Formulation and evaluation of mebendazole dosage forms

Kobus Buys

B. Pharm.

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Supervisor: Prof A.P. Lötter

Co-supervisor: Dr. J.L. du Preez

Assistant Supervisor: Dr. W. Liebenberg

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Abstract

Parasites in the restricted sense are those members of the animal kingdom which derive their means of well-being from other members of the animal kingdom, at the same time depriving their host of some (sometimes all) of its well-being. Parasitic diseases are much more widespread than many people realise. These diseases affect not only impoverished people in remote countries but they can be important health problems for rich and poor throughout the world. Different parasites infect our domestic animals and cause great losses; they have a great influence on the growing, production and overall resistance against other diseases. The best solution to the problem rests in preventing these infections rather than in curing them. They are never beneficial and we must control them effectively.

Mebendazole is a synthetic benzimidazole with a wide spectrum of anthelmintic activity. Three polymorphic forms of mebendazole, identified A, B and C can be formed through controlled crystallisation procedures. Polymorph C is apparently pharmaceutically favoured. It has been clearly demonstrated that a correlation exist between the polymorphism of the active substance and the bioavailability of the finished product. The characterisation of the active substance was done by means of infrared spectroscopy, DSC and X-ray powder diffraction.

The first aim of this study was to formulate a chewable tablet appropriate for the multi-dosage of children during a deworming program. Chewable tablets present an attractive alternative for children who have not yet learned to wash tablets down with water.

The second aim was to formulate a gel for dogs and domestic animals which is more viscous than the products on the market. Dosing an animal with a liquid can be difficult, and a sudden movement of the animal often results in spillage. A

drug in a gel form is a convenient means for administration to pets, to reduce spillage.

Stability tests were carried out over a test period of three months at 5°C, 25°C + 60% RH and 40°C + 75% RH storage conditions for both dosage forms. All the tests complied with the acceptable criteria except for the loss on drying tests done on the chewable tablets. Therefore silica gel should accompany the tablets to prevent this problem.

An HPLC method was developed and validated for the simultaneous determination of the preservative, potassium sorbate and the active substance, mebendazole.

Uittreksel

Parasiete is daardie lede van die diereryk wat afhanklik is van hulle voorspoed en vooruitgang deur op ander lede van die diereryk te teer. Parasiete verminder die gesondheid en welstand van die gasheer waarop hulle teer. Parasitiese siektes is baie wyer versprei as wat die meeste mense besef. Hierdie siektes beïnvloed nie net minderbevoorregte mense in arm lande nie, maar is ook 'n belangrike gesondheidsprobleem vir ryk en arm regoor die wêreld. Verskillende parasiete infesteer ons diere en veroorsaak groot verliese. Hulle het 'n groot invloed op groei, produksie en algemene weerstand teen ander siektes. Die beste oplossing vir hierdie probleem lê in die voorkoming eerder as in die behandeling. Parasiete is nooit voordelig nie en moet dus effektief beheer word.

Mebendasool is 'n sintetiese bensimidasool met 'n wye spektrum aktiwiteit teen wurms. Drie polimorfiese vorme van mebendasool, geïdentifiseer as A, B en C kan volgens beheerde kristallisasiemetodes berei word. Polimorf C is die mees farmaseuties geskikte vorm. 'n Defnitiewe verwantskap bestaan tussen die polimorfisme van die aktief en die biobeskikbaarheid van die finale produk. Die karaktereienskappe van die aktiewe middel was geanaliseer deur middel van infrarooi spektroskopie, DSC en XRPD.

Die eerste doel van die studie was om 'n koubare tablet te formuleer wat geskik sou wees vir die multidosering van kinders in 'n ontwurmingsprogram. Koubare tablette is 'n aantreklike alternatief vir kinders wat nog nie geleer het hoe om tablette met behulp van water af te sluk nie.

Die tweede doel was om 'n gel vir diere te formuleer met 'n meer viskeuse tekstuur as die oplossings wat huidig beskikbaar is. Vloeistof dosering kan moeilik wees en gereeld word medisyne gemors as gevolg van 'n skielike

beweging van die dier. 'n Geneesmiddel in die vorm van 'n gel is 'n baie meer betroubare manier om diere te behandel. Weens die meer klewerige tekstuur word minder van die medisyne gemors.

Stabiliteitstoetse is uitgevoer oor 'n tydperk van drie maande op beide produkte wat blootgestel was aan die volgende bergings kondisies: 5°C, 25°C + 60% RH en 40°C + 75% RH. Al die toetse het die aanvaarbare kriteria geslaag behalwe die toets op die koubare tablette om die vogverlies daarop te bepaal. Silika gel behoort daarom die tablette te vergesel om die probleem te voorkom.

'n HPLC metode is ontwikkel en gevalideer vir die gelyktydige bepaling van die preserveermiddel, kaliumsorbaat en die aktief, mebendasool.

Introduction and aim of study

Parasitic helminthes affect people and animals around the world. They are widespread and are causing health problems and overall resistance against other diseases. Dogs and children are some of the most important hosts of helminthes, causing these parasites to spread and contaminate the environment. They are never beneficial and therefore we must control them effectively.

The aim of this study was to formulate and test an anthelmintic chewable tablet and a gel. The chewable tablet is planned to serve as a palatable dosage form for children who could serve well in a deworming program. The formulations currently on the South African market don't serve the conditions in the country enough, specifically in a multidosage deworming program.

The second aim of this study was to formulate a gel for the deworming of domestic animals. As, the sudden movement of the animal's head lead to spillage of medicine, the gel could prevent this problem during the deworming of animals like dogs. The more viscous texture of the gel results in a more sticky formulation to prevent spillage and therefore inappropriate dosaging. Underdosing because of spillage could lead to an increased resistance against anthelmintics.

It is of great importance to use mebendazole polymorphic form C in the formulations. Polymorph C is the pharmaceutically favoured form. Throughout the formulation process further steps must be taken to assure that the formulations still contain the polymorph C. Transformations to the more stable form A could lead to false deworming results because polymorph A is too insoluble for almost any anthelmintic effect.

The chewable tablet and gel were subjected to accelerated stability studies. A validated HPLC method for the simultaneous analysis of mebendazole and potassium sorbate should be developed.

Chapter 1

Parasites and diseases effectively controlled by anthelminthics

1.1 Introduction

Parasites in the strictest sense are those members of the animal kingdom which derive their means of well-being from other members of the animal kingdom, at the same time depriving their host of some (sometimes all) of its well-being (Hall, 1985:13).

Parasitic diseases are much more widespread than many people realise. These diseases affect not only impoverished people in remote countries but they can be important health problems for rich and poor throughout the world (Comeunity, 1998). Different parasites infect our domestic animals and cause great losses. They have a great influence on the growing, production (wool, eggs, milk, etc.) and overall resistance against other diseases. The best solution to the problem is preventing these infections rather than in curing them. The same parasites exist on relevant animals. The animal or person in which the parasites live is called the host. Sometimes there are other animals except the hosts where the parasites develop. These animals are called the intermediate hosts and the most effective way to control these parasites is to kill the intermediate hosts (Mönnig & Veldman, 1989). Although parasitic helminths in many instances produce little serious damage to the host, they are never beneficial and can sometimes produce severe and even fatal disease (Jones & Hunt, 1983). Parasitic helminths, or worms, are important for all species. We must control them effectively. In this chapter only a few of the parasitic helminths and diseases are discussed, to give a background on helminths, so that we could understand the role of the anthelminthic drug, mebendazole, better.

1.2 Helminths in dogs

Dogs are the victims of several internal parasites referred to as worms, the most common ones are the roundworms, hookworms, whipworms and tapeworms. Most worm infestations cause any or all of the following symptoms: diarrhoea, weight loss, dry hair, general poor appearance and vomiting. Some infestations cause few or no symptoms, some worm eggs or larvae can be dormant in the dog's body and activated only in times of stress, or in the case of roundworms activate and infest the puppies in the last stages of pregnancy (Woolf, 2003).

1.2.1 Roundworms

Roundworms are active in the intestines of puppies, often causing a pot-bellied appearance and poor growth. The worms may be seen in vomit or stool. A roundworm can grow to seventeen centimetres in length. Females can produce two hundred thousand eggs in a day. The eggs are protected by a hard shell and can exist in the soil for years. Ingesting worm eggs from contaminated soil infects dogs. The eggs hatch in the intestine and the resulting larva is carried to the lungs by the bloodstream. The larva crawls up the trachea and gets swallowed, often causing the puppy to cough. Once the larvae return to the intestine, they grow into adults. Roundworms do not typically infest adults. However, the larvae can encyst in body tissue of adult bitches and activate during the last stages of pregnancy to infecting the puppies (Woolf, 2003). The lifecycle of roundworms is described in figure 1.1.

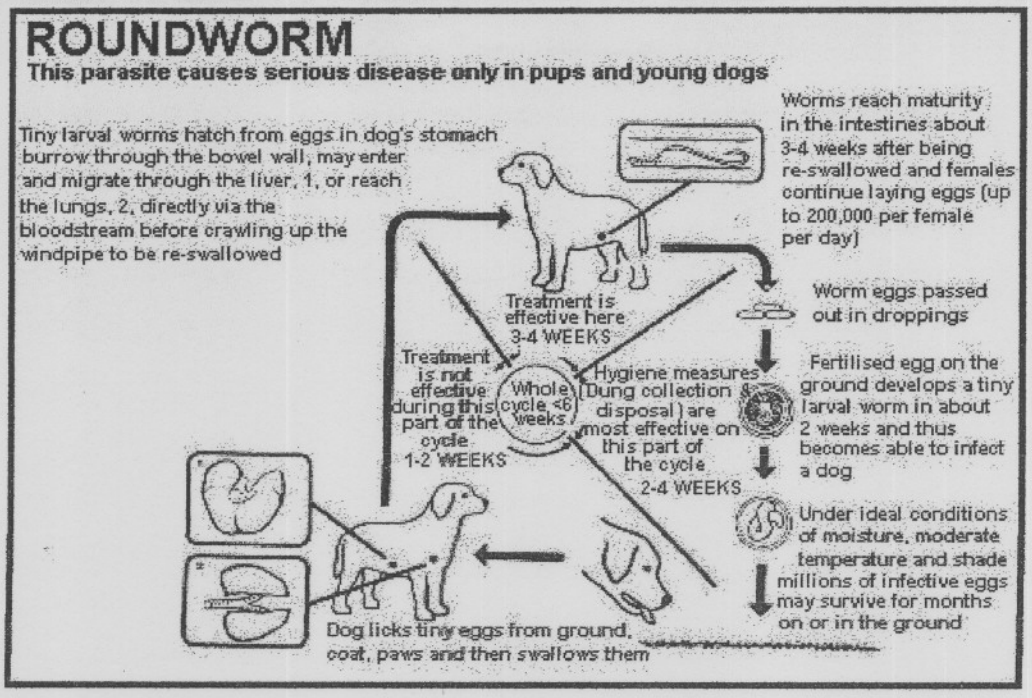


Figure 1.1 Lifecycle of roundworms.

1.2.2 Hookworms

These are small, thin worms that fasten to the wall of the small intestine and suck blood. Dogs get hookworms if they come in contact with the larvae in contaminated soil. The hookworm larvae become an adult in the intestine. The pups can contract hookworms in the uterus and the bitch can infest the puppies through her milk. A severe hookworm infestation can kill puppies but hookworm infections are usually not a problem in adult dogs (Woolf, 2003). The lifecycle of hookworms is described in figure 1.2.

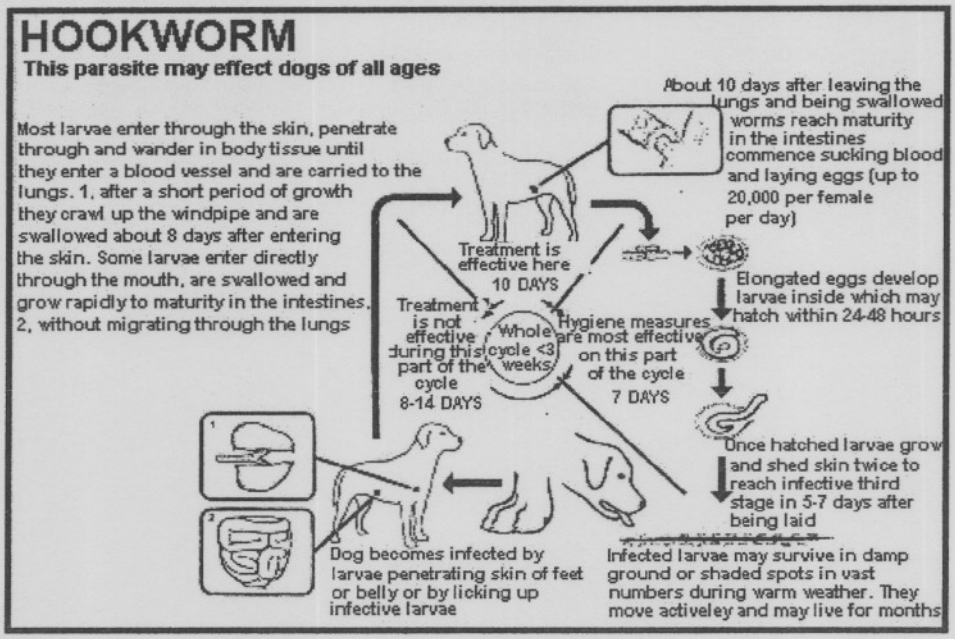


Figure 1.2 Lifecycle of hookworms.

1.2.3 Tapeworms

Tapeworms are small intestine parasites. The tapeworm is transmitted to dogs who ingest fleas or who hunt and eat wildlife infested with tapeworms or fleas. The dog sheds segments of the tapeworm containing the eggs in its faeces. The segments are flat and look like grains of rice when dried. The segments can be found either in the dog's stool or stuck to the hair around his anus (Woolf, 2003). The lifecycle of tapeworms is described in figure 1.3.

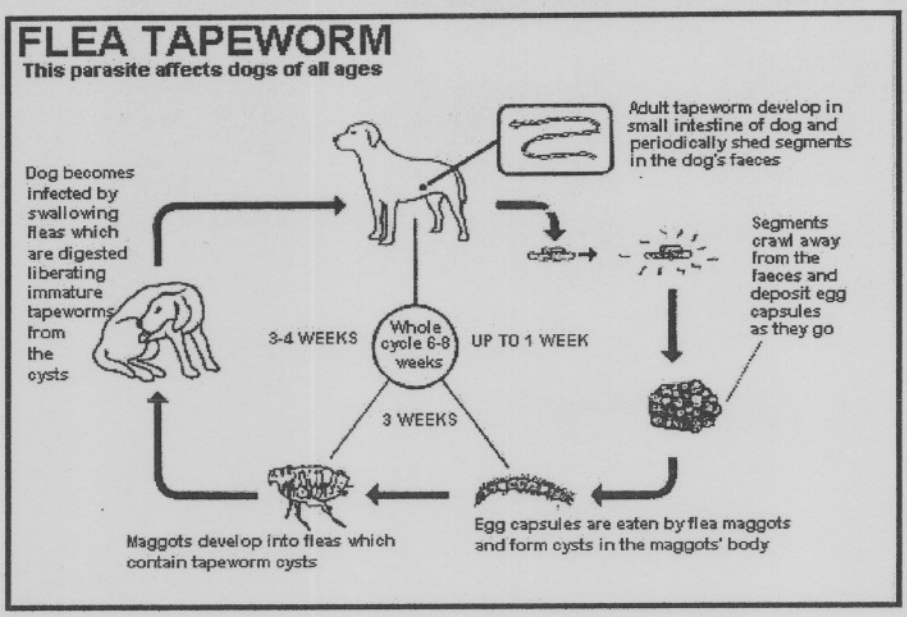


Figure 1.3 Lifecycle of the tapeworm.

1.2.4 Whipworms

Adult whipworms look like pieces of thread with one end enlarged. They live in the cecum, the first section of the dog's large intestine. Infestations are usually light, so an examination of faeces may not reveal the presence of eggs. Several checks may be necessary before a definitive diagnosis can be made (Woolf, 2003). The lifecycle of whipworms is described in figure 1.4.

