

**THE FORMULATION OF DIFFERENT DOSAGE FORMS
WITH THE ANTHELMINTICS: LEVAMISOLE,
NICLOSAMIDE AND OXYCLOZANIDE**

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ABSTRACT

Different formulations of dosage forms of niclosamide, levamisole HCl or levamisole HCl and oxcyclozanide have been used on the local market for a very long time. Unfortunately, the spectrum of each of the drugs alone has a very narrow therapeutic index. Together, however, they have a broad spectrum of action and it is a solution to most of the helminths infestations today. Because of the increasing cost to produce livestock, it is important to formulate a product that is not only pharmacologically effective but also cost effective. A combination dosage form plays a big role in decreasing the cost of live stock production in that only one product is bought instead of more.

A solution, suspension and tablet were formulated and tested. Niclosamide is very slightly soluble in water and is difficult to solubilise. A solution with niclosamide 3%, oxcyclozanide 3.4%, and levamisole HCl 2,5% was formulated. This was done because of the assumption that niclosamide is better absorbed in a solution and that the effective concentration needed is much lower. A suspension and a dispersible tablet with niclosamide 20%, oxcyclozanide 3.4%, and levamisole HCl 2,5% was formulated where the tablet is specially formulated to accommodate the smaller live stock farmers.

All the above dosage forms were tested under accelerated stability conditions. Physical and chemical tests have been conducted on the dosage forms before and after storage at accelerated stability conditions. An assay was done on each of the three dosage forms where a HPLC method was developed and validated as there is no method in the literature that describe the simultaneous analyses of levamisole HCl, niclosamide and oxcyclozanide in a dosage form.

The results of al three dosage forms were very encouraging. The solution proved to be the most promising of all the formulations, and it might be worth while to put such a product on the market.

UITTREKSEL

Verskillende formulerings van die doseervorme vir die geneesmiddels niklosamied, levamisool HCl of levamisool HCl en oksiklosanied is al vir 'n geruime tyd op die plaaslike mark beskikbaar. Ongelukkig het die geneesmiddels alleen 'n baie nou terapeutiese indeks. Saam het dit egter 'n breër spektrum van werking en kan vandag teen meeste van die helmint infestasies effektief wees. As gevolg van die hoë produksiekoste van vee is dit belangrik om 'n produk te formuleer wat nie net farmakologies effektief is nie, maar ook koste-effektief. 'n Kombinasie doseervorm het die voordeel dat dit die produksiekoste van vee afbring deurdat net een geneesmiddel produk aangekoop kan word in plaas van meer.

'n Oplossing, 'n suspensie en 'n tablet is geformuleer onder versnelde stabiliteitstoestande. Niklosamied is swak oplosbaar in water en daarom is dit moeilik om die niklosamied oplosbaar te maak. 'n Oplossing van niklosamied 3%, oksiklosanied 3.4% en levamisool HCl 2.5% is geformuleer. Dit is gedoen as gevolg van die aanname dat niklosamied beter geabsorbeer word in oplossing en dat die effektiewe konsentrasie benodig, baie laer sal wees. 'n Suspensie en 'n dispergeerbare tablet met niklosamied 20%, oksiklosanied 3.4% en levamisool HCl 2.5%, is ook geformuleer waar die tablet spesiaal geformuleer is om kleiner veeboere se behoeftes te akkommodeer.

Stabiliteitstoetse is op al die bogenoemde doseervorme gedoen. Die fisiese en chemiese parameters is beide voor en na die versnelde stabiliteitstoetse in ag geneem. 'n HPLC metode is ontwikkel en gevalideer aangesien daar nog nie enige data beskikbaar is oor die gelyktydige analise van levamisool HCl, niklosamied en oksiklosanied in 'n enkel doseervorm nie.

Die resultate van al drie die doseervorme lyk belowend. Van al drie formulerings was die oplossing die indrukwekkendste

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THE AIMS AND OBJECTIVES OF THIS STUDY

BACKGROUND

The labour and the cost of treating animals are becoming an increasingly important factor in the production of animals for purposes of meat and milk. It is therefore important that the formulator's formulations can be adapted to mass medication techniques using mechanical equipment.

In this study, it was decided to take the oral route of administration as the preferred dosage form.

According to Pope and Baggot (1983:37), once the route of administration and the pharmacokinetics have been established, the advantages and disadvantages of the different formulation types should be considered before initiating a pilot study.

SOLUTIONS

This dosage form presents the drug component in a form most suitable for absorption. Thus, if there is no complexation or micellisation with components of the formula, and if there is no degradation in the stomach, this dosage form will give maximal bioavailability and a rapid onset of action (Pope & Baggot, 1983:37).

SUSPENSIONS

A suspension may be considered the most desirable dosage form for a particular drug for a number of reasons. The suspension may be preferred over a unit dose solid dosage form as it presents most of the advantages of the solution dosage form: it provides ease in swallowing, it gives greater ease in administration of

usually large doses and it also provides infinite variability of dosage (Pope & Baggot, 1983:41).

TABLETS

A tablet or bolus offers many advantages over other dosage forms and should be one of the first considerations for oral dosing. The tablet offers ease of administration of an "accurate" dose, and is readily adaptable to various dose sizes of medicinal substances. Most formulations have two or more dose strengths. Tablets are portable and compact. They do not have as large a bulk as a liquid or suspension dosage form. They usually present the fewest problems with respect to stability and they are quite economical compared to other dosage forms (Pope & Baggot, 1983:45).

CONCLUSION AND CONTRIBUTION

Levamisole is water-soluble and niclosamide and oxclozanide are poorly water-soluble drugs. The poor solubility makes it more difficult to formulate into a solution but easier into a tablet and a suspension. Formulating a solution would be a priority to possibly increase absorption. Taking all considerations into account, formulating a wide spectrum anthelmintic can assist in the battle against worms and worm infestations.

The aim of this study is to:

- review the anatomy of the ruminant digestive tract.
- review the parasites causing helminthiases in ruminants.
- identify the most effective type of dosage form to treat helminthiases.
- review the pharmacology and characteristics of the anthelmintics levamisole HCl, niclosamide and oxclozanide.
- do a preformulation study

- formulate and develop a dispersible tablet, a solution and a suspension of a combination of levamisole HCl, niclosamide and oxclozanide.
- develop and validate a HPLC method to assay the combination of the above mentioned three drugs.
- do accelerated stability trials on the developed dosage forms.

Every livestock farmer has an infestation of worms occasionally in his livestock. These helminth infections cause a loss of condition and a loss of weight, which in turn causes lower prices and less money. Careful observation of the symptoms can help in diagnosing the problem at an early stage. Early treatment or cyclic treatments can lessen or stop the degree of the helminth infections.

Although these anthelmintic drugs have been used alone or in different combinations in the veterinary market for several years, there is always a need and a search for cheaper, newer and better anthelmintics and anthelmintic combinations

Chapter 1

1.1. Background

Since the earliest times man tried to heal the sick, be it humans, or animals. Knowledge was carried over from generation to generation. Although the medication they used in earlier times worked well, the parasites that infected man and animal over the years developed resistance and it became more difficult to treat the patient and cure the disease. This started the search for newer methods and better drugs to treat disease.

In the early years of drug development, the compounds discovered were usually effective only against some of the parasites in one of the major groups, such as the helminths. In this case, the drugs were collectively called anthelmintics.

Anthelmintics are drugs used to rid the body of helminths (a helminth is a worm or a wormlike parasite). The term anthelmintic applies to agents that act either locally to expel worms from the gastrointestinal tract or systemically to eradicate species and developmental forms of helminths that invade organs and tissues. Anthelmintics are used to treat acute infections but it is mostly used as a prophylactic measure to prevent infections.

Anthelmintics have been on the market for many years now in the battle to control endo- and ecto-parasites in animals. There are lots of different parasites that affect livestock. Although these parasites seem to be of less importance, it can cause a lot of damage and can lead to the death of livestock. It is important in this struggle against parasites that the medicine must not only kill the parasites, it must also inhibit further growth and its multiplication. It is essential to control endoparasites for the welfare of the animal and to promote optimal growth and production.

In a recent joke of the history of medicine the following was said:

"Doctor, I have an ear ache."

2000 BC - Here, eat this root.

1000 BC - That root is heathen. Here, say this prayer.

1805 AD - That prayer is superstition. Here, drink this potion.

1940 AD - That potion is snake oil. Here, swallow this pill.

1985 AD - That pill is ineffective. Here take this antibiotic.

2000 AD - That antibiotic doesn't work anymore. Here, eat this root.....

In South Africa the leaf of the *Cotyledon orbiculata* (pigs ear or plakkie) is chewed to expel worms. The *Albizia anthelmintica* is also commonly used as an effective anthelmintic for tapeworm and other worms in humans and stock animals. Other plants used as anthelmintics include: *Acorus Calamlis*, *Albizia Adianthifolia*, *Aster Bakeranus*, *Ballota Africana*, *Ekebergia Capensis*, *Embelia Ruminata*, *Heteromorpha Arborescens*, *Punica Granatum*, *Rumex Lanceolatus* and *Sansevieria Hyacinthoides* (Van Wyk *et al.*, 2000:28-224).

Over the years, pharmaceutical dosage forms have developed into the medicine we all know today. Where the bark, leaves or root were once chewed or boiled and drank, it changed to highly developed dosage forms of injections, suspensions, solutions, tablets, capsules, etc.

This dissertation addresses the development and the formulation of anthelmintic dosage forms for sheep, cattle and goats.

1.2. Special considerations in veterinary formulation design

1.2.1. Introduction

Renewed interests in the formulation and design of new veterinary drugs, combinations and delivery systems have emerged in the last few years. It is said that the veterinary market is as hungry for new products now as it has been in the human pharmaceutical market ten years ago. The future in the formulation of veterinary products in South Africa and other parts of the world are bright.

Different species and the different disease states in animals provide an exciting challenge to the formulator. To develop a drug and its delivery system for use in veterinary medicine requires special precautions and considerations (Pope & Baggot, 1983:124-125).

1.2.2. Dietary habit

The dietary habit provides a broad basis for the grouping or classification of domestic animal species. They may be grouped as herbivores, omnivores and carnivores. In terms of physiological function, the digestive system is the principal distinguishing feature between herbivorous and omnivorous species. Based on limited available information, it appears that the half-lives of drugs, which undergo extensive hepatic metabolism, are considerably shorter in herbivorous species than in carnivorous species. . The urinary pH is determined by the diet. Carnivores excrete acidic urine, herbivores excrete alkaline urine and the pH of pigs may vary depending on the diet (Pope & Baggot, 1983:124-125).

1.2.3. Gastro intestinal tract

1.2.3.1. Anatomy of domestic animals

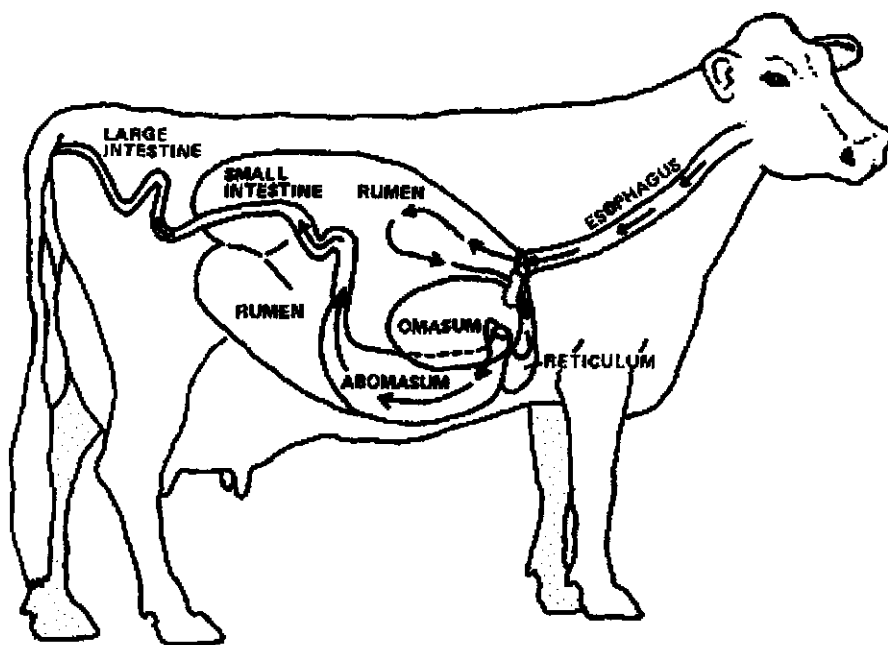


Figure 1. Anatomy of the adult digestive tract of the cow (University of Minnesota: 1996).

For the most part, the digestive system of ruminants is very similar to that of other mammals, but the stomach is considerably different from the so-called "monogastric" condition. The word "ruminant" comes from the Latin *ruminare*. In monogastric animals, the stomach's functions are limited to temporary storage and preliminary mastication of the food into a liquid mass; little or no absorption of nutrients takes place. In ruminants,

however, the stomach has an absorption function in addition to the usual functions of mastication and acidification. The key to the ruminant digestive system's efficiency is the presence of symbiotic microorganisms in it, particularly in the forestomachs (Caceci^a, 2002).

The Forestomach Components

The four divisions of the ruminant stomach are the rumen, the reticulum, the omasum, and the abomasum.

The stomach

The stomach is very large and occupies nearly three quarters of the abdominal cavity. It consists of four parts, viz., rumen, reticulum omasum and abomasum. The first three parts are lined with mucous membranes which is covered with squamous stratified epithelium and is non glandular. The abomasum has a glandular mucous membrane and is therefore called the "true stomach". The abomasum joins the small intestine (Caceci^a, 2002).

The Ruminant Animal

Due to the unique physiological characteristics of the digestive system of ruminants, these food-producing animals have offered and continue to offer many new opportunities for the development of dosage forms and delivery devices.

Physiological Principles

Ruminant animals have evolved an anterior digestive organ called the rumenoreticulum, which functions as the initial site for the breakdown of less digestible cellulose food sources. An active fermentation by symbiotic bacteria and protozoa accomplishes this. The animal to fulfill its own metabolic requirements for growth and maintenance utilises the fermentation metabolites as well as the microorganisms themselves. The rumenoreticulum can be envisioned as a "fermentation vat". typically 100 to 200 liters in the cow and 10 to 15 liters in the sheep. It has an entrance port at the proximal end of the esophagus through which food and buffering saliva enter and an exit sphincter through which the rumen contents enter the abomasum, or "true stomach", after passing through a "strainer", the omasum. The remaining digestive tract is similar to that of most other monogastric mammals (Caceci^a, 2002).

1.2.3.1.1. General Anatomy and Physiology of the Ruminant Digestive System

Forestomach (Rumeno reticulum)

This fermentation vat is composed of two areas called the reticulum and the rumen. The reticulum has a distinctive "honeycomb" appearance. It aids to help bring boluses of feedback up to the mouth for rechewing. It also serves as a receptacle for heavy foreign objects that it eats (Umphrey & Staples, 1992).

Rumen

The rumen is, by far, the largest compartment. Its purpose is to store large quantities of feed, to keep the feed mixing by strong contractions, and to provide a suitable environment for the bacteria and protozoa to live in. This environment is kept agreeable to the microorganisms by maintaining a relatively constant temperature and pH and by removing many of their waste products. Most of the waste products are volatile fatty acids. These volatile fatty acids are the primary sources of energy for the ruminant. They are absorbed through the rumen wall (Umphrey & Staples, 1992).

Omasum

Once the feed has been reduced in size by chewing and digested by the bacteria and protozoa, it can pass into a third compartment called the omasum. It has the appearance of an open book with three sides bound. The tissues within are likened to the pages of a book and are called leaves. Up to 100 leaves can be found in the omasum. These leaves have small papillae on them, which absorb a large portion of the volatile fatty acids that were not absorbed through the rumen wall. Water and electrolytes such as potassium and sodium are likely absorbed here as well, thus drying out the feedstuffs before they enter the next compartment (Umphrey & Staples, 1992).

Abomasum

This fourth and last compartment which makes up the ruminant's stomach is the abomasum or "true" stomach as it is called because it functions in a very similar way to the stomach of a man or pig. As in the omasum, the abomasum contains many folds to increase its surface area. These folds are called leaves that enable the abomasum to be in contact with the large amounts of feed passing through it daily. The walls of the abomasum secrete enzymes and hydrochloric acid. The pH of the digesta coming into the

abomasum is around 6.0 but is quickly lowered to about 2.5 by the acid. This creates a proper environment for the enzymes in which to function. The main digestive function of the abomasum is the partial breakdown of proteins. The enzyme pepsin is responsible for this. Proteins from the feed and the microorganisms coming from the rumen are broken down into peptides (Umphrey & Staples, 1992).

Small intestine

Next in the digestive process is the small intestine, a 4.5 m long, 5 cm wide tube. As the feed enters the small intestine, it mixes with secretions from the pancreas and liver, which elevate the pH of the digesta from 2.5 to between 7 and 8. This higher pH is necessary for enzymes in the small intestine to work. In order for the ingested feed to become available to the cow, they must be broken down into smaller molecules. These enzymes reduce any remaining proteins to amino acids, starch to glucose, and complex fats into fatty acids. Much of that occurs in the small intestine using enzymes and hormones from the pancreas, liver, and small intestine. Absorption of these nutrients also occurs in the lower half of the small intestine. The intestinal wall contains numerous "finger-like" projections called villi that increase the surface area of the intestine to aid in the absorption process. Muscular contractions aid in mixing the digesta and moving it down to the next section (Umphrey & Staples, 1992).

Large intestine

The cecum, colon, and rectum make up the rest of the digestive tract. They are collectively referred to as the large intestine. Its primary purpose is to absorb water from the digesta, thus making it more solid. Bacteria living in the intestine work at digesting any feedstuffs which escaped digestion earlier. Usually this contributes less than 15% of the total digestion. Between these bacteria and those that passed out of the rumen, up to 50% of the dry weight of the faeces can be of microbial origin (Umphrey & Staples, 1992).

1.2.3.1.2. Rumen

The rumen has numerous finger-like projections projecting from its wall. These are the ruminal papillae, which are somewhat conical in three-dimensional view as seen in Figure 2. There are several interesting histological features. First, the papillae are covered with stratified squamous epithelium. Moreover, it is keratinised, which is a little unusual by the standards of non-ruminant animals, but it is normal here. Despite keratinisation, absorption

occurs across the epithelium in the rumen. There are no muscularis mucosae in the rumen: the lamina propria and the submucosa combine to form a single underlying support for the epithelium. Nor are there any glands present in either the mucosa or submucosa (Caceci^d, 2002).



Figure 2. Rumen papillae (Caceci^d, 2002).

1.2.3.1.2.1. Rumen fluid

In a recent study done by Biro *et al.* (1998:77-90) the dissolution of albendazole in an anthelmintic veterinary suspension was investigated. The purpose of their study was to examine the liberation of active substance from albendazole containing suspensions in artificial rumen and abomasal fluids of cattle.

According to Biro *et al.* (1998) It is important to select the correct dissolution medium for in vitro measurements and dissolution. The problem with rumen fluid for dissolution is that neither the European Pharmacopoeias nor the USP23 prescribe dissolution media suitable for rumen modelling. They tested several buffer solutions to select a suitable rumen model (Biro *et al.*, 1998:77-90).

The first of these was a phosphate buffer solution (see Table 1.) analogous with the intestine model of the Pharmacopoeias.

Table 1. Michaelis' phosphate buffer (Biro *et al.*, 1998:77-90).

Michaelis' phosphate buffer	
KH ₂ PO ₄	(1/15 mol/dm ³)
Na ₂ HPO ₄ ·2H ₂ O	(1/15 mol/dm ³)
7:03	

The second (see Table 2) and the third (see Table 3) buffer solutions approximate natural conditions more closely since the buffer capacity of the rumen fluids is mostly influenced by three short chained aliphatic organic acids. These are: acetic acid, propionic acid and butyric acid.

Table 2. Britton-Robinson's acetic acid/sodium acetate buffer (Biro *et al.*, 1998:77-90).

Britton-Robinson's	
Acetic acid/sodium acetate buffer	
CH ₃ COOH 96%	(2.3 cm ³ /1000 cm ³)
NaOH	(0.1 mol/dm ³)
100:46:00	

Table 3. Buffer taken from the literature in Biro *et al.* (Biro *et al.*, 1998:77-90).

Buffer taken out of the literature	
CH ₃ COOH	65 mmol/l
CH ₃ CH ₂ COOH	21 mmol/l
CH ₃ CH ₂ CH ₂ COOH	14 mmol/l
NH ₄ OH	5 mmol/l
NaOH	98 mmol/l
1:1:1:1:1	

It was concluded that the third buffer solution taken from the literature was the most similar to the natural medium (Biro *et al.*, 1998:77-90).

1.2.3.1.3. Reticulum

In Figure 3, it can be seen why the reticulum is sometimes described as a "honeycomb". In the reticulum, there is a muscularis mucosa, of rather odd type. It consists of bands of smooth muscle which run through the tops of the ridges of the honeycomb, and which are

more or less isolated from the lower levels, nearer the wall. It can be shown in gross specimens that this smooth muscle is continuous with that of the esophagus. The contraction of the muscularis mucosa serves to contract the openings of the honeycomb, somewhat like a purse-string closes a purse. The epithelium on the surface is a keratinised stratified squamous type, and there are no glandular elements (Caceci^b, 2002).



Figure 3. The mucosa of the reticulum (Caceci^b, 2002).

1.2.3.1.4. Omasum

The omasum has a muscularis mucosa, which underlies the epithelium, but in addition to muscularis mucosa, there is an excursion of smooth muscle from the tunica muscularis up between the folds (as seen in Figure 4) of the muscularis mucosa. What at first glance appears to be a "spine" of muscle running up through each of the "leaves" of the mucosa, can be resolved on careful examination as a three-layered structure: muscularis mucosa on the outside, with tunica muscularis inside it. If you follow the central strand of muscle outwards, you should find where it comes off the inner layer of the tunica muscularis. There is a very scanty bit of submucosa separating the two types of smooth muscle, but it's so tenuous that you may not be able to make it out.

If you were to pass a rod through a mucosal fold from side to side, you would go through the following sequence: epithelium / lamina propria / muscularis mucosae / submucosa / tunica muscularis / submucosa / muscularis mucosae / lamina propria / epithelium. The epithelium is stratified squamous, and there are no glands (Caceci^c, 2002).



Figure 4. The folds of the omasum (Caceci^c, 2002).

1.2.3.1.5. Abomasum

The surface epithelium here is simple columnar, not stratified squamous. There are gastric pits (foveolae) and below those, there are gastric glands of the fundic type. The glands contain parietal cells (which make hydrochloric acid) and chief or zymogenic cells (which make digestive enzymes). The submucosa of this region is much more developed than in the previous divisions of the stomach. The abomasum, unlike the rumen, reticulum, and omasum, does not absorb nutrients. As with monogastric animals, it prepares food for enzymatic breakdown and absorption in the small intestine (Caceci^a, 2002).

