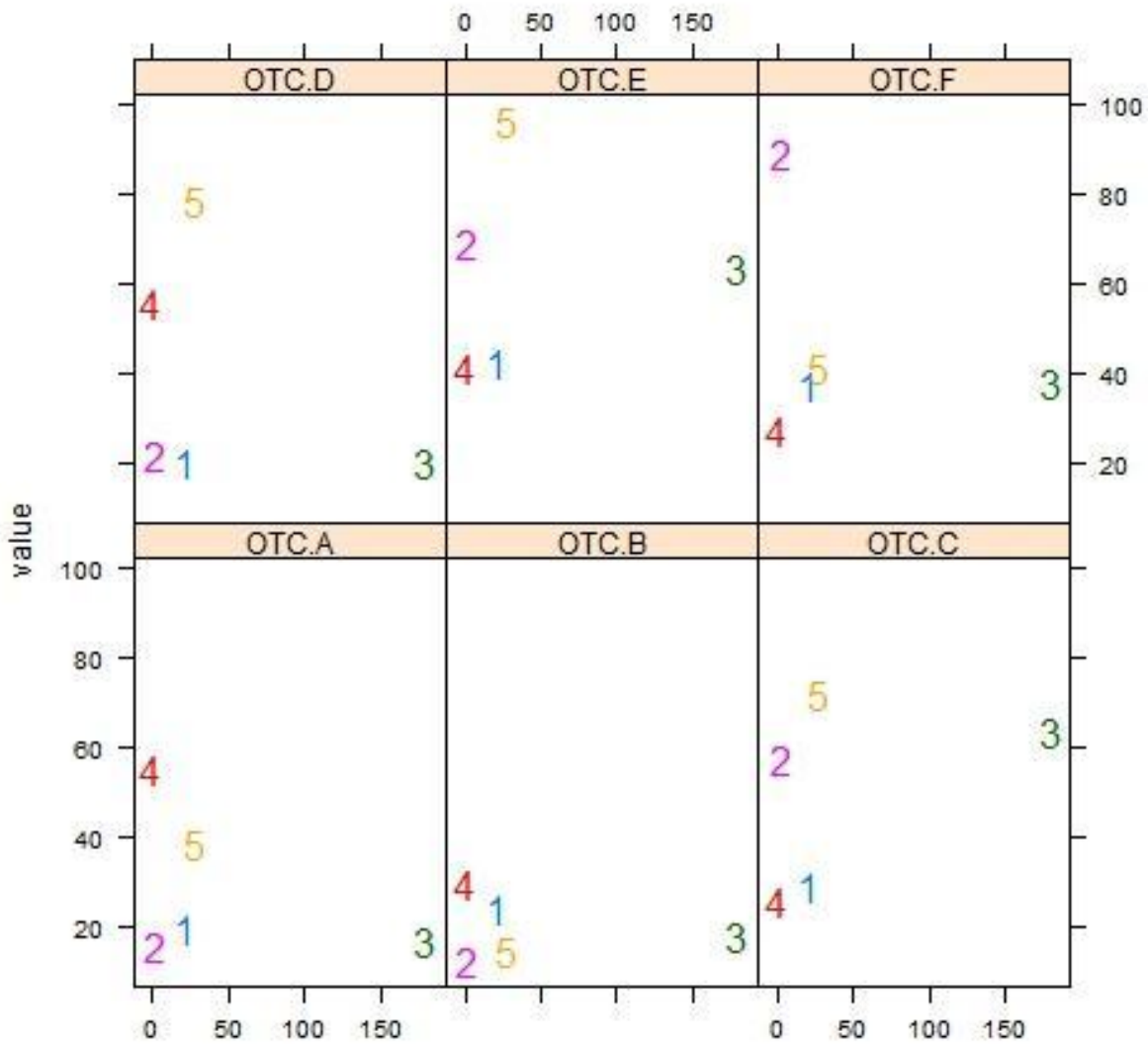
Graphic 54. Conductivity and based-tylosin products.