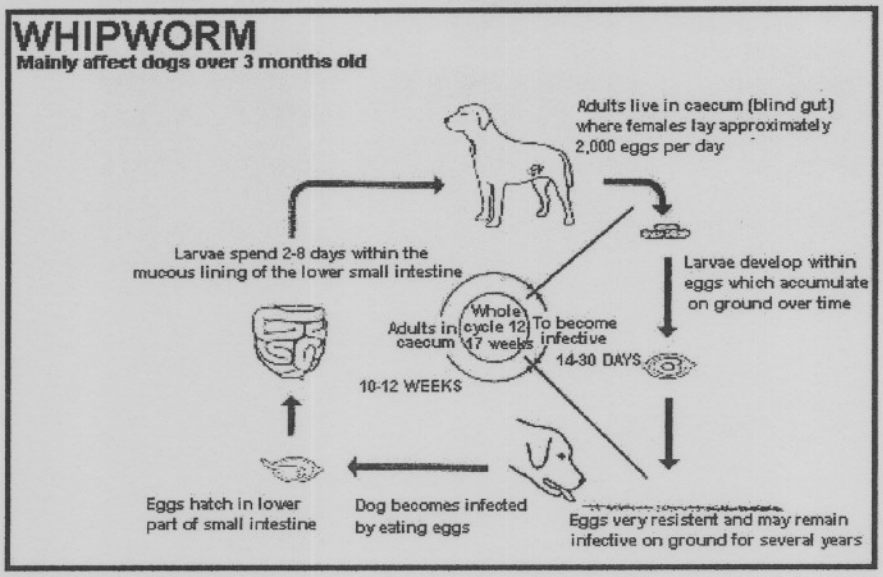


Figure 1.4 Lifecycle of whipworms.

1.3 Helminths and parasitic diseases in humans

Many parasitic diseases result from human carelessness and a lack of appropriate personal hygiene and sanitation measures. Thus, the best solution to the problem rests in preventing these infections rather than in curing them (Comeunity, 1998). It has been estimated that 3 billion humans suffer from parasitic infections, plus a much greater number of domestic and wild animals. Although these diseases constitute the most widespread human health problem in the world today, they are for various reasons also been the most neglected. In theory, the parasitic infections should be relatively easy to treat because the etiologic agents are known in almost all cases. However, many problems remain to be solved before effective chemotherapeutic agents will be available for all the parasitic diseases (Wang, 1998). Nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes) are the three main helminthic groups which affect people (Comeunity, 1998).

Pinworm

It is the most common roundworm parasite. In the Unites States it affects up to one-third of the country's children. Pinworm infection is spread mainly by children; therefore it is most prevalent in family groups, day-care centers, schools, and camps. The eggs may be scattered into the air from bed linen and clothing, and can cling to doorknobs, furniture, tubs and even food. Enterobiasis is caused by *Enterobius vermicularis* (Comeunity, 1998).

Ascariasis

Ascariasis is caused by *Ascaris lumbricoides,* a large intestinal roundworm. Infections are common throughout the world. Heavy infection may cause partial or complete blockage of the intestine resulting in severe abdominal pain, vomiting, restlessness and disturbed sleep. Occasionally, the first sign of infection may be the presence of a worm in the vomitus or in the stool (Comeunity, 1998).

Hookworms

One of the most common roundworm infections is hookworm. Hookworms are endemic in some of the tropical and subtropical countries of the world. The infection is usually contracted by persons walking barefoot over contaminated soil. Persons in good health and on a diet containing adequate iron can tolerate the presence of these worms in small or moderate numbers with no ill effects. Serious anemia can occur in chronic infections, if the number of parasites become great enough. Necator and Ancylostoma are two types of hookworms that cause ancylostomiasis (Comeunity, 1998).

Whipworms

Although the incidence of whipworm infection is high, its intensity is usually light. The name whipworm comes from the parasite's long, very thin, whip like shape. Severe infections in young children can result in serious disease with bloody

diarrhoea and a condition called rectal prolapse. The whipworm, *Trichuris trichiura* causes trichuriasis (Comeunity, 1998).

Strongyloidiasis

Humans are the principle hosts of the parasitic roundworm called *Strongyloides stercoralis* which causes strongyloidiasis. Autoinfection may produce heavy infection and severe disease, especially in patients with reduced immunity such as those receiving corticosteroids or other immunosuppressive drug treatment (Comeunity, 1998).

Trichinosis

Trichinosis is an infection by the larvae of a most versatile roundworm, *Trichinella spiralis*. This parasite can infect virtually every meat-eating mammal. Trichinosis is not an intestinal infection like the other parasitic roundworms. The migration of *T.spiralis* larvae through the body and their encystment in a muscle creates serious problems. The disease occurs in humans when they eat undercooked infected pork (Comeunity, 1998).

1.3.1 Hydatid disease

Hydatid disease is produced by cysts that are the larval stages of the tapeworm, *Echinococcus* (Public health, 1999). *Echinococcus granulosus* is a very small tapeworm, up to 9 mm long. The larval stage, hydatid cysts, is found in numerous intermediate hosts, i.e. humans, domestic livestock and many wild animals. It is therefore a very efficient tapeworm (Hall, 1985). Symptoms depend on the location of the cyst, and develop as a result of pressure, leakage or rupture. The most common sites for the cysts are the liver, brain, lungs, kidneys, heart, thyroid and bone. Cysts remain viable or die and calcify. Prognosis is generally good and depends on the site and potential for rupture and spread. Sudden rupture of the brood capsules and liberation of the daughter cysts may cause fatal anaphylaxis. The occurrence is worldwide and mainly associated with sheep

farming. Diagnosis can occur by X-ray, ultrasound or CT. If a cyst ruptures, examine for protoscolices, brood capsules and cyst wall in sputum, vomitus, faeces and urine. Infection occurs by hand-to-mouth transfer of tapeworm eggs from dog faeces. The larvae penetrate the intestinal mucosa, enter the portal system and are carried to various organs where they produce cyst in which infectious protoscolices develop. The incubation period varies from months to years. It is not communicable through person-to-person and children are more often infected than adults (Public health, 1999). Humans serve as inadvertent intermediate hosts for cestodes of *Echinococcus* spp., which are carried as tapeworms by canines such as dogs. In South Africa, *E. granulosus* is prevalent and sheep, in which the larval cysts are found, are the intermediate hosts. The lifecycle of the hydatid tapeworm is described in figure 1.5.

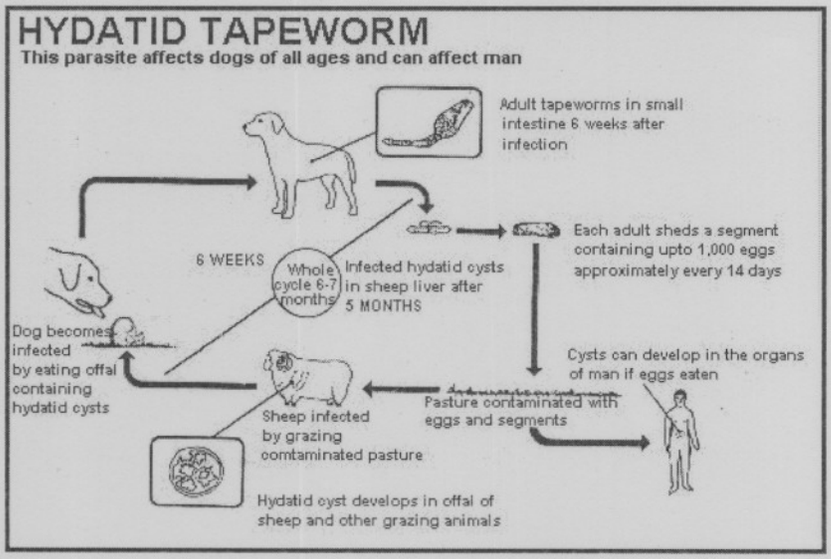


Figure 1.5 Lifecycle of the hydatid tapeworm.

Optimal treatment of symptomatic cysts is by surgical resection to remove the complete intact cyst. Because of the risk of spreading infection if the cyst ruptures, the recommended approach is to visualise the cyst, remove a fraction of the fluid, and instill a cysticidal agent (e.g. hypertonic (30%) saline, iodophor,

or 95% ethanol), to kill the germinal layer and daughter cysts prior to resection. Thirty minutes after instillation, the cyst should be removed intact. It may be prudent to treat the patient perioperatively with an anthelminthic active against Echinococcus larvae (e.g. mebendazole, albendazole) to further limit the risk of intraoperative dissemination of daughter cysts. Medical therapy for inoperable cysts with either albendazole or mebendazole has provided improvement in most patients. The alternative agent, mebendazole, is poorly absorbed, and must be taken at higher doses for several months to achieve a therapeutic effect (Ampath, 2000). Mebendazole is a very insoluble drug. Although the poor solubility of mebendazole is advantageous in the treatment of intestinal helminth infections, this low solubility means that the drug is poorly bioavailable for the treatment of systemic infections like hydatid disease. It has been the subject of a considerable amount of research as an agent for the chemotherapy of echinococcosis. It was found that there was a 30% increase in the percentage of the dose excreted in the urine with the administration of mebendazole suspension dispersed in olive oil (Dawson & Watson, 1985). Sunflower oil as well as other lipids increased bioavailability and prolongend the maintenance of therapeutic levels of mebendazole (Lur'e *et al.*, 1987). The successful treatment of this disease with mebendazole requires that a sufficient quantity of the administered dose is absorbed to achieve therapeutic plasma concentrations. Such a concentration is estimated to be approximately 100 ng/ml (Witassek *et al.*, 1981; Bryceson *et al.*, 1982).

Preventive measurements should be taking to control hydatid disease. Wash your hands after contact with dogs. Treat infected dogs. Control the slaughter of animals, particularly sheep. Prevent the access of dogs to the area. Dispose animal carcasses as soon as possible. Control dogs on farms at all times and do not allow them to have access to vegetable gardens. Treat all your dogs for *E.granulosis* regularly in rural or endemic areas (Public health, 1999).

1.3.2 Toxocariasis

Toxocariasis is a zoonotic (animal to human) infection caused by the parasitic roundworms commonly found in the intestine of dogs (*Toxocara canis*) and cats (*T. cati*). There are two major forms of toxocariasis: ocular larva migrans (OLM) and visceral larva migrans (VLM). *Toxocara* infections can cause OLM, an eye disease that can cause blindness. OLM occurs when a microscopic worm enters the eye. Each year more than 700 people infected with *Toxocara* experience permanent partial loss of vision.

Heavier or repeated *Toxocara* infections can cause VLM, a disease that cause swelling of the body's organs or central nervous system. Symptoms of VLM, which are caused by the movement of the worms through the body, include fever, coughing, asthma, or pneumonia. The most common *Toxocara* parasite of concern to humans is *T. canis,* which puppies usually contract from the mother before birth or from her milk. The larvae mature rapidly in the puppy's intestines. When they are 3 weeks old, they begin to produce large numbers of eggs, that contaminate the environment through there faeces. The eggs soon develop into infective larvae. People and especially children can become infested after accidentally ingesting infective *Toxocara* eggs from larvae in soil or other contaminated surfaces (Division of parasitic diseases, 2002). The popularity of pets together with high ascarid and hookworm infection rates in dogs and cats, especially pups and kittens, result in widespread contamination of soil with infective-stage larvae. Epidemiologic studies have implicated the presence of dogs, particularly pups, in the household and pica (dirt eating) as the principle factors for human toxocaral disease. Children's play habits and attraction to pets put them at high risk for ascarids and hookworm infection (Kalkofen, 1987). The lifecycle of *Toxocara canis* is shown in figure 1.6.

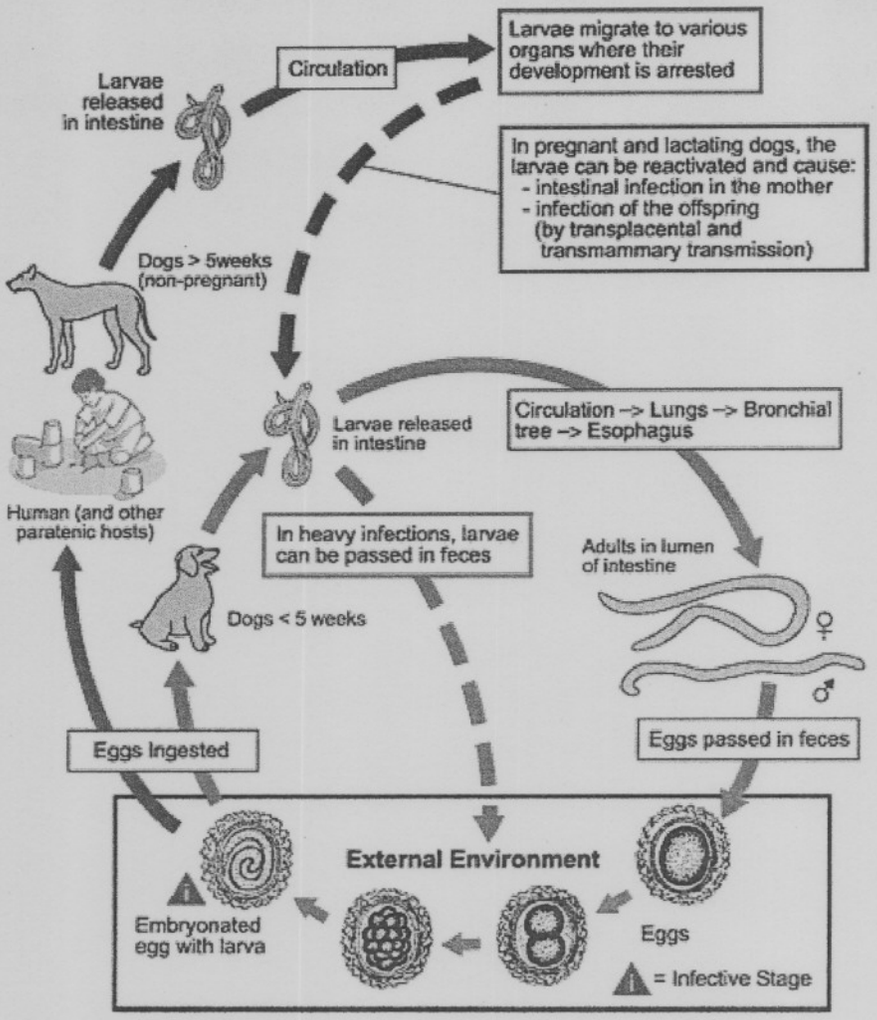


Figure 1.6 The lifecycle of *Toxocara canis* (DPD, 2001).

1.3.3 Cysticercosis

Cysticercosis is tissue infections with larval cysts of the cestode, *Taenia solium*. Diagnosis and treatment of cysticercosis depends on the site of involvement and the symptoms experienced. Cysts outside the CNS tend not to be symptomatic. These eventually die and calcify, to be detected incidentally on plain radiographs of the limbs. Surgical resection is the optimal approach for symptomatic cysts outside the CNS. Deep tissue and CNS lesions are more difficult to diagnose and

treat surgically. For most patients with neurocysticercosis, drug therapy is the treatment of choice (Ampath, 2000).

1.4 Preventive management

"An ounce of prevention beats a pound of cure"

Health and performance flourish when minimising an internal parasitic burden in animals. Controlling internal parasites with anthelminthics is an essential part of management, but should be combined with intelligent husbandry (Loving, 2000). Several worms that infect and reinfect dogs can also infect humans, so treatment and eradication of the worms in the environment is important (Woolf, 2003). Most cases of human toxocariasis and zoonotic ancylostomiasis can be prevented by simple measures, such as practising good personal hygiene, eliminating intestinal parasites from pets, and making potentially contaminated environments off limits to children (Glickman & Schantz, 1981). Remove dog faeces, use appropriate vermicides and have the dog's faeces checked frequently in persistent cases. Do not mix anthelminthics or use them when your dog is currently taking any other medication (Woolf, 2003). New animals should not be introduced to a kennel immediately, but should be isolated. Deworm new arrivals two or three times, at three-to-four-week intervals. This practise protects those animals that have received excellent deworming management from reinfection. All animals' young and old should be dewormed at the same time. It serves little purpose to deworm only a small percentage of the animals in a kennel. The untreated animals continue to excrete eggs in their faeces, recontaminating not only themselves, but the treated animals as well. Careful pasture management prevents overgrazing. Removing manure twice weekly will control parasite populations (Loving, 2000).