1.2.4. Metabolism

Most drugs are eliminated by a combination of biotransformation, mainly hepatic metabolism and renal excretion. From knowledge of the functional group in a compound, probable pathways for biotransformation can be predicted. Although pathways may be predicted, biotransformation rates may vary between species and will therefore govern the rate of elimination. These biotransformation rates may also determine the principal pathway for metabolism (Pope & Baggot, 1983:124-125).

1.2.4.1. Biopharmaceutics

The concept of bioavailability

The therapeutic response of a drug is normally dependent on an adequate concentration of the drug being achieved and then maintained at the site or sites of action of the drug. In

the case of systemically acting, it is generally accepted (for clinical purposes) that a dynamic equilibrium exists between the concentration of the drug at its site of action and the concentration of the drug in blood plasma.

An important consequence of this dynamic equilibrium is that it permits a therapeutically effective concentration of the drug to be achieved at its site of action by adjustment of the concentration of the drug in blood plasma.

The concentration of the drug in blood plasma depends on numerous factors.

These include: The relative amount of an administered dose that enters the systemic circulation.

The rate at which this occurs.

The rate and extent of distribution of the drug between the systemic circulation and other tissues and fluids.

The rate of elimination of the drug from the body.

Apart from the intravenous route of drug administration, where a drug is introduced directly into the blood circulation, all other routes of administering systemically acting drugs involve the absorption of the drug from the place of administration into the blood. The drug must be absorbed in a sufficient quantity and at a sufficient rate in order to achieve a certain blood plasma concentration, which, in turn, will produce an appropriate concentration of the drug at its site(s) of action to elicit the desired therapeutic response. It follows that there are two aspects of drug absorption which are important in clinical practice: the rate and the extent to which the administered dose is absorbed (Proudfoot, 1988:132).

The administered dose of a particular drug in an oral dosage form will be 100% bioavailable only if the drug is completely released from the dosage form into solution in the gastrointestinal fluids. The released drug must also be completely stable in solution in the gastrointestinal fluids and all of the drug must pass through the gastrointestinal barrier into the mesenteric circulation without being metabolised. All of the absorbed drug must pass into the systemic circulation without being metabolised on passing through the liver (Proudfoot, 1988:131).

Thus, any factor which adversely affects either the release of the drug from the dosage form, its dissolution in the gastrointestinal fluids, its stability in the gastrointestinal fluids, its permeation through and stability in the gastrointestinal barrier, or its stability in the hepatic portal circulation will influence the bioavailability exhibited by that drug from the dosage form in which it was administered (Proudfoot, 1988:132).

The concept of biopharmaceutics

Many factors have been found to influence the time course of a drug in the plasma and hence at its site(s) of action.

These include: the foods eaten by the animal,

the effect of the disease state on drug absorption,

the age of the animal, the site of absorption of the administered drug,

the administration of other drugs,

the physical and chemical properties of the administered drug,

the type of dosage form,

the composition and method of manufacture of the dosage form and the size of the dose and frequency of administration of the dosage form (Proudfoot, 1988:132).

A given drug may also show differences in its bioavailability from one type of dosage form to another when given by the same route, e.g. a tablet, a hard gelatin capsule and an aqueous suspension administered by the peroral route. A given drug might show different bioavailabilities from different formulations of the same type of dosage form given by the same route of administration, e.g. different formulations of aqueous suspensions of a given drug administered by the peroral route. Variability in the bioavailability exhibited by a given drug from different formulations of the same type of dosage form or from different types of dosage forms etc., can cause animals to be under- or overmedicated. The result may be therapeutic failure or serious adverse effects particularly in the case of drugs that have a narrow therapeutic range (Proudfoot, 1988:133).

The entry of a drug into the systemic circulation following the administration of a drug product usually involves: the release of the drug from its dosage form into solution in the biological fluids at the absorption site and the movement of the dissolved drug across biological membranes into the systemic circulation (Proudfoot, 1988:133).

Drug absorption from the gastrointestinal tract

It is evident that the rate and extent of appearance of intact drug into the systemic circulation depends on a succession of rate processes. The slowest step in this series of rate processes, which is known as the rate-limiting step, will control the overall rate and extent of appearance of intact drug in the systemic circulation. The particular rate-limiting step may vary from drug to drug. Thus, for a drug, which exhibits a very poor aqueous solubility, the rate at which the drug dissolves in the gastrointestinal fluids is often the slowest step and therefore exhibits a rate-limiting effect on a drug bioavailability. In contrast, for a drug which has a high aqueous solubility, its dissolution rate will be rapid and the rate at which the drug crosses the gastrointestinal membrane may be the rate-limiting step. Other potential rate-limiting steps include: the rate or release of the drug from the dosage form, the rate at which the stomach empties the drug into the small intestine, the rate at which the drug is metabolised by enzymes in the intestinal mucosal cells during its passage into the mesenteric blood vessels and the rate of metabolism of the drug during its initial passage through the liver (Proudfoot, 1988:133-137).

The gastrointestinal tract consists of three major anatomical regions: the stomach, the small intestine and the large intestine (colon). The small intestine includes the duodenum, jejunum and ileum. As a drug descends through these regions of the gastrointestinal tract, it encounters different environments with respect to pH, enzymes, electrolytes, fluidity and surface features, all of which can influence drug absorption (Proudfoot, 1988:137).

The gastrointestinal tract is basically a hollow muscular tube composed of four concentric layers of tissue named from the innermost to the outermost as the mucosa (or mucous membrane), the submucosa, the muscularis externa and the serosa.

Of these four layers, the mucosa is the most important with respect to the absorption of drugs from the lumen of the gastrointestinal tract. The mucosa contains the cellular membranes and regions through which a drug must pass in order to reach the blood. The mucosa itself consists of three layers: the lining epithelium, the lamina propria and the muscularis mucosa. The epithelium lining and the lumen of the gastrointestinal tract

comprises of a single layer of columnar and some specialized secretory cells (Proudfoot, 1988:137).

In the stomach, the mucosa contains many folds which increase the total surface area over that afforded by a flat smooth lining. Although the stomach does not function primarily as an absorption organ, its excellent blood supply and the fact that a drug can potentially reside in the stomach for 30 minutes up to several hours in contact with a reasonably large surface area, is conducive to the absorption of certain drugs, e.g. weak acidic drugs.

The small intestine is the most important site for drug absorption in the gastrointestinal tract. The outstanding anatomical feature of the small intestine is the tremendously large epithelial surface area through which drug absorption can take place. This large epithelial surface area results from the existence of folds in the intestinal mucosa known as the folds of Kerckring, villi and microvilli.

Villi are finger-like projections which arise from the entire mucosal surface of the small intestine. Each villus is covered by a single continuous layer of epithelium which is made up primarily of the columnar absorption cells and the mucus-secreting goblet cell. In terms of drug absorption from the small intestine, the columnar cells are extremely important since it is the anatomical structure of the apical surface of each columnar cell which further increases the epithelial surface area of the small intestine that is available for drug absorption.

The large intestine like the stomach lacks villi. However, the large intestine serves as a site for the absorption of the drug which has not been completely absorbed in the more proximal regions of the gastrointestinal tract, i.e. the stomach and small intestine. Incomplete drug absorption in the more proximal regions may be due to the physicochemical properties of the drug itself, or a result of the intended slow release of the drug from a prolonged/sustained/controlled release dosage form. In general, if a large proportion of an orally administered dose of drug reaches the large intestine, it is likely that the drug will exhibit poor bioavailability (Proudfoot, 1988:138).

1.2.5. Renal excretion

When a significant fraction of the dose is eliminated by renal excretion, the urinary pH reaction will influence the excretion rate of a weak organic electrolyte. Urinary pH will affect the rate of excretion of acids as elucidated by the pH portion hypothesis which relates dissociation constant, lipid solubility and the pH at the absorption with the absorption characteristics at that site. Herbivorous species excrete a slightly alkaline urine, pH 5.5-7.0. In any species, however, urinary pH depends mainly upon diet (Pope & Baggot, 1983:127).

1.2.6. Age

The age of an animal affects a number of parameters. Studies conducted in this specific field show that drugs may be more widely distributed in neonatal animals than in mature animals of the same species. In addition, the structure of the gastrointestinal tract may be different in the neonate as compared to the adult. At birth, in ruminants, the capacity of the rumen and the reticulum is smaller in relation to the abomasum in the adult. The development of these organs is highly dependent upon diet. The onset of rumination is considerably slower in animals subsisting on milk alone than in free ranging animals (Pope & Baggot, 1983:128-129).

1.2.7. Disease state

Drug distribution and elimination is likely to be affected in disease states, such as scours, impaired renal function, congestive heart failure, fever and in physiological conditions such as pregnancy and dehydration (Pope & Baggot, 1983:129).

1.2.8. Drug residues

Ruminant animals, since they are raised primarily as a food source, present the additional consideration of residual drug in tissues. When drugs are administered to meat-producing animals, a waiting period, called the withdrawal time, is prescribed by the regulatory agencies. The meat may not be slaughtered for human consumption before this period. The duration of this withdrawal period varies depending on the drug and the dose used (Pope & Baggot, 1983:129-130).

Table 4. The withdrawal times of levamisole HCl, niclosamide and levamisole & oxclozanide from milk and meat (Swan, 2000:40-43).

	Meat	Milk
--	------	------

Levamisole	7 days	Zero days
Niclosamide	8 days	Not used
Levamisole & Oxyclozanide	7 days	Zero days
Oxyclozanide	7 days	Zero days

Table 4 shows that the withdrawal times for meat are between 7 and 8 days. In milk, however, it (niclosamide) is either not used or it has no withdrawal time at all. This means that it can be given to pregnant cows and ewes with safety.

1.3. The ideal anthelmintic

Control measures against the helminths of our domestic animals depend to a large extent on the use of anthelmintic drugs. There is a large number of anthelmintics available for use today. The number of the different products is a testament to the widely accepted view that any level of parasitism is bad and that infected animals must be treated.

In developing anthelmintics, new products are measured against an ideal standard. While no anthelmintic has ever met all the criteria we might consider ideal, some of the more recently developed anthelmintics have come very close.

An ideal anthelmintic should have the following properties:

It should have a broad spectrum of activity; it should be non-toxic; it should be easy to administer; it should be cost effective; it should have a wide safety margin and it should be rapidly metabolised and excreted (Johnstone^d, 1998).

Broad Spectrum of Activity

An anthelminthic should be effective against all parasitic stages of a nematode. There is a clear need for an anthelmintic to be effective against developing larval stages (including arrested L4's) as well as adults. From both a practical and an economic point of view, it would be beneficial to have a drug that is effective against a broad spectrum of parasites (Johnstone^d, 1998).

Non-Toxic/Broad Safety Margin

An anthelmintic should be relatively non-toxic and have a wide safety margin. A number of organophosphates have been found to be useful as anthelmintics. The margin of safety in these products is relatively narrow compared to some of the anthelmintics in use today. In

practice, most anthelmintics in larger animals are given to a herd of animals and most of the times the handlers do not have formal education. This can bring forth the problem of under- and overdosing, which may lead to toxicity in drugs with a narrow safety margin (Johnstone^d, 1998).

Ease of Administration

Anthelmintics should be easy to administer under practical conditions. Oral anthelmintics have the biggest segment of sales in the South African market. This is followed by injectable and pour-on anthelmintics. Although the products given orally should be palatable to dosed animals, it is not always easy to formulate such a product. In ruminants the most common products are oral and injectable anthelmintics. In cats and dogs, tablets are the dosage form of choice and in pigs and poultry the easiest dosage form is either feed or water additives (Johnstone^d, 1998).

Cost

The cost of anthelmintics should be reasonable. They must be considered an investment in food producing animals where return on investment is a major consideration. However, two factors should also be considered. First, veterinary services are one of the smallest components of the total cost of production. Therefore, the impact of anthelmintic treatments on total production costs will be minimal. Second, the positive impact on the productivity of broad spectrum anthelmintics in parasite control programs is considerable. Therefore, the return on investment is significant (Johnstone^e, 1998).

Rapid metabolism and excretion

Anthelmintics should either be rapidly metabolised after absorption or act locally by not being absorbed at all. Some anthelmintics require a long withdrawal period for milk and meat and this renders them unsafe for human consumption (Johnstone^e, 1998).

1.4. Classification of anthelmintics

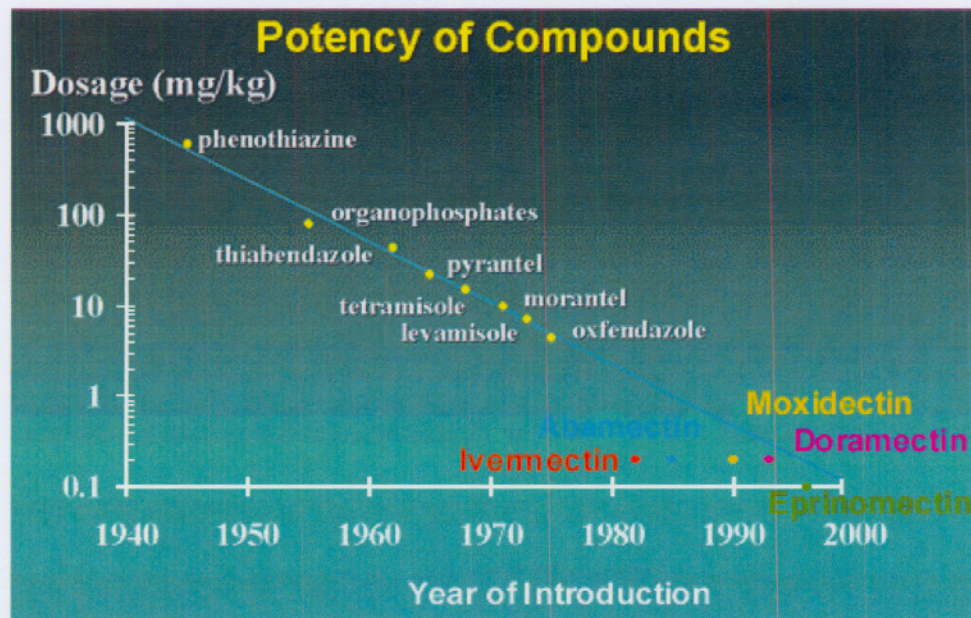


Figure 5. The potency of anthelmintic compounds (Johnstone^d, 1998).

In figure 5 it is shown how the potency of discovered anthelmintics has increased with time. The earliest of the modern anthelmintics was phenothiazine. This drug was primarily used against nematodes in ruminants. It was a drug that not only had a narrow spectrum of activity but also a high therapeutic dose of more than 600 mg/kg, as well as a narrow margin of safety. As other groups of anthelmintics were discovered, their effective dosages were found to be less than previous compounds and their spectra of activity were broader. The most recently discovered macrocyclic lactones (Ivermectin, Abamectin, Moxidectin, Doramectin and Eprinomectin) has a broad spectrum of activity in all our domestic animals. They also have a very small therapeutic dose of less than 200 µg/kg. In addition, they are extremely safe to use in most domestic animals (Johnstone^d, 1998).

The proper choice of an anthelmintic is important, as most drugs are more effective against some species than others (Swinyard, 1990:1242).

1.5. Anthelmintic resistance

A worldwide resistance towards anthelmintics have been documented in sheep, goats, cattle and horses. Resistance has evolved in step with the commercial release and

extensive use of the broad spectrum anthelmintics like phenothiazines, benzimidazoles, levamisole and morantel.

Anthelmintic resistance was first reported in *Haemoncus contortus* in 1957 against phenothiazine, a drug that is rarely used today. In 1964, 3 years after the introduction of thiabendazole, a resistant strain of *Haemoncus contortus* was reported. Since then, anthelmintic resistance has been widely and reliably reported in many currently available anthelmintics and in a number of nematodes (Johnstone^d, 1998).

Detecting anthelmintic resistance

Faecal egg depression test:

Nematode egg counts are made pre- and post-treatment. They are assessed 5-10 days before and 5-10 days after treatment. If there is not at least an 80% reduction in the egg counts after treatment, then one should suspect anthelmintic resistance. One problem is that only one species out of a mixed infection of many may be resistant to the particular anthelmintic being used. Therefore, it may be necessary to do pre- and post-treatment larval culture to detect the species showing resistance. Otherwise, more extensive and costly experiments need to be done in terms of killing of treated and control animals and the counting of worm burdens, including the differentiation of species to accurately determine the affected species of nematode.

To minimize anthelmintic resistance, it is important to stop using resistant anthelmintics. Since the intensity of selection pressure depends primarily on frequency of treatments, the resistant nematode population should, theoretically, revert to a susceptible one when selection pressure is discontinued. To avoid too frequent treatments, the correct doses of anthelmintics should be administered. Genes expressing resistance are normally at a low frequency in a population of nematodes. Frequent use of an anthelmintic increases the frequency of these resistant genes since the resistant survivors of treatment become more numerous in succeeding generations and eventually become the dominant strain in a population.

Drug control programs should be based on a minimum number of treatments. It is therefore important to rotate drugs. The principle here is to avoid exposing a single generation of parasites to multiple classes of anthelmintics. Thus, treatment strategies should be based, as far as possible, on the rotation of drug classes *between* years rather

than *within* years. The practice of treating and moving to clean pastures have it's disadvantages. Contamination of this new pasture will result with eggs from nematodes surviving the treatment. Therefore, selection pressure for resistance would be increased (Johnstone^d, 1998).

1.6. Lack of response to treatment

Failure of animals to respond to treatment with anthelmintics may be due to one of several causes.

Under dosing: this usually results from badly guessing weights of animals for computing dosages. Because of our fear of giving too much and the potential toxic effects, we tend to guess under rather than over the real weight of an animal. The other reason for under dosing is because of manufacturers' dosage recommendations for a broad weight range. In treating groups of animals, overdosing tends to occur at the low end of the weight range and, conversely, under dosing tends to occur at the high end of the weight range. If animals are treated and left on heavily contaminated pastures, they will become rapidly reinfected. The drug used may be ineffective against developing parasitic stages and against hypo-biotic larvae.

Administration may be faulty, e.g. orally administered boluses may be "spit out" by sheep and cattle. The wrong anthelmintic might be chosen, e.g. Levamisole is highly effective against arrested larvae of *H. contortus* in sheep. However, the same drug is not effective at all against arrested larvae of *O. ostertagi* of cattle. If you didn't remember this, you might use Levamisole in cattle with unrealistic expectations (Johnstone^g, 1998).

1.7. Safety considerations

Anthelmintics are basically poisons and their safe use in animals is directly dependent on the therapeutic dose against the parasite being much lower than the toxic dose in treated animals.

Before the discovery of the avermectins, the benzimidazoles such as oxfendazole were often combined with an organophosphate like Trichlorfon to broaden the spectrum of activity, thus including the bots. In doing so it had to be remembered that the safety factor of the combination equaled that of the most toxic component, namely trichlorfon – a property that seems obvious but easily forgotten in the whirl of a busy practice. The

organophosphates are problematic in terms of the fact that they are acetylcholinesterase inhibitors in mammalian as well as parasite neuromuscular junctions. Therefore, their mode of action is one of the reasons why they have a narrow margin of safety when used in mammals.

It is particularly important to use anthelmintics with wide safety margins in horses, ruminants and pigs, since the weight of these animals for computing doses is usually estimated for convenience and speed. The use of drugs with narrow safety margins becomes problematic if weight is overestimated – animals will be given a dose that is not only greater than the therapeutic dose but also may be close to or at the toxic dose (Johnstone^c, 1998).

2. CHAPTER 2

2.1. Veterinary parasitology

2.1.1. Helminths

Classification of helminths

The name “helminth” is derived from the Greek word *helmins* or *helminthos*, which refers to a worm. At the present time, it is usually applied to parasitic and non-parasitic species belonging to the phyla Plathelminthes (flukes, tapeworms and other flatworms) and Nematelminthes (roundworms and their relatives) (Lapage, 1962:31-31).

Diagnosis of helminthiases

The digestive tract is usually inhabited by several species of parasites. There is also clinical evidence that the larval stages of many parasites do more harm than the adult worms. Many anthelmintics remove only adult parasites, but if administered at the proper times, they may reduce the degree of pasture contamination by larvae of the parasitic worms (Siegmund, 1961:860).

Symptoms

Young animals are affected more often, but mature animals frequently show symptoms and succumb to infection. Digestive disturbances are common in infections with all the stomach worms and may develop in the prepatent period through the activity of the larvae. Other symptoms include progressive loss of weight, anaemia, weakness, rough haircoat and anorexia. The latter is of utmost importance in prognosis and therapy (Siegmund, 1961:862).

Clinical diagnosis

The clinical diagnosis based on history and symptoms can usually be confirmed by identifying eggs on faecal examination. In interpreting the results of faecal examinations, the following points should be remembered: The number of eggs is not always an accurate indication of the severity of the infection because of the presence of large numbers of immature worms or the suppression of egg production by immune reaction or by previous anthelmintic treatment. The egg producing capacity of different worms varies greatly. Specific identification of eggs is often difficult and in some instances impossible.

On post-mortem examination, some of the worm species can easily be seen. When no worms are seen, samples of the contents and scrapings of the mucosa should be examined microscopically. Multifactorial etiology should be considered in evaluating clinical, laboratory and microscopy findings (Siegmund, 1961:860-863). Bath (1994:86-87) listed the helminths in table 5 and 6 as parasites pathogenic to cattle, sheep and goats.

Table 5. Helminths in cattle (Bath, 1994:86-87).

Latin names	Common names
Roundworms	
<i>Haemonchus contortus</i>	Wireworm
<i>Ostertagia spp.</i>	Brown stomach worm
<i>Cooperia spp.</i>	Bankrupt worm
<i>Oesophagostomum spp.</i>	Nodular worm
<i>Bunostomum spp.</i>	Hookworm
Tapeworms	
<i>Moniezia spp.</i>	Milk tapeworm
Flukes	
<i>Fasciola hepatica</i>	Liver fluke
<i>Fasciola gigantica</i>	Giant liver fluke
<i>Paramphistomum microbothrium</i>	Conical fluke

Table 6. Helminths in sheep and goats (Bath, 1994:86-87).