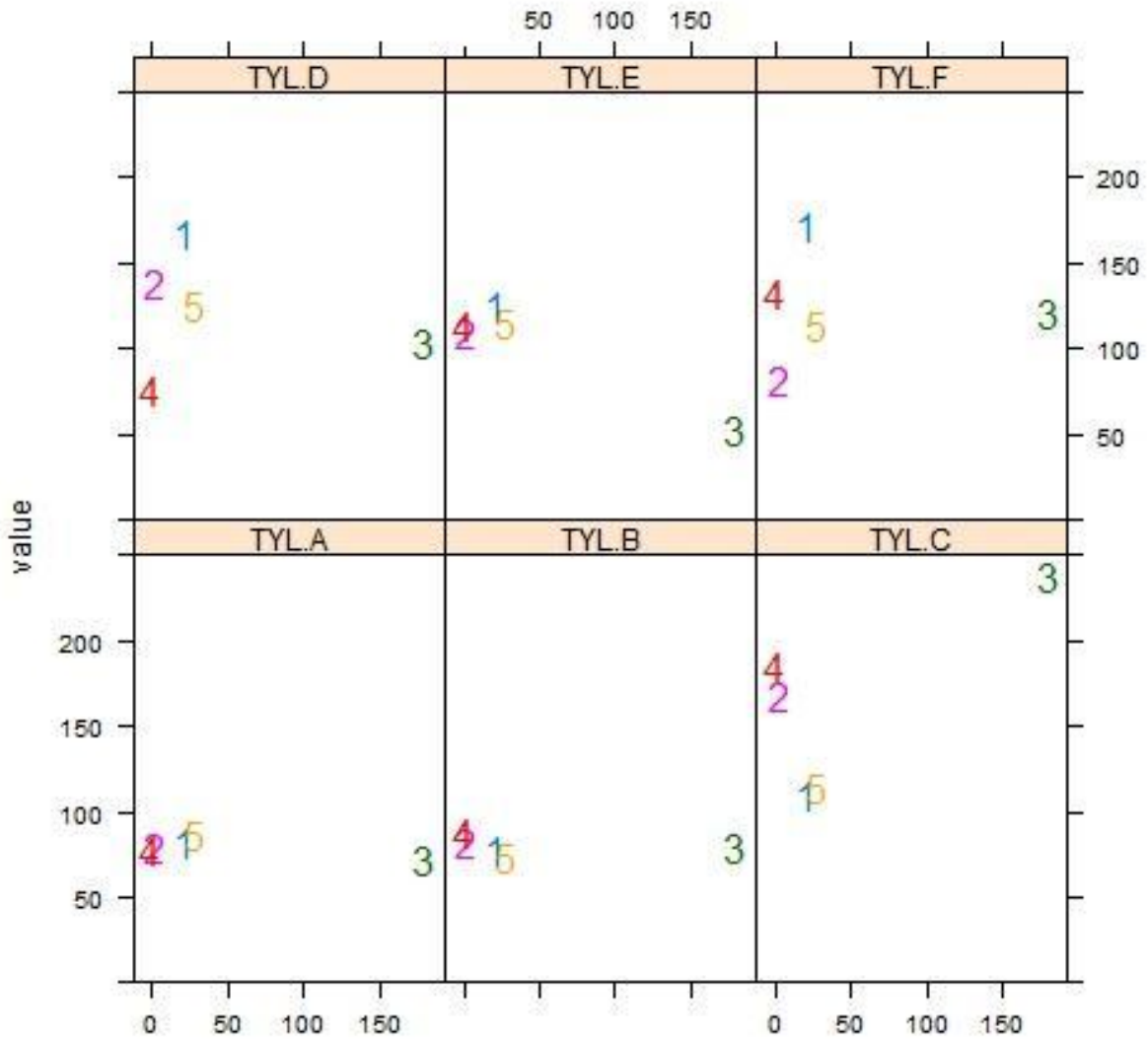
Graphic 55. Chlorides and based-oxytetracycline products.