1.5 Anthelminthics

One of the most important ways to control parasitic helminths is the usage of anthelminthics.

1.5.1 History

For man and animals alike, the plant kingdom was the first medicine chest. The bark, berries, roots, leaves, flowers and seeds of all sorts of plants were used against worms. Most have only limited activity or work only as a purgative. In the 19th century the search for new anthelminthics was a matter of uncritical empiricism i.e. the 'glass pill'. Fragments of crushed glass were mixed with gat or ginger to form a worming pill. The idea was that the glass splinters would fatally wound all the worms without penetrating the mucosal layer of the stomach and intestine. In the early 20th century the anthelminthics available for dogs were arsenic compounds, ground male fern root, finely chopped pumpkin seeds, fig tree sap, alkaloids, calomel and garlic in milk. All these remedies showed fairly poor activity. The accurate evaluation of anthelminthics began in the early part of the 20th century with the use of critical tests. In these tests the nature of the infection was established by faecal examination. The experimental animals were killed by euthanasia in order to count the number of worms remaining in the intestine after treatment. The first narrow-spectrum anthelminthics such as phenothiazine and piperazine (1953) were evaluated in tests of this type. Although the efficacy of piperazine against adults was nearly eighty percent, it remained the most used anthelminthic for years. The anthelminthics of the seventies and later such as pyrantel, nitroscanate and the benzimidazoles had a broad spectrum (Rochette, 2002).

Less than two decades ago, veterinary medicine only offered an arsenal of potent chemical adult anthelminthics. These chemicals were not without hazard. Many of them were toxic and they needed to be given in large quantities to be effective. The best way to administer the medication rapidly was to give it by

stomach tube. Before safe paste formulas, and with extreme risk of drug toxicity reactions, stomach deworming was the only way to go (Loving, 2000).

1.5.2 Classification

The pressures of urban living have promoted intensive research over the last decade into newer, more efficient and safer dewomers in the form of pastes and powders. Many anti-parasite products are available on the market. By simplifying the list of anthelminthics, a strategy can be devised to limit the parasite burden on animals by reducing the number of infective larvae.

The following six classes are some of the important anthelminthics available: benzimidazoles, pro-benzimidazoles, tetrahydropyrimidines, avermectins, organophosphates and piperazines. Benzimidazoles include products with chemical names of oxibendazole, oxfendazole, mebendazole, fenbendazole, thiabendazole and cambendazole. Benzimidazoles interfere with the worms energy metabolism, they die of starvation. Febantel is the only drug in the pro-benzimidazole class and has similar effects as the benzimidazoles. Pyrantel is an example of the tetrahydropyrimidines, it interferes with neuromuscular activity which causes the spastic paralysis of the worm. Ivermectin is an example of the avermectins which is produced by fermentation of certain bacteria. Ivermectin interferes with neuromuscular coordination of the worm, causing flaccid paralysis. Organophosphates have active ingredients of either dichlorvos or trichlorfon. This drug class specifically targets bot fly larvae in the stomach. Piperazine belongs to the sixth class and is effective against ascarids. Although the organophosphates and piperazine are effective against their specific target worms, these two classes are obsolete due to the development of safer, broad-spectrum products found in the other drug classes that are effective against all parasites (Loving, 2000).

1.5.3 Treatment facts and schedules of helminthic infections

The administration of an anthelminthic should not disrupt the precious relationship between a dog owner and his pet. Finding a formulation that most dogs would accept should make routine deworming easy (Rochette, 2002). Not all worms respond to the same treatment and no single anthelminthic works against all kind of parasites. Some non-prescription anthelminthics are quite ineffective in removing worms (Dunn, 2003). Therefore stool samples should be taken for microscopic examination if worms are suspected (Woolf, 2003). Deworming is most effective in preventing environmental contamination and human illness when it is aimed at pups and kittens and their dams. For optimal prevention initiate anthelminthic treatment of pups and kittens soon after birth. Where both ascarids and hookworms are commonly transmitted, anthelminthic drugs should be given to pups at 2, 4, 6, and 8 weeks of age. If only ascarids are present, preventive anthelminthic treatments may begin at 3 weeks. Different climatic conditions dictate how a management program should be approached. For instance, moisture and warm temperatures speed larval development into infective stage. They can survive freezing temperatures, emerging in the spring with warmth and moisture. Aggressive deworming programs of monthly treatments in the summer will kill most internal parasites. During the winter, due to dormancy and reduced maturation of worms in the body, deworming every two months is usually enough. Using ivermectin twice a year at six-month intervals in addition to other anthelminthics should eliminate damaging migratory forms of the parasites. Ivermectin can also be incorporated into a system of slow rotation, but should not be used exclusively and cause resistance to develop. Overcrowding or excessively unsanitary conditions may also require a deworming schedule to be increased. Each animal's immune response is different. A sick or unthrifty animal may have trouble ridding its body of parasites even with the aid of anthelminthics, especially if it is continuously re-exposed to infective larvae in mounds of uncollected manure (Loving, 2000). In table 1.1 is an example of a deworming program.

Table 1.1 Deworming program

Year	Type and frequency of anthelminthic
1 Ivermectin	Every 2 months
2 Oxibendazole	Every 2 months, use ivermectin alternatively twice a year to kill bots
3 Pyrantel	Every 2 months, use ivermectin alternatively twice a year to kill bots
4 Ivermectin	Every 2 months

The recommended dose of mebendazole (100 mg mebendazole twice a day for three consecutive days) is shown to be very effective in the treatment of hookworm and trichuris infections (Charoenlarp *et al.*, 1993). Due to the complicated life cycle of some of the worm species i.e. *Toxocara canis*, a deworming program should be based on the life cycle of the worms and at certain critical moments in the dog's life. The efficacy of anthelminthics, especially the nearly insoluble benzimidazoles, can be improved by giving the dose in intervals over several days. The worms in the gut cannot fast that long (Rochette, 2002). To monitor the parasite control program's effectiveness, faecal analysis can be preformed, comparing a faecal sample before deworming treatment with a faecal sample obtained exactly two weeks after treatment. Effective deworming depends on knowledge of body weight. Adjust upwards of suspected weight, but keep out of the toxic range (Loving, 2000).

1.5.4 Allergic reaction

When an animal with an overwhelming infection is dewormed for the first time, the destruction and breakdown of the worm expose the animal to foreign proteins. This exposure can result in an allergic reaction, causing edema and thickening of the intestine. These reactions decrease absorption of nutrients and fluids and may be accompanied by temporary diarrhoea. An overwhelming

infection produces a similar response, resulting in chronic diarrhoea or colic, common signs of intestinal parasitism.
It is far better to have a consistent deworming program than to subject an animal to continual internal damage or to side effects associated with deworming an older animal for the first time (Loving, 2000).

1.5.5 The myth of one single treatment

It is a myth to believe one can free his dog of roundworms with one single treatment. Even the best anthelminthics available at this moment can't do the job 100 percent like it should be. To get rid of all the roundworms in your dog, more than a 'one day treatment' is required. The efficacy of one single treatment is insufficient against the dog roundworm and not all the worms are excreted. The variability after a single treatment is too wide. In every case, several worms are still present in the treated dog. Due to the zoonotic potential it is a real danger for the dog owner and his children (Rochette, 2002). The gut transit is fast in cases of diarrhoea, a common symptom in dogs with worms. Different experiments with pyrantel elucidate differences in the uptake of pyrantel palmoate. Adult worms can limit or even reduce the ingestion of the anthelminthic for more than 4 hours. This leads to the assumption that repeated treatments with lower concentrations of the anthelminthic will be more effective than high concentrations given only once (Mackenstedt *et al.*, 1993). This phenomenon explains maybe the wide variability in the anthelminthic activity of a single treatment. So, to ensure sufficient contact time between the anthelminthic and the worms it is better to spread the dose over more than one day (Rochette, 2002).

1.6 Diagnosis and symptoms of helminthic infections

"A negative faecal examination does not mean there are no worms in the dog" (Rochette, 2002). The anthelminthic activity evaluated with faecal examination for the presence of worm eggs, is valuable only as a general indication of the

efficacy, but is not a scientific reliable test. Even the results of faecal examination, done by the best laboratories, underestimate the real infection with 20 to 25 percent for roundworms and even 4 to 5 times for tapeworms (Nichol *et al.*, 1981). One should not forget that male worms, the immature and somatic larvae are not laying eggs. The number of eggs in the faeces further depends of the consistency of the faeces, the time of the day, the age of the worms, the worms species involved and the technique used (Rochette, 2002). Young animals acquire new infections continuously from dam's milk and from the environment and many worms are not yet fully mature, faecal examinations are often falsely negative in pups and kittens (Hall, 1985). If an animal does not respond to a regular parasite control program, a faecal exam analysed two weeks or more after deworming determines the number of parasite eggs, per gram of faeces. Parasites such as ascarids may produce 100,000 eggs per day, while large strongyles may only produce 5,000 eggs per day. Pinworms are not normally seen in the faeces, but are obtained by pressing cellophane tape against the anus. Large numbers may mean the worms are resistant to an anthelminthic product. Faecal analysis and observation of hair coat, body condition, weight gain, attitude and performance provide other clues (Loving, 2000). Only two helminths are commonly seen in the stool with the unaided eye, roundworms and tapeworms. Roundworms can assume different sizes and when they are fresh they are whitish in appearance. Only small segments from the end of the tapeworms might be seen in the stool. Hook- and whipworms are so small that they are seldom seen in the stool. Occasionally adult whipworms can be seen in the stool when the infestation has already caused some debilitation or weight loss in the dog. The eggs of all these worms can be seen under microscope and that is how their presence is detected. Early diagnosis for the presence and type of intestinal parasite is very important. The type of anthelminthic depends on the kind of worm found in the stool (Woolf, 2003).

1.7 Important facts to remember

Controlling parasites with anthelminthics are very important but some facts like immunity and resistance should also be take in consideration.

1.7.1 Immunity against parasites

Normally over time, a healthy animal develops some degree of immunity to certain parasites and can fend off massive infestation. The body's immune system recognises the parasite's proteins (antigens) as foreign and launches an immune attack by forming antibodies. The more antigens in the animal's body, the more antibodies are formed. Anthelminthic efficacy of 100 percent may not be advantageous because it eliminates the source of the antigens. Then an animal's immune system cannot defend against future parasite infections. Animals younger than two years that have not yet developed immunity may succumb to overwhelming parasite loads by ascarids or large strongyles if not regularly dewormed. The objective is to allow an animal's normal immune system to deal with a very small load of parasites (Loving, 2000).

1.7.2 Resistance against anthelminthics

Resistance allows the worm to tolerate anthelminthic doses that previously killed them. Different strategies can be used to maximise the effect of anthelminthics. Currently, it is feared that by exposing parasites to a rapid rotation of different drug classes every few months, we may inadvertently select parasites that develop resistance to many of these chemicals. Based on current research, an optimal strategy is one of slow rotation of anthelminthics at one-year intervals (Table 1.1). Rotation that is more frequent may result in multiple-drug resistance to several different classes at once. Ideally, one product should be used during the season of maximum egg transmition. In this way, a single generation of parasites (of one year) is not exposed to different and multiple-drug classes. By

slowly rotating at yearly intervals, each generation is only subjected to one mechanism of action by a drug and is subsequently less likely to develop drug resistance. The next year, another drug class is used, the third year a third drug class, the fourth year returns to the first year's product and so on. To date, many of the 40 species of small strongyles have developed resistance to the benzimidazoles. Not only are the adult worms able to develop resistance, but they genetically pass resistance genes along to future generations. Of the benzimidazoles, the only drug currently available that the small strongyles cannot resist is oxibendazole. No resistance has yet developed to either pyrantel or ivermectin. Consistent underdosing can lead to larger problems than not deworming at all. Constant exposure to doses not large enough to kill, but large enough to stress the worm, promotes the worm's drug resistance. When finally exposed to adequate levels of a drug, resistance capabilities protect the worm from dying (Loving, 2000).

1.8 Conclusion

Parasitic helminths affect almost everyone around the world; they are never beneficial and can sometimes produce severe and even fatal diseases. The prevention, controlling and treatment of parasitic infections are of great importance to all human beings. The controlling and correct treatment of parasitic worms with anthelminthics like menbendazole could be valuable in this difficult task.

Chapter 2

Mebendazole, a broad-spectrum anthelmintic

2.1 Introduction

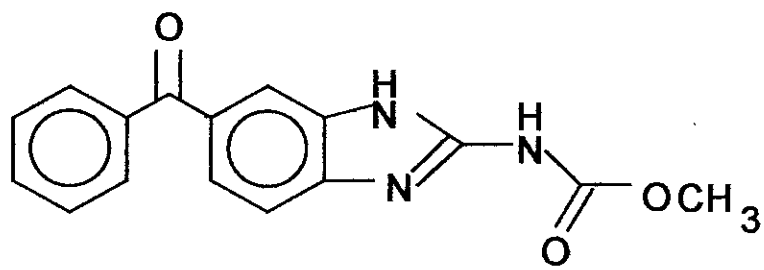
Mebendazole is a synthetic benzimidazole with a wide spectrum of anthelmintic activity. Three polymorphic forms of mebendazole, identified A, B and C can be formed through controlled crystallisation procedures. Polymorph C is apparently pharmaceutically favoured (Himmelreich *et al.*, 1977:123).

2.2 History

Mebendazole is a broad-spectrum anthelmintic agent synthesised and developed by Janssen Pharmaceutica, Research Laboratory, Beerse, Belgium. After its introduction in 1972, the drug became available in numerous countries around the world (AL-Badr & Tariq, 1987:293).

2.3 Physico-chemical properties

The structure of mebendazole according to Budavari (1996:982) is as follows:



The chemical name of mebendazole is (5-Benzoyl-1H-benzimidazol-2-yl)-carbamic acid methyl ester. The molecular weight is 295.29 and the empirical formula is $C_{16}H_{13}N_3O_3$ (USP, 2002:1054). The elemental composition of

mebendazole is C, 65.08%; H, 4.44%; N, 14.23% and O, 16.25% (AL-Badr & Tariq, 1987:294). Mebendazole is a white or faintly yellowish powder, practically insoluble in water, alcohol, ether and methylene chloride. It shows polymorphism (EP, 1997:1151), which will be discussed in chapter 3.

Mebendazole appears to be minimally absorbed from the gastro intestinal tract following oral administration. Limited data indicate that about 2-10% of an oral dose is absorbed. Peak plasma concentrations of mebendazole occur 0.5-7 hours after oral administration of the drug and exhibit wide interpatient variation. Mebendazole is highly bound to plasma proteins. The elimination half-life has been reported to be about 2.8-9 hours. The drug is metabolised via decarboxylation to 2-amino-5 (6)-benzimidazolyl phenylketone, this metabolite does not have anthelmintic activity (McEvoy, 1988:39).

Mebendazole undergoes extensive first-pass elimination, being metabolised in the liver, eliminated in the bile as unchanged drug and metabolites, and excreted in faeces. Only 2% of a dose is excreted unchanged or as metabolites in the urine (Reynolds, 1989:57). Absorption of mebendazole is increased if the drug is ingested with a fatty meal (Goldsmith, 1998:869).

2.4 Clinical uses

Mebendazole is used for the treatment of trichuriasis (whipworm infection), enterobiasis (pinworm infection), ascariasis (roundworm infection), and hookworm infections caused by *Ancylostoma doudenale* or *Necator americanus*. The drug's broad spectrum of activity makes it useful in the treatment of mixed helminthic infections. Mebendazole has also activity against cestodiasis (tapeworm infection) caused by *Hymenolepis nana* (dwarf tapeworm), *Taenia saginata* (beef tapeworm), and *Taenia solium* (pork tapeworm); strongyloidiasis (threadworm infection), cutaneous larva migrans (creeping eruption), toxocariasis (visceral larva migrans), capillariasis, trichostrongylosis, and draculiasis (guinea worm disease). The drug has been effective in a limited number of patients for the treatment of hydatid cysts caused by *Echinococcus granulosus* and therapy

can be attempted with the drug when surgical resection is contraindicated or when the cysts rupture spontaneously during surgery. Some clinicians currently consider mebendazole as an alternative for the treatment of trichinosis or onchocerciasis (*filariasis caused by Onchocerca volvulus), gnathostomiasis and Angiostrongylus* cantonensis (McEvoy, 1988: 39).