Latin names	Common names
Roundworms	
<i>Haemonchus contortus</i>	Wireworm
<i>Trichostrongylus spp.</i>	Bankrupt worm
<i>Nematodinus spp.</i>	Long necked Bankrupt worm
<i>Teladorsagia</i>	Brown stomach worm
<i>Oesophagostomum spp.</i>	Nodular worm
<i>Galgeria spp.</i>	Hookworm
<i>Chabertia ovina</i>	Large mouthed bowel worm
<i>Stongyloides papillosus</i>	White Bankrupt worm
<i>Dictyocaulus filarial</i>	Lungworm
<i>Trichuris spp.</i>	Whipworm
<i>Oestrus ovis</i>	Nasalworm
Tapeworms	
<i>Moniezia spp.</i>	Milk tapeworm
<i>Avitellina centripunctata</i>	Narrow tapeworm
<i>Thysanotria giardi</i>	Serrated tapeworm
Flukes	
<i>Fasciola hepatica</i>	Liver fluke
<i>Fasciola gigantica</i>	Giant liver fluke
<i>Paramphistomum microbothrium</i>	Conical fluke

2.1.2. Classification and taxonomy of helminths

Table 7. Representation of the classes of parasitic helminths (Bath, 1994:86-87).

Helminth Parasites			
Phylum: <i>Platyhelminthes</i>	Class I:	<i>Turbellaria</i>	Non-parasitic species living in fresh water
	Class II:	<i>Trematoda</i>	Flukes. With an alimentary tract
	Class III:	<i>Cestoda</i>	Tapeworms. With no alimentary tract
Phylum: <i>Nemathelminthes</i>	Class I	<i>Nematoda</i>	Roundworms. Cylindrical worms with pointed ends.
	Class II	<i>Nematomorpha</i>	Hairworms. Non-parasitic, long threadlike worms
	Class III	<i>Acanthocephala</i>	Thorny headed worms. Thick cuticle and retractable proboscis

A simplified taxonomy of the parasitic helminths of importance in Southern Africa.

There is no real consensus on the taxonomy of the helminths – different textbooks show different groupings, particularly with regards to the nematodes. The term “helminth” itself contains a number of phyla, many of which are completely unrelated. However, for the sake of convenience, the term is still used to describe four groups with superficial similarities, namely the phyla Annelida, Platyhelminthes, Nematoda and Acanthocephala. A fifth group, the phylum Pentastomida (the Tongue worms) is not usually included here, but a brief description of this group is provided in Table 7 (Stewart, 2002).

Introduction to the Phylum Platyhelminthes

(Flatworms)

The Platyhelminthes are dorso-ventrally flattened worms, with solid acoelomate bodies, (i.e. no body cavities), the organs and muscle fibres being embedded in parenchymal tissue. The respiratory and circulatory systems are absent. They are mostly parasites (except for free living members of the Class Turbellaria) and, with the important exception of the schistosomes, are mostly hermaphroditic. The outer layer of the body consists of a biologically active syncytial layer called a tegument, which varies in structure between the different classes of Platyhelminthes, while the Cestodes are covered in numerous microtriches.

The Platyhelminthes are divided into four classes. The two most important classes in terms of human or veterinary pathology are the Class Trematoda, which may be further

divided into the following: the Sub Class Digenea (including the schistosomes); the Sub Class Aspidogastrea; the Class Cestoda which is divided into many sub classes with the Subclass Eucestoda being the most important of these (Stewart, 2001).

The Trematodes

(Flukes)



Figure 6. The liver fluke (Anon, 2002^c).

Trematodes are flukes of the class Trematoda; phylum Platyhelminthes. Important trematodes affecting man belong to the genera *Schistosoma* (blood fluke), *Echinostoma* (intestinal fluke), *Fasciolopsis* (liver fluke), *Gastrodiscoides* (intestinal fluke), *Heterophyes* (intestinal fluke), *Metagonimus* (intestinal fluke), *Clonorchis* (Asiatic liver fluke), *Fasciola* (liver fluke), *Dicrocoelium* (liver fluke), *Opisthorchis* (liver fluke), and *Paragonimus* (lung fluke). Man usually becomes infected after ingesting insufficiently cooked fish, crustaceans, or vegetables that contain their larvae. The cycle begins when larvae are released into freshwater by infected snails. The free-swimming larvae can then directly penetrate the skin of humans while swimming or be ingested after encysting in or on various edible vegetation, fish, or crustaceans.

The oval-shaped fluke, as seen in Figure 6, has a tough outer body layer called a tegument that covers layers of circular, longitudinal, and diagonal muscles that protects it from the human digestive tract. Some can inhabit the liver, bile duct, or lymph vessels. They can be several inches long, an inch or so wide and only thick enough to hold themselves together (Anon, 2002^c).

The Cestodes

(Tapeworms)



Figure 7. The tapeworm (Anon, 2002^a).

Cestodes are tapeworms: class Cestoidea; phylum Platyhelminthes; subclass Cestoda. They are specialized flatworms, looking very much like a narrow piece of adhesive tape. Tapeworms are the largest, and among the oldest of the intestinal parasites that have plagued humans and other animals since time began. Found all over the world, tapeworms exist in many different forms, but they have no close relatives living outside of animal hosts. Tapeworms do not have a mouth like the fluke, nor do they have a head or a digestive tract with digestive enzymes. The ends differ, but neither has any organs or sensors that could be associated with what is commonly thought of as being a "head." However, through a segment called a scolex, they are able to absorb predigested food. The scolex attaches to the intestinal wall by hooks or suckers. The body contains hundreds of segments called proglottids which can be clearly seen in Figure 7. Each segment is a sexually complete unit that can reproduce, if necessary. Some tapeworms have reached lengths of more than ten meters (thirty feet) with a lifespan, inside a host, of thirty years or more. Tapeworms are dependent on two hosts for their development, a human and an animal. Larvae are found in animal hosts, while the adult worm is found in humans (Anon, 2002^a).

Phylum Nematoda

(The round worms)

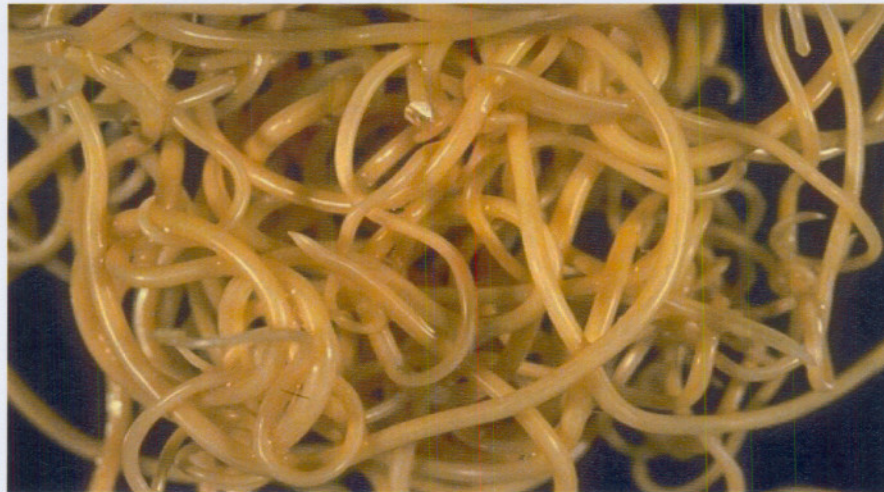


Figure 8. The roundworm found in dogs -*Toxocara canis* (Anon, 2002^b).

Although the roundworm in figure 8, *Toxocara canis* is not of medical importance in livestock, it is however a very good illustration of roundworm in general.

Nematodes are commonly called roundworms because, as the name suggests, they are round when viewed in cross section. However, they are in fact cylindrical in structure and taper towards their anterior and posterior ends. They are bilaterally symmetrical, and while the sexes are separate in most species, a few are hermaphrodite. Nematodes that parasitize our domestic animals are found in all parts of the body, but are most commonly found in the digestive and respiratory tracts and the circulatory system.

Nematode parasites of domestic animals vary greatly in size ranging from small hair-like worms (up to 2 cm long) in the Superfamily Trichostrongyloidea, to large, robust worms (up to 40cm long) in the Superfamily Ascaridoidea (Johnstone, 1998).

3. CHAPTER 3

3.1. Drugs to be used in the formulation

3.1.1. Levamisole Hydrochloride

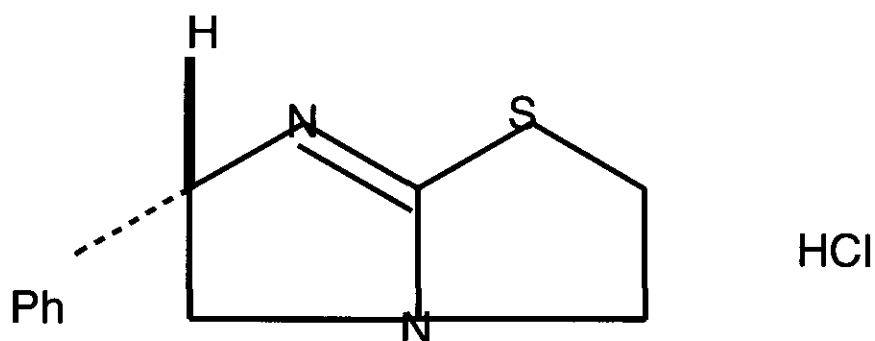


Figure 9. Structure of Levamisole Hydrochloride.

Physical properties

Molecular structure: C₁₁H₁₂N₂S HCl

Molecular weight: 240.8

Chemical name: (-)-(S)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole.

Description: It is a white to pale cream-coloured, crystalline powder; odourless to almost odourless.

Solubility: Soluble in 2 parts of water and 5 parts of methyl alcohol; practically insoluble in solvent ether.

Melting point: 228°C

(British Pharmacopoeia, 1977:44)

Pharmacological action

It acts by interfering with parasite nerve transmission by selective inhibition of succinate dehydrogenase, causing muscular paralysis, and rapid expulsion (Reynolds, 1977:104).

Spectrum

It is effective in the treatment of roundworm (*Ascaris*) infestation and moderately effective against hookworms (*Ancylostoma* and *Necator*) and strongyloids (*Strongyloides*), but ineffective against *Trichuris* (Reynolds, 1977:104).

Absorption

Levamisole is absorbed from the gastro- intestinal tract and appears in the blood. It is excreted in the faeces and urine (Reynolds, 1977:104).

Dosage

Cattle: By mouth or by subcutaneous injection 7.5 mg/kg

Sheep and goats: By mouth or by subcutaneous injection 7.5 mg/kg

(Anon, 1991:114)

Side-effects

In a small proportion of patients, mild and transient effects may occur, including nausea and vomiting, abdominal discomfort, headache, dizziness and hypotension (Reynolds, 1977:104).

Route of administration

Oral, injection and pour-on.

3.1.2 Niclosamide

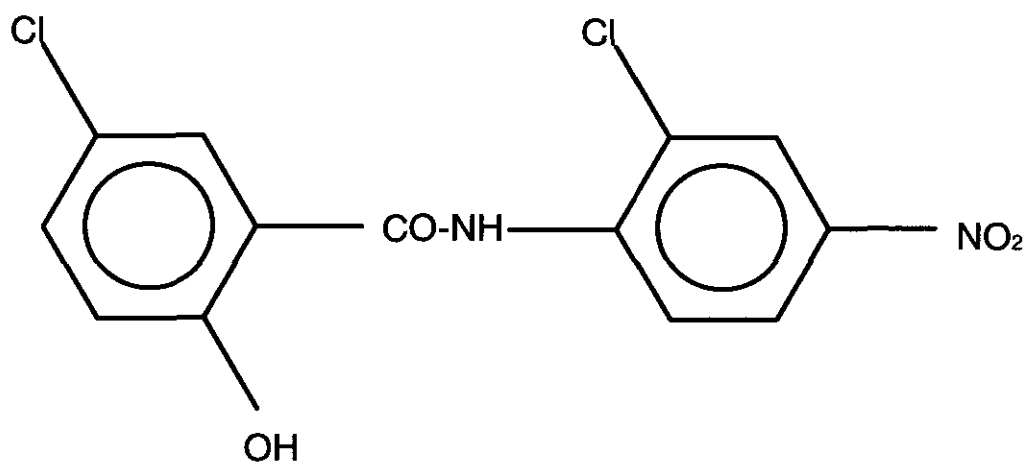


Figure 10. Structure of Niclosamide.

Physical properties

Molecular structure: C₁₃H₈Cl₂N₂O₄

Molecular weight: 327.1

Chemical name: 2',5-dichloro-4'-nitrosalicylanilide

Description: An odourless, tasteless cream-coloured powder.

Solubility: Very slightly soluble in water, soluble at 20°C in 150 parts of alcohol, in 350 parts of ether.

Melting point: 228°C

(Lund, 1979:592)

Pharmacological action

Niclosamide exerts its anthelmintic effect by inhibiting mitochondrial oxidative phosphorylation in worms. The drug also markedly decreases the anaerobic generation of ATP via inhibition of the glucose uptake in susceptible cestodes (McEvoy, 1988:40-41).

Spectrum

Niclosamide is active against most pathogenic cestodes (tapeworms). This includes the fish tapeworm (*Dipyllobothrium latum*), the dog and cat tapeworm (*Dipylidium canium*), the rat tapeworm (*Hymenolepis diminuta*), the dwarf tapeworm (*Hymenolepis nana*), the beef tapeworm (*Taenia saginata*) and the pork tapeworm (*Taenia solium*). The drug has also been shown to be active against the intestinal nematode (roundworm) *Enterobius vermicularis* (pinworm) (McEvoy, 1988:40-41).

Absorption

Niclosamide is virtually unabsorbed from the GI tract following oral administration. Studies in animals indicate that only minimal amounts of Niclosamide reach the systemic circulation. Limited evidence suggests that the drug is metabolised in the GI tract. It is excreted in faeces (McEvoy, 1988:40-41).

Dosage

Cattle: 50 mg/kg

Sheep and goats: 75 mg/kg

Dogs and cats: 100 mg/kg
(British Pharmacopoeia, 1977:54)

Side effects

At the recommended dosage, Niclosamide is generally well tolerated. Adverse effects are usually transient. Most frequent side effects are nausea, vomiting, abdominal discomfort, diarrhoea, drowsiness, dizziness and headache.

Route of administration

Oral

3.1.3 Oxyclozanide

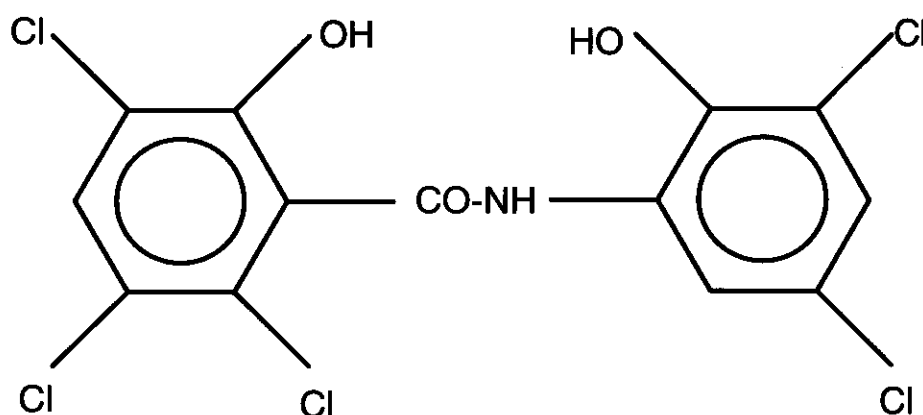


Figure 11. Structure of Oxyclozanide.

Physical properties

Molecular structure: C₁₃H₆Cl₅NO₃

Molecular weight: 401.5

Chemical name: 3,3',5,5',6-pentachloro-2'-hydroxysalicylanilide.

Description: An odourless cream coloured powder.

Solubility: Very slightly soluble in water; soluble at 20°C in 20 parts of alcohol, in 600 parts of chloroform and in 5 parts of acetone.

Melting point: 208°C

(Lund, 1979:623)

Pharmacological action

Oxyclozanide causes serious alterations in the structure and functions of the absorptive surfaces of susceptible organisms. The changes may be described as destructive, degenerative and necrotic processes. The organelles that are affected are the surface plasmalemma, the mitochondria and the lysosomes (Chemical abstracts, 1992:75734q).

Spectrum

Oxyclozanide is mainly active against adult flukes. It kills adult *Fasciola* and tapeworms in ruminants and immature *Fasciola* in sheep.

(Anon., 1991:120-121).

Absorption

Oxyclozanide is absorbed in the GI tract and is distributed to the liver, kidney and intestines. It is excreted in the bile.

Dosage

Cattle, sheep and goats: 10 mg/kg - 15 mg/kg.

Side effects

Occasional loose stools and inappetance in cattle

(Anon., 1991:121).

Route of administration

Oral

3.2. Available products on the market

Levamisole HCl

Levamisole HCl is the laevo-isomer of tetramisole hydrochloride.

Actions and uses: Used in the treatment of gastro intestinal ascariasis, ancylostomiasis, and strongyloids. Levamisole HCl enhances cell-mediated immunity in certain cases associated with immunodeficiency states (Lund, 1979:489-490).

Table 8. Tradenames of registered Levamisole products in South Africa (Swan, 2000:42).

Tradenname	Concentration	Company
Levicon	Levamisole HCl 25% m/v	Milborrow
Levisole	Levamisole HCl 2,5% m/v	Milborrow
Prodose Red	Levamisole HCl 2,56% m/v	Logos Agvet
Rippercol-L	Levamisole HCl 30mg/ml	Janssen
Telskoot	Levamisole HCl 99% m/v	Logos Agvet
Tramisol	Levamisole HCl 2,5% m/v	Hoechst Roussel Vet
Tramisol Conc	Levamisole HCl 25% m/v	Hoechst Roussel Vet.
Vetdose 3	Levamisole HCl 2,56% m/v	Logos Agvet

Niclosamide

Actions and uses: Used in the treatment of cestodiasis (tapeworm infections) caused by all pathogenic adult or intestinal cestodes (McEvoy, 1988:40).

Table 9. Tradenames of registered Nicosamide products in South Africa (Swan, 2000:41).

Tradenname	Concentration	Company
Ex-a-lint	Niclosamide 20% m/v	Hoechst Roussel Vet
Lintex L	Niclosamide 20% m/v	Bayer A.H.
Lintex M (Powder)	Niclosamide 75% m/m	Bayer A.H.
Prodose Lint	Niclosamide 20% m/v	Logos Agvet

Oxyclozanide

Actions and uses: Used in the treatment of adult flukes (Lund, 1979:623).

Table 10. Tradename of a discontinued Oxyclozanide product in South Africa (Swan, 2000:41)

Tradenname	Concentration	Company
Milborrow Liver fluke Remedy	Oxyclozanide 3,4% m/v	Bayer A.H

Combinations of levamisole HCl and oxyclozanide have been used together in different formulations. The joint combination is very effective against roundworm and liver fluke.

Combinations of levamisole HCl and niclosamide are also available as a tablet. It is used as a broad-spectrum anthelmintic against round-, hook- and tapeworms.

Table 11. Tradenames of registered combination products in South Africa (Swan, 2000:42).

Tradename	Concentration	Company
Atenas Poultry	Niclosamide 32% m/v Levamisole HCl 12% m/v	Vine-Vet
Dairy Drench	Oxyclozanide 3,4% m/v Levamisole HCl 2,5% m/v	Bayer A.H.
Diworm	Niclosamide 500mg/tab Levamisole HCl 25mg/tab	Bayer A.H.
Levisan	Oxyclozanide 3,4% m/v Levamisole HCl 2,5% m/v	Bayer A.H.
Tramizan	Oxyclozanide 3,4% m/v. Levamisole HCl 2,5% m/v	Hoechst Roussel Vet

When looking at Tables 8 to 11, it is clear that all the active ingredients are either alone or in combination on the shelves. Although this illustrates the mass of products, it also confirms that these three active ingredients are still effective in treating helminthiases in livestock.

4. CHAPTER 4

4.1. Veterinary Pharmaceutical Formulation

4.1.1. Pharmacological considerations

Gastric emptying is an important physiological factor controlling the rate of drug absorption. The emptying rate of multi-stomach animals can vary as greatly as the consistency of the material in each of the stomachs, which in its turn have an effect on absorption.

The distribution of drugs will differ between animals because they have different body compositions. The body composition with regards to the percentage of live weight of various animals differs greatly. The skin of a horse comprises 7.4% of the bodyweight compared to that of the goat, which is 9.2%, and the bone is 14.6% in the horse to that of the goat, which is 6.3%. This can influence the distribution of drugs in animals either positively or negatively.

The urinary pH of herbivores is alkaline (pH 7-8), but the urinary pH of carnivores is acidic (pH 5.5-7). This difference can affect the excretion rate of certain drugs, especially those with pKa values in the close vicinity of these ranges.

The rate at which drugs are metabolised also differs from animal to animal. Thus, in some animals the synthesis of drugs can be slower or faster than in others. It is therefore important to select a drug with a dosage that is appropriate for a particular species of animal (Allen, 1998:278-279).

4.1.2. Physiochemical consideration

Unstable drugs are even more unstable in solution. Poorly soluble drugs must be solubilised

or suspended. Bad-tasting drugs can be masked to produce a palatable product. The formulation of a successful product thus depends upon a combination of scientific acuity and pharmaceutical art.

Factors to be considered in the formulation of an oral liquid dosage form include the following:

- Physical and chemical properties of the ingredients.
- Order of mixing and adjuvant.
- Pharmaceutical techniques required.
- Incompatibilities in preparation and storage.
- Stability and potency of the ingredients.

When preparing an oral liquid, the formulator should consider the drug's concentration, solubility, pKa, taste and stability. Vehicle considerations include pH, flavour, sweetener, colour, preservative, viscosity, compatibility and, if indicated, suspending and emulsifying agents (Allen, 1998:159).

4.1.3. Considering the type of formulation

The labour and the cost of treating animals are becoming an increasingly important factor in the production of meat. It is therefore important that the formulator's formulations can be adapted to mass medication techniques using mechanical equipment.

In a study conducted by Pope and Baggot, findings show that once the route of administration and the pharmacokinetics have been established, the advantages and disadvantages of the different formulation types should be considered before initiating a pilot study.

The questions that the formulator should ask about the type of formulation to be considered:

1. Would the release characteristics and bioavailability from the considered formulation correspond to what is required?
2. Could the formulation be manufactured economically?
3. Could the formulation type only be made by a contract manufacturer or could it be made inhouse without additional space or equipment?
4. Would the formulation type be stable or would it be likely to give problems?
5. Would the formulation type provide convenience in handling to the administrator?
6. Has the formulation type been generally accepted by the veterinary profession in the country in which it is to be marketed?

7. Can the formulation type be administered by existing equipment or should a new dosing apparatus be developed?
 8. Will the route of administration have any effect?
- (Pope & Baggot, 1983:37).

Solutions

This dosage form presents the drug component in a form most suitable for absorption. Hence, if there is no complexation or micellisation with components of the formula, and if there is no degradation in the stomach, this dosage form will give maximal bioavailability and a rapid onset of action (Pope & Baggot, 1983:38).