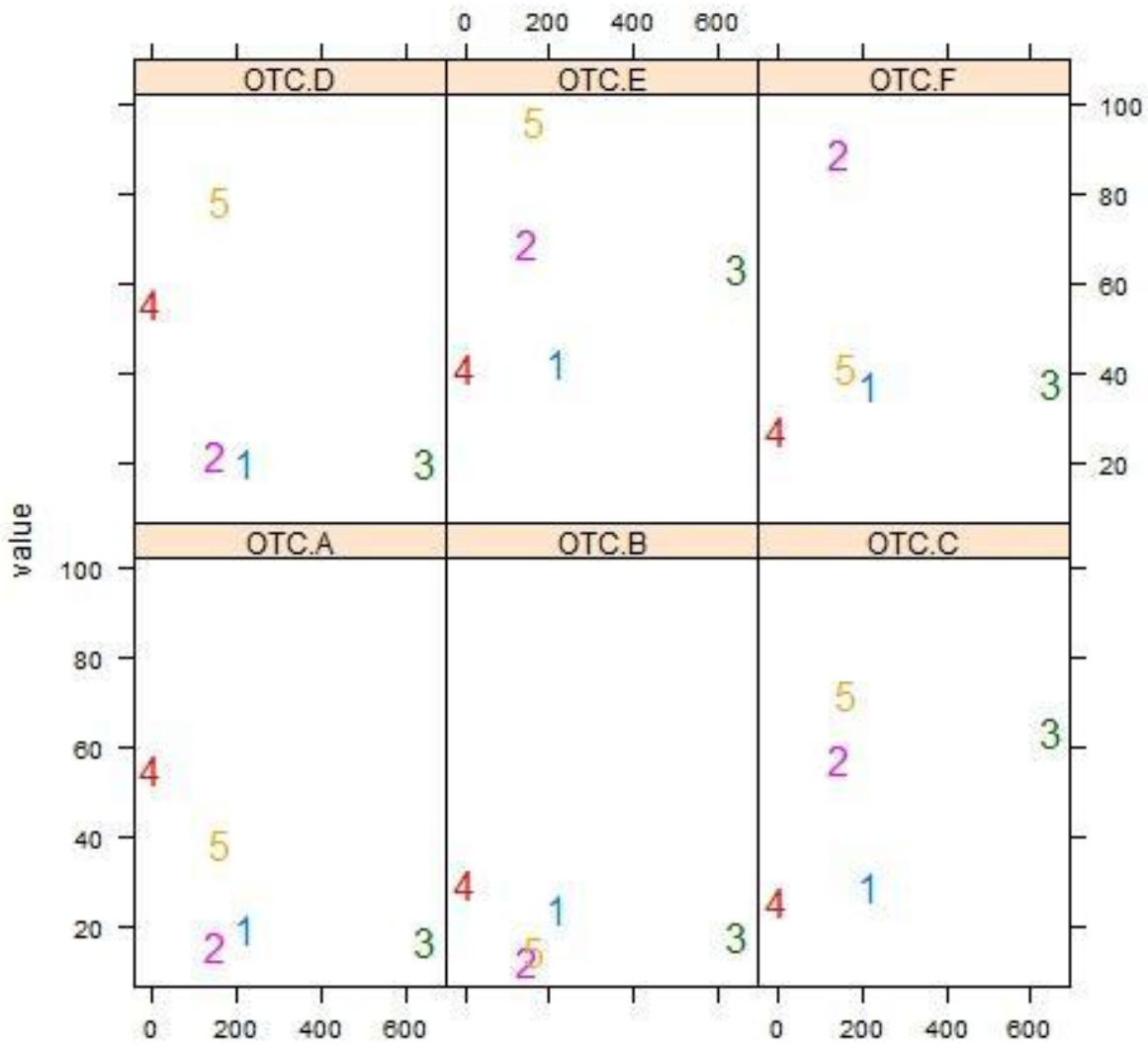
Graphic 56. Chlorides and based-tylosin products.