Mebendazole kills malignant human lung cancer cells without toxicity to normal cells, it reduces the size and number of lung tumors in mice (Pharmawatch Communications LLC, 2002). Infections with *Capillaria philippinesis* are responsible for serious diarrhoea and malabsorption among the inhabitants of south East Asia. A 100% cure rate was reported in 33 new cases treated with mebendazole (Dollery, 1999:M13).

2.5 Mechanism of action

The drug appears to cause selective and irreversible inhibition of the uptake of glucose and other nutrients in susceptible helminths. The inhibition of glucose uptake results in the endogenous depletion of glycogen stores in the helminths. Mebendazole does not inhibit glucose uptake in mammals. Mebendazole appears to cause degenerative changes in the intestine of nematodes and in the absorptive cells of cestodes. The principal anthelmintic effect of the drug appears to be degeneration of cytoplasmic microtubules within these intestinal and absorptive cells (McEvoy, 1988:38).

2.6 Cautions

2.6.1 Adverse effects

Since mebendazole is poorly absorbed from the gastro-intestinal tract at the usual therapeutic doses, side effects have generally been restricted to gastro-intestinal disturbances such as abdominal pain and diarrhoea (Reynolds, 1989:57). Other adverse effects appear to occur more frequently when higher

doses are used. Nausea, vomiting, headache, tinnitus, numbness, and dizziness have been reported occasionally during mebendazole therapy. Fever, reversible neutropenia, alopecia, rash, pruritus, flushing, hiccups, cough, weakness, drowsiness, chills, hypotension, transient abnormalities in liver function tests, decreased hemoglobin concentration and/or hematocrit, leucopenia, thrombocytopenia, eosinophilia, hematuria, and cylinduria are some of the rarely reported adverse effects of mebendazole (McEvoy, 1988: 39).

2.6.2 Drug interactions

Limited data suggest that carbamazepine and phenytoin may enhance the metabolism of mebendazole. This interaction is unlikely to be clinically important in patients receiving mebendazole for the management of intestinal helminth infections. It could however prevent adequate therapeutic response in patients receiving mebendazole for the management of extraintestinal infections like hydatid disease (McEvoy, 1988:39).

2.6.3 High risk groups

Mebendazole has been shown to be embryotoxic and teratogenic in rats when given at single oral doses as low as 10 mg/kg. Mebendazole should only be used during pregnancy, especially during the first trimester, when the potential benefits justify the possible risks to the fetus. No information on secretion into breast milk is available (McEvoy, 1988:39).

Mebendazole should not be given to neonates and children under the age of 2 years. The drug may be used in the elderly in normal adult doses (Reynolds, 1989: 57).

2.7 Conclusion

Mebendazole polymorph C is apparently pharmaceutically favoured. It is therefore important to make sure that polymorph C is used in mebendazole formulations. Mebendazole is poorly soluble in water, which benefits the action against gastro-intestinal helminths. However, the low solubility leads to low bioavailability for systemic diseases like hydatid disease. Further studies to improve the solubility of mebendazole in formulations to get better bioavailibilty should be investigated.

Chapter 3

Polymorphic forms of mebendazole

3.1 Introduction

Many pharmaceutical solids exhibit polymorphism, which is frequently defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice (Grant, 1995:1,2). It has clearly demonstrated that a correlation exists between the polymorphism of the active substance and the bioavailability of the finished product. Examples of studies of these correlations are presented in Table 3.1.

Table 3.1 Examples of correlation between polymorphism of active principle and bioavailability of finished products (Andriollo *et al.*, 1998:140)

Active principle	**Type of measurement of bioavailability**
Ampicillin	Different plasma levels for anhydrous and trihydrate forms in suspension
Aspirin	Subcutaneous implantation, plasma levels
Carbamazepine	Similar plasma levels in human for anhydrous and trihydrate forms
Cimetidine	Plasma levels in rats
Griseofulvine	Plasma levels in dogs
Hydrocortisone acetate	Percutaneous absorption
Insulin	Amorphous and crystalline
Mebendazole	Acute toxicity and activity in mouse
Methylprednisolone	Identical pellets for two forms implanted in rat
Chloramphenicol palmitate	No therapeutic effect in some commercial forms
Pentobarbital	Plasma levels in rabbit

Toxic effects may also be linked to polymorphism (e.g. mebendazole). Polymorphism may be transformed with the influence of different parameters or events, such as being put into solution, or following mechanical effects, such as crushing or compression. Climatic conditions such as storage may also have an influence (Andriollo *et al.*, 1998:141). Therefore is it of great importance that polymorphic transformations are studied during the preformulation and formulation phases of drug development.

3.2 Polymorphism

Polymorphism is the capability of any compound or element to emerge in more than one crystal form. Pharmaceuticals may exist in different solid forms; these include true polymorphs, solvates (pseudopolymorphs), desolvates and amorphous solids. Polymorphs of the same compound have the same vapour, liquid or solution phase but differ in crystal structure (Yu *et al.*, 1998:118). Polymorphs are pharmaceutically important because different polymorphs feature different physical and chemical properties like different crystal sizes, shapes, hardness, density, solubility, dissolution rates, solid-state stability and compaction behaviour (Haleblian & McCrone, 1969:911). It was observed that different batches of mebendazole showed variations in relation to its physical and physical-chemical characteristics (such as solubility, infrared spectrum and microscopic appearance). Three polymorphic forms of mebendazole, identified as A, B and C can be formed using controlled crystallisation procedures. There are significant therapeutic differences between the different polymorphic forms, which support the fact that solubility and poor rate of solution are important factors limiting its use in treatment of several diseases (Himmelreich *et al.*, 1977:123).

3.3 Identification methods

Three different polymorphs (A, B, C) of mebendazole are available on the market of which polymorph C is pharmaceutically favoured. Therefore a complete

preformulation study is necessary before a decision on the use of any mebendazole raw material can be made. The use of only one technique might not clearly identify the polymorphic form (Liebenberg *et al.*, 1998:488).

3.3.1 Infrared spectrophotometry (IR)

IR spectra were recorded on a Nexus™ 470 spectrophotometer (Nicolet Instrument Corporation, Madison, USA) over a range of 4000-400 cm^{-1} with the Avatar Diffuse Reflectance smart accessory. Samples weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle. The IR absorption spectra of the various polymorphic forms show characteristic differences in the detailed shape and intensities of some of the major absorption bands. The carbonyl stretching frequency (1700 – 1730 cm^{-1}) and the –NH stretching frequency (3340 – 3410 cm^{-1}) were different in each form and could be used to identify each of the polymorphs (Himmelreich *et al.*, 1977:123). In table 3.2 the different characteristic frequencies as described by Himmelreich *et al.* are shown. The infrared spectra of mebendazole polymorphs are given in figure 3.1.

Table 3.2 Stretching frequencies of mebendazole polymorphs (Himmelreich *et al.*, 1977:123)

	- NH	> C=O
Polymorph A	3370	1730
Polymorph B	3340	1700
Polymorph C	3410	1720

In figure 3.1 and table 3.3 the different frequencies of three mebendazole polymorphs tested are shown. The results are similar to the results of (Himmelreich *et al.*, 1977:123).

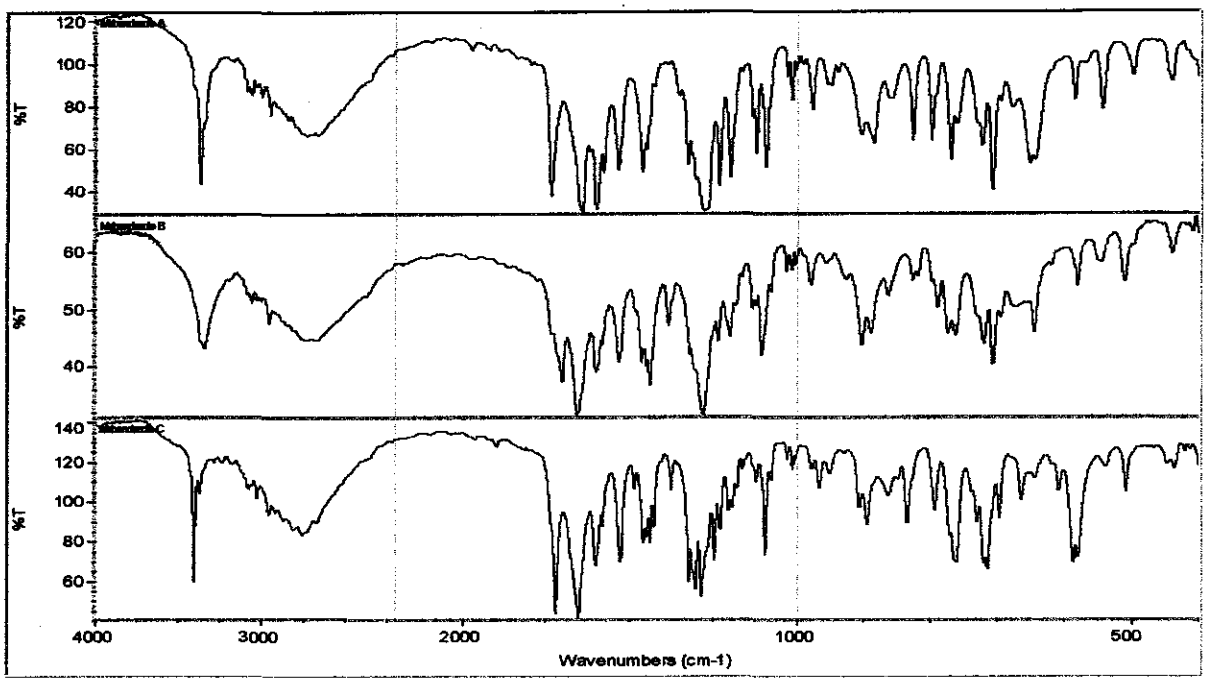


Figure 3.1 Infrared spectra of mebendazole polymorph A, B and C.

Table 3.3 Stretching frequencies of the three mebendazole polymorphs

	Batch number	- NH	> C=O
Polymorph A	M-27942	3369.74	1732.55
Polymorph B	TD076Q	3340	1700
Polymorph C	1870	3404.35	1716.69

3.3.2 X-ray powder diffractometry (XRPD)

All the X-ray powder diffraction patterns (XRPD) were obtained at room temperature using a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; receiving slit, 0.2 mm; monochromator; detector slit, 0.1 mm; scanning speed, 2°/min (step size 0.025°, step time, 1.0 sec). Approximately 300 mg samples were weighed into aluminium sample holders. The XRPD diffractograms of mebendazole polymorph A, B and C are illustrated in figure 3.2.

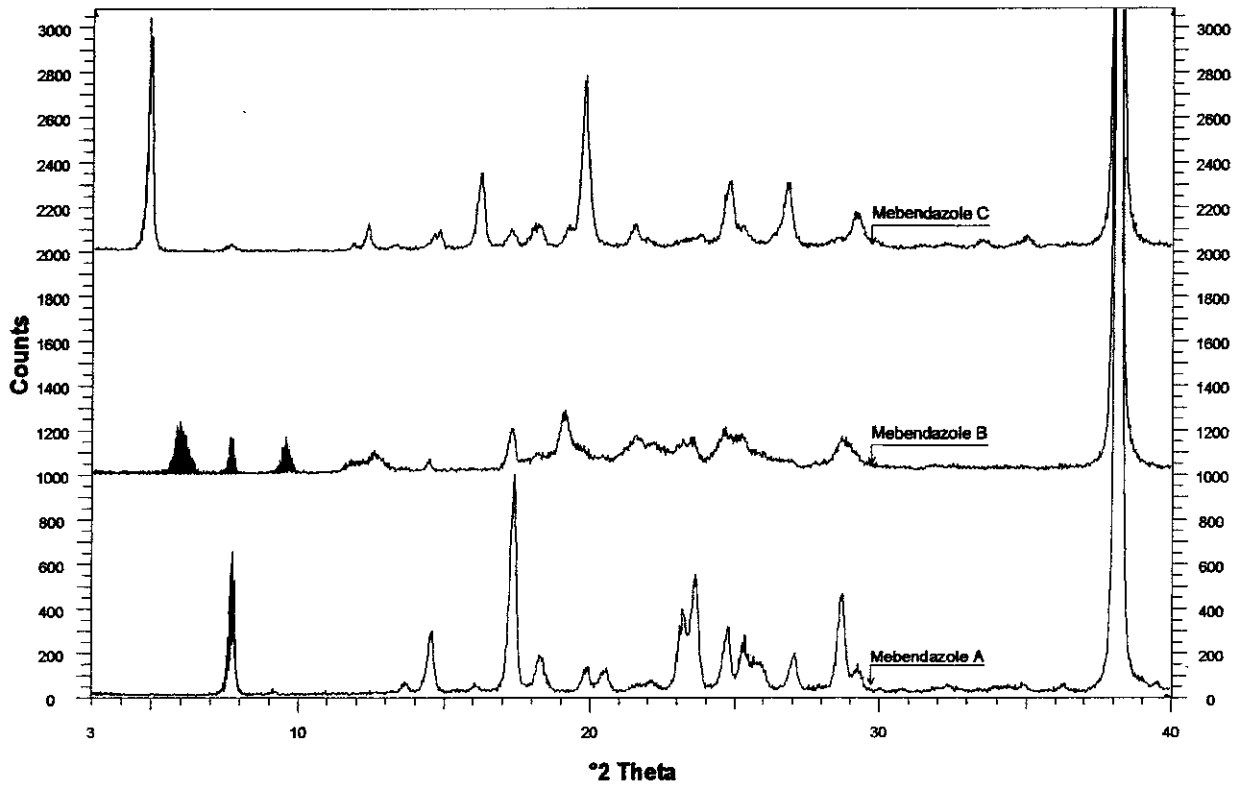


Figure 3.2 X-ray powder diffraction (XRPD) patterns of mebendazole polymorphs.

3.3.3 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) thermograms were recorded with a Shimadzu DSC-50 instrument (Shimadzu, Kyota, Japan). Samples weighing 3-5 mg were heated in closed aluminium crimp cells at a rate of 10°C/minute under nitrogen gas flow of 35 ml/minute. According to Himmelreich *et al.* (1977:124) each polymorph showed a common endotherm at 320°C, which was initially attributed to the melting of the drug since mebendazole is quoted as melting "above 280°C with decomposition" (Janssen Pharmaceutica, 1974). There was also a common endotherm at 325°C for each of the polymorphs. In a study done by Himmelreich *et al.* (1977:124) polymorphs were heated to 270°C and allowed

to cool without exceeding the temperature of 320°C. The resulting solids were different from any of the original forms of mebendazole. Thin layer chromatography confirmed that mebendazole was absent from the samples. Mebendazole undergoes solid phase pyrolysis, which was confirmed with chemical ionisation mass spectroscopy. These experiments indicate that the endotherm at 235°C in the thermogram of mebendazole represents the thermal decomposition of the compound, which results in the production of a mixture of three compounds. The thermograms of polymorph B and C show additional exotherms at 210°C and 170°C respectively. When heating of these forms was ceased above these temperatures but below 235°C, the solid was found to consist entirely of polymorphic form A. This proofed that at high temperatures polymorph A is the most stable crystalline form. The conversion of C to A occurs at a lower temperature (170°C) than B to A (210°C). These are the temperatures at which relaxation of crystal energies permit the transition to polymorphic form A which has the lowest chemical potential over the range of temperature to 235°C (Himmelreich *et al.* 1977:124). All the polymorphic forms had a final endotherm above ±300°C, which can be attributed to the melting point of the resultant products of the earlier decomposition. The DSC thermograms of the three mebendazole polymorphs tested are given in figure 3.3. In figure 3.3 it is visible that polymorph C exhibit three thermal events very similar to the events described by Himmelreich *et al.* (1977:124). A small endothermic event (± 187°C) followed by two sharply defined endotherms. The first sharply defined endotherm (± 253°C) is followed by a second and final endotherm (± 325°C). According to Himmelreich *et al.* (1977:124) the 187°C event is the conversion from polymorph C to A due to an internal rearrangement of the crystal structure. Therefore DSC could also be a convenient way to identify the polymorphic forms.