Suspensions

A suspension may be considered the most desirable dosage form for a particular drug for a number of reasons: the suspension may be preferred over a unit dose solid dosage form as it presents most of the advantages of the solution dosage form, namely ease in swallowing, greater ease in the administration of usually large doses which are often more stable than solutions, and infinite variability of dosage (Pope & Baggot, 1983:41).

Tablets

A tablet or bolus offers many advantages over other dosage forms and should be one of the first considerations for oral dosing. The tablet offers ease of administration of an "accurate" dose, and is readily adaptable to various dose sizes of medicinal substances. Most formulations have two or more dose strengths. Tablets are portable and compact. They do not offer as large a bulk as a liquid or suspension dosage form. They usually present the fewest problems with respect to stability and they are quite economical compared to other dosage forms (Pope & Baggot, 1983:45).

4.1.4. Drug delivery devices

As in humans, a variety of dosage forms can be prepared for animals, using several routes of administration. The routes that are mostly used for the administration of veterinary dosage forms, are the oral, parenteral and topical routes of administration (Allen, 1998:276-278).

Anthelmintics are administered to animals by a variety of methods and formulations. The three routes of administration are oral, injection and topical.

Oral formulations are marketed in a variety of forms - liquids, pastes, hard pills, boluses (large pills), chewable soft pills, medicated salt blocks for licking and premedicated feed or salt mixtures. Palatability is very important in orally administered products, particularly to horses and cats which can be difficult to treat with drugs that have unpleasant tastes.

Tablets in their various forms are most commonly used in dogs and cats because they are clearly single dose preparations and are relatively easy to give.

A more recent innovation in oral administration is the use of intra ruminal sustained release devices in cattle and sheep (Johnstone, 1998). They allow a slow release of active drug over a period of time, usually the complete grazing season. The aim is to prevent the establishment of nematode populations in hosts during periods of maximum availability of third stage infective larvae.

Injectables are most commonly given to cattle and swine, which can be easily crowded into restricted spaces. They are given as sterile solutions and the subcutaneous route is the most favoured because of the risks involved in hurting carcass quality when the intramuscular route is used.

Topical pour-on macrocyclic lactones, absorbed through the skin, are also available for treatment of internal and external parasites in cattle. This formulation is clearly preferred by producers and veterinarians because of the ease of use, which markedly reduces stress for treated animals. The active compound is absorbed via the sebaceous glands and hair follicles.

Feed additives are particularly useful and popular in controlling parasites in pigs. These compounds allow the stress-free, economical treatment of large numbers of animals. Feed additives are also useful in treating range cattle as additives to mineral mixes and salt blocks (Johnstone, 1998).

4.1.4.1. Oral administration

Oral dosage forms present the greatest challenge in administering medication to animals.

Balling gun: it is a device that has a barrel through which passes a plunger capable of dislodging a bolus into the gullet of the animal.

Esophageal delivery device: it is a syringe or a tube that is designed to deliver medication directly into the stomach.

Drench syringe: it is a single or multiple dose device that is capable of delivering preset volumes of liquid into the gullet.

Liquid drench gun: it is a single or multiple dose device that is capable of delivering oral solutions or suspensions of an aqueous or oily nature.

Powder drench gun: it is a device that is capable of delivering a required amount of powder into the back portion of the animal's mouth where it adheres and it is subsequently swallowed.

Paste dispensers: it includes devices such as; paste guns, paste syringes, squeeze bottles and squeeze tubes that are capable of delivering a specified dose to an animal.

Water medication metering device: this type of dosage provides a method of adding medication into a water supply of numerous animals. The amount of medication released depends on the average daily water intake of the animals.

Rumen-lodging devices: these are incorporated into medications that have a controlled release delivery system. These devices aid the product by adhering to the mucosa and releasing the drug at the desired rate (Allen, 1998:276).

4.2. Solutions

4.2.1. Types of solutions

Solutions are liquid preparations containing one or more drug substances that are molecularly dispersed in a suitable solvent or a mixture of mutually miscible solvents. Oral

liquids are intended for oral administration and contain one or more substances with or without flavouring, sweetening, or colouring agents dissolved in water or co-solvent water mixtures. They may be either formulated for direct oral administration to the patient or dispensed in a concentrated form that requires dilution prior to dispensation or administration.

Topical solutions are generally aqueous but may contain co-solvent systems such as various alcohols or other organic solvents with or without added active ingredients. The term "lotion" is often used to designate solutions or suspensions that are applied topically.

Syrups are concentrated aqueous preparations of a sugar or sugar-substitute with or without flavouring agents and medicinal substances. Syrups can serve as pleasant-tasting vehicles for active drugs.

Elixirs are clear, sweetened, hydro-alcoholic solutions that are usually flavoured and are suitable for drugs that are insoluble in water alone, but soluble in water-alcohol mixtures. Less sweet and less viscous than syrups, elixirs are generally less effective in masking taste. Elixirs may also contain different solvents as co-solvent systems, for example water, alcohol, glycerine, sorbitol, propylene glycol, and polyethylene glycol 400.

Aromatic waters can be used for both internal and external purposes. They are clear, saturated aqueous solutions of volatile oils or other aromatic or volatile substances. For compounding purposes, they are generally prepared using volatile oils and water (Allen, 1998:157).

4.2.2. Historical use

Solutions are one of the oldest dosage forms. Myrrh, laudanum, and other tinctures are mentioned in literature dating back to Biblical times. The composition and preparation of earlier solutions (e.g. fluid extracts, tinctures, spirits, and potions) were simpler than that of contemporary preparations. Solutions prepared today may be buffered, preserved, flavoured, sweetened, adjusted for pH and osmolality range, and protected against oxidation (Allen, 1998:158).

4.2.3. Application and advantages

An oral liquid may be the most appropriate dosage form for an animal for a number of reasons. The most common reasons are as follows:

Many drug products are not commercially available as oral liquids.

Some products are therapeutically better in liquid form.

The bulk of some preparations make oral liquids more feasible.

Oral liquid dosage forms are diverse and have varying dosage strengths

Drugs are often more bioavailable from oral liquids than from solids (Allen, 1998:158).

Toraddo *et al.* conducted a study in 1997 where they looked at the formulation of an albendazole solution to improve bioavailability and efficacy. They found that there was a relative increase in bioavailability of albendazole solution in relation to the suspension. This increase in bioavailability may be due to the increase in solubility. When they performed efficacy tests, they found that the albendazole suspension at a dosage of 10 mg/kg was only 62.75% effective whereas the solution with a dosage of 2.5 mg/kg presented a 100% efficacy. Thus, the antiparasitic effect of the albendazole solution was 25 fold greater than the albendazole suspension (Torrado *et al.*, 1997:185).

Niclosamide and oxclozanide are similar to albendazole in that they are poorly water-soluble drugs. This implies that both niclosamide and oxclozanide are poorly absorbed from the gastrointestinal tract. Although this property is ideal for the treatment of geohelminths and other intestinal parasites, it might not be advantageous in the treatment of systemic helminthiases.

4.3. Suspensions

4.3.1. Types of suspensions

A suspension is a two-phased system consisting of a finely divided solid dispersed in a solid liquid or gas. A suspension is the dosage form of choice when the drug to be incorporated is not sufficiently soluble in an ordinary solvent or co-solvent system.

Oral suspensions are liquid preparations in which solid particles of the active drug are generally dispersed in a sweetened, flavoured and sometimes viscous vehicle.

Topical suspensions are liquid preparations containing solid particles dispersed into a suitable liquid vehicle that are intended for application to the skin (Allen, 1998:167).

4.3.2. Historical use

The first suspensions were possibly simple preparations of vegetable or earth products mixed with water. Mixing the drug with water made it easier to take orally or apply topically. This dosage form was a simple way of converting powdered leaves, clays and other medicaments of the time into a fluid preparation that would not only be easier to administer but would also increase patient compliance (Allen, 1998:167).

4.3.3. Application and advantages

As with people, animals also have difficulty swallowing solid dosage forms and therefore require the drugs to be dispersed in a liquid dosage form. Suspensions have the advantage of smaller and more accurate dosages for smaller animals like lambs and calves. Another advantage might be that you have a "depo" effect with a suspension, especially if the drug is poorly absorbed. If a drug is insoluble or poorly soluble (like niclosamide and oxyclozanide), then the formulation of a suspension is usually required. The normal concentration of niclosamide products on the shelves today are 20% of the total volume. Experimental work (the formulation of a solution) showed that the maximum solubility of niclosamide is 3%. Therefore, a concentration higher than that has to be formulated in a suspension.

A suitable vehicle for such as a suspension would have the viscosity to keep the drug particles suspended separately from each other, but would be sufficiently fluid to allow the preparation to be poured from the container to be taken. Suspensions can also enhance the stability of a drug that is poorly stable in a solution. Using an insoluble form of the drug places more of the drug in the suspended form and not into a solution, which benefits the system in that the degradation of the solution is virtually non-existent (Allen, 1998:167).

4.4. Tablets

Tablets are the most frequently prescribed commercial dosage form today. This type of dosage form is stable and effective.

4.4.1. Types of tablets

Sublingual moulded tablets are placed under the tongue. They dissolve rapidly, releasing the medication for absorption sublingually, or they can be swallowed.

Buccal moulded tablets are administered in the cheek pouch. They dissolve rather quickly, and the released medication can be absorbed or swallowed.

Sintered tablets can be prepared to either dissolve in the mouth, if they are flavoured, or to be swallowed.

Compressed tablets are designed to be swallowed. Although they are generally prepared using large, expensive tableting machines, they can be extemporaneously prepared using pellet punches or single-punch tableting machines.

Chewable tablets must be chewed and swallowed. Formulated to be pleasant tasting, they should not leave a bitter or unpleasant aftertaste.

Soluble effervescent tablets are prepared by compression and contain mixtures of acids and sodium bicarbonate, which releases or forms carbon dioxide when dissolved in water.

Implants or pellets are small, sterile, solid masses containing a drug with or without excipients (Allen, 1998:107).

4.4.2. Historical use

The tablet originated in England in the 1800s as a compressed pill. In 1878 Burroughs Wellcome and Company applied the term "tablet" to this dosage form. John Wyeth and his brother introduced compressed tablets to the United States in 1882 in the form of compressed hypodermic tablets and compressed tablet triturates. In 1892, a British manufacturer was granted use of the term "tablet" for biconvex discs. Today, tablets make up the majority of the drug dosage forms on the market because of their economy, ease of preparation, accuracy of dosage and ease of administration (Allen, 1998:108).

4.4.3. Application and advantages

Because of the many small farmers, especially in rural areas, it was decided to look at a tablet formulation that is dispersible. In most cases there are only 2 or 4 animals that have to be dosed. To buy a litre of an anthelmintic would not be cost effective for such a farmer. To give the dose, the farmer has to know the approximate weight of the animals to be dosed. He then takes one tablet for a fixed weight, takes the plunger out of a 20ml syringe, inserts the tablets, adds water and shakes well. This contributes to easy administration and cost-effectiveness. One of the major advantages of tablets is that it enables an accurate and easy dosage form and it is easier to transport than the liquid dosage forms. Tablets can be mass-produced and the manufacturing cost is much lower than with other dosage forms.

5. CHAPTER 5

5.1. Preformulation

Preformulation studies may be defined as investigations designed to identify those physiochemical properties of drug substances and formulation excipients which may influence the pharmacokinetic properties of subsequent formulations; their design and method of manufacture (Jones, 1977:29-43).

This data must be obtained at an early stage of identification of an active molecule as part of the chemical identification of an active compound. This can be done by DSC, Infra Red, Nuclear Magnetic Resonance and Ultra Violet spectroscopy and melting point. These are analytical procedures used to determine drug stability, incompatibility, dissolution, solubility etc. It can also be used to predict potential problems in the formulation of a new drug (Jones, 1977:29-43).

Preformulation studies provide a set of physico-chemical parameters against which detailed formulation design can be carried out (Jones, 1977:35-36).

Further goals are to establish the drug's kinetic rate profile, its physical characteristics and its compatibility with common excipients. With increasing specificity in drug design and a greater understanding of drug absorption, it is likely that this type of formulation will increasingly be used for oral dosage products. Each type of oral dosage form will demand a careful study of the physico-chemical parameters relevant to the nature of the product.

All the formulation work must follow a logical progression of scientific investigation. The formulator is subjected to a variety of constraints during the development of any particular drug delivery system (Jones, 1977:35-36).

5.1.1. Compatibility

It is necessary to evaluate which excipients can be used with a drug early in development programs. For solutions and suspensions, the solution kinetic studies will frequently give the necessary optimisation information. For solids, compatibility studies are more extensive. The purpose of such a compatibility study is to examine which excipients could be used with a new drug (Carstensen, 1990:263-300).

5.1.2. Thermal analysis

Thermal analysis can be used to examine and predict the physiochemical interactions in a formula. It can therefore be used to choose chemically compatible excipients.

5.1.2.1. Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA)

In the interpretation of the data, the thermal properties of a physical mixture is the sum total of the individual components. The thermogram of the formula is compared with each of the thermograms of the active ingredients and the excipients.

An interaction with the ingredients normally shows as a change in the melting point, the shape of the peaks or the surface of the peaks. Chemical interaction shows as new peaks with the excessively broad or long change of the endo- or exotherm. It might also be the formation of an eutectic point (Lötter, 1989:66-67).

DSC thermograms were recorded with a Shimadzu DSC-50 instrument under the following conditions:

- Sample holder: Aluminium crimp cell
- Sample weight: Approximately 2 mg
- Gas: Nitrogen at a flow rate of 45 ml/min
- Maximum temperature: 300 °C
- Heating rate: 10 °C/min

TGA thermograms were recorded with a Shimadzu TGA-50 instrument under the following conditions:

- Sample holder: Platinum cell
- Sample weight: Approximately 5.00 mg
- Gas: Nitrogen at a flow rate of 20 ml/min
- Maximum temperature: 250 °C
- Heating rate: 10 °C/min

5.1.2.1.1. Results

The DSC thermograms were obtained in the preformulation study. Thermograms of all the excipients have been done. Only the three active substances in the dosage form have been tested as a combination.

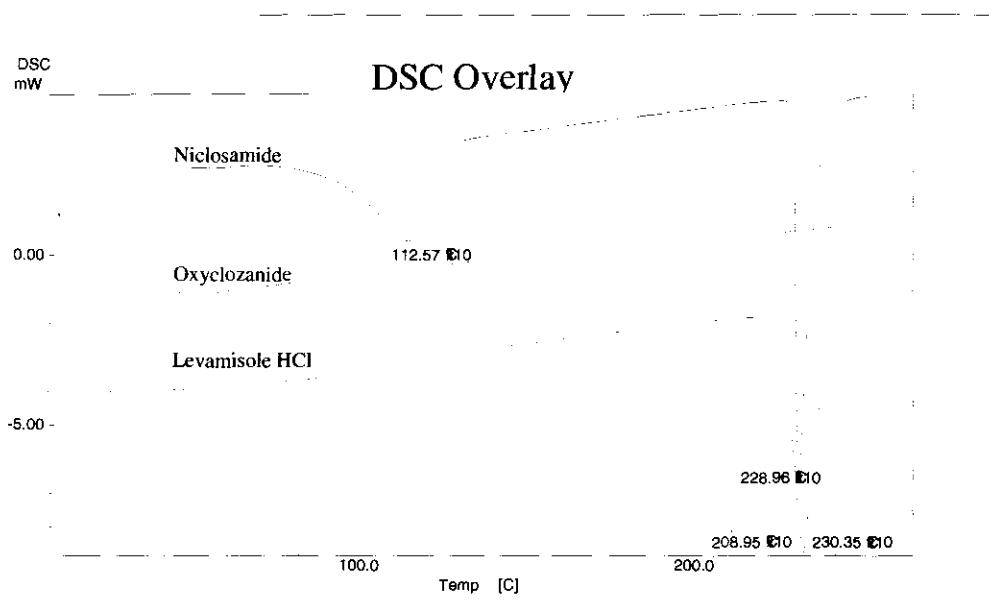


Figure 12. The DSC Thermogram of Levamisole HCl, Niclosamide and Oxytocanide.

As seen in Figure 12, the melting point of levamisole HCl was at 230.35°C, niclosamide at 228.96°C and oxytocanide at 208.95°C.

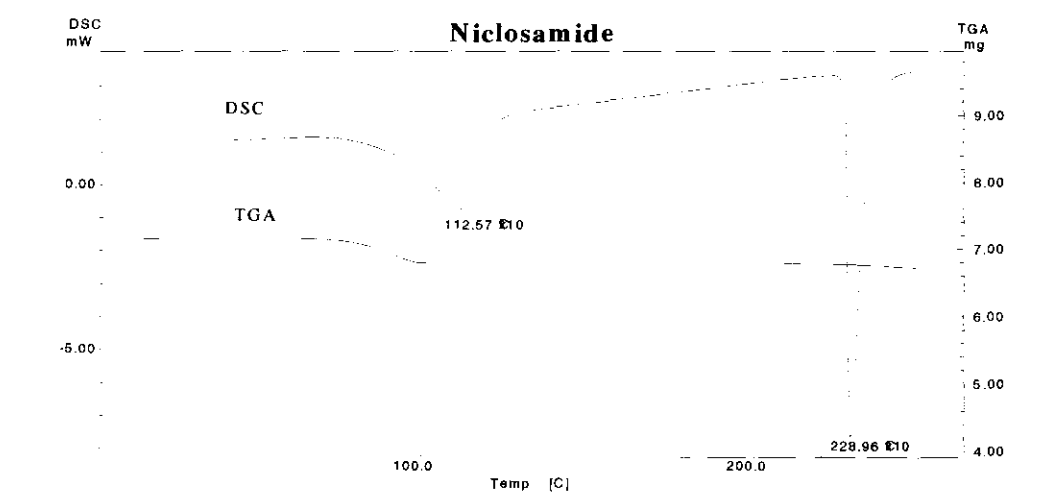


Figure 13. The DSC and TGA thermograms of niclosamide.

In Figure 13 a peak was noticed at 112.57°C. After doing a TGA, it was concluded that it was a hydrate peak. It also indicated that the niclosamide used in this formulation is niclosamide monohydrate.

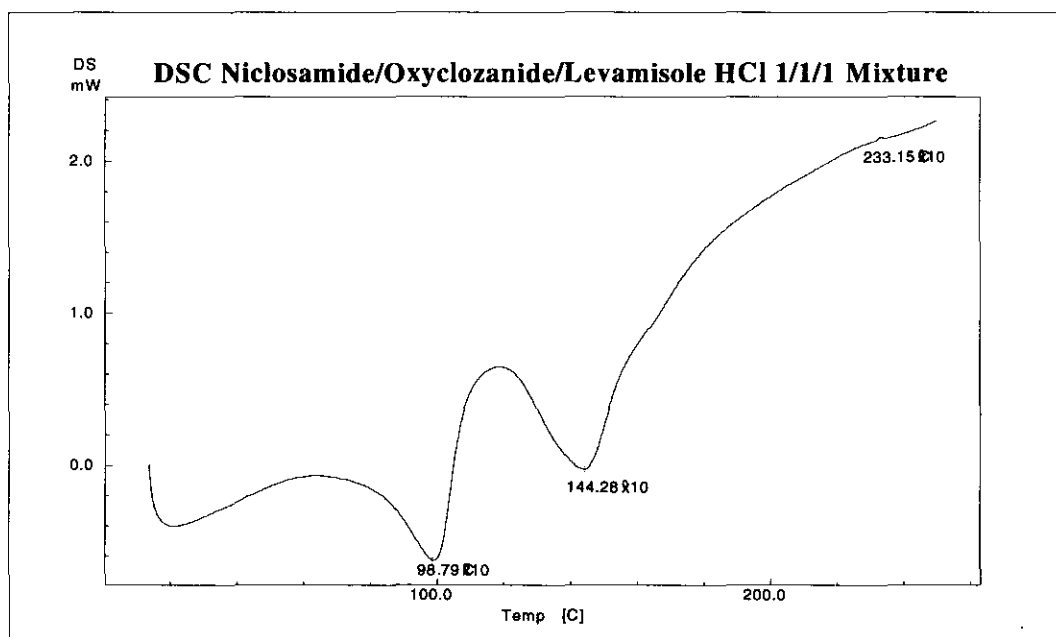


Figure 14. The DSC thermogram of a 1:1:1 mixture of levamisole HCl, niclosamide and oxyclozanide.

The 1:1:1 mixture, extra peaks appeared at 98.79°C and 144.28°C (Figure 14). The peaks that gave an idea of the incompatibility of the combination of the active substances are the peaks that formed at 98.79°C and 144.28°C. In a mixture of 1:7.7:1.3 of levamisole HCl, niclosamide and oxyclozanide a thermogram appeared with a possible hydrate of niclosamide at 112.57°C; another peak appeared around 135°C. In a study done by Maleka, findings show that niclosamide anhydrate hydrated in all the stability tests performed on the monohydrate (Maleka, 2000:58-77). This was confirmed in a conducted TGA, as seen in Figure 13.

A peak also appeared at 233.15°C. Although these peaks showed a possible incompatibility, it was decided to continue with the tests to determine if there were other incompatibilities. The assay of the combination will be discussed later in Chapter 5 (see section 5.3).

5.2. Formulation

An extensive amount of literature has been published about the formulation of tablets, solutions and suspensions. Instead of reproducing what has been written in other textbooks and articles, an account of the experiments, as well as the results of the experiments will be provided in this study.

The final product will be called **Nivanide**. This name is derived from **niclosamide**, **levamisole** and **oxyclozanide**. Although it is not a registered trade name, it is used to streamline logistics in this study.

5.2.1. Solution

5.2.1.1. Composition

Maximum solubility

The maximum solubility was determined to find the solubility parameters in which to formulate the solution. Solubility tests of niclosamide were done, using PEG 400, Tween 80® and propylene glycol. These solvents were chosen according to the solubility of niclosamide, as discussed in Chapter 3. Table 12 gives a summary of the formulas and the quantities used to determine the maximum solubility of levamisole HCl, niclosamide and oxyclozanide.

Formula 1X

The PEG 400, propylene glycol and Tween 80® were measured in a glass beaker. It was heated at 70-75°C and stirred at a speed of 250 rpm. Both the oxyclozanide and niclosamide were added at 15.0 g each. The reason for this was to determine the maximum solubility of both the actives. The concentration was approximately 4 times higher than the concentration decided on. This combination did not solubilise. Undissolved particles settled on the bottom of the beaker.

Formula 2X

The PEG 400, propylene glycol and Tween 80® were measured in a glass beaker. It was heated at 70-75°C and stirred at a speed of 250 rpm. Levamisole, oxyclozanide and niclosamide were added at 20.0 g, 15.0 g, and 15.0 g respectively. In a separate phase, distilled water was added to solubilise the levamisole. With the addition of the levamisole

solution to the water levamisole HCl precipitated and a thick paste formed. This proved, once again, that levamisole HCl could not be pre-dissolved in water. It had to be solubilised in another solubilising agent, like the propylene glycol being used in the final formula.