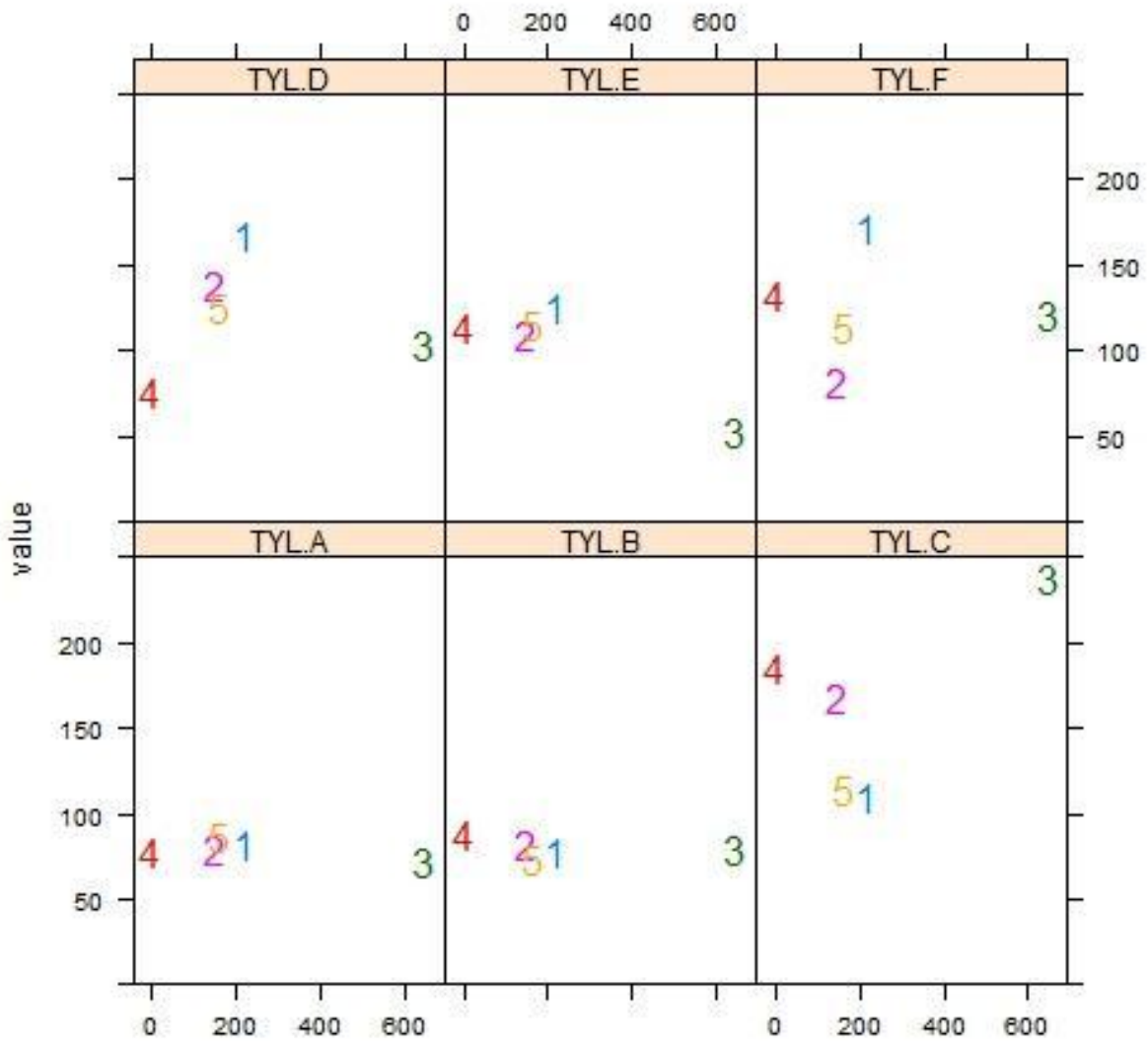
Graphic 57. Sulphates and based-oxytetracycline products.