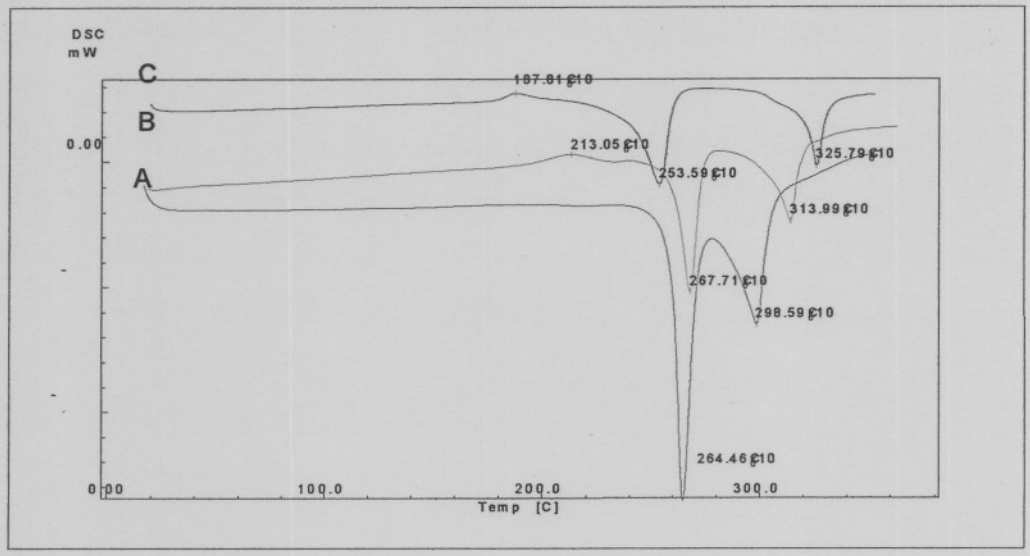


Figure 3.3 DSC thermograms of the three mebendazole polymorphs.

3.3.4 Dissolution rate

Dissolution of drugs from solid oral dosage forms is a necessary criterion for drug availability. Therefore, the dissolution test for solid oral drug products has emerged as the single most important control test for assuring batch-to-batch bioequivalence once its bioavailability has been defined (Skelly, 1976:539). Drug solubility studies and clinical trials have shown that form C of mebendazole is preferred. Unfortunately, the high concentration of sodium lauryl sulphate in the USP dissolution medium does not allow the use of this test to determine if form C is used or not (Swanepoel *et al.*, 2003:120). When sodium lauryl sulfate was removed from the dissolution medium, the profiles changed dramatically. Polymorph C went into solution faster (70% in 120 min) compared to polymorph B (37% in 120 min) and polymorph A (20% in 120 min). The order of the dissolution rate (A < B < C) does not correlate with the reported differences in solubility but does correlate with the reported in vivo effectiveness of the polymorphs. This suggest that the dissolution rate of the polymorphs depended

on more than just the inherent solubility of each polymorph and the degree of dispersion of the drug in the medium in which it is dissolving (Swanepoel *et al.*, 2003:4). Mebendazole polymorphs spontaneously aggregate to form large, poorly wettable aggregates. The mean volume particle sizes of the three polymorphs were: A = 3.56±1.54 µm, B = 6.18±1.72 µm and C = 3.35±1.02 µm. For particles of the same size, the dissolution rate decreased as the level of flakiness and irregularity increased (Mosharraf and Nystrom, 1995:35). Mebendazole is poorly water soluble and has a slow dissolution rate. Low solubility and poor rate of solution of the drug are important factors limiting its use in the treatment of several diseases (Himmelreich *et al.*, 1977:123). Surfuctants like sodium lauryl sulphate enhance the dissolution rate of poorly water-soluble drug products due to wetting, micellar solubilisation or deflocculation (Shah *et al.*, 1989: 612). The sodium lauryl sulphate present in the dissolution medium reduces the discriminative power between the three polymorphic forms of mebendazole. Therefore, discriminating between the polymorphic forms of mebendazole is not significant when using the USP dissolution medium containing sodium lauryl sulphate (Swanepoel *et al.*, 2003:4). Dissolution studies, without sodium lauryl sulphate could be a very useful method for the identification of polymorphic forms. The order of thermodynamic stability is A > C > B. The observation that the conversion of C to A occurs at a lower temperature than B to A; is not inconsistent with this conclusion, since these temperatures do not represent thermodynamically reversible phase transitions (Himmelreich *et al.*, 1977:124). The solubility of the three polymorphs in both water and 0.3 M hydrochloric acid is in the order B > C > A (Himmelreich *et al.*, 1977: 124).

3.4 Solubility study between mebendazole polymorph A and C in water and 0.1 M hydrochloric acid

Solubility was determined after saturated water and 0.1 M hydrochloric acid with polymorph A and C. Ten tubes contained water and ten tubes 0.1 M hydrochloric acid. Five tubes of each were saturated with polymorph A and the other five with

polymorph C. The samples were rotated for 48 hours in a thermostated water bath at 30°C. The bath was covered with foil to protect the sample against UV-light. The saturated solutions were centrifuged for five minutes at 3000 rpm. The samples were analysed by means of high performance liquid chromatography (HPLC) under the following conditions:

Analytical instrument: HP1050 series HPLC equipped with a HP1050 quaternary gradient pump, HP1050 auto sampler, HP1050 diode array detector and Chemstation Rev. A.06.02 data acquisition and analysis software.

Column:	USP 25 (2005) packing L1, p1058 (Luna C18-2 column, 150 x 4.6 mm, 5 µm, Phenomenex, Torrance, CA was used)
Mobile phase:	A mixture of methanol and 0.05 M monobasic potassium phosphate (60:40), adjust to a pH of 5.5
Flow rate:	1.0 ml/min
Injection volume:	10 µl
Wavelength:	UV at 247 nm

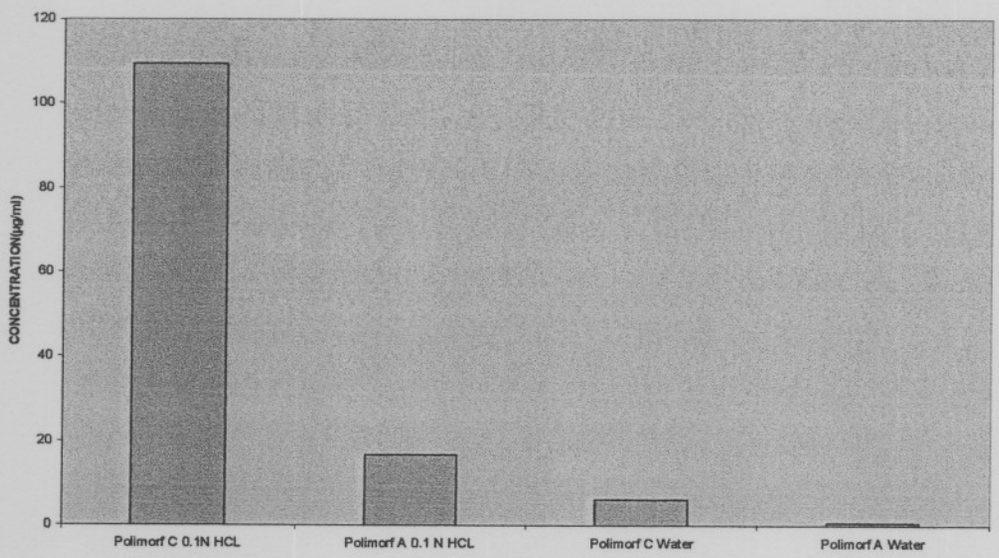


Figure 3.4 Solubility differences between polymorph A and C in water and 0.1 M hydrochloric acid.

According to the results (figure 3.4) the solubility of polymorph C is significantly higher than that of polymorph A in both 0.1 N HCl and water. Both polymorphic forms were more soluble in the hydrochloric acid than in the water.

3.5 A study to determine the polymorphic forms of mebendazole products on the South African market

Four mebendazole products from different companies in South Africa were tested by means of a infrared spectrophotometer to identify the polymorphic form (Table 3.4). IR spectra were recorded on a Nexus^{TM} 470 spectrophotometer (Nicolet Instrument Corporation, Madison, USA) over a range of 4000-400 cm^{-1} with the Avatar Diffuse Reflectance smart accessory. Samples of the crushed tablets, weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle.

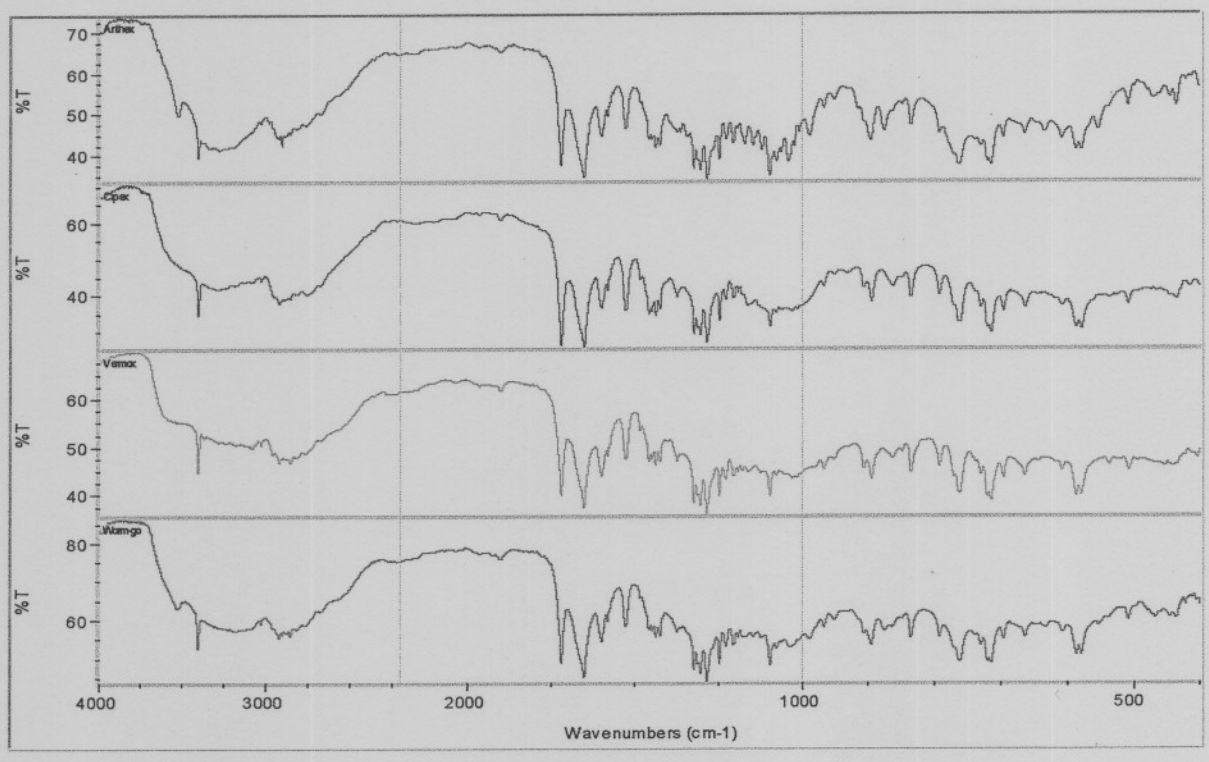


Figure 3.5 Infrared spectra of mebendazole products on the market.

Table 3.4 Stretching frequencies of mebendazole products on the market

	- NH	> C=O
Anthex®	3403.99	1717.32
Cipex®	3403.77	1717.21
Vermox®	3404.50	1716.93
Worm-go®	3403.84	1716.88

According to the results in figure 3.5 and table 3.4, it is confirmed that all the mebendazole products tested contain polymorph C. The stretching frequencies of the products tested, compare well with the published frequencies (Himmelreich *et al.*, 1977:124) of polymorph C.

3.6 Polymorphic forms of mebendazole raw materials tested

Different polymorphic forms of mebendazole are available on the market; therefore a complete characterisation study is necessary as part of the preformulation study. The following raw material powders were obtained from different suppliers: Mebendazole B/N M-27942 from Sigma and B/N F10958 from Rolab.

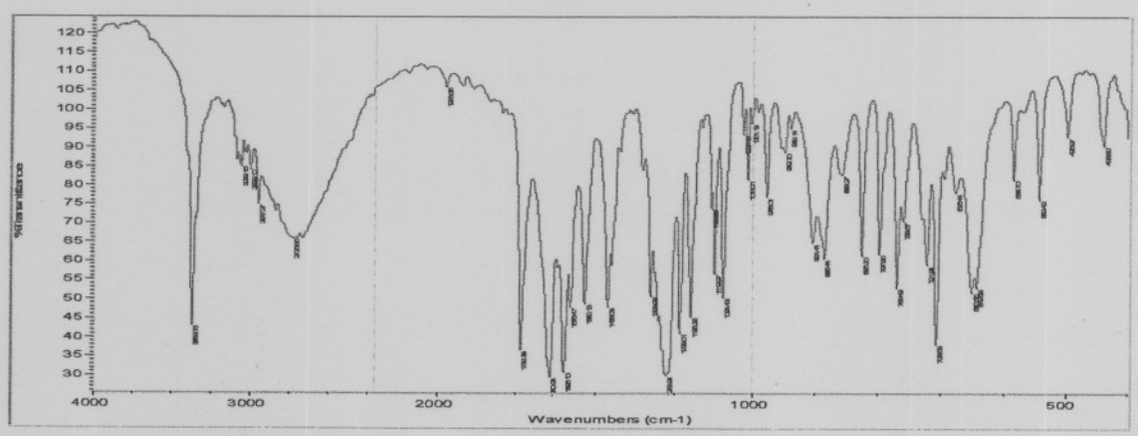


Figure 3.6 Infrared spectra of mebendazole M-27942.

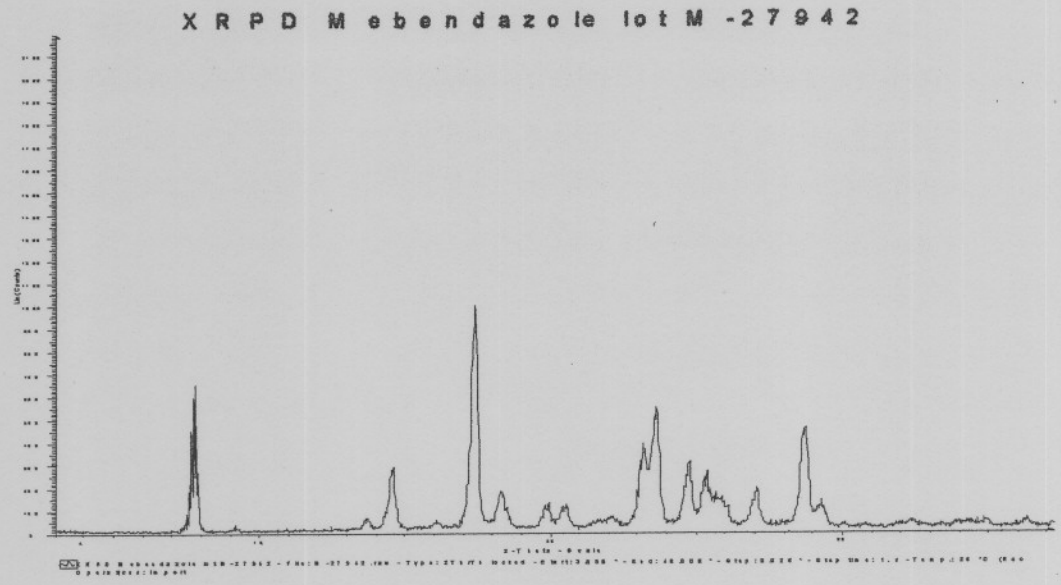


Figure 3.7 X-ray powder diffraction patterns (XRPD) of mebendazole M-27942.