Formula 3X

The PEG 400, propylene glycol and Tween 80[®] were measured in a glass beaker. It was heated at 70-75°C and stirred at a speed of 250 rpm. Levamisole HCl, oxyclozanide and niclosamide were added at 20.0 g, 10.0 g, and 15.0 g respectively. Levamisole was added to the propylene glycol to establish maximum solubility. It did not solubilise completely in this solution. After adding it to the other solution it did not completely dissolve. The particles of both solutions were not completely soluble. A mass of crystals formed and settled on the bottom of the beaker.

Formula 4X

The PEG 400 and Tween 80[®] were measured in a glass beaker. It was heated at 70-75°C and stirred at a speed of 250 rpm. Levamisole HCl, oxyclozanide and niclosamide were added at 20.0 g, 10.0 g, and 15.0 g respectively. In a separate phase, distilled water and propylene glycol were added to solubilise the levamisole. With the addition of the levamisole solution, it stayed in solution for a short and then crystallised.

Table 12. A summary of the maximum solubility of levamisole HCl, niclosamide and oxyclozanide.

Ingredients in g/200 g	F1X	F2X	F3X	F4X
Levamisole HCl		20.0	20.0	20.0
Niclosamide	15.0	15.0	10.0	10.0
Oxyclozanide	15.0	15.0	15.0	15.0
Cremophor RH40 [®]				
Distilled Water		20.0		10.0
Ethanol				
Glycerol				
PEG 300				
PEG 400	130.0	100.0	130.0	120.0
PEG 1000				
PEG 4000				
Propylene Glycol	30.0	20.0	15.0	15.0

Tween 80®	10.0	10.0	10.0	10.0
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Formula 1 to Formula 11 shows the different formulas in which the solubilising of the active ingredients was attempted. In Formula 1 to Formula 8 it was attempted to solubilise niclosamide. Formula 8 is the final formula for the niclosamide solution and Formula 11 is the final formula for the Nivanide solution. Because of the difficulty solubilising niclosamide, it was decided to formulate a solution of niclosamide first, and then to add the rest of the active substances.

Formula 1

The PEG 300 was weighed in a glass beaker. It was heated to 70-75°C and stirred at a speed of 250 rpm. The niclosamide was accurately weighed and added. This was then stirred for approximately 30 min and then the Tween 80® and propylene glycol was added. The sample was made up to volume with glycerol.

In this solution the niclosamide solubilised only partly. There were still undissolved particles of the active ingredient in the solution. When the glycerol was added there was an immediate clouding effect on the solution. When left for one day, the particles formed small crystals and sediment formed on the bottom. This formula was indicative to the fact that niclosamide can be solubilised.

Formula 2

The PEG 300, Tween 80® and Cremophor RH40® were weighed in a glass beaker. It was heated to 70-75°C and stirred at a speed of 250 rpm. The niclosamide was accurately weighed and added. The distilled water and the glycerol were mixed. The sample was made up to volume with the glycerol mixture. This formula only solubilised partly. Upon addition of the glycerol-water mixture, it immediately formed a solid mass. This solid mass was so thick that it could not be decanted.

A positive point with the result in this formula was the fact that it might be used as a type of a long acting dosage form because of the solid mass it formed.

Formula 3

The PEG 300, the Tween 80[®] and Cremophor RH40[®] were weighed in a glass beaker. It was heated to 70-75°C and stirred at a speed of 250 rpm. The niclosamide was accurately weighed and added. The ethanol was added and upon the addition, it turned into a clear solution. The sample was made with distilled water and the glycerol were mixed. When making the sample up to volume with the propylene glycol, it turned into an opal colour. After a while, it formed a sediment of crystals on the bottom.

Addition of ethanol gave a clear solution, but it would not be permitted to use in veterinary medicine because of its potential toxicity.

Formula 4

A 10% m/m solution of PEG 4000 and distilled water were made. About 150 g of this solution was heated to 70-75°C and stirred at a speed of 250 rpm. Tween 80[®] was added, and then the accurately weighed niclosamide was added. It was then made up to volume with the rest of the PEG solution. This sample formed a suspension from the beginning. It did not stay in suspension for very long. It did, however, have very small particles of the niclosamide.

This combination might work as a base formula to formulate suspensions, but better thickeners should be used.

Formula 5

The PEG 300, the Tween 80[®] and propylene glycol were weighed in a glass beaker. It was heated to 70-75°C and stirred at a speed of 250 rpm. The niclosamide was accurately weighed and added. The water was added slowly. It immediately formed a thick mass. The thought of a slow release dosage form came to mind again. The addition of the water to the solution proves that water will precipitate niclosamide out of a solution. The effect was a micro fine precipitated thick mass.

Formula 6

The PEG 400 and the Tween 80[®] were weighed in a glass beaker. Half of the propylene glycol was also added. It was heated to 70-75°C and stirred at a speed of 250 rpm. The niclosamide was accurately weighed and added. Solubility was very good. Clarity was good except for a few undissolved particles.

Formula 7

A 10% m/m solution of PEG 1000, propylene glycol and distilled water were made. About 150 g of this solution was heated to 70-75°C and stirred at a speed of 250 rpm. The niclosamide was accurately weighed and added. A suspension formed that was easily resuspendable.

Although it is a very watery suspension, it might be worth considering when doing future formulations.

Formula 8

The PEG 400, the propylene glycol and the Tween 80® were weighed in a glass beaker. It was heated to 70-75°C and stirred at a speed of 250 rpm. The niclosamide was accurately weighed and added. This sample was clear and thus in a total solution.

The solubility of levamisole HCl and oxyclozanide was determined first to make the final solution.

Formula 9

The propylene glycol was weighed in a glass beaker. It was heated to 70-75°C and stirred at a speed of 250 rpm. The levamisole was accurately weighed and slowly added. Although levamisole HCl is water-soluble, it went into solution with propylene glycol.

This sample was made to see if the levamisole HCl would go into solution without the addition of water.

Formula 10

The PEG 400, the Tween 80® and propylene glycol were weighed in a glass beaker. It was heated to 70-75°C and stirred at a speed of 250 rpm. The oxyclozanide was accurately weighed and slowly added. Oxyclozanide was soluble in this combination, which meant that it could be solubilised together with the niclosamide.

Formula 11

The PEG 400 and the Tween 80® were weighed in a glass beaker. The propylene glycol was measured separately to solubilise the levamisole HCl first. It was heated to 70-75°C and stirred at a speed of 250 rpm. The niclosamide, levamisole HCl and oxyclozanide were accurately weighed and added. The niclosamide and oxyclozanide was solubilised. Then the solubilised levamisole was added. This sample was in solution without any crystallisation or sedimentation. This combination would be used as the final formula to be used in the stability tests.

In Table 13 the formulas are given in a summary to illustrate the above formulas and quantities. The final or working formula is discussed in Table 14, where the precise method of manufacture is described.

Table 13. A summary of the composition of the different formulas of the solution.

Ingredients in g / 200 g	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Levamisole HCl									20.0		5.0
Niclosamide	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0			6.0
Oxyclozanide										6.8	6.8
Cremophor RH40®		20.0	20.0								
Distilled Water		20.0		165.6	141.0		50.0				
Ethanol			50.0								
Glycerol	44.0	98.0									
PEG 300	100.0	50.0	50.0		50.0						
PEG 400						100.0		100.0		100.0	100.0
PEG 1000							20.0				
PEG 4000				18.0							
Propylene Glycol	20.0		67.0			88.0	124.0	84.0	180.0	83.0	72.0
Tween 80®	30.0	6.0	7.0	10.0	3.0	6.0		10.0		10.0	10.0

5.2.1.1.1. Discussion

In a study done by Torrado in 1997, it was concluded that a solution with a concentration of 25% albendazole was more effective than the 100% albendazole suspension. (Torrado *et al.*, 1997:184-185).

With this study in mind, it was decided to make the concentration of niclosamide 3% m/m, levamisole 2.5% m/m and the oxyclozanide 3.4% m/m as seen in Table 14 where the final

formula is given for Nivanide solution. Most of the solutions or suspensions on the market consist of levamisole 2.5% and oxyclozanide 3.4%. This combination subsisted, partly due to the calculation problems when dosing the animals and partly due to the fact that the levamisole HCl and oxyclozanide are proven to be effective at that dosage. Oxyclozanide in solution would probably also have a better bioavailability, which might increase effectivity. Neither the niclosamide nor the oxyclozanide have been tested for efficacy in a solution, thus we are assuming that it will increase bioavailability and effectivity.

5.2.1.2. Working formula

The total concentration of the active ingredients.

Levamisole: 2.50%

Niclosamide: 3.00%

Oxyclozanide: 3.40%

Table 14. The formula of Nivanide solution per 100 g.

Phase A	
PEG 400	50.00 g
Niclosamide	3.00 g
Tween 80®	5.00 g
Oxyclozanide	3.40 g
Propylene glycol	7.50 g
Phase B	
Levamisole HCl	2.5 g
Propylene Glycol	28.6 g

5.2.1.3. Preparation

Take the PEG 400, propylene glycol and Tween 80® in Phase A and heat them together at 70-75°C, stirring at a speed of 250 rpm. Add the niclosamide slowly. Keep on stirring until all the crystals are dissolved. Add the oxyclozanide and keep on stirring until the solution is clear.

Heat the Propylene Glycol as in Phase B in a different container and add the levamisole HCl. Keep on stirring until the solution is clear.

Mix Phase A and Phase B together and keep on stirring until the solution is clear.

5.2.1.4. Excipients and preservatives.

PEG 300, PEG 400, PEG 1500 and PEG 4000 – Solvent

PEG 300 and 400 have very good solvent power for numerous substances that are sparingly soluble in water. For this reason, the liquid Lutrol® E products are mainly used as solvents in the production of liquid preparations. The very good solvent power can be ascribed to the formation of a complex between polyethylene glycol and the active substance. Lutrol® E 1500, 4000, and 6000 can be used as carriers for active substances, solubilisers, and absorption improvers for substances that are insoluble or sparingly soluble in water. Polyethylene glycols are substances with very little acute toxicity and very good compatibility with the skin (BASF, 1998).

Propylene Glycol - Solvent

Propylene glycol is used as a solvent in the extraction of crude drugs and in preparation of solutions. Preparations made with propylene glycol are less viscous than those made with glycerol. In certain instances it is a better solvent than glycerol, and its power of inhibiting mould growth and fermentation is equal to that of alcohol. (Reynolds, 1982:709)

Tween 80®– Solubilising agent

Polysorbate 80 is used as a solubilising agent in a lot of substances like fish liver oils, vegetable oils and mineral oils. It is also used as a wetting agent in the formulation of oral and parental suspensions (Reynolds, 1982:378).

Preservation

No preservative was added because of the antimicrobial action of propylene glycol. The concentration of propylene glycol as a preservative is between 15–30% (Wade & Weller, 1994:407).

5.2.2. Suspension

5.2.2.1. Composition

The next descriptions are given for the preparation for the three formulas in Table 15. It was decided to stay with these concentrations because of the fact that it has been used at these concentrations in other registered products, as seen in Tables 8 to 11. Another motivation for keeping the concentrations as they are, is the fact that these products have been tried and tested in the market and the field.

Formula 1

The citric acid, potassium sorbate, sodium benzoate and sodium citrate were weighed accurately and dissolved in part of the distilled water. The levamisole HCL was added and the solution was stirred until all the crystals dissolved. The Kollidon CLM[®], niclosamide and oxyclozanide were weighed accurately, wetted with the propylene glycol and added slowly to the levamisole gel solution. The solution was filled up to 100 g with distilled water.

Formula 2

The citric acid, potassium sorbate, sodium benzoate, sodium citrate and sodium metabisulphite were weighed accurately and dissolved in part of the distilled water. The levamisole HCl was added and the solution was stirred until all the crystals dissolved. The niclosamide and oxyclozanide were weighed accurately, wetted with the propylene glycol and added slowly to the levamisole gel solution. The suspension was filled up to 100 g with distilled water and was stirred until smooth.

Formula 3

The citric acid, potassium sorbate, sodium benzoate, sodium citrate, sodium lauryl sulphate and sodium metabisulphite were weighed accurately and dissolved in part of the distilled water. The levamisole HCl was added and the solution was stirred until all the crystals dissolved. The niclosamide and oxyclozanide were weighed accurately, wetted with the propylene glycol and added slowly to the levamisole gel solution. The suspension was filled up to 100 g with distilled water and stirred until smooth.

Table 15. The composition of different formulas of the suspension.

Ingredients in g/100 g	F1	F2	F3
Levamisole HCl	2.50	2.50	2.50
Niclosamide	20.00	20.00	20.00
Oxyclozanide	3.40	3.40	3.40
Citric acid	0.49	0.49	0.49
Kollidon CLM®	10.00	0.00	0.00
Distilled water	57.02	66.72	66.67
Potassium sorbate	0.02	0.02	0.02
Propylene glycol	5.00	5.00	5.00
Sodium benzoate	0.10	0.10	0.10
Sodium citrate	1.47	1.47	1.47
Sodium lauryl sulphate	0.00	0.00	0.05
Sodium meta bisulphite	0.00	0.10	0.10
Xanthan gum	0	0.2	0.2

5.2.2.1.1. Discussion

Although a decrease in the concentration of the Kollidon® CLM will help in making the suspension less viscous in Formula 1, it is still more expensive than the 0.2% Xanthan gum used in Formula 2 and 3 as seen in Table 15. The resultant suspension was a viscous liquid that was redispersable. Although the suspension with Kollidon® CLM was redispersable, the Xanthan gum produced better results as a suspending agent.

The suspension in Formula 2 was too difficult to disperse and a surface active agent was necessary for two reasons: 1) It will help break the surface tension of the suspension and stop foaming and 2) it will help with flocculation.

The resuspendability of the suspension in Formula 3 was very good. The suspension did not foam, and flocculation was good. Of the three formulas, this combination is the most suitable.

5.2.2.2. Working formula

Total concentration of the active ingredients:

Levamisole HCl: 2.5%,

Niclosamide: 3.4%,

Oxyclozanide: 20.0%.

The excipients in the formula in Table 16 will be discussed under the heading: Excipients and preservatives.

Table 16. The formula of Nivanide suspension per 100 g.

Phase A	
Citric acid	0.49
Distilled water	66.67
Potassium sorbate	0.02
Sodium benzoate	0.10
Sodium citrate	1.47
Sodium lauryl sulphate	0.05
Sodium meta bisulphite	0.10
Phase B	
Levamisole HCl	2.50
Phase C	
Xanthan gum	0.2
Phase D	
Niclosamide	20.00
Oxyclozanide	3.40
Propylene glycol	5.00

5.2.2.3. Preparation

Method

Accurately weigh and dissolve the excipients in Phase A in 50 ml of water. Stir until dissolved. Add phase B the (levamisole HCl) to the solution and stir until the solution is clear and all the levamisole HCl is dissolved. Add Phase C to gel the solution and stir well. Wet the powders in Phase D, add it to the gelled solution and keep on stirring until the suspension is smooth. Add water to make up to 100 g.

5.2.2.4. Excipients and preservatives

According to Ofner *et al.* (1996:151), specific desired attributes of a suspension as a dosage form may depend on the physical/chemical nature of the drug. A typical suspension may contain the following: a wetting agent, a suspending agent, a protective colloid, a flocculating agent, a sweetener, a preservative, a buffer system, a flavour, a colour, a sequestering agent and an antifoaming agent (Ofner *et al.*, 1996:151).

Xanthan gum - Suspending agent

The use of xanthan gum in the formulation of suspensions are increasing for a number of reasons: it is a high molecular weight natural polysaccharide produced through microbial fermentation; it has a good batch-to-batch uniformity; it has few microbial contamination problems because it is unusually resistant to enzymes; and it is soluble in both hot and cold water. Xanthan gum is particularly useful as a suspending agent because of its shear thinning or pseudoplastic behavior. Its solution viscosity is independent of temperature and pH. Another major advantage is that it is used in a pH range from 2-13 and at a concentration of 0.3-3%. Xanthan gum helps as a flocculating agent (Ofner *et al.*, 1996:165).

Propylene glycol - Wetting agent

Propylene glycol has become widely used as a solvent, extractant and preservative in a variety of parenteral and non-parenteral pharmaceutical formulations. It is a better general solvent than glycerin and it dissolves a wide variety of materials. Propylene glycol consists of less than 0.2 % water. This makes it an ideal wetting agent, not only for the suspending agent but also for the active ingredients (Wade & Weller, 1994:407).

Xanthan gum, sodium lauryl sulphate - Flocculating agent

It is necessary for the suspension to be flocculated. This may be achieved by the addition of electrolytes, surfactants and/or hydrophilic polymers. Flocculating agents enable particles to link together in loose aggregates or flocs. These flocs settle rapidly, but must be redispersed easily (Ofner *et al.*, 1996:166).

Citric acid, Sodium citrate - Buffer system

The inclusion of buffers may be necessary in order to maintain chemical stability, control and tonicity or to ensure physiological compatibility. Buffers are used to control the pH of the formulation. The pH is often adjusted to ensure that the drug stays insoluble (Ofner *et al.*, 1996:167).

Sodium benzoate, potassium sorbate - Preservative

Preservatives are required in most suspensions because the suspending agents and sweeteners are very good growth media for microorganisms.

A preservative efficacy test was done as described by the USP (USP 24, 2000:1809, 1818). The test result showed no bacterial, fungal or any other growth that would pose a threat to the stability of the suspension.

5.2.3. Tablet

5.2.3.1. Composition

The next descriptions are given for the preparation for the three formulas in Table 17.

Formula 1

All the ingredients were accurately weighed in glass beakers. All the powders were added together and mixed in a Turbula® mixer for 10 minutes. The tablets were compressed by means of direct compression. The tablet dispersed, but not fast enough. It was decided to increase the concentration of the Explotab® to see if the tablet will break up faster. It was also decided to increase the concentration of Aerosil® to accommodate, by means of adsorption, any liquid that may interfere with the stability of the tablet.

Formula 2

All the ingredients were accurately weighed in small glass beakers. All the powders were added together and mixed in a Turbula® mixer for 10 minutes. The tablets were compressed by means of direct compression. The main concern with the tablets in formula 2 was picking and capping. Most of the tablets of this batch split. Although the Aerosil® works well at lower concentrations, it caused the picking because of the dryness of the tablet. The tablet did, however, disperse faster because of the increased concentration of Explotab®.

Formula 3

All the ingredients were accurately weighed in small glass beakers. All the powders were added together and mixed in a Turbula® mixer for 10 minutes. The tablets were compressed by means of direct compression. The Aerosil® concentration was changed back to 3 mg per tablet and this, together with the increased concentration of Explotab® gave a good balance for dispersibility.

The total concentration of the active ingredient:

Levamisole: 3.00%

Niclosamide: 24.00%

Oxyclozanide: 4.08%

Table 17. The combination of different formulas for the tablet.

Ingredients per tablet	F1	F2	F3
Levamisole HCl	75.00	75.00	75.00
Niclosamide	600.00	600.00	600.00
Oxyclozanide	102.00	102.00	102.00
Aerosil®	3.00	10.00	3.00
Explotab®	80.00	100.00	100.00
Magnesium Stearate	35.00	35.00	35.00
PEG 6000	35.00	35.00	35.00
PVP 25	540.00	54.00	54.00
Tabletose	1030.00	1489.00	1496.00

One tablet weighs 2500 mg.

5.2.3.1.1. Discussion

The tablet was manufactured with direct compression. The technology involved in direct compression is of great importance in tablet formulations, because it is often the cheapest means.

As previously discussed, the idea was to formulate a dispersible tablet that could be dispersed in a syringe with water. Two of the key advantages are that the tablets could be sold separately for use by livestock farmers in rural or under-developed areas, and that it is easily dispersed and administered. The excipients were carefully chosen to give maximum dispersability for this specific combination. Furthermore, the Explotab® volumises the water in the syringe and the dispersed end product gives a loosely flocculated suspension in the syringe that is easy to administer.

5.2.3.2. Working formula

Table 18. The final formula for Nivanide tablets.

Ingredients in (mg) per tablet	
Levamisole HCl	75.00
Niclosamide	600.00
Oxyclozanide	102.00
Aerosil®	3.00
Explotab®	100.00
Magnesium Stearate	35.00
PEG 6000	35.00
PVP 25	54.00
Tabletose ®	1496.00

5.2.3.3. Preparation

Weigh all the ingredients accurately in small glass beakers. Add all the powders together and mix in a Turbula® mixer for 10 minutes. Compress the tablets by means of direct compression to a weight of ± 2.50 g, a hardness of ± 60 N, a diameter of ± 20.20 mm and a thickness of ± 6.30 mm.

5.2.3.4. Excipients and preservation

Aerosil® – Glidant and adsorbent

Adsorbents such as silicon dioxide (Aerosil®) are capable of retaining large quantities of liquid without becoming wet. This allows many oils, fluid extracts, and eutectic melts to be incorporated into tablets. Silicon dioxide can also exhibit glidant properties and can play both a glidant and an adsorbent role in the formula (Banker *et al.*, 1980:98).

Explotab® – Disintegrant

The purpose of a disintegrant is to facilitate the breakup of a tablet after administration. Modified starches with dramatic disintegration properties have recently become available as Primogel® and Explotab®, which are low substituted carboxy methyl starches. While natural pre-dried starches swell to the extent of 10 to 25%, these modified starches increase in volume by 200 to 300% in water (Banker *et al.*, 1980:87).

Magnesium stearate Lubricant and antiadherent

The primary function of tablet lubricants is to reduce the friction arising at the interface of the tablet and the wall during compression and ejection. The lubricants may also possess

antiadherent or glidant properties. Magnesium stearate is a water insoluble lubricant and is usually incorporated at 0.25 to 2.00 % in the formula (Banker *et al.*, 1980:98).

PEG 6000 – Lubricant and binder

PEG 1500, 4000, and 6000 can be used as carriers for active substances, solubilizers, and absorption improvers for substances that are insoluble or sparingly soluble in water, e.g. steroids and fat-soluble vitamins. They are usually processed in the form of a melt in this case. They also act as lubricants and binders for the production of tablets. In particular, the flexibility of tablets and cores of sugarcoated tablets are increased by PEG 4000 and 6000 (BASF, 1998).

PVP 25 – Binder and adhesive

Binders and adhesives are added to tablet formulations to add cohesiveness to powders. Polyvinylpyrrolidone (PVP) is an alcohol soluble material, which is used in concentrations between 3 and 15% (Banker *et al.*, 1980:85).

Tabletose® – Diluent

Lactose is the most widely used diluent in tablet formulation. It displays good stability in combination with most drugs. Lactose is freely soluble in water. Lactose formulations usually show good drug release rates, is easy to dry and is not sensitive to moderate variation in tablet hardness. The cost of lactose is low relative to other diluents (Banker *et al.*, 1980:76).