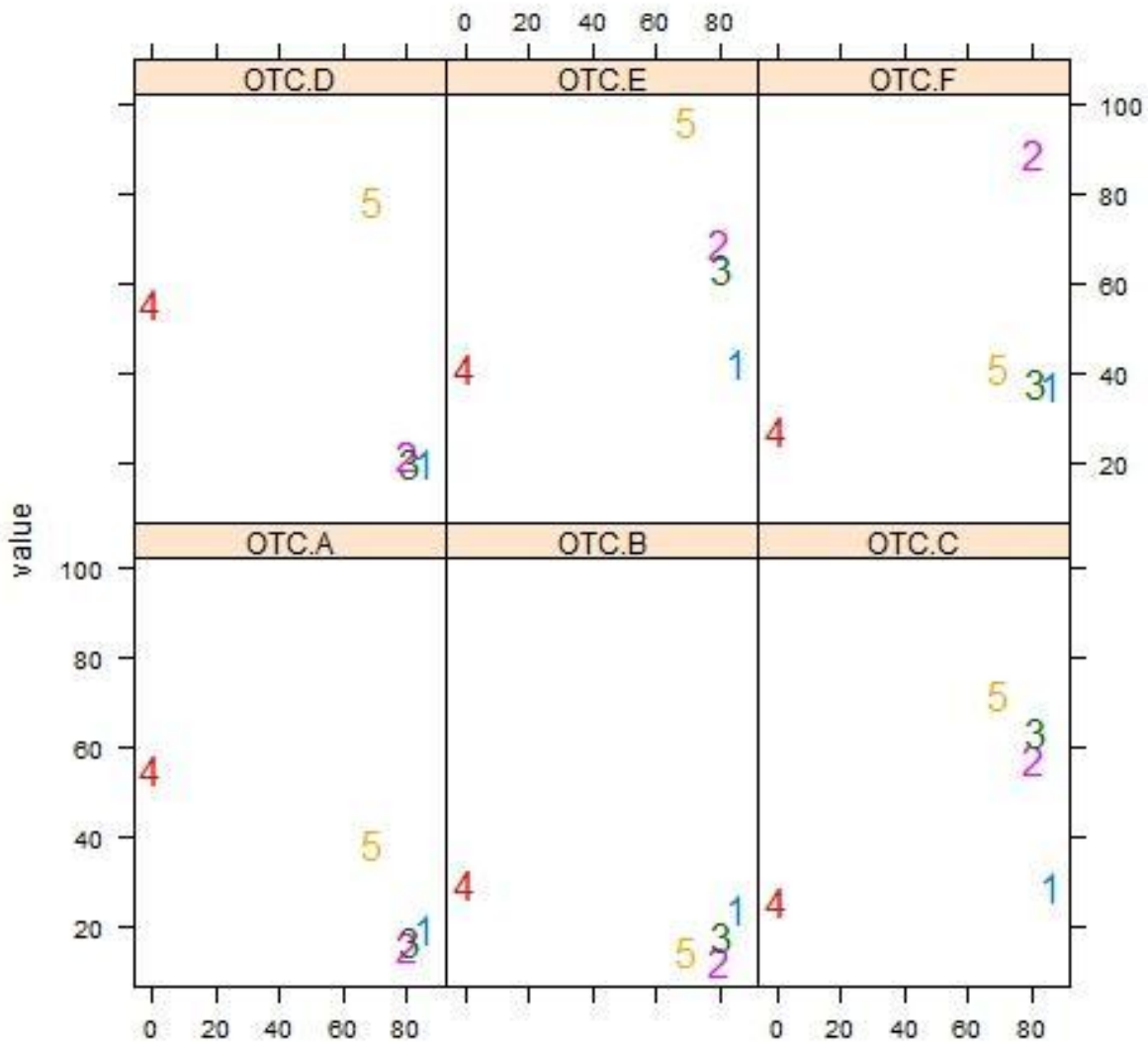
Graphic 58. Sulphates and based-tylosin products.



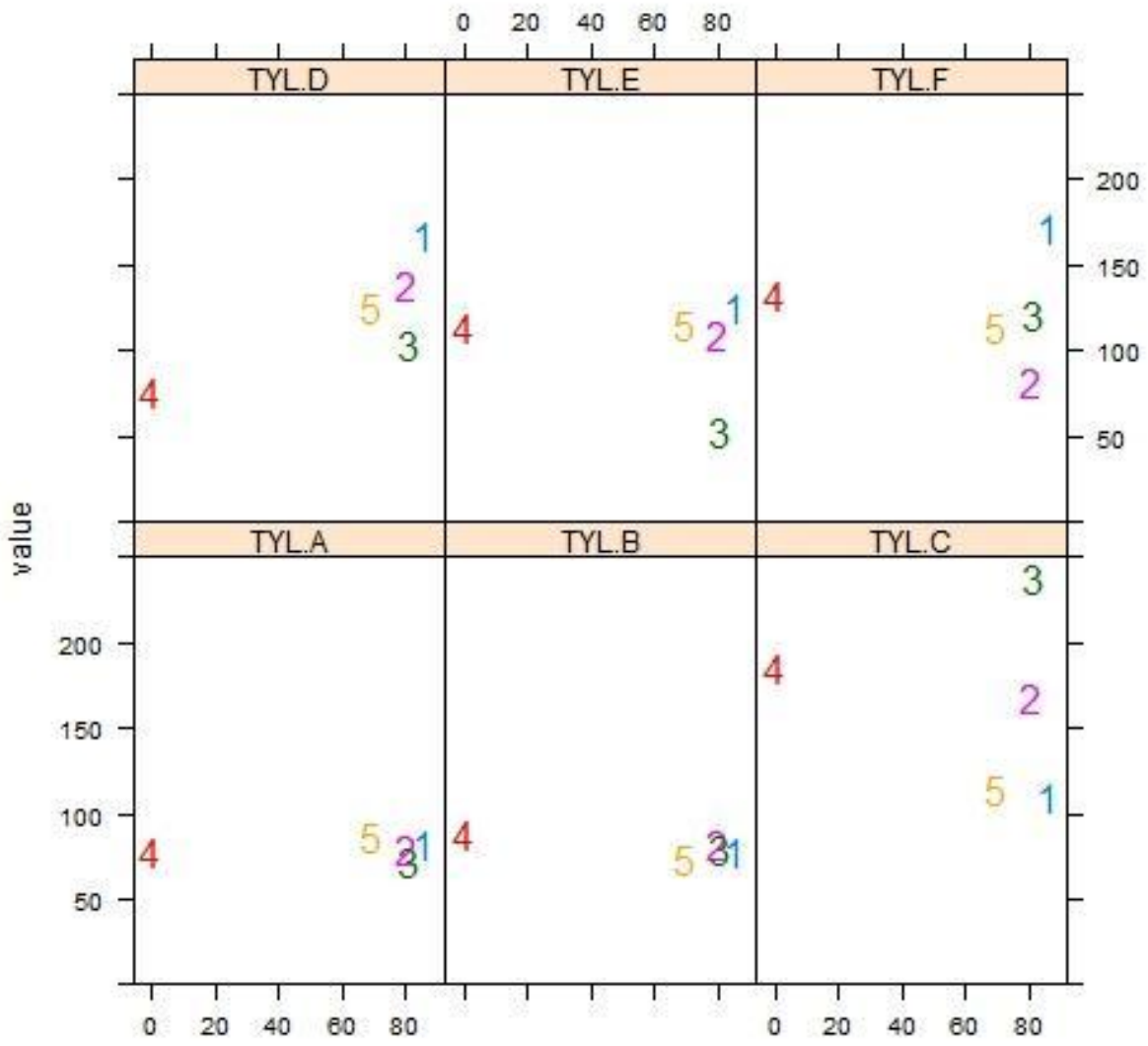
Graphic 59. Total dissolved Solids at 180° C and based-oxytetracycline products.