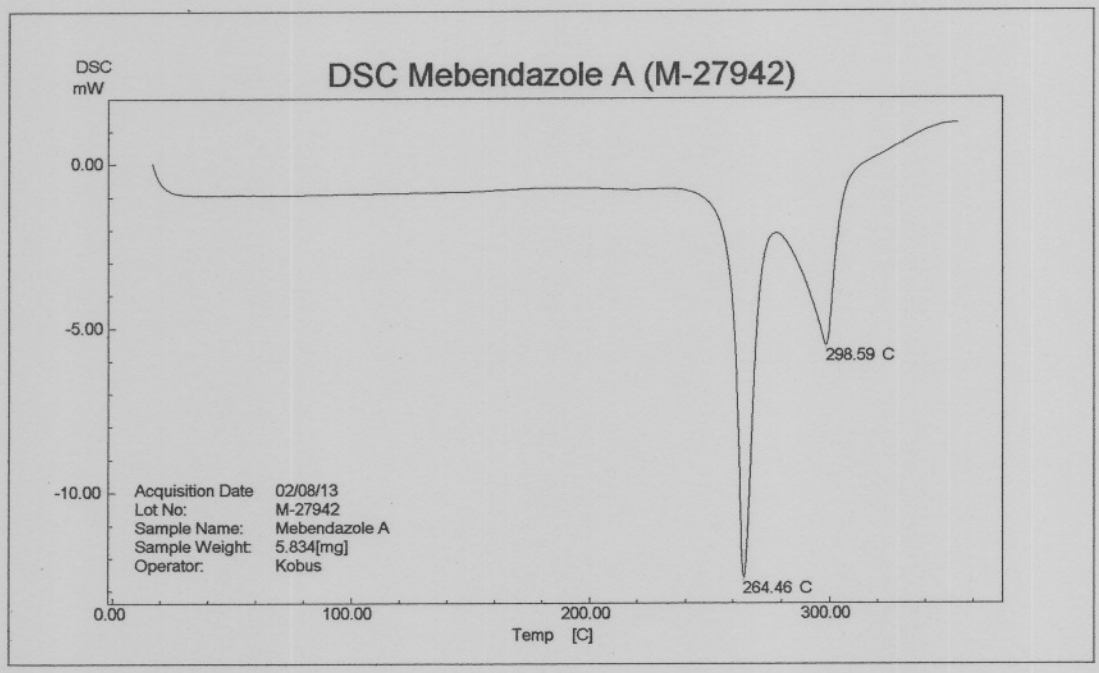


Figure 3.8 DSC thermogram of mebendazole M-27942.

According to the infrared data in figure 3.6 the –NH and the C=O stretching frequencies were 3369.75 cm^{-1} and 1732.33 cm^{-1} respectively. These frequencies compare well with published results of mebendazole polymorph A, in table 3.1. The X-ray diffraction patterns of M-27942 in figure 3.7 and the DSC thermograms compare well with that of polymorph A in figures 3.2 and 3.3. These results confirm that mebendazole batch M-27942 indeed contains polymorphic form A.

Mebendazole batch number F10958 was also tested by means of IR and DSC for the identification of the polymorphic form. The infrared spectrum of batch F10958 that was recorded in May 2002 is shown in figure 3.9.

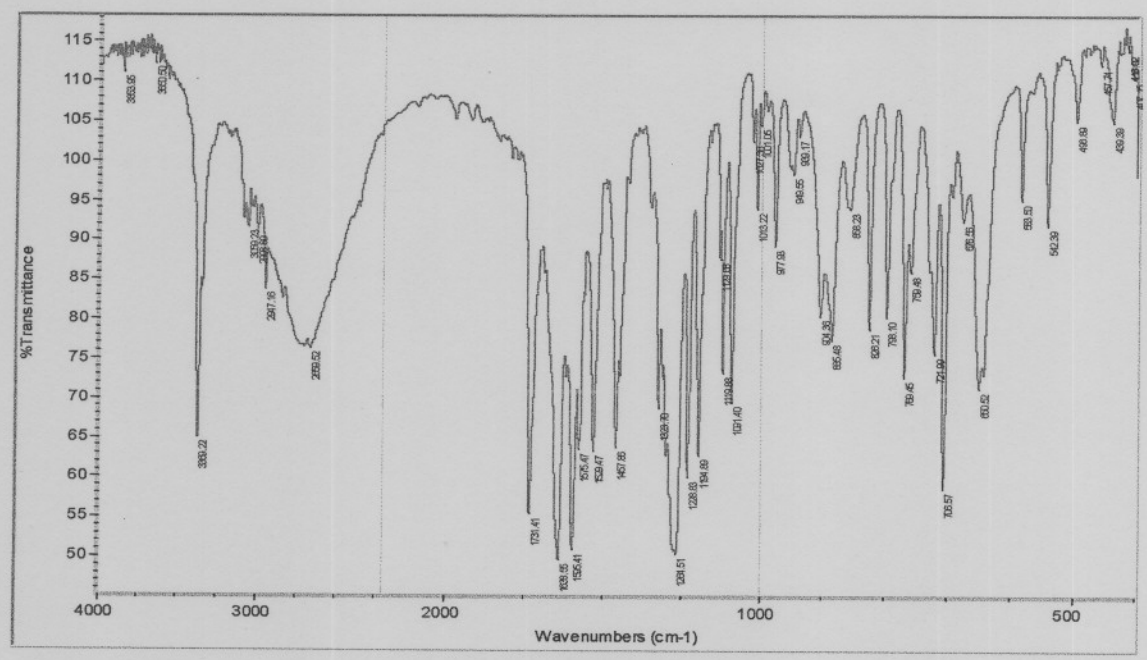


Figure 3.9 Infrared spectra of mebendazole F10958 (8/05/2002).

The infrared data in figure 3.9 showed that the –NH (amide) and the C=O (carbonyl) stretching frequencies were 3369.22 cm^{-1} and 1731.41 cm^{-1}, which indicated polymorph A.

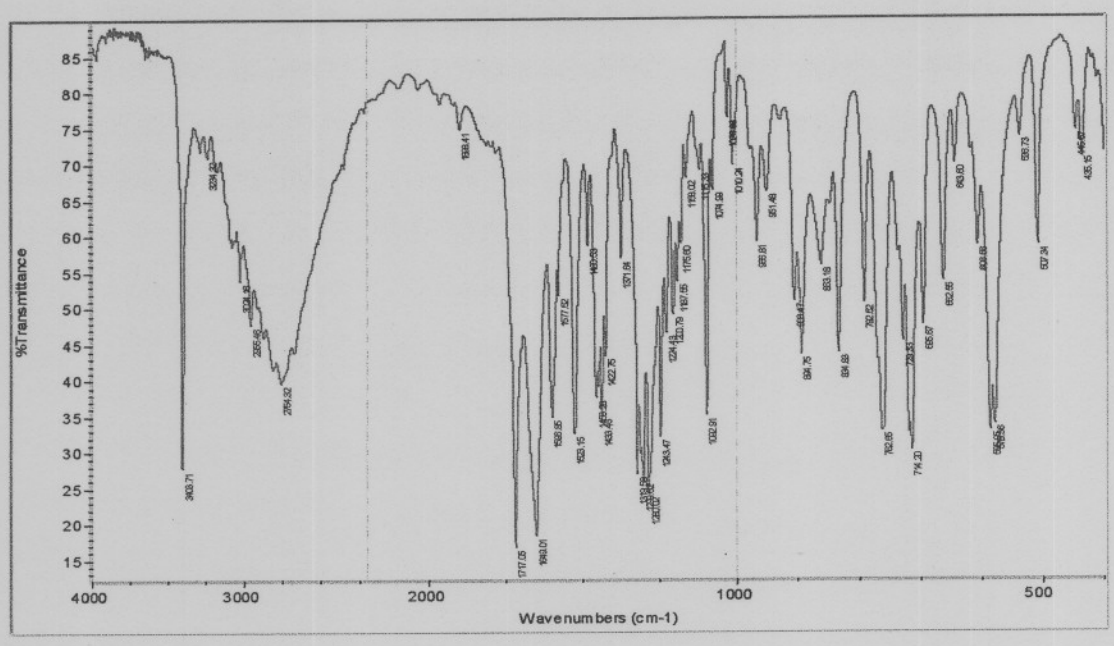


Figure 3.10 Infrared spectra of mebendazole F10958 (13/08/2002).

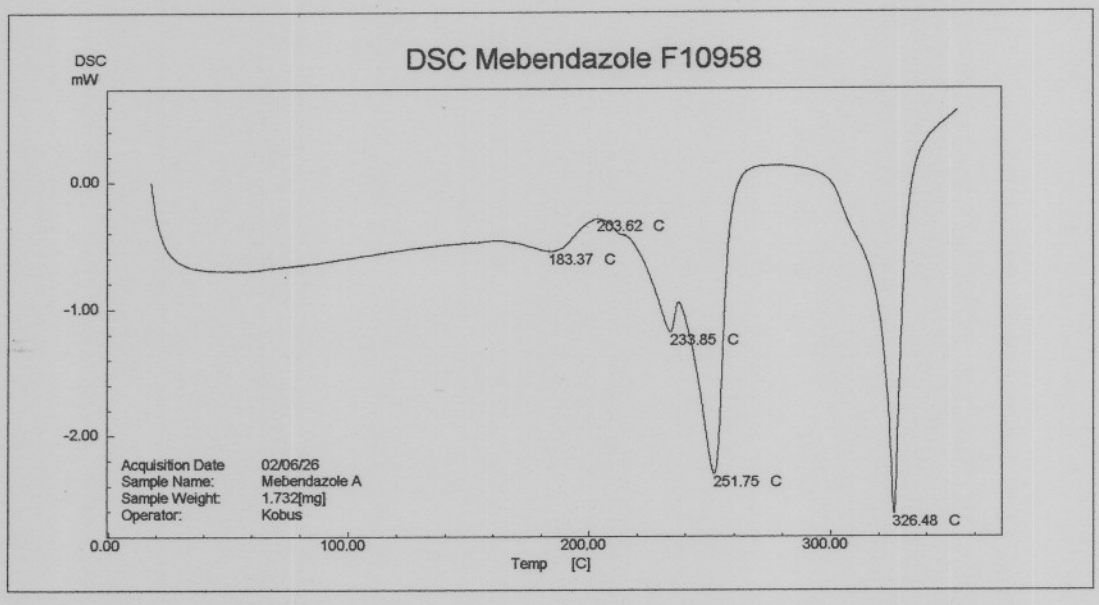


Figure 3.11 DSC thermogram of mebendazole F10958 (26/06/2002).

The same mebendazole batch F10958 was tested 3 months later. It was found that the –NH and the C=O stretching frequencies changed to 3403.71 cm^{-1} and

1717.05 cm^{-1} (figure 3.10) indicative of polymorph C. According to the DSC thermogram in figure 3.11 it was clear that the thermogram was not comparable with any of the thermograms of polymorph A or C in figure 3.3. Several factors and environmental conditions can lead to polymorphic transformation. These factors include formulation processes such a mixing, granulation, drying, grinding and compression (Wadke and Jacobson, 1980:31). Mebendazole is expected to be sensitive to light like other drugs containing an imidazole ring. Mebendazole is a thermal and photosensitive drug. The rate of photodecomposition was found to be dependent on temperature, pH, intensity of radiation, solvent and drug concentration (Karim *et al.*, 1996:252). The raw material (B/N F10958) was stored at room temperature protected from light and humidity. Polymorph A is the most stable polymorphic form and changes usually occurred from form C and B to A. This could be an example of a mixture of polymorphic forms, which showed that the IR can't be use alone in identification of polymorphs. This is subjected to further investigation. In table 3.5 the different stretching frequencies of mebendazole F10958 are shown.

Table 3.5 Stretching frequencies of mebendazole F10958

Date tested	**- NH (cm^{-1})**	**> C=O (cm^{-1})**
8 May 2002	3369.70	1732.40
13 Aug 2002	3403.71	1717.05

3.7 Effect of increased temperature on mebendazole polymorph C

Mebendazole raw material, polymorph C, was subjected to a heating process to 260°C, whereafter it was cooled down. The resulting product was evaluated by means of IR and DSC. Figure 3.13 shows the endotherm, which represents the melting point of the resultant products from the earlier decomposition of mebendazole at 253°C. The infrared spectra of mebendazole after being exposed to 260°C is shown in figure 3.12. The data confirmed that the spectra are not comparable to any of the polymorphic forms given in figure 3.1. Mebendazole is absent in the resultant products after being exposed to 260°C. These results are compatible with the results from (Himmelreich *et al.*, 1977:124) which were discussed in paragraph 3.3.3.

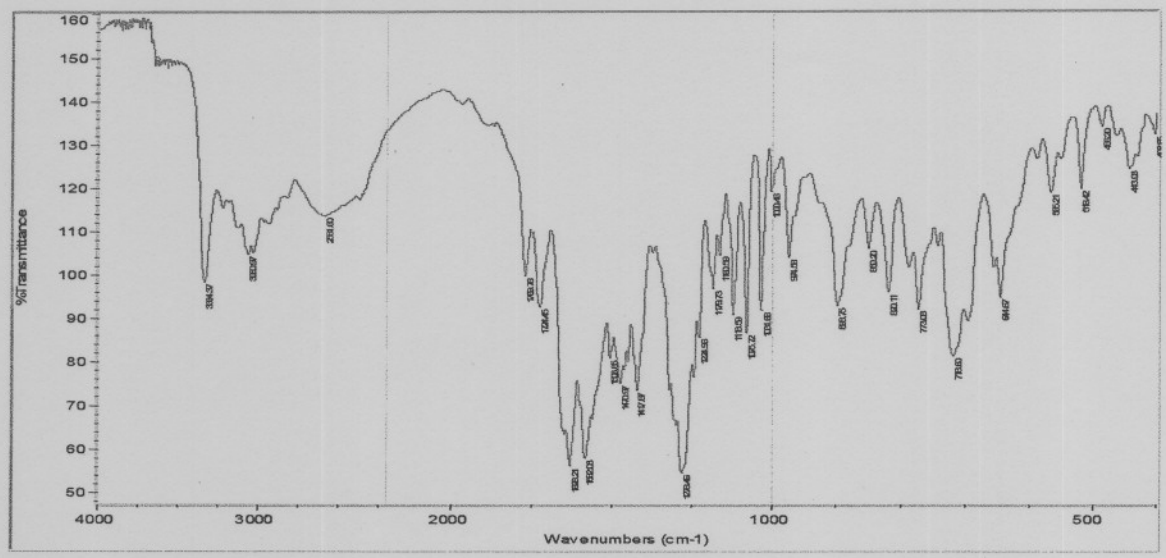


Figure 3.12 Infrared spectra of mebendazole after being exposed to 260°C.

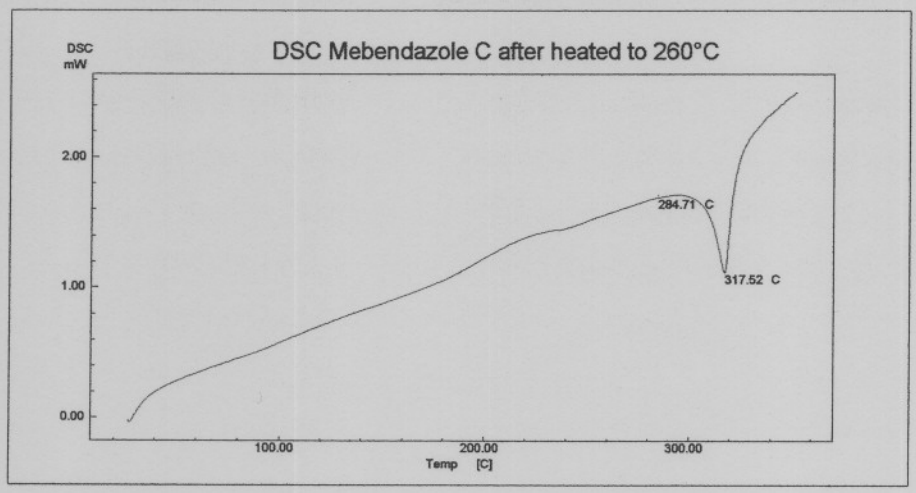


Figure 3.13 DSC thermogram of mebendazole after exposed to 260°C.

3.8 Stability

It is described that mebendazole undergoes possible crystal transformation during trituration (Chan and Doelker, 1985:315). Several factors and environmental conditions can lead to polymorphic transformation. These factors include formulation processes such a mixing, granulation, drying, grinding and compression (Wadke and Jacobson, 1980:31). Compression does not lead to any significant changes in the crystal structure of mebendazole polymorph C. This can be deduced from comparable XRPD patterns and DSC thermograms before and after compression (Terblanche *et al.*, 2003).