5.3. Stability test and test method

All three of the formulated products have been stored in accelerated conditions. Stability tests have been done at 0 weeks and after 12 weeks. This was done for two reasons: to determine stability and the regulatory authority in South Africa suggests an accelerated stability test of 12 weeks for anthelmintics.

Each of the products was divided in three batches. One batch of each product was stored at 25°C with a Relative Humidity (RH) of 60%, 30°C with a Relative Humidity (RH) of 60% and 40°C with a Relative Humidity (RH) of 75%.

Tests performed on the solution and suspension before and after the stability test included viscosity, colour, clarity, taste, pH, density and an assay.

Tests performed on the tablets before and after the stability tests included tablet hardness, diameter and thickness, average mass and disintegration, loss on drying (LOD) and an assay.

5.3.1. Solution

5.3.1.1. Physical tests

This is a description of the methods and the instruments used to do the physical tests at 0 and 12 weeks.

Viscosity

The viscosity was measured with a Brookfield Model DV-II rotational viscometer using method 911 in the USP (USP24, 2000:2002). Spindle number LVT2 was used for the solution and LVT3 for the suspension. Nine measurements were taken at 2-minute intervals at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The value was taken at 60 rpm after 18 minutes.

Colour, clarity, odour and taste

The colour and clarity was evaluated visually at a viewing station and recorded as such. The odour and taste was also recorded by means of tasting and smelling.

pH

The pH was measured with a calibrated Mettler Toledo MP220 pH meter using method 791 in the USP (USP24, 2000:1977). The measurements were made at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The sample was shaken before the measurement was made.

Density

The density was measured according to method 841 in the USP (USP24, 2000:1990) at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The weight of exactly 25 ml of freshly prepared distilled water and 25 ml solution was measured on a calibrated and tarred Sartorius balance. The relative density was calculated according to the following formula:

$$\text{relative density} = \frac{\text{mass of sample}}{\text{volume of sample}}$$

where the mass of the sample is measured in gram (g) and volume of sample measured in millilitre (ml).

The results of the physical tests done at 0 and 12 weeks on Nivanide solution are given in Table 19.

Table 19. The results of the physical tests done on Nivanide solution.

Viscosity in (cP)	Nivanide at 0 weeks	102.5
	Nivanide 25°C at 12 weeks	103.0
	Nivanide 30°C at 12 weeks	103.0
	Nivanide 40°C at 12 weeks	101.0
Colour	No difference in colour after 12 weeks.	
Clarity	No difference in clarity after 12 weeks.	
Odour	No difference in odour after 12 weeks.	
Taste	No difference in taste after 12 weeks.	
pH	Nivanide at 0 weeks	3.25
	Nivanide 25°C at 12 weeks	2.56
	Nivanide 30°C at 12 weeks	2.50
	Nivanide 40°C at 12 weeks	2.24
Density in (g/ml)	Nivanide at 0 weeks	1.128
	Nivanide 25°C at 12 weeks	1.135
	Nivanide 30°C at 12 weeks	1.137
	Nivanide 40°C at 12 weeks	1.133

5.3.1.1.1. Discussion

There seems to be no difference of relevance in the viscosity. The average viscosity was 102.4 cP. The viscosities remained between 101.0 and 103.0 cP for each of the batches. Thus, the viscosity of the solution was not affected by the stability conditions.

No difference in the colour was detected. Before the stability tests, the solution had a light brown almost amber colour, it stayed the same in all three test batches. The colour was not affected by the stability conditions.

No difference in clarity was detected in any of the three test batches. Before and after the stability test, the solution was perfectly clear without any undissolved particles. The clarity was not affected by the stability conditions as no crystals or sediment formed.

Although taste would not really be one of the physical parameters if you formulate an anthelmintic solution for livestock, it was very interesting to observe it as a parameter. It had a slightly bitter taste with a local anaesthetic effect on the tongue. Looking at the lighter side of this phenomenon is that even though it might not be palatable for the animal, the local anaesthetic effect will definitely take away the after taste. The three test batches had exactly the same taste and numbness of the tongue. Before and after the stability test, the solution had the same taste. The taste was not affected by the stability conditions.

The pH dropped from 3.25 to 2.24. Although this is different in all three test batches, it did not affect the stability of the solution. A buffer can be included in the formula to try and regulate the pH. Although there was a slight change in density it was not enough to adversely affect the stability.

5.3.1.2. Chemical tests

5.3.1.2.1. Assay

Although a lot of assays have been done on the active substances in Nivanide solution, the simultaneous assay has not been done before. This is a short summary to describe the method and instruments used to do the assay as well as the validation. To see the validation method and results, refer to Appendix 1.

Chromatographic conditions:

Analytical instrument: HP1050 series HPLC equipped with a HP1050 ternary pump, HP1050 autosampler, HP1050 diode array detector and Chemstation Rev. A.06.03 data acquisition and analysis software or equivalent

Column: USP 24 (2000) packing L1, p1924, Luna C18-2 column, 250 x 4.6 mm, 5 μ m, 100 Å pores, 17.8% carbon load, Phenomenex, Torrance, CA.

Mobile phase:	Acetone	25%
	Acetonitrile	25%
	Methanol	50%
Flow rate:	1.0 ml/min.	
Injection volume:	20 ul.	
Detection:	UV at 254 nm.	
Solvent:	Mobile phase.	
Retention time:	Levamisole:	2.0-3.2 min
	Oxyclozanide:	8.5-13.8 min
	Niclosamide:	16.2-23.3 min
	Acetone:	2.5-3.5 min

Because of the solubility problems of niclosamide and oxyclozanide, it was difficult to get the right mobile phase. A mixture of methanol and 10% formic acid solution and phosphate buffer with methanol was used, but the niclosamide immediately precipitated. This left only volatile solvents to use both as mobile phase and solvent. Acetonitrile and methanol was used at first but no definite niclosamide peaks eluated. Out of experience, it is known that acetone dissolves niclosamide and it was decided to add it to the mobile phase. Acetone peaks between 2.5 and 3.5 minutes. Fortunately, the active substances in this sample give definite and distinct peaks that are easily recognisable.

The following instructions are followed to prepare a sample for analysis:

1. Place a 100 ml volumetric flask on an analytical balance and tare.
2. Accurately weigh 1 g of the solution into a 100 ml volumetric flask.
3. Add about 70 g of mobile phase into the flask and shake well.
4. Check that all the suspension or tablet powder has dissolved. If solid pieces are still visible, shake the flask and sonicate it again until it is completely dissolved.
5. Allow the flask to cool to room temperature and fill to 100 g with solvent.
6. Filter the solution into an autosampler vial and analyse.

To prepare a standard for the solution, the following instructions are performed:

1. Weigh approximately 2.5 g of levamisole HCl, 3.0 g of niclosamide and 3.4 g of oxyclozanide accurately.
2. Transfer into a 100 ml volumetric flask and dissolve, and make up to 100 g with solvent.
3. Dilute 1 g of this solution to 100 g with solvent.
4. Transfer this solution into an autosampler vial and inject into the chromatograph.

The following formula was used to calculate the final concentration in % w/w:

$$\text{\% of moisture} = \frac{\text{Mass of sample before drying} - \text{Mass of sample after drying}}{\text{Mass of sample before drying}}$$

The assay results of Nivanide solution are given in Table 20 and Figure 15.

Nivanide at 0 weeks is equal to Fresh sample in Figure 15.

Nivanide 25°C at 12 weeks is equal to 25°C RH 60% in Figure 15.

Nivanide 30°C at 12 weeks is equal to 30°C RH 60% in Figure 15.

Nivanide 40°C at 12 weeks is equal to 40°C RH 75% in Figure 15.

Table 20. The assay of Nivanide solution.

	% of Levamisole HCl	% of Oxyclozanide	% of Niclosamide
Nivanide at 0 weeks	105.40	104.17	106.73
Nivanide 25°C at 12 weeks	102.35	109.07	108.74
Nivanide 30°C at 12 weeks	101.75	107.27	106.61
Nivanide 40°C at 12 weeks	98.27	95.69	95.16

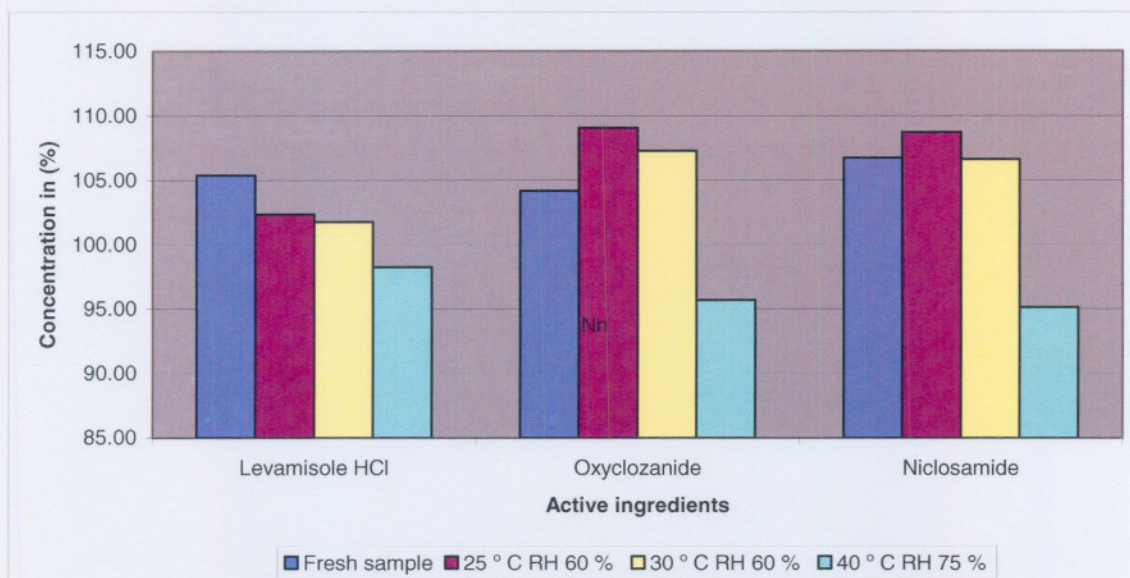


Figure 15. The assay results of Nivanide solution.

5.3.1.2.1.1. Discussion

Although there were different concentrations for each of the active ingredients, it stayed well within the parameters of 95% to 105%. Looking at the concentration of the actives at 40°C RH 75%, it is clearly visible that the higher temperature and humidity have a negative effect on the product. It would therefore be advisable to keep the product in a cool dry place to extend the shelf life. The solution was stable under the test conditions.

5.3.2. Suspension

5.3.2.1. Physical parameters

This is a short description of the methods and the instruments used to do the physical tests at 0 and 12 weeks.

Viscosity

The viscosity was measured with a Brookfield Model DV-II rotational viscometer using method 911 in the USP (USP24, 2000:2002). Spindle number LVT3 was used for the suspension. Nine measurements were taken at 2-minute intervals at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The value was taken at 60 rpm after 18 minutes.

Colour, clarity, odour and taste

The colour and clarity was evaluated visually at a viewing station and recoded as such. The odour and taste was also recorded by means of tasting and smelling.

pH

The pH was measured with a calibrated Mettler Toledo MP220 pH meter using method 791 in the USP (USP24, 2000:1977). The measurements were made at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The sample was shaken before the measurement was made.

Density

The density was measured according to method 841 in the USP (USP24, 2000:1990) at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The weight of exactly 25 ml of freshly prepared distilled water and 25 ml solution was measured on a calibrated and tared Sartorius balance. The relative density was calculated according to the following formula:

$$\text{relative density} = \frac{\text{mass of sample}}{\text{volume of sample}}$$

where the mass of sample is measured in gram (g) and the volume of the sample in millilitre (ml).

Resuspendability and sedimentation rate

The resuspendability and sedimentation rates were determined using the 24-hour method, where a sample is shaken and exactly 100 ml of the suspension is transferred to a glass measuring cylinder. The cylinder was closed and left to stand for 24 hours. The volumes of both the sediment and the supernatant were measured. After the measurements, the cylinder was shaken. This is to determine the resuspendability of the sediment and to see if the sediment would be resuspendable in 1 minute.

Preservative and efficacy test

The preservative efficacy test was done as prescribed by the USP (USP24, 2000:1809,1818). The test was performed by the Wits Health Consortium (Pty) Ltd trading as Adcibact.

The results of the physical tests done at 0 and 12 weeks on Nivanide suspension are given in Table 21.

Table 21. The physical parameters for Nivanide suspension.

Viscosity in (cP)	Nivanide at 0 weeks	707.8
	Nivanide 25°C at 12 weeks	813.8
	Nivanide 30°C at 12 weeks	941.8
	Nivanide 40°C at 12 weeks	1030.0
Colour	After 12 weeks the colour changed slightly colour.	
Odour	No difference	
Taste	No difference	
pH	Nivanide at 0 weeks	4.91
	Nivanide 25°C at 12 weeks	4.86
	Nivanide 30°C at 12 weeks	4.82
	Nivanide 40°C at 12 weeks	4.67
Density in (g/ml)	Nivanide at 0 weeks	1.102
	Nivanide 25°C at 12 weeks	1.097
	Nivanide 30°C at 12 weeks	1.109
	Nivanide 40°C at 12 weeks	1.105
Resuspendability and Sedimentation rate.	Nivanide at 0 weeks	comply
	Nivanide 25°C at 12 weeks	comply
	Nivanide 30°C at 12 weeks	comply
	Nivanide 40°C at 12 weeks	comply

5.3.2.1.1. Discussion

There is a difference in the viscosity of the suspension. The average viscosity was 873.35 cP. The change in viscosities did not adversely affect the stability of the suspension. No difference in the colour was detected. Before the stability tests, the suspension had a light yellowy to cream colour. It stayed the same in all three test batches. The colour changed slightly but the taste were not affected by the stability conditions.

There was a slight decline in the pH. Although there is a difference in all three test batches, it did not affect the stability of the solution. A buffer was included in the formula and it regulated the pH. It did not affect the stability of the suspension.

There was almost no change in the density of the suspension.

Resuspendability and sedimentation rate complied in all three samples.

Preservative efficacy complies.

5.3.2.2. Chemical tests

5.3.2.2.1. Assay

This is a short summary to describe the method and instruments used to do the assay and the validation. To see the full validation method and results go to Appendix 1.

Chromatographic conditions:

Analytical instrument: HP1050 series HPLC equipped with a HP1050 ternary pump, HP1050 autosampler, HP1050 diode array detector and Chemstation Rev. A.06.03 data acquisition and analysis software or equivalent

Column: USP 24 (2000) packing L1, p1924, Luna C18-2 column, 250 x 4.6 mm, 5 μ m, 100 Å pores, 17.8% carbon load, Phenomenex, Torrance, CA.

Mobile phase: Acetone 25%
Acetonitrile 25%
Methanol 50%

Flow rate: 1.0 ml/min.

Injection volume: 20 μ l.

Detection: UV at 254 nm.

Solvent: Mobile phase.

Retention time: Levamisole: 2.0-3.2 min
Oxyclozanide: 8.5-13.8 min
Niclosamide: 16.2-23.3 min
Acetone: 2.5-3.5 min

The following instructions are followed to prepare a sample for analysis:

1. Place a 100 ml volumetric flask on an analytical balance and tare.
2. Accurately weigh 1 g of the suspension into a 100 ml volumetric flask.
3. Add about 70 g of mobile phase to the flask and put the flask in an ultrasonic bath, with lukewarm water for 15 to 45 minutes.
4. Check that the suspension has dissolved. If solid pieces are still visible, shake the flask and sonicate it again until it is completely dissolved.
5. Allow the flask to cool to room temperature and fill to 100 g with solvent.
6. Filter the solution into an autosampler vial and analyse.

To prepare a sample for analysis the following instructions are performed:

1. Weigh approximately 2.5 g of levamisole HCl, 20.0 g of niclosamide and 3.4 g of oxyclozanide accurately.
2. Transfer it into a 100 ml volumetric flask and dissolve, and make up to 100g with solvent.
3. Dilute 1 ml of this solution to 100 g with solvent.
4. Transfer this solution to an autosampler vial and inject into the chromatograph.

To get to the final concentration in % w/w the following formula was used in the calculations:

$$\text{\% of moisture} = \frac{\text{Mass of sample before drying} - \text{Mass of sample after drying}}{\text{Mass of sample before drying}}$$

Nivanide at 0 weeks is equal to Fresh sample in Figure 16.

Nivanide 25°C at 12 weeks is equal to 25°C RH 60% in Figure 16.

Nivanide 30°C at 12 weeks is equal to 30°C RH 60% in Figure 16.

Nivanide 40°C at 12 weeks is equal to 40°C RH 75% in Figure 166.

The assay results of Nivanide suspension are given in Table 22 and Figure 16.

Table 22. The assay of Nivanide suspension.

	% of Levamisole HCl	% of Oxyclozanide	% of Niclosamide
Nivanide at 0 weeks	97.75	97.75	98.83
Nivanide 25°C at 12 weeks	103.22	108.84	97.89
Nivanide 30°C at 12 weeks	99.34	100.1	93.45
Nivanide 40°C at 12 weeks	97.84	110.92	103.92

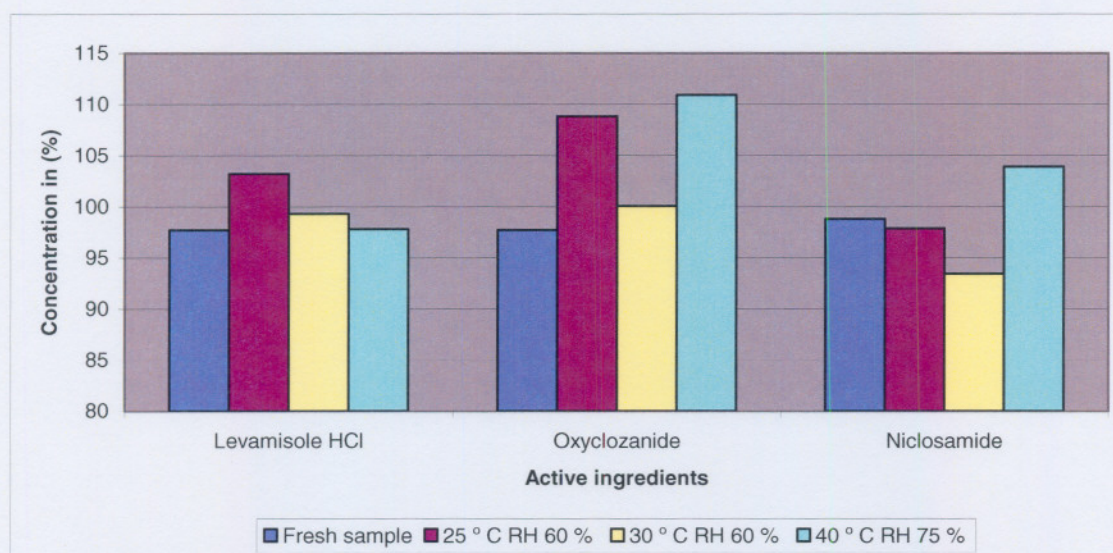


Figure 16. The assay results of Nivanide suspension.

5.3.2.2.1.1. Discussion

When looking at the graphic results of the suspension's assay, it is clear that it stayed within the limits. The concentration of oxyclozanide was higher than 110% but the other active ingredients were not affected. It might be because of the plastic bottles in which the samples were tested, in that the water in the suspension might have diffused through the plastic or evaporated because of the heat and humidity at 40°C. Taking all factors into account, the suspension was stable and might be more stable than the solution at warmer temperatures.

5.3.3. Tablets

5.3.3.1. Physical tests

5.3.3.1.1. Tablet dimension and hardness

The hardness, the thickness and the diameter of each batch of the tablets were determined using a PharmaTest PTB-311 tablet hardness tester. The 10 tablets were chosen randomly out of each batch, dusted and measured. Table 23 provides the results of the hardness, diameter and thickness of the tablets. The humidity during the 12 week period was RH 60% at 25°C, RH 60% at 30°C and RH 75% at 40°C.

Table 23. The dimensions and hardness of Nivanide tablets.

Hardness in (N)											
Nivanide at 0 weeks	53.50	46.60	67.40	48.20	67.40	51.50	55.60	44.90	49.90	66.60	55.16
Nivanide 25°C at 12 weeks	59.2	66.6	67.9	56.0	54.8	49.4	57.2	62.1	63.7	53.5	59.04
Nivanide 30°C at 12 weeks	56.0	57.6	52.7	77.6	46.2	78.5	54.3	61.7	71.5	51.1	60.72
Nivanide 40°C at 12 weeks	79.3	64.6	68.2	67.0	77.3	74.8	66.2	71.5	75.6	74.4	71.89
Diameter in (mm)											
Nivanide at 0 weeks	20.21	20.21	20.20	20.21	20.21	20.22	20.22	20.21	20.22	20.21	20.21
Nivanide 25°C at 12 weeks	20.2	20.22	20.21	20.21	20.2	20.22	20.21	20.22	20.22	20.22	20.21
Nivanide 30°C at 12 weeks	20.24	20.24	20.22	20.2	20.22	20.2	20.22	20.21	20.22	20.22	20.22
Nivanide 40°C at 12 weeks	20.3	20.25	20.21	20.22	20.21	20.22	20.22	20.23	20.2	20.21	20.23
Thickness in (mm)											
Nivanide at 0 weeks	6.27	6.32	6.27	6.27	6.31	6.25	6.28	6.28	6.35	6.29	6.29
Nivanide 25°C at 12 weeks	6.28	6.34	6.31	6.30	6.30	6.27	6.31	6.30	6.28	6.31	6.30
Nivanide 30°C at 12 weeks	6.28	6.31	6.30	6.27	6.36	6.29	6.34	6.32	6.29	6.30	6.31
Nivanide 40°C at 12 weeks	6.34	6.27	6.32	6.30	6.33	6.33	6.31	6.32	6.31	6.35	6.32

5.3.3.1.1.1. Discussion

The tablet hardness increased over a period of time, from 55.16 N to 71.89 N. This might be due to evaporation of moisture from plastic container. However, this does not affect the physical stability of the tablet.

Although there are an increase in values of both the tablet diameter and thickness, it does not affect the stability of the tablet.

5.3.3.1.2. Average mass and disintegration

Average mass

Twenty tablets are randomly chosen and dusted, and the average weight is determined as described in The British Pharmacopoeia (2000:A231). The results are given in Table 24.

Table 24. The average mass of Nivanide tablets.