Graphic 60. Total dissolved Solids at 180° C and based-tylosin products.



Graphic 61. Calcium and based-oxytetracycline products.



Graphic 62. Calcium and based-tylosin products.

9. Discussion and Conclusion

The water samples used in this experimental test reflect, in their own diversity, the heterogeneity of water used to dissolve pharmaceutical products in breeding farms; there are a large number of water samples intrinsic variables that may affect the drugs solubility that was the objective of this experimental trial.

The graphs 37, 38, 41, 42 show the based-oxytetracycline products dissolution in different water samples at 0h and 24h respectively, as well as the variability associated to the different determinations. For all based-oxytetracycline products considered, it was possible to observe a tendency to decrease in solubility between 0h and 24 h. In fact, for the different products, at 24h, active ingredient percentages between 20 and 62.8% (compared to that ones detected at 0h) were observed. The already expired products (OTC A, OTC B, OTC D and OTC F) showed active ingredient concentrations much lower as they were far from the expiration date. This occurrence has been noticed in all water samples analyzed both at 0 hours and at 24 hours. After 24 hours, in 4 products expired liquids OTC A, OTC B, OTC D and OTC F, it was possible to detect an oxytetracycline average percentage of 33.9% compared to that one detected at 0h. At 24 h, the not expired products (OTC C and OTC E)

showed an active ingredient average of 56.3% compared with that one rileved at 0h so maintaining a higher over time.

At 0h it was possible to observe how some products (OTC A, B OTC, OTC C) were less affected by the water effect: there was a lower results dispersion but in other cases (OTC E) the variability was higher. In the OTC E case, the variability even in the determinations carried out at 24h was maintained, as well as the variability lack of OTC A and OTC B products over 24 hours was maintained. The OTC C product had an intermediate situation, with little variability at 0h and increased determinations variability at 24h from dissolution.

The graphs 39, 40, 43, 44 show the based-tylosin products dissolution in different water samples at 0h and 24h respectively, as well as the variability associated to the different determinations. About the based-tylosin products, the results were different. In fact, for some products was possible to observe a trend of solubility increase between 0h and 24h results. At 24h, for all based-tylosn different products, active ingredient percentages between 80% and 164% (compared to that ones detected at 0h) were detected. At 0h it was possible to observe how some products (TYL D, TYL E, TYL F) were less affected by the water effect: there was a lower results dispersion but in other cases (TYL C) the variability was higher. In the case of TYL D, TYL E, TYL F such variability even in

determinations carried out at 24h was maintained. In contrast, TYL C variability decreases at 24 hours. In 24 hours, all these products showed solubility increase. The in liquid form TYL C, TYL D, TYL E and TYL F based-tylosin products (only 2 over expiration date), at 0h showed active principle concentrations lower than those registered at 24h. In fact, after 24 hours it was possible to detect a tylosin percentage average of 128.5% compared to that one detected at 0h. These results could be occurred because of the low and slow solubility of these products in all 5 water samples selected. The TYL A and TYL B products (in powder form) showed variability intermediate and similar results both at 0h and at 24h, with a tendency to decrease in solubility; after 24h for TYL A and TYL B products active principle percentages of 80.4% and 80.6% (compared to that one detected at 0h) respectively were detected.