Mebendazole is expected to be sensitive to light like other drugs containing an imidazole ring. Mebendazole is a thermal and photosensitive drug. The rate of photodecomposition was found to be dependent on temperature, pH, intensity of radiation, solvent and drug concentration. The drug was found to be stable against photodecomposition at an acidic pH, however at a pH greater than 6 the drug started to decompose rapidly on irradiation. Additives like sucrose, sodium carboxymethyl cellulose (CMC), povidone (PVP), sodium benzoate, sodium saccharin, keltrol and glycerol were found to decrease the rate of

photodecomposition. CMC was the most effective additive (Karim *et al.*, 1996:252).

3.9 Solubility

Mebendazole is an important drug against hydatid disease but it presents low solubility and absorption from the intestinal tract. Cyclodextrins increase the solubility and dissolution rate of poorly soluble drugs and as drug carriers they control the drug release at the gastrointestinal level (Diaz *et al.*, 1999:111). In human investigations it has been established that its absolute bioavailability is 22%, which is a consequence of its low solubility. The cyclodextrin derivates are suitable for increasing the dissolution characteristics of apolar drugs. It has been established that the dissolution of the products containing mebendazole and ß-cyclodextrins depends on the percentage composition and on the methods of preparation of the products (Kata and Papp, 1987: 65). The dielectric constant of the solution has an influence on mebendazole solubility.

3.10 Conclusion

Manufacturers should take care when buying mebendazole raw material. This is important since all three polymorphic forms of mebendazole are available on the market. Care should also be taken when using IR spectral data alone to identify mebendazole polymorphic forms since a mixture of these forms could exhibit an IR spectrum comparable to either of the components of the mixture. XRPD and DSC analysis should be used in combination with IR analysis.

Chapter 4

Preformulation, formulation and stability of mebendazole products

4.1 Introduction

In this chapter the preformulation, formulation and evaluation of mebendazole products are discussed. The first aim of this study was to formulate a chewable tablet appropriate for the multidosage of children during a deworming program. Chewable tablets present an attractive alternative for children who have not yet learned to wash tablets down with water, with the added advantage that this type of medication can be taken at any time or place when water is not available (Rubenstein, 1988:314). The world's population exceeds six billion individuals, half of whom are children. The majority of these children live in developing countries where there is a problem not only with off-label use of drugs but with access to any drugs at all. Children have the right by necessity and thus the right to have access to drugs labelled for use in children (Milne, 2000:193). The International Conference on Harmonization (ICH) of Technical Requirements for Registration of pharmaceuticals for human use has recently directed efforts toward the global development of safe and effective therapeutic drugs for children. Development of each new formulation for children is a formidable task. (Simar, 2000:809).

The second aim was to formulate a gel for dogs and domestic animals which is more viscous than the suspensions on the market. Dosing an animal with a liquid can be difficult, and a sudden movement of the animal often results in spillage. A drug in a gel form is a convenient means for administration to pets, to reduce spillage.

4.2 Preformulation and compatibility studies

Preformulation studies (Table 4.1) properly carried out, have a significant part to play in anticipating formulation problems and identifying logical paths in liquid and solid dosage form technology. Solid-state stability by DSC and HPLC and in the presence of tablet excipients will indicate the most acceptable vehicles for solid dosage form formulations (Wells and Aulton, 1988:251). The assessment of incompatibilities between active ingredients and excipients, forms an important part of the preformulation stage during the development of new dosage forms (Wells and Aulton, 1988:227). In figure 4.1 the relationship between preformulation and formulation is illustrated.

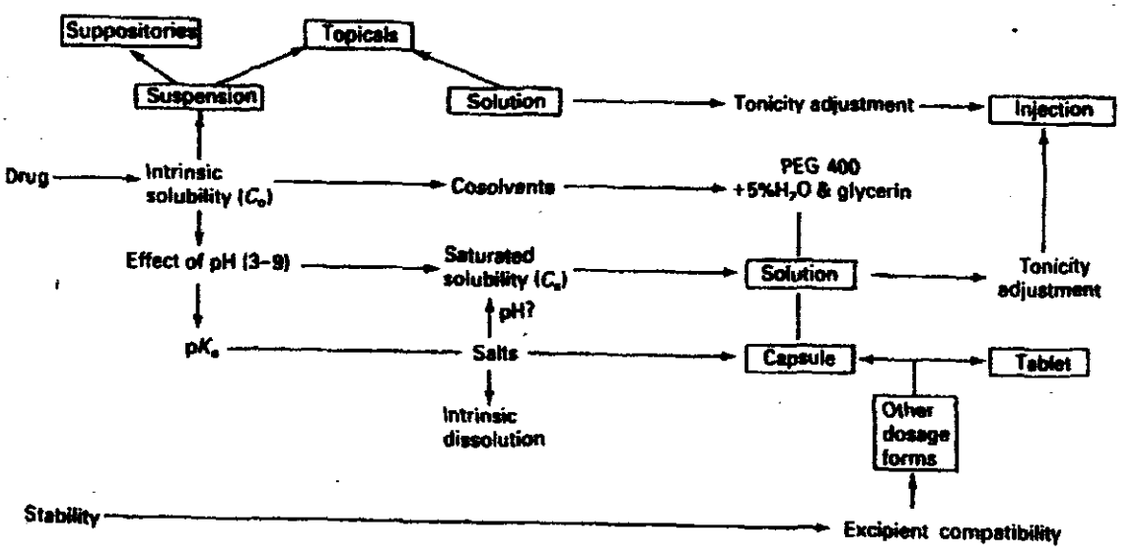


Figure 4.1 Relationship between preformulation and formulation in generic dosage form development. (The formulation stages are shown in boxes and the preformulation stages are unboxed) (Wells and Aulton, 1988:251).

Table 4.1 Preformulation drug characterisation (Wells and Aulton, 1988:224)

Test	**Method/function/characterisation**
1. Spectroscopy	Simple u.v. assay
2. Solubility	Phase solubility, purity
aqueous	Intrinsic solubility, pH effects
pKa	Solubility control, salt formation
salts	Solubility, hygroscopicity, stability
solvents	Vehicles, extraction
partition coeff K_w^o	Lipophilicity, structure activity
dissolution	Biopharmacy
3. Melting point	DSC- polymorphism, hydrates, solvates
4. Assay development	u.v., t.l.c., h.p.l.c.
5. Stability (in solution and in solid state)	Thermal, hydrolysis, oxidation, photolysis, metal ions, pH
6. Microscopy	Morphology, particle size
7. Powder flow	
bulk density	Tablet and capsule formulation
angle of repose	Tablet and capsule formulation
8. Compression properties	Aids excipient
9. Excipient compatibility	Preliminary screening by DSC, confirmation by t.l.c.

4.2.1 Preformulation study using differential scanning calorimetry (DSC)

In this study the compatibility between mebendazole and excipients was assessed prior to the formulation of a tablet. DSC is an easy and effective method to determine possible incompatibilities between the active substance and the excipients used.

Differential thermal analysis has been developed by Le Chatelier in 1887. In 1960-1970 the pharmaceutical industry became interested in the method, with the appearance of the first differential scanning calorimeter (Giron, 1986: 755).
Two ovens are linearly heated. One oven contains the sample in a closed pan, the other one contains an empty pan, called a reference pan. Two measurements are generally used. In the first method called "heat flux DSC" the instrument measures the temperature difference between the sample pan and the reference pan. Through calibration this temperature is transformed into heat flow. In the second method, which is called DSC, two individual heaters are used in order to monitor the individual heating rates. A control system regulates the temperature difference between sample and reference. If any difference is detected, the individual heating will be corrected such that the temperature is kept the same in both pans. That is, when an endothermic or exothermic process occurs, the instrument delivers the compensation energy, which must be given in order to maintain equal temperatures in both pans (Giron, 1986:756).

Differential scanning calorimetry (DSC) thermograms were recorded with a Shimadzu DSC-50 instrument (Shimadzu, Kyota, Japan). Samples weighing 3-5 mg were heated in closed aluminium crimp cells at a rate of 10°C/minute under nitrogen gas flow of 35 ml/minute. Castor sugar, sodium cyclamate, dextrose, lemon flavour, magnesium stearate were mixed in a 1:1 (w/w) mebendazole:excipient relationship.

In figure 4.2 – 4.12 the DSC thermograms of the samples containing mebendazole and the excipients used in the formulations are shown.

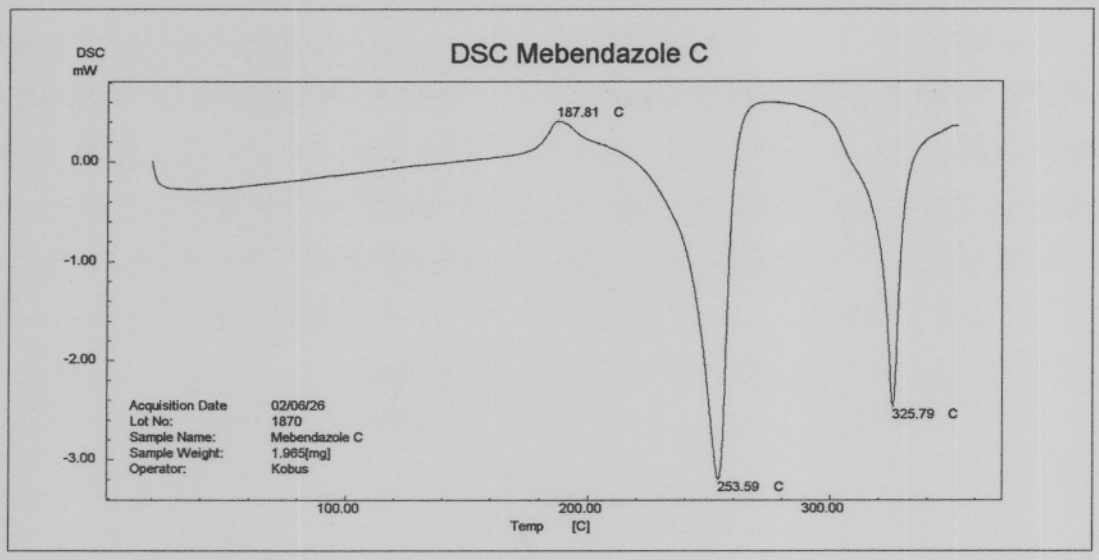


Figure 4.2 DSC thermogram of mebendazole polymorph C.

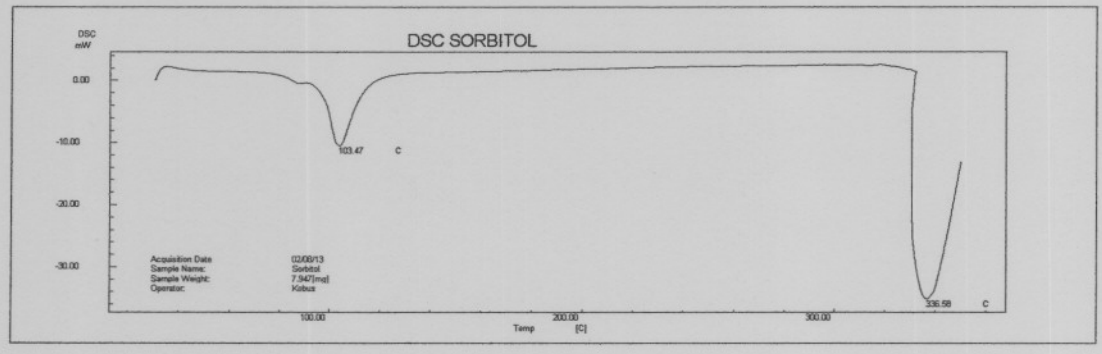


Figure 4.3 DSC thermogram of sorbitol.

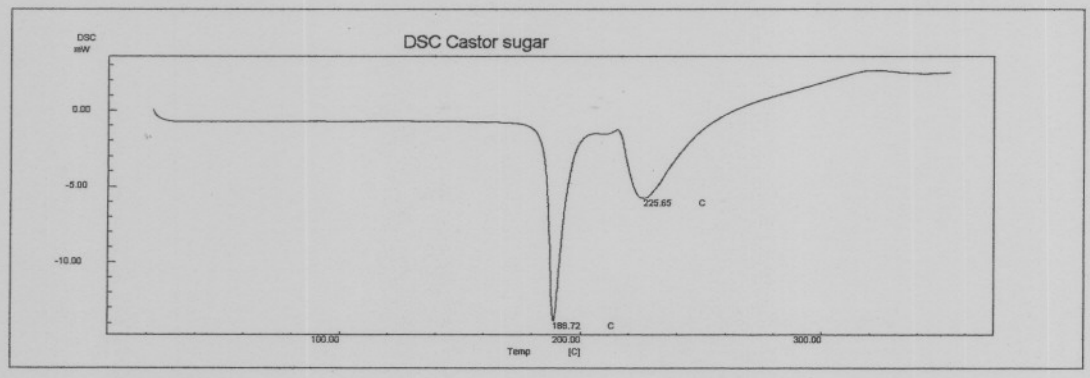


Figure 4.4 DSC thermogram of castor sugar.

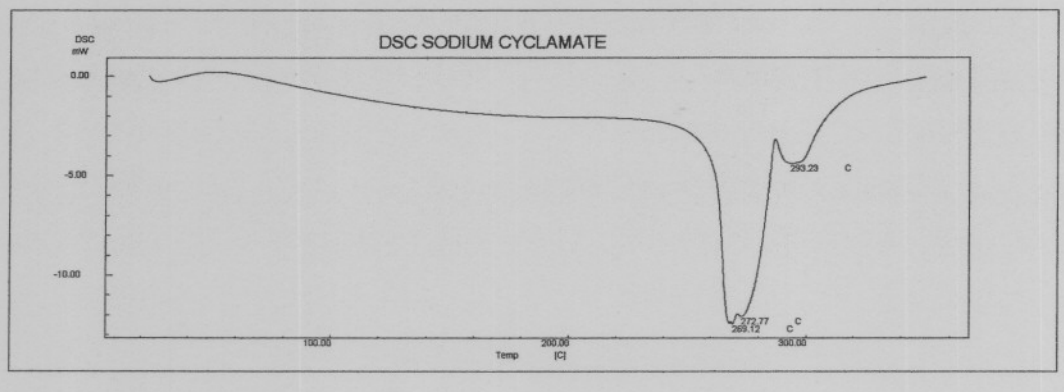


Figure 4.5 DSC thermogram of sodium cyclamate.

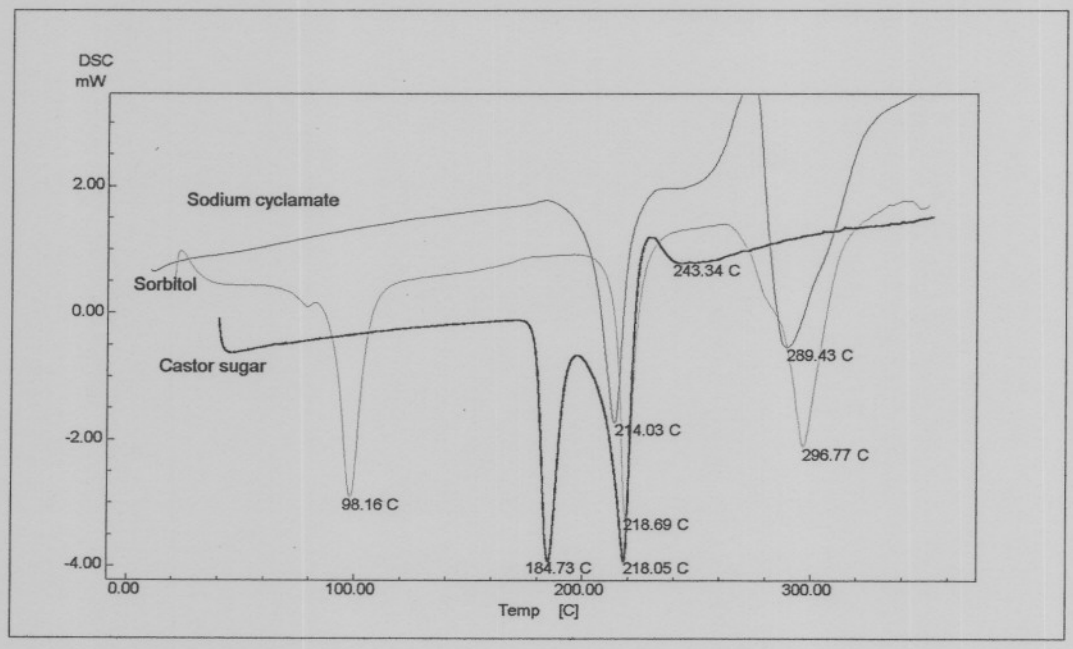


Figure 4.6 DSC thermograms of mebendazole and sorbitol, sodium cyclamate and castor sugar.