Average mass of the tablets in mg	
Nivanide at 0 weeks	2504.00
Nivanide 25°C at 12 weeks	2496.00
Nivanide 30°C at 12 weeks	2497.00
Nivanide 40°C at 12 weeks	2489.00

Disintegration

Six tablets were selected randomly from each batch. The disintegration tests were performed with a PharmaTest PTZ Tablet and Capsule disintegrator, described in method 701 in the USP (USP24, 2000:1941). The procedure used for uncoated tablets was followed, where one tablet was put in each of the six tubes. It was immersed in water with a temperature of $37 \pm 2^\circ\text{C}$. The disintegration time of the tablets were noted. All the tablets complied with the specifications. Nivanide at 0 weeks, Nivanide 25°C at 12 weeks, 30°C at 12 weeks and 40°C at 12 weeks all disintegrated under 2 minutes and 12 seconds.

5.3.3.1.2.1. Discussion

The average mass decreases with warmer stability conditions. This may be the case because of the evaporation of liquid from the plastic containers as previously discussed. Disintegration times were fast. This has a very big advantage in the use and administration of the dosage form. These quick-dispersing tablets would disperse even quicker in a syringe when vigorously shaken. This means that the quicker the tablet disperses the quicker it can be administered and the less time and effort would be needed.

5.3.3.1.3. Moisture content

The moisture content was determined according to method 731 in the USP (USP24, 2000: 1954). Twenty tablets from each batch were crushed. Of this, a duplicate of 1 g was accurately weighed in a tarred glass beaker. The samples in the beaker were then dried over phosphorous pentoxide for two hours under a pressure of 112.5 mmHg at 50°C. After

drying, the samples were placed in a dessicator until it reached room temperature before it was measured again.

The formula below was used to give the % of moisture as given in Table 25.

$$\% \text{ of moisture} = \frac{\text{Mass of sample before drying} - \text{Mass of sample after drying}}{\text{Mass of sample before drying}}$$

Table 25. The % of moisture of Nivanide tablets.

	Sample 1	Sample 2	Average
Nivanide at 0 weeks	4.47	4.43	4.45
Nivanide 25°C at 12 weeks	4.20	3.76	3.98
Nivanide 30°C at 12 weeks	3.24	3.40	3.32
Nivanide 40°C at 12 weeks	3.60	3.23	3.41

5.3.3.1.3.1. Discussion

The percentage of moisture in the tablets was determined, with values between 4.47% and 3.23%. It indicates that the tablets are not inclined to absorb moisture.

5.3.3.2. Chemical tests

5.3.3.2.1. Assay

Chromatographic conditions:

Analytical instrument: HP1050 series HPLC equipped with a HP1050 ternary pump, HP1050 autosampler, HP1050 diode array detector and Chemstation Rev. A.06.03 data acquisition and analysis software or equivalent

Column: USP 24 (2000) packing L1, p1924, Luna C18-2 column, 250 x 4.6 mm, 5 µm, 100 Å pores, 17.8% carbon load, Phenomenex, Torrance, CA.

Mobile phase: Acetone 25%
Acetonitrile 25%
Methanol 50%

Flow rate: 1.0 ml/min.

Injection volume: 20 µl.

Detection: UV at 254 nm.

Solvent:	Mobile phase.	
Retention time:	Levamisole:	2.0-3.2 min
	Oxyclozanide:	8.5-13.8 min
	Niclosamide:	16.2-23.3 min
	Acetone:	2.5-3.5 min

Sample preparation

1. Place a 100 ml volumetric flask on an analytical balance and tare.
2. Accurately weigh 1 g of the tablet powder into a 100 ml volumetric flask.
3. Add about 70 g of mobile phase to the flask and put the flask in an ultrasonic bath, with lukewarm water for 15 to 45 minutes.
4. Check that all the tablet powder has dissolved. If solid pieces are still visible, shake the flask and sonicate it again until it is completely dissolved.
5. Allow the flask to cool to room temperature and fill to 100 g with solvent.
6. Filter the solution into an autosampler vial and analyse.

Standard solution

1. Weigh approximately 3.0 g of levamisole HCl, 4.08 g of niclosamide and 24.0 g of oxyclozanide accurately.
2. Transfer into a 100 ml volumetric flask and dissolve, sonicate if necessary and make up to 100 g with solvent.
3. Dilute 1 ml of this solution to 100 g with solvent.
4. Transfer this solution to an autosampler vial and inject into the chromatograph.

Calculations

$$\% \text{ of moisture} = \frac{\text{Mass of sample before drying} - \text{Mass of sample after drying}}{\text{Mass of sample before drying}}$$

Nivanide at 0 weeks is equal to Fresh sample in Figure 17.

Nivanide 25°C at 12 weeks is equal to 25°C RH 60% in Figure 17.

Nivanide 30°C at 12 weeks is equal to 30°C RH 60% in Figure 17.

Nivanide 40°C at 12 weeks is equal to 40°C RH 75% in Figure 17.

The assay results of Nivanide tablets are given in Table 26 and Figure 17.

Table 26. The assay of Nivanide tablets.

	% of Levamisole HCl	% of Oxytoclozanide	% of Niclosamide
Nivanide at 0 weeks	103.87	103.99	104.33
Nivanide 25°C at 12 weeks	99.94	108.75	109.28
Nivanide 30°C at 12 weeks	98.40	98.82	101.41
Nivanide 40°C at 12 weeks	90.19	94.34	97.66

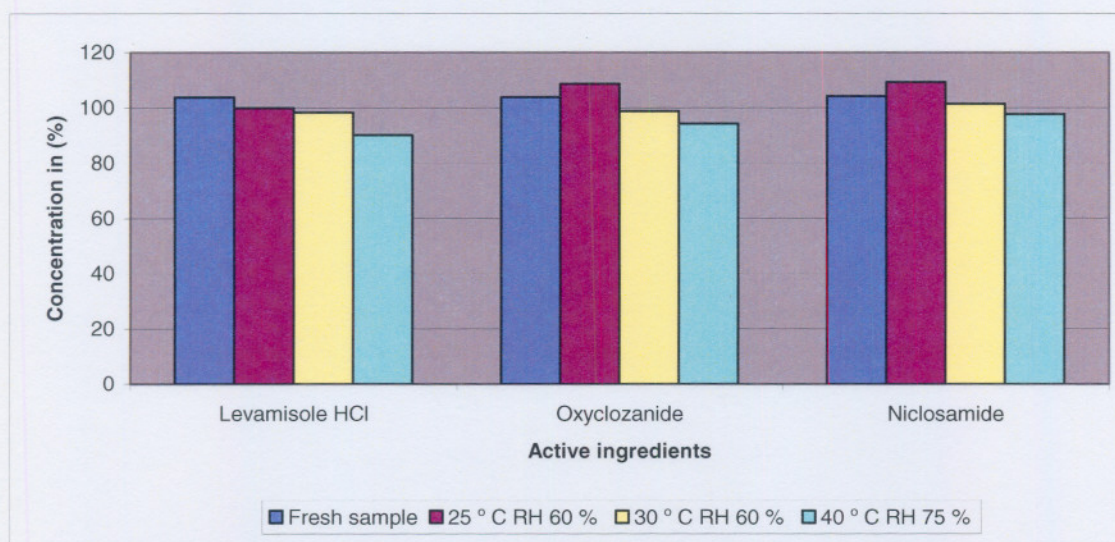


Figure 17. The assay results of Nivanide tablets at 0 and 12 weeks.

5.3.3.2.1.1. Discussion

Of all the dosage forms the tablets have the most constant stability profile. Although the niclosamide and oxytoclozanide have a higher concentration at 25 °C after 12 weeks than the standard, it proved to be stable.

6. CHAPTER 6

6.1. Summary and conclusion

Even though new products and new concepts appear on the market almost every month, it is far from the end of helminthiases. It will always be a threat to the livestock farmers.

Another important factor in product design and formulation is the cost. There is a general tendency that people want the best available on the market, but they are not willing to pay the price. Therefore, products with new combinations of “old” drugs that will have a synergistic effect or that will broaden the therapeutic index might be a new line of thinking when formulating new products.

There will always be a place in the market for a new, effective and cost-effective product.

The aim of this study was:

- the formulation and development of the anthelmintic drugs levamisole, niclosamide and oxclozanide into a solution, suspension and a tablet
- the anatomy of ruminants
- the parasites causing helminthiases
- the most effective type of dosage form
- the pharmacology and characteristics of the above mentioned anthelmintics
- preformulation
- results

In Chapter 1, the background with regards to helminthiases is discussed. It also addresses the special considerations the formulator must keep in mind when formulating a new product. The general anatomy, physiology, biopharmaceutics and an ideal anthelmintic is also discussed. The background with regards to the anatomy sketches a picture of the pathway the dosage will travel to reach the places of absorption.

In Chapter 2 classifications of helminths as well as the veterinary parasites are discussed. Although most of the infections or infestations can be controlled today, it will never be eradicated. So many farmers will under-dose instead of giving the correct dosage. This is making the parasites more resistant. The bright side to this difficult position the livestock

farmers are facing is that newer drugs and newer drug combinations will always be needed.

Chapter 3 examines the drugs that were used in this study with reference to the pharmacology and the absorption of the drugs. The available products on the market are also discussed to determine the competition and to identify what they consist of. Since this study is based on the development of a better medicinal option preventing or fighting helminthiasis in animals (specifically live stock), substantial research was essential before any formulation could commence. It is important to have a vision of what you want to formulate. It is also important to ask the following questions:

What do you want to do?

Why do you want to do it?

How do you want to do it?

If you can visualise what you want to formulate and how you want to formulate your product it will shed light on even the most difficult formulating problem.

Although all three active substances might be seen as “old” drugs, if combined and formulated correctly it might still have devastating effects on helminth populations. The fact that the combination is in solution and that it might have a better effect than suspensions also comes to mind.

Chapter 4 discusses the pharmaceutical formulation, the pharmacological and physiochemical considerations, the devices with which it can be administered and an overview of solutions, suspensions and tablets as dosage forms. Of the three dosage forms, niclosamide in solution presents promising future possibilities. Since it is now in solution form, it can be combined with a whole lot of other active ingredients to get better and optimum results. Although the niclosamide precipitates when it comes in contact with water, the ideal would be to prevent precipitation altogether and to use it as a systemic anthelmintic.

Chapter 5 addresses the preformulation and the possible prediction of interactions. It also discusses the results of the physical tests as well as the assay of each of the three formulated products. Keeping the DSC results in mind, it might not have worked, putting

these three products with the excipients together in a dosage form. The results, however, were both pleasing and promising.

The aims and objectives of this study comply with what was set out to do.

Looking back at writing this dissertation; it has been both a wonderful experience and privilege to learn more about formulation and product development.

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Discovery consists of looking at the same thing as everybody else and thinking something different.

Albert Szent-Gyorgi

APPENDIX 1

VALIDATION OF AN HPLC ASSAY FOR THE SIMULTANEOUS DETERMINATION OF LEVAMISOLE, NICLOSAMIDE AND OXYCLOZANIDE IN SOLUTION, SUSPENSION AND IN TABLET FORM

SUMMARY

Test	Result
Specificity	Complies
Range	Levamisole HCl 188.3 – 301.3µg Oxyclozanide 255.5 – 408.8µg Niclosamide 225.2 – 360.4µg
Linearity	Levamisole HCl $R^2 = 0.9922$ Oxyclozanide $R^2 = 0.9974$ Niclosamide $R^2 = 0.9998$
Accuracy	Levamisole HCl 100.7% Oxyclozanide 100.2% Niclosamide 100.1%
Precision	Levamisole HCl 97.61% Oxyclozanide 100.53% Niclosamide 100.57%
Ruggedness	Complies
Robustness	Complies

1. ORIGIN OF METHOD

The method was developed and validated in co-operation with Dr. J.L. du Preez at the Research Institute for Industrial Pharmacy.

2. CHROMATOGRAPHIC CONDITIONS

Analytical instrument: HP1050 series HPLC equipped with a HP1050 quaternary gradient pump, HP1050 autosampler, HP1050 diode array detector and Chemstation Rev. A.06.03 data acquisition and analysis software or equivalent.

Column: USP 24 (2000) packing L1, p1924, Luna C18-2 column, 250 x 4.6 mm, 5 µm, 100 Å pores, 17.8% carbon load, Phenomenex, Torrance, CA.

Mobile phase:	Acetone	25%
	Acetonitrile	25%
	Methanol	50%
Flow rate:	1.0 ml/min.	
Injection volume:	20 ul.	
Detection:	UV at 254 nm.	
Solvent:	Mobile phase	
Retention time:	Levamisole:	2.0-3.2 min
	Oxyclozanide:	8.5-13.8 min
	Niclosamide:	16.2-23.3 min
	Acetone:	2.5-3.5 min
Stop time:	30 minutes	

3. SAMPLE PREPARATION

1. Place a 100 ml volumetric flask on an analytical balance and tare.
2. Accurately weigh 1g of the solution, suspension or tablet powder into a 100 ml volumetric flask.
3. Add about 70 g of mobile phase to the flask and put the flask in an ultrasonic bath, with luke warm water for 15 to 45 minutes (suspension and tablet powder).
4. Check that all the suspension or tablet powder has dissolved. If solid pieces are still visible, shake the flask and sonicate it again until it is completely dissolved.
5. Allow the flask to cool to room temperature and fill to 100 g with solvent.
6. Filter the solution into an autosampler vial and analyse.

4. STANDARD PREPARATION

Solution

1. Weigh approximately 2.5 g of levamisole HCl, 3.0 g of niclosamide and 3.4 g of oxyclozanide accurately.
2. Transfer into a 100 ml volumetric flask and dissolve, and make up to 100 g with solvent.
3. Dilute 1 ml of this solution to 100 g with solvent.

4. Transfer this solution to an autosampler vial and inject into the chromatograph.

Suspension

1. Weigh approximately 2.5 g of levamisole HCl, 20.0 g of niclosamide and 3.4 g of oxyclozanide accurately.
2. Transfer into a 100 ml volumetric flask and dissolve, sonicate if necessary and make up to 100g with solvent.
3. Dilute 1 ml of this solution to 100 g with solvent.
4. Transfer this solution to an autosampler vial and inject into the chromatograph.

Tablet

1. Weigh approximately 3.0 g of levamisole HCl, 4.08 g of niclosamide and 24.0 g of oxyclozanide accurately.
2. Transfer into a 100 ml volumetric flask and dissolve, sonicate if necessary and make up to 100g with solvent.
3. Dilute 1 ml of this solution to 100 g with solvent.
4. Transfer this solution to an autosampler vial and inject into the chromatograph.

5. CALCULATIONS

$$\frac{\text{Sample area} \times \text{mass of standard (g)} \times \text{potency of standard}}{\text{Standard area} \times \text{Sample mass} \times 50} = \% \text{ w/w}$$

6. SYSTEM SUITABILITY PARAMETERS

- Make duplicate injections of a standard solution.
- Calculate the relative standard deviation of the peak areas obtained.
- Calculate the number of theoretical plates for the paraben peaks.

The system is suitable to perform the analysis if the following criteria are met:

- RSD of 2 injections not more than 2%.
- The column must have more than 3000 theoretical plates for all the peaks.

7. VALIDATION TEST PROCEDURE AND ACCEPTANCE CRITERIA

7.1 Specificity

Method

1. Prepare a sample from a placebo of the tablet, solution and suspension that does not contain the substances being tested with this method.
2. Dilute a standard solution 1:1 with water, 1M hydrochloric acid and 1M sodium hydroxide respectively.
3. Store these solutions overnight in closed test tubes at 40°C to degrade.
4. Inject the samples into the chromatograph and examine the chromatograms to determine whether any additional peaks were formed.

ACCEPTANCE CRITERIA:

- The placebo should not contain any peaks that will interfere with the determination of the actives.
- Extra peaks formed in the stressed samples should be discernible from those of the active components.

7.2 Linearity

Preparation of standards.

1. Prepare a standard solution as described under standard preparation.
2. Inject variable volumes into the chromatograph to obtain standards from 75-125% of the expected sample concentration.

ACCEPTANCE CRITERIA:

- Linear regression analysis should yield a regression coefficient (R squared) of ≥ 0.99 .

7.3 Accuracy

1. Spike with known amounts of active at concentrations of approximately 80%, 100% and 120% of the expected sample concentration.
2. Inject into the chromatograph in duplicate.

ACCEPTANCE CRITERIA:

- Recovery must be between 98-102%.

7.4 Precision**7.4.1 Intra-day precision (repeatability).**

1. Measure approximately 3 x 2.0 g (80%), 3 x 2.5 g (100%) and 3 x 3.0 g (120%) of the solution into 100 ml volumetric flasks and fill to volume with solvent.
2. Inject into the chromatograph in duplicate.

7.4.2 Inter-day precision

1. Analyse the same homogeneous sample in triplicate as described above for intra-day precision (at 100% of the sample concentration) on two more occasions to determine the between-day variability of the method. On one occasion (day 3) a different analyst should perform the analysis on a different set of equipment.

ACCEPTANCE CRITERIA:

- Repeatability must be better than 2%. (n = 9)
- Inter-day precision must be better than 5%. (n = 9)

7.5 Ruggedness

7.5.1 Stability of sample solutions

1. Prepare a sample as described under sample preparation in the method.
2. Inject the sample into the chromatograph.
3. Leave the sample in the autosampler tray and reanalyse over a period to determine the stability of the sample.

ACCEPTANCE CRITERIA:

- Sample solutions should not be used for a period longer than it takes to degrade by 2%, and in the case of degradation, special precautions should be followed to compensate for the loss.

7.5.2 System repeatability

1. Inject a sample six times consecutively in order to test the repeatability of the peak area as well as the retention time.

ACCEPTANCE CRITERIA:

- The peak area and retention times should have an RSD of 2% or less.

7.6 Robustness

1. Make deliberate changes to the chromatographic conditions to determine the method's tolerance towards changes.
2. Change the flow rate, wavelength, injection volume, acetonitrile concentration and use a similar column from a different manufacturer.

ACCEPTANCE CRITERIA:

- The method should be able to tolerate a 5% variance in the chromatographic conditions.

7.7 System and method performance characteristics (system suitability)

1. Calculate the chromatographic performance characteristics of the separation, like retention time, USP peak tailing factor, capacity factor and resolution between peaks and repeatability of multiple injections.
2. Use the data obtained to set realistic performance limits that should be met before the analysis can be performed.

ACCEPTANCE CRITERIA:

- The USP tailing factor must be less than 1.5 and the RSD must be less than 2%.

8. VALIDATION RESULTS

8.1 Specificity

A standard of the solution was prepared with levamisole, oxyclozanide and niclosamide. All the samples have been analysed at 254.4 nm. In figure 1 the levamisole, niclosamide and oxyclozanide shows clearly at 2.107 min, 8.537 min and 16.555 respectively. Figure 2 and 3 are chromatograms that have been obtained after the samples have been stressed for 24 hours in 1M of sodium hydroxide and 1M of hydrochloric acid.

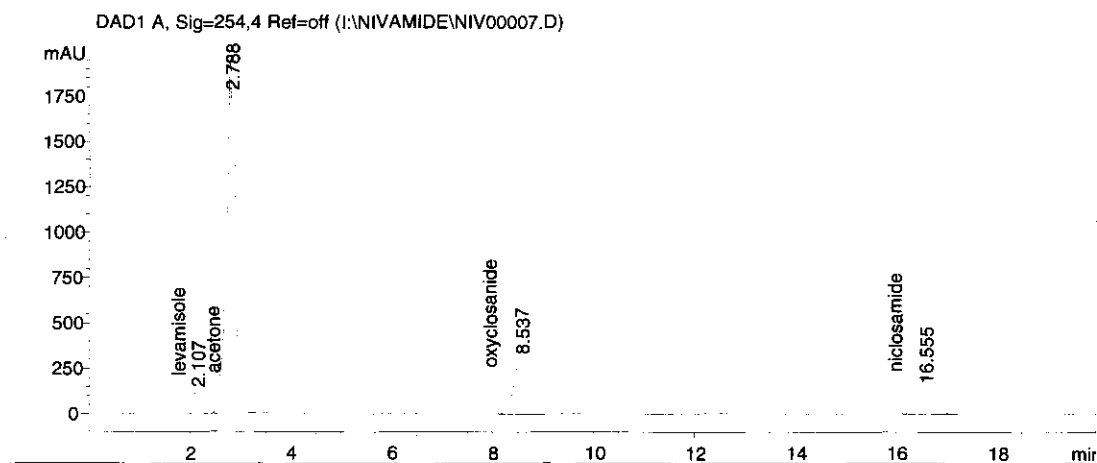


Figure 1. A sample of the solution with levamisole, oxyclozanide and niclosamide.

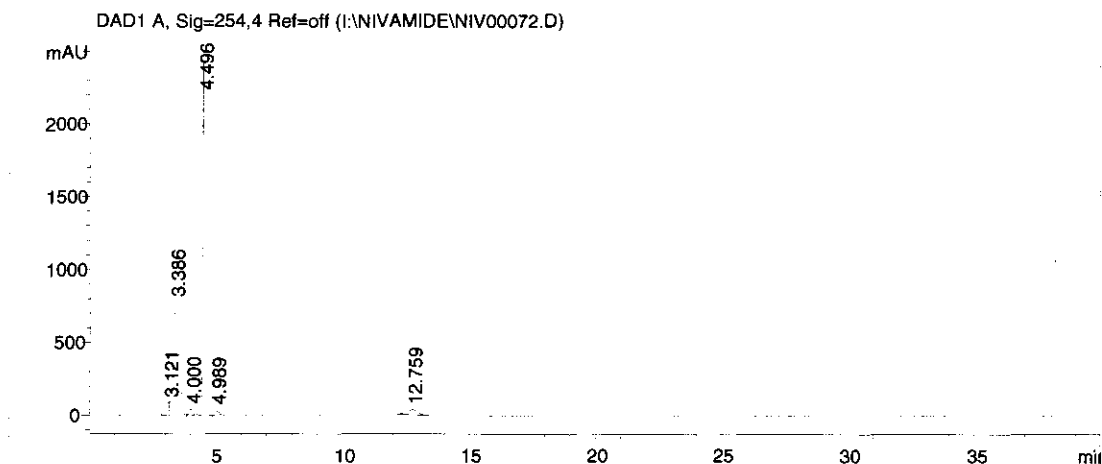


Figure 2. A sample stressed in 1M of sodium hydroxide at 25°C for 24 hours.