The graphs 45-62 show how the intrinsic characteristics of the water such as pH, hardness, conductivity and calcium may affect the dissolution of drugs tested in water. The results suggest that it would be appropriate to test the products in water samples under challenging conditions dissolution, in order to identify in advance possible problems.

In general, is possible to conclude that powder products showed a best dissolution kinetics then liquid form products.

Finally the data obtained in this study show how the water quality is critical for the therapeutic success, especially in light of the growing use of water as vehicle for immunological treatments. In effect, as requested by the Summary Product Characteristics of these vaccines, a good quality of the water is crucial for the proper functioning of the immunological products dissolved in. Therefore, data obtained in the present study may represent a starting point for further investigations, bearing in mind that a drugs poor solubility in drinking water can cause beyond a lower efficacy of treatments, an antibiotic-resistance increase, episodes of toxicity, problems to water distribution systems, drug residues presence and finally environmental pollution.

10. References

Adams H.R. (1999) Farmacologia e terapeutica veterinaria, 2a Ed. italiana a cura di Beretta C., EMSI, Roma.

Allen L.V. Jr (2001). Veterinary compounding, Secundum artem. *Current & Practical Compounding Information for the Pharmacist*, 7 (2):-87-92.

Almond G. and Monahan K. (2011) Water medication with antibiotics. North Carolina Swine Veterinary Group.

Arduin M. (2004) L'acqua di bevanda. *Veneto Agricoltura*. Scheda di aggiornamento n. 72

Associazione Farmaceutici dell'Industria (1991). L'acqua nell'industria farmaceutica. Manuale tecnico n° 3, 1a Ed., 1-46, OEMF, Milano.

Carr J. (2002) Water systems – troubleshooting common mistakes. *Proc ISU Swine Dis Conf Swine Pract*.,99–110.

Canadian Water Quality Guidelines for Livestock: 1987. Task force on water quality guidelines.

Centennials (1989) The Merck Index, 11th Ed, Merck and Co Inc., Rahway, N.J., U.S.A.

Chemical and physical properties of water. http://www.62.co.za/water_properties.html (visited 07/03/2013).

Consiglio dell'Unione europea (1998). DIRETTIVA 98/83/CE DEL CONSIGLIO concernente la qualità delle acque destinate al consumo umano. Gazzetta ufficiale delle Comunità europee, pagg. 32-54.

Council Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption as amended by Council Directives 81/858/EEC and 91/692/EEC (further amended by Council Regulation 1882/2003/EC).

Croubels S., Vrielinck J., Baert K. (2001) A special case of acute tiamulin-salinomycin intoxication by tiamulin four months after medications. *VI. Diergen. Tijdschr.*, 70: 54-58.

DECRETO LEGISLATIVO 2 febbraio 2001, n. 31. Attuazione della direttiva 98/83/CE relativa alla qualità delle acque destinate al consumo umano

Doerschuk A., Bitler B., McCormack J. (1955) Reversible isomerizations in the tetracycline family. *J. Amer. Chem. Soc.,* 77: 4687-4695.

Dorr P.M (2005). Residue and pH effects on therapeutic products administered through the water. *Abstract presented at Healthy Hogs Seminar; October 2005*; Sampson Community College, Clinton, North Carolina.

Dorr, P.M., Madson D., Spencer W., Scheidt A. B., Almond G. W. (2009) Impact of pH modifiers and drug exposure on the solubility of pharmaceutical products commonly administered through water delivery systems. *Journal of Swine Health and Production.* 17(4), p. 217-222.

D.P.R. 24 maggio 1988, n. 236 Attuazione della direttiva n. 80/778/CEE concernente la qualità delle acque destinate al consumo umano ai sensi dell'art. 15 della legge 16 aprile 1987, n. 183 Pubblicato nella G.U. 30 giugno 1988, n. 152, S.O.

European Agency for the Evaluation of Medicinal Products (2002). Note for guidance on quality of water for pharmaceutical use, CPMP/QWP/158/01 Revision – EMEA/CVMP/115/01 Revision, London.