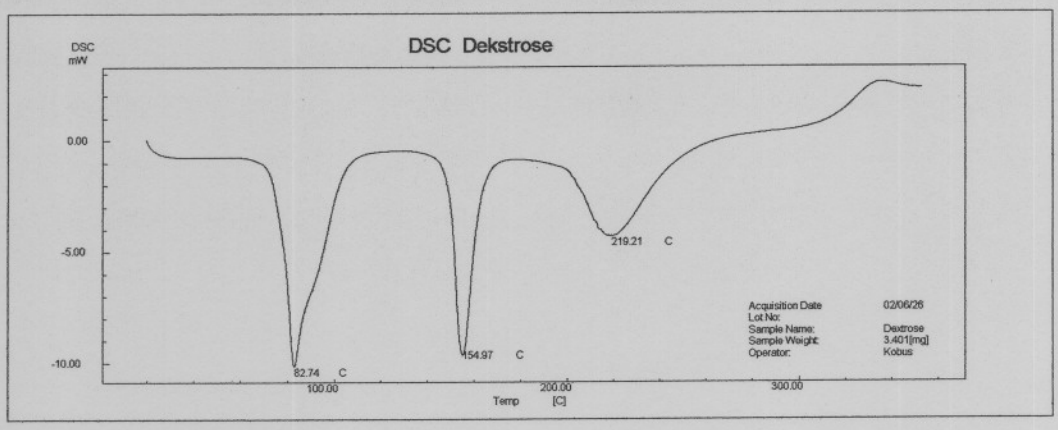


Figure 4.7 DSC thermogram of dextrose.

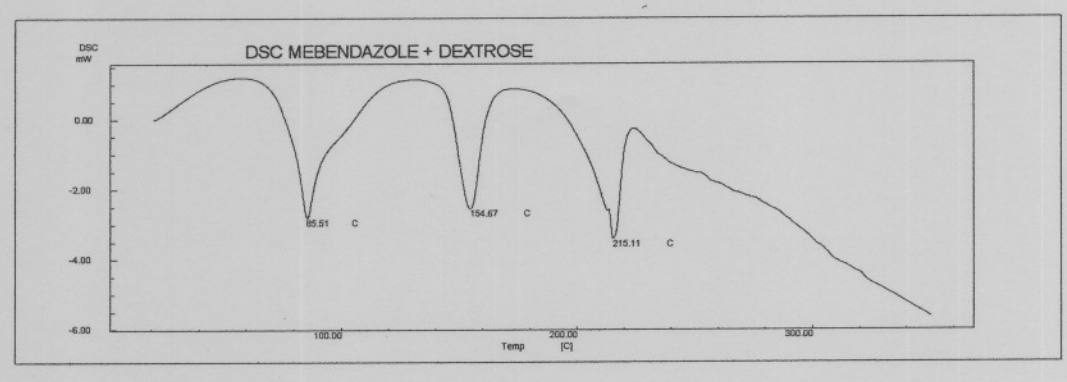


Figure 4.8 DSC thermogram of mebendazole and dextrose.

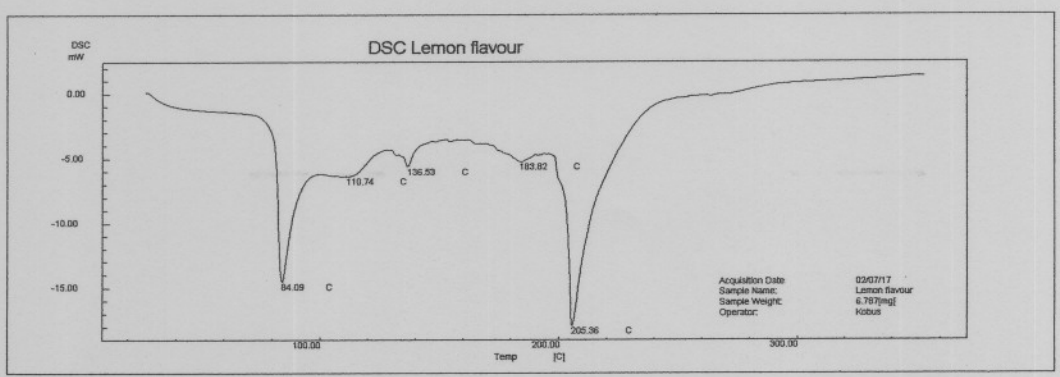


Figure 4.9 DSC thermogram of lemon flavourant.

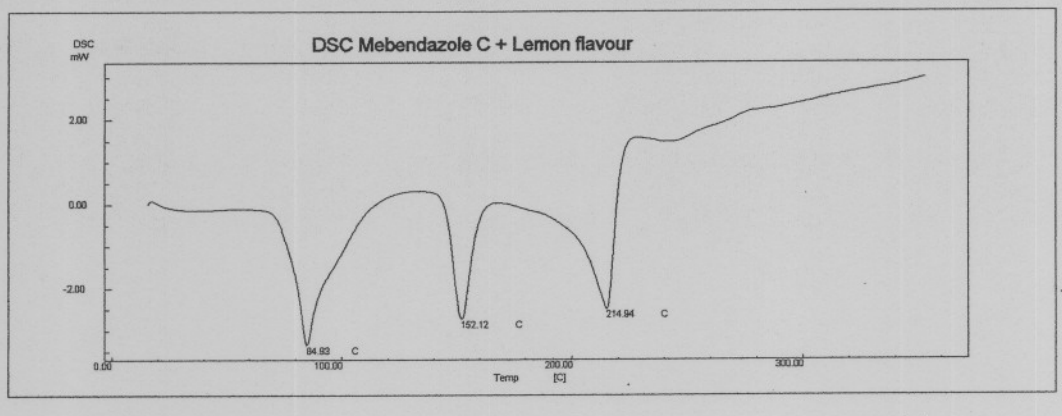


Figure 4.10 DSC thermogram of mebendazole and lemon flavourant.

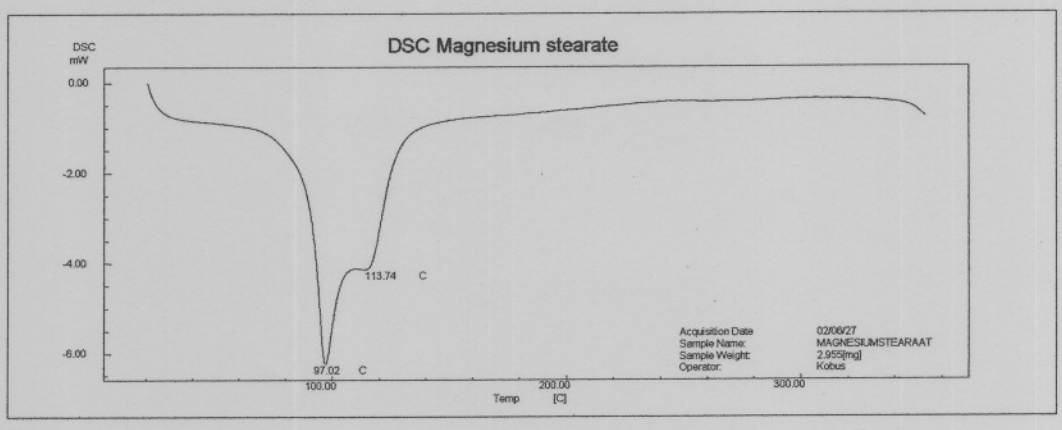


Figure 4.11 DSC thermogram of magnesium stearate.

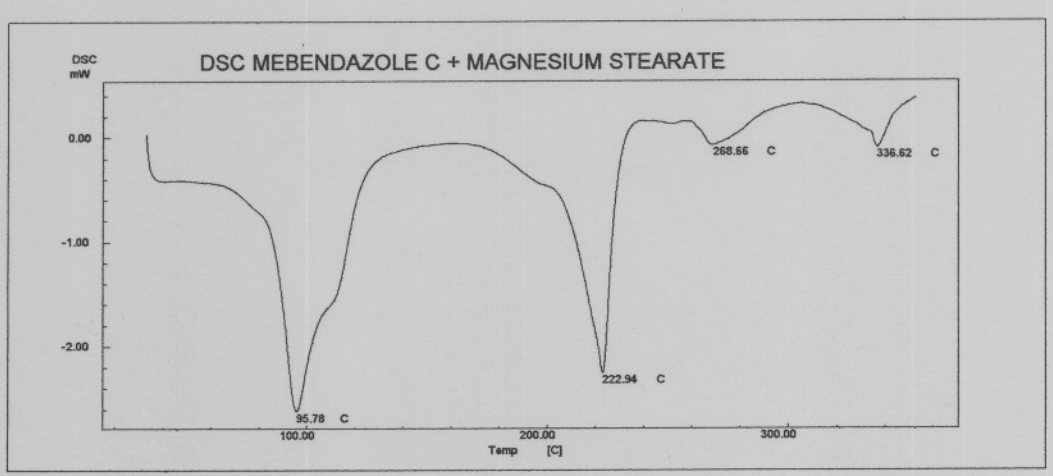


Figure 4.12 DSC thermogram of mebendazole and magnesium stearate.

4.2.1.1 Discussion

DSC allows the fast evaluation of possible incompatibilities between the formulation compounds derived from appearance, shift or disappearance of peaks and variations in the corresponding ΔH (Van Dooren, 1983:43-55). According to Van Dooren (1983:43-55) all the above mentioned excipients are incompatible with mebendazole. From the thermograms in figures 4.6, 4.8, 4.10 and 4.12 it is clear that the peaks from the mebendazole/excipients combinations shift and/or disappear from the original thermogram peaks. In figure 4.2 it is clear that polymorph C exhibit an exotherm at ±187°C and two endotherms at ± 253°C and ±325°C. The exotherm and the two endotherms in the thermogram of mebendazole polymorph C (figure 4.2) and the peaks from the excipients disappear or change considerably. A possible indication of incompatibilities between mebendazole and the excipients tested.

Vermox® is one of the best known mebendazole products on the South African market. The thermogram of Vermox® in figure 4.13 shows a major endotherm at 218.15°C. The exotherm and endotherm of mebendazole disappear or shift significantly in the Vermox® thermogram, which would also indicate a possible incompatibility between mebendazole and the excipients used in the formulation of the Vermox® tablet. The endotherm at 218.15°C from Vermox® in figure 4.13 could be the shifted endotherm which represents the thermal decomposition of mebendazole. The excipient/mebendazole samples tested also showed the "decomposition peak" at ± 220°C, the same as was the case with the Vermox® tablets.

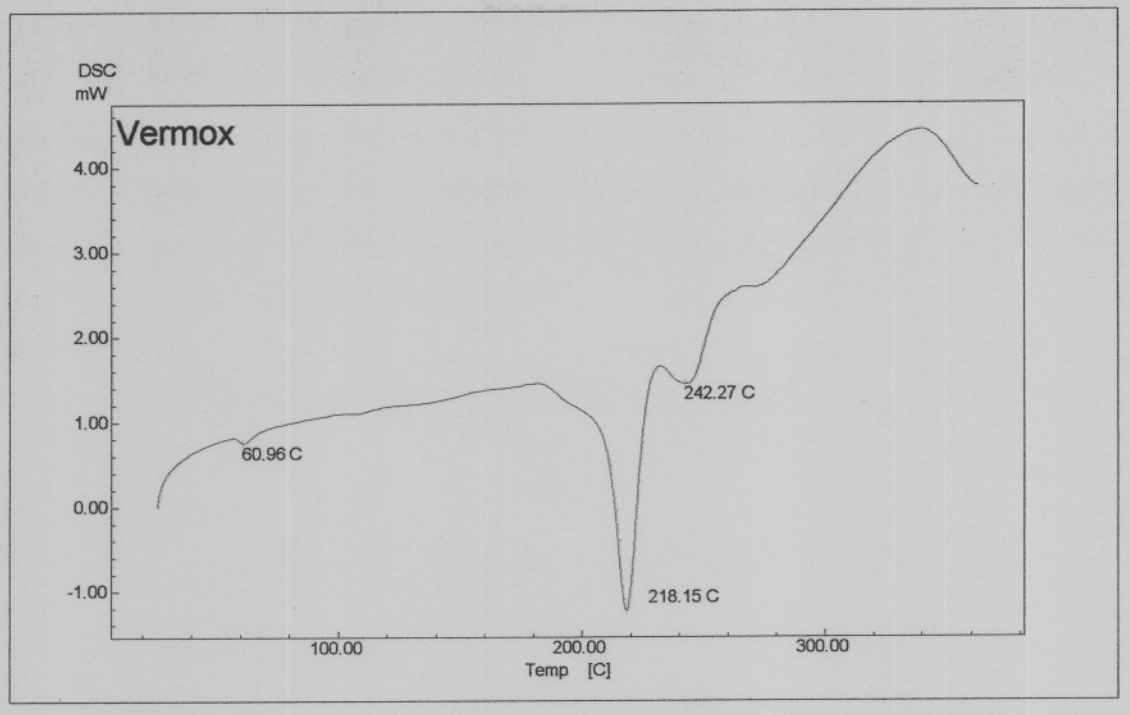


Figure 4.13 DSC thermogram of Vermox®.

The thermograms of polymorphs B and C show additional exotherms at 210°C and 170°C respectively. When heating above these temperatures but below 235°C occurred, the solid was found to consist entirely of polymorph A (Himmelreich *et al.*, 1977:124). The decomposition peak contains only polymorphic form A. Because of the complex thermal behaviour of mebendazole, differential scanning calorimetry (DSC) proofed to be an insufficient method to study incompatibilities between excipients and mebendazole polymorph C. HPLC will be the best and most accurate method to study possible incompatibilities.

4.2.2 Content uniformity

This test was preformed to determine if the excipients were mixed properly. The test was done by means of high performance liquid chromatography (HPLC) under the following conditions:

Analytical instrument: HP1050 series HPLC equipped with a HP1050 quaternary gradient pump, HP1050 auto sampler, HP1050 diode array detector and Chemstation Rev. A.06.02 data acquisition and analysis software.

Column:	USP 25 (2002) packing L1, p1056 (Luna C18-2 column, 150 x 4.6 mm, 5 µm, Phenomenex, Torrance, CA was used)
Mobile phase:	A mixture of methanol and 0.05 M monobasic potassium phosphate (60:40), adjust to a pH of 5.5
Flow rate:	1.0 ml/min
Injection volume:	10 µl
Wavelength:	UV at 247 nm

The label claim of the mebendazole chewable tablets was 100 mg for each tablet with a tablet mass of 1 gram. Therefore 250 mg of tablet powder should contain 25 mg of mebendazole. A standard solution containing 0.1 mg/ml of mebendazole was prepared and analysed. Different samples were taken out of the left, middle and right-hand sides of the batch. Samples containing 250 mg of tablet powder were transferred into a 250 ml volumetric flask, 20 ml of methanol and 5 ml of acetic acid were added. The flask was shaken for a few seconds. Methanol was added almost to volume and then sonicated for 10 minutes. After 5 minutes the solution was finally made up to 250 ml with methanol. This solution was transferred into HPLC vials and analysed. The results are shown in appendix 1.

Calculations:

$$C_{std} = \frac{\text{mass mebendazole weighed (mg)} \times \text{potency (\%)}}{250 \times 100}$$

$$\% \text{ Mebendazole} = \frac{SAR \times C_{std} \times 250 \times 255 \times 100}{STR \times EqM_{sample} \times 25}$$

SAR = sample peak area

STR = standard peak area

EqM_{sample} = powder weighed (mg) containing equivalent mass of mebendazole

4.3 Formulation

Due to the importance of using polymorph C, the prepared formulations were analysed throughout the study, to determine the stability of polymorph C in the tablets, gel and suspension. In figure 4.14 the infrared spectra of the mebendazole raw material, tested initially and