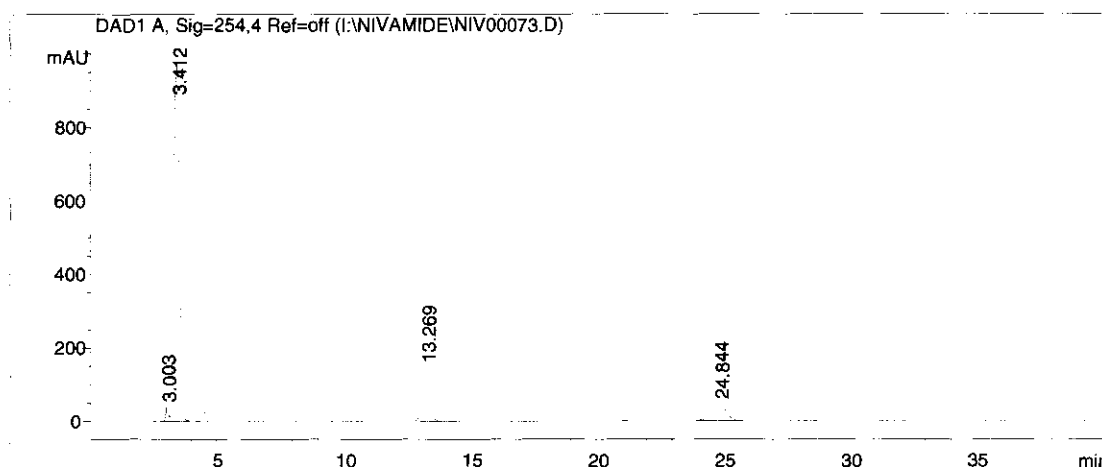


Figure 3. A sample stressed in 1 M of hydrochloric acid at 25°C for 24 hours.

Conclusion

None of the ingredients in the placebo interfered with the levamisole, oxyclozanide or niclosamide peaks. Although the degradation process formed extra peaks, it did not interfere with the simultaneous detection of levamisole, niclosamide and oxyclozanide.

8.2 Linearity and range

8.2.1 Levamisole HCl

Concentration µg/ml	Peak area		
	Area 1	Area 2	Mean
188.3	629.4	629.0	629.2
200.9	670.6	668.3	669.5
213.4	705.4	707.7	706.6
226.0	748.4	746.2	747.3
238.5	790.0	786.4	788.2
251.1	829.9	825.8	827.8
263.7	866.5	866.1	866.3
276.2	910.9	910.0	910.4
288.8	946.6	947.1	946.8
301.3	991.9	988.2	990.0

Table 1: Peak area and concentration for Levamisole HCl

REGRESSION PARAMETERS			
R squared	0.999849	Lower 95%	Upper 95%
Intercept	27.5977	19.69997	35.49542
Slope	3.188494	3.156579	3.220408

Table 2: Regression parameters for Levamisole HCl

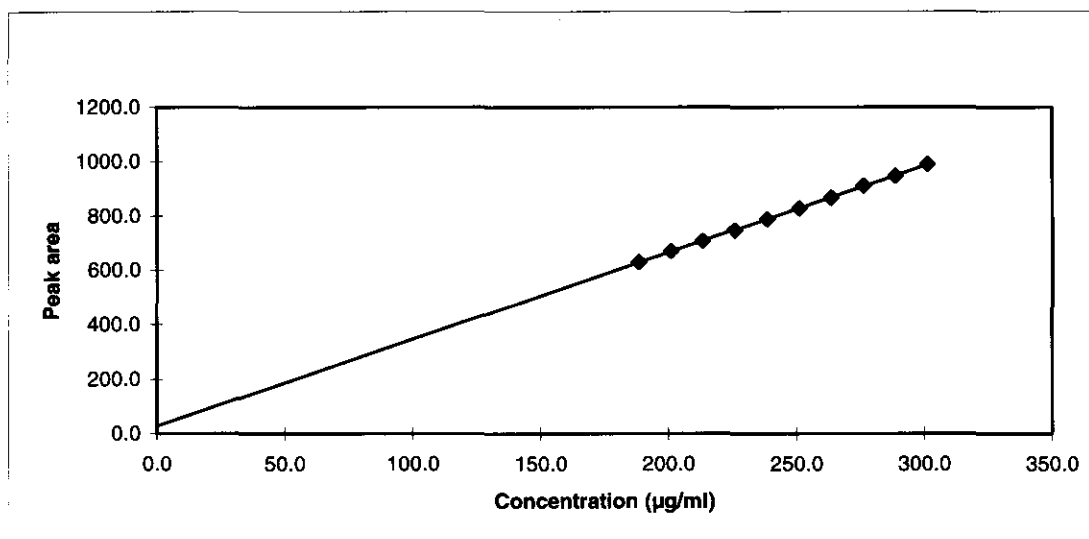


Figure 4: The linear regression curve for levamisole HCl

8.2.2 Oxyclozanide

Concentration µg/ml	Peak area		
	Area 1	Area 2	Mean
255.5	3515.1	3511.2	3513.2
272.6	3739.7	3740.2	3740.0
289.6	3952.5	3951.9	3952.2
306.6	4185.4	4170.3	4177.9
323.7	4417.3	4397.1	4407.2
340.7	4631.8	4618.9	4625.3
357.7	4853.5	4844.6	4849.0
374.8	5085.4	5085.4	5085.4
391.8	5303.8	5304.3	5304.0
408.8	5554.6	5539.0	5546.8

Table 3: Peak area and concentration for oxyclozanide

REGRESSION PARAMETERS			
R squared	0.999884	Lower 95%	Upper 95%
Intercept	129.7927	90.9027	168.6828
Slope	13.21654	13.10072	13.33236

Table 4: Regression parameters for oxyclozanide

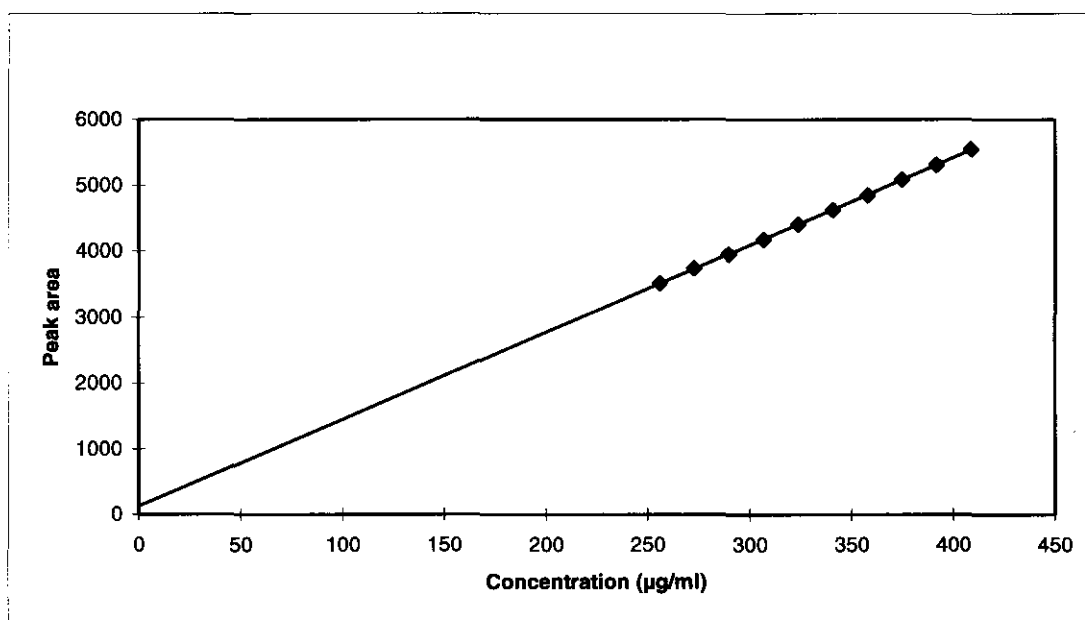


Figure 4: The linear regression curve for oxyclozanide

8.2.3 Niclosamide

Concentration		Peak area	
µg/ml	Area 1	Area 2	Mean
225.2	2403.8	2401.3	2402.5
240.2	2538.1	2538.1	2538.1
255.3	2684.8	2695.4	2690.1
270.3	2838.2	2843.3	2840.7
285.3	3026.0	2999.7	3012.8
300.3	3155.2	3152.7	3153.9
315.3	3320.1	3326.1	3323.1
330.3	3465.5	3456.1	3460.8
345.3	3626.1	3632.9	3629.5
360.4	3799.5	3787.6	3793.6

Table 5: Peak area and concentration for niclosamide

REGRESSION PARAMETERS			
R squared	0.999582	Lower 95%	Upper 95%
Intercept	58.96527	7.948218	109.9823
Slope	10.33339	10.16101	10.50578

Table 6: Regression parameters for niclosamide

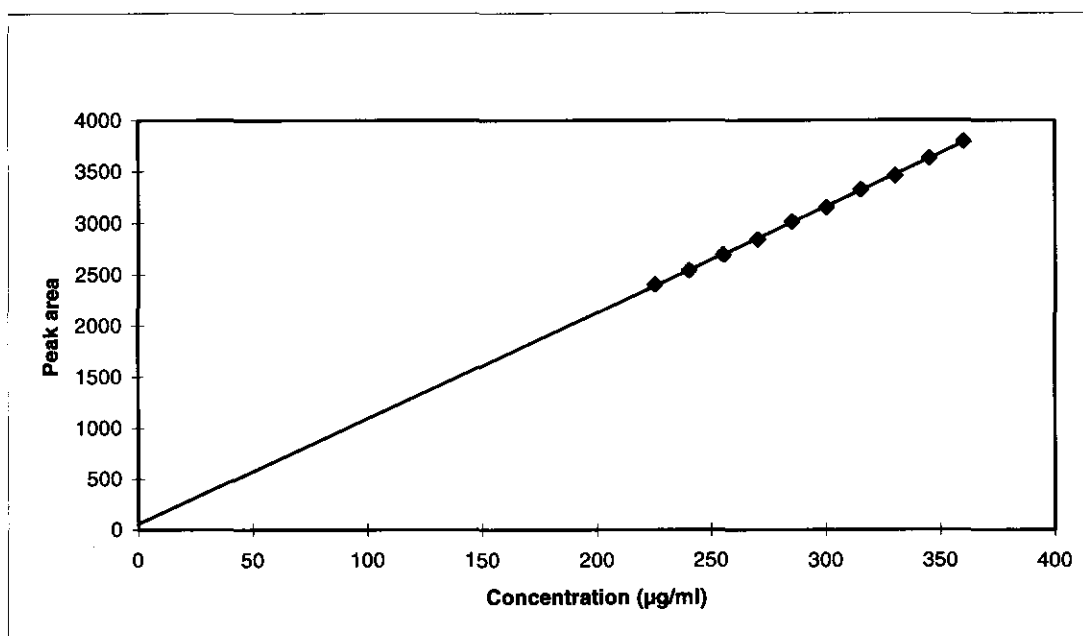


Figure 5: The linear regression curve for niclosamide

Conclusion:

This method is linear over the concentration range of 188.3 – 301.3 µg/ml for levamisole, 255.5 – 408.8 µg/ml oxytetracycline and 225.2 – 360.4 µg/ml for niclosamide. This method meets the criteria for acceptance and is therefore suitable for single point calibration.

8.3 Accuracy

8.3.1 Levamisole HCl

Conc. spiked µg/ml				Recovery µg/ml	Recovered %
	Area 1	Area 2	Mean		
200.0	666.4	665.1	665.8	200.2	100.1
200.0	660.8	659.7	660.3	198.4	99.2
200.0	662.8	662.3	662.6	199.1	99.6
250.0	832.3	831.7	832.0	252.3	100.9
250.0	826.3	826.0	826.2	250.5	100.2
250.0	826.1	828.7	827.4	250.8	100.3
300.0	1008.2	997.1	1002.7	305.8	101.9
300.0	987.1	996.2	991.7	302.4	100.8
300.0	1014.4	1015.6	1015.0	309.7	103.2

Table 7: Percentage levamisole HCl recovered

Statistical analysis	
Mean	100.7
SD	1.2
% RSD	1.2
95% confidence intervals	
Lower limit	99.7
Upper limit	101.6
Estimated median	100.3
Confidence	1.0

Table 8: Confidence intervals for levamisole HCl

Oxyclozanide

Conc. spiked µg/ml				Recovery µg/ml	Recovered %
	Area 1	Area 2	Mean		
272.00	3698.5	3701.9	3700.2	270.1	99.3
272.00	3671.4	3668.0	3669.7	267.8	98.5
272.00	3681.5	3689.5	3685.5	269.0	98.9
340.00	4640.9	4654.5	4647.7	341.8	100.5
340.00	4620.6	4625.1	4622.8	340.0	100.0
340.00	4640.9	4614.9	4627.9	340.3	100.1
408.00	5638.7	5593.5	5616.1	415.1	101.7
408.00	5503.1	5570.9	5537.0	409.1	100.3
408.00	5663.6	5676.0	5669.8	419.2	102.7

Table 9: Percentage oxyclozanide recovered

Statistical analysis	
Mean	100.2
SD	1.3
% RSD	1.3
95% confidence intervals	
Lower limit	99.2
Upper limit	101.3
Estimated median	100.1
Confidence	1.0

Table 10: Confidence intervals for oxyclozanide

Niclosamide

Conc. spiked µg/ml	Area 1	Area 2	Mean	Recovery µg/ml	Recovered %
240.00	2524.5	2511.3	2517.9	238.0	99.2
240.00	2503.1	2501.4	2502.2	236.4	98.5
240.00	2514.6	2522.9	2518.7	238.0	99.2
300.00	3171.3	3173.0	3172.1	301.3	100.4
300.00	3158.1	3161.4	3159.8	300.1	100.0
300.00	3168.0	3146.6	3157.3	299.8	99.9
360.00	3839.6	3829.7	3834.6	365.4	101.5
360.00	3768.6	3786.8	3777.7	359.9	100.0
360.00	3866.0	3867.6	3866.8	368.5	102.4

Table 11: Percentage niclosamide recovered

Statistical analysis	
Mean	100.1
SD	1.1
% RSD	1.1
95% confidence intervals	
Lower limit	99.2
Upper limit	101.2
Estimated median	100.0
Confidence Level	0.9

Table 12: Confidence intervals for niclosamide

Conclusion

Over the range of 80-120% for levamisole HCl, oxyclozanide and niclosamide, the method yielded a mean recovery of 100.70%, 100.20% and 100.10% respectively.

8.4 Precision

8.4.1 Intra-day precision

8.4.1.1 Levamisole HCl

Mass (g)	Area 1	Area 2	Mean	Concentration $\mu\text{g/ml}$	Recovered (%)
0.889	727.9	727.6	727.8	247.08	98.8
0.949	777.1	776.8	777.0	247.66	99.1
0.963	773.7	772.1	772.9	242.69	97.1
0.973	791.8	792.9	792.4	246.48	98.6
0.996	803.9	803.9	803.9	244.55	97.8
0.998	805.3	805.8	805.5	244.37	97.7
1.059	847.4	845.8	846.6	242.66	97.1
1.192	955.0	953.9	954.5	243.97	97.6
1.365	1059.5	1056.3	1057.9	236.73	94.7

Mean	97.61
SD	1.24
RSD %	1.27

Table 13: Intermediate (intraday) precision results for levamisole HCl

8.4.1.2 Oxyclozanide

Mass (g)	Area 1	Area 2	Mean	Concentration $\mu\text{g/ml}$	Recovered (%)
0.889	4183.4	4187.0	4185.2	345.25	101.5
0.949	4457.9	4472.5	4465.2	345.66	101.7
0.963	4414.0	4424.9	4419.5	336.99	99.1
0.973	4589.6	4586.0	4587.8	346.63	102.0
0.996	4629.9	4633.6	4631.7	342.12	100.6
0.998	4633.6	4651.9	4642.7	342.01	100.6
1.059	4864.1	4878.8	4871.5	338.94	99.7
1.192	5544.9	5537.6	5541.2	343.64	101.1
1.365	6159.8	6181.7	6170.8	334.85	98.5

Mean	100.53
SD	1.13
RSD %	1.13

Table 14: Intermediate (intraday) precision results for oxyclozanide

8.4.1.3 Niclosamide

Mass (g)	Area 1	Area 2	Mean	Concentration µg/ml	Recovered (%)
0.889	2849.0	2854.4	2851.7	304.10	101.4
0.949	3026.7	3025.6	3026.2	302.58	100.9
0.963	3021.3	3019.7	3020.5	297.56	99.2
0.973	3116.9	3119.0	3118.0	304.21	101.4
0.996	3161.7	3157.9	3159.8	301.39	100.5
0.998	3134.7	3136.9	3135.8	298.23	99.4
1.059	3302.1	3303.7	3302.9	296.58	98.9
1.192	3741.7	3743.3	3742.5	299.17	99.7
1.365	4456.6	4452.8	4454.7	311.64	103.9

Mean	100.57
SD	1.46
RSD %	1.46

Table 15: Intermediate (intraday) precision results for niclosamide

Conclusion

Precision was satisfactory with a RSD of 1.27%, 1.13 % and 1.46% respectively.

8.4.2 Inter-day precision

8.4.2.1 Levamisole HCl

	Day 1	Day 2	Day 3	Between days
	98.6	97.2	95.0	
	97.8	96.4	95.5	
	97.7	98.1	95.3	
Mean	98.05	97.22	95.28	96.85
SD	0.38	0.66	0.22	1.16
RSD %	0.39	0.68	0.23	1.20

Table 16 : Inter-day precision for levamisole HCl

Anova: Single
Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	3	294.1613	98.05377	0.219733
Column 2	3	291.6607	97.22024	0.660794
Column 3	3	285.8451	95.2817	0.070051

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	12.13709	2	6.068543	19.15216	0.002484	5.143249
Within Groups	1.901156	6	0.316859			
Total	14.03824	8				

8.4.2.2 Oxyclozanide

	Day 1	Day 2	Day 3	Between days
	102.0	101.6	101.6	
	100.6	99.6	100.9	
	100.6	100.3	102.1	
Mean	101.05	100.49	101.54	101.03
SD	0.63	0.83	0.46	0.43
RSD %	0.63	0.83	0.46	0.42

Table 17: Inter-day precision for oxyclozanide

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	2	201.6447	100.8223	0.107679
Column 2	2	200.7935	100.3967	0.018887
Column 3	2	203.5967	101.7984	0.128503

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.065504	2	1.032752	12.14673	0.036441	9.552082
Within Groups	0.255069	3	0.085023			
Total	2.320573	5				

8.4.2.3 Niclosamide

	Day 1	Day 2	Day 3	Between days
	101.4	100.3	100.8	
	100.5	100.0	101.5	
	99.4	101.4	101.6	
Mean	100.43	100.58	101.31	100.77
SD	0.81	0.60	0.36	0.38
RSD %	0.81	0.59	0.35	0.38

Table 18: Inter-day precision for niclosamide

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	3	301.2787	100.4262	0.994949
Column 2	3	301.7361	100.5787	0.532328
Column 3	3	303.9228	101.3076	0.192099

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.331372	2	0.665686	1.161503	0.374639	5.143249
Within Groups	3.43875	6	0.573125			
Total	4.770123	8				

SS = sum of squares

df = degrees of freedom

MS = mean squares

F = F ratio

Conclusion:

Precision was satisfactory with RSD's of less than 4%. The repeatability is still within acceptable limits, and the assay should perform well, even when executed by other personnel in a different laboratory.

8.5 Ruggedness

8.5.1 Stability of sample solutions

8.5.1.1 Levamisole HCl

Time (hours)	Peak Area	%
0	824.4	100.0
1	828.3	100.5
2	826.6	100.3
4	827.3	100.4
6	829.4	100.6
8	831.1	100.8
10	830.4	100.7
12	831.7	100.9
Mean	828.65	100.52
SD	2.32	0.28
RSD %	0.28	0.28

Table 19: Ruggedness for levamisole HCl

8.5.1.2 Oxyclozanide

Time (hours)	Peak Area	%
0	3997.8	100.0
1	3988.5	99.8
2	3987.2	99.7
4	4003.3	100.1
6	4026.3	100.7
8	4023.2	100.6
10	4015.1	100.4
12	4025.0	100.7
Mean	4008.30	100.26
SD	15.19	0.38
RSD %	0.38	0.38

Table 20: Ruggedness of oxyclozanide

8.5.1.3 Niclosamide

Time (hours)	Peak Area	%
0	2986	100.0
1	2985	100.0
2	2990	100.1
4	2995	100.3
6	3006	100.7
8	3012	100.9
10	3004	100.6
12	3012	100.9
Mean	2998.75	100.43
SD	10.45	0.35
RSD %	0.35	0.35

Table 21: Ruggedness of niclosamide

Conclusion:

The levamisole HCL, oxyclozanide and niclosamide are stable over a period of 12 hours.

8.5.2 System repeatability

8.5.2.1 Levamisole HCl

	Area	Retention time
	828.3	2.244
	828.3	2.244
	827.8	2.242
	826.1	2.242
	827.2	2.239
	826.1	2.242
Mean	827.3	2.242
SD	0.93	0.00
RSD %	0.11	0.07

Table 22: System repeatability for levamisole HCl

Conclusion:

System performance proved well with RSD values for levamisole HCl of 0.11% for peak area and 0.07% for retention time respectively.

8.5.2.2 Oxyclozanide

	Area	Retention time
	4529.8	11.462
	4547.4	11.452
	4517.0	11.578
	4529.8	11.468
	4545.2	11.468
	4524.9	11.446
Mean	4532.3	11.479
SD	10.76	0.05
RSD %	0.24	0.39

Table 23: System repeatability for oxyclozanide

Conclusion:

System performance proved well with RSD values for oxyclozanide of 0.24% for peak area and 0.39% for retention time respectively.

8.5.2.3 Niclosamide

	Area	Retention time
	3688.0	23.317
	3692.0	23.355
	3693.0	23.317
	3714.0	23.264
	3698.0	23.523
	3697.0	23.302
Mean	3697.0	23.346
SD	8.29	0.08
RSD %	0.22	0.36

Table 24: System repeatability for niclosamide

Conclusion:

System performance proved well with RSD values for levamisole HCl of 0.11% for peak area and 0.07% for retention time respectively.

8.6 Robustness

Changes were made to the chromatographic conditions to determine the robustness of the method.

The following changes were made and found to be acceptable:

Column: Luna C18-2 column, 150 x 4.6 mm, 5 μ m, 100 Å pores, 17.8% carbon load, endcapped, Phenomenex, Torrance, CA, and Lichrospher 100-5 RP-18ec cartridge column, 125 x 4.0 mm, 5 μ m, 100 Å pores, 21.5% carbon load, endcapped, Machery-Nagel, Düren, Germany were found to be suitable. The latter column should be operated at a flow rate of 0.7-1.0 ml/min.

Mobile phase: Concentrations of 47-53% acetonitrile is still suitable.

Flow rate: 0.7 – 1.2 ml/minute.

Wavelength: The wavelengths can be altered by ± 2 nm without any ill effect.

9. CONCLUSION

The method performed well and should be suitable to analyse levamisole HCl, oxyclozanide and niclosamide in tablets, solutions and suspensions for stability testing, quality control and batch release purposes. No interference was encountered from stressed samples or known related substances. The method can therefore be regarded as being stability-indicating.