European Agency for the Evaluation of Medicinal Products (2003). EMEA/CVMP/540/03-CONSULTATION. Note for Guidance on "Quality aspects of pharmaceutical veterinary medicines for administration via drinking water".

Food and Drug Administration (FDA). http://www.fda.gov/default.htm (visited 21/01/2013).

Food and Drug Administration – Department of Health and Human Services, Oral dosage form new animal drugs, Soluble bacitracin methylene disalicylate. http://law.justia.com/cfr/title21/21-6.0.1.1.9.0.1.33.html. (visited 28/02/2013).

Food and Drug Administration – Department of Health and Human Services, Oral dosage form new animal drugs, Gentamicin sulfate oral

solution http://www.gpo.gov/fdsys/pkg/CFR-2011-title21-vol6/xml/CFR-2011-title21-vol6-part520.xml. (visited 02/03/2013).

Kunesh J., Anderson P., Runnels L., Larson R., Communications Specialist-Agriculture, Iowa State University. http://www.ca.uky.edu/agc/pubs/id/id69/id69.htm. (visited on 06/03/2013).

Kunesch, J. P. (1986). Therapeutics. In: A. D. Leman, B.Straw, R. D. Glock, W. L. Mengeling, RHE. Penny and E. Scholl (Ed.) Diseases of Swine (6th Ed.). pp 8M12. Iowa State Univ. Ress, Ames.)

La stabilità dei farmaci. http://www.galenotech.org/stabilit.htm (visited 21/01/2013).

Lawhorn B. (1998) Swine pneumonia. *Texas Agricultural Extension Service*. Publication L-5203.

Manual of water medication (2011) Published by Elanco Animal Health.

Marco, E. (2009) Efecto del pH sobre las medicaciones y possibile correcciones. *Marco i Colell S. L.* España.

Ministero della Sanità (1988), D.P.R. 24 Maggio 1988, n° 236. Attuazione della direttiva CEE n° 80/778 concernente la qualità delle acque destinate al consumo umano, ai sensi dell'art. 15 della L. 16 aprile 1987, n° 183. Gazzetta Ufficiale, serie generale, n° 152 del 30 Giugno 1988.

McCormack J., Fox S., Smith L., Bitler B., Reichenthal J., Origoni V., Muller W., Winterbottom R., Doerschuk A. (1957) On the nature of reversible isomerization of the tetracycline family. *J. Amer. Chem. Soc.*, 79: 2849-2857.

Moisescu S., Antonescu V., Vanghelie I. (1975). Farmacia, 23: 43-48.

Osborn E., Baum JL, Ernst C, Koch P. (1976) The stability of ten antibiotics in artificial tear solutions. *Am. J. Opththalmol.*, 82: 775-780.

Pijpers A. Schoevers E. J., van Gogh H., van Leengoed L. A., Visser I. J., van Miert A. S., Verheijden J. H. (1991) The influence of disease on feed

and water consumption and on pharmacokinetics of orally administered oxytetracycline in pigs. Journal of Animal Science 69,
p. 2947-2954.

Reeve-Johnson L. (1998) Water medication and the treatment of respiratory diseases in pigs. *The Pig Journal (Proceedings Section)*, p. 74-86

Schneider P. (2003). The why's and how's of eliminatine clinical Salmonella. London Swine Conference – Maintaining Your Competitive Edge, 9-10 April, 9-16.

Servais H., Tulkens M.P. (2001) Stability and compatibility od ceftazidime administered by continuous infusion to intensive care patients. *Antimic. Ag. Chemother.*, 45: 2643-2647.

The Extra Pharmacopoeia "Martindale", 26th Edition, The Pharmaceutical press, London.

USGS, “The USGS Water Science School http://ga.water.usgs.gov/edu/waterproperties.html (visited 09/03/2013)

Van Heugten, E. (2011) Guidelines for water quality in pigs. *Animal science facts.* Pubblication number ANS00.811S.

Vervaet C., Voorspoels J., Kiekens F., Remon J.P. (2003) Reduced availability of trimethoprim/sodium sulfadiazin and doxycyclin hyclate in drinking water systems, *Int. J. appl. Res. Vet. Med.*, 1 (1): 50-55.

Zaghini, A. (2005). Water as a vehicle for applying drugs orally in pig production: advantages and defects. *Large Animals Review* Vol. 11 No. 2 pp. 3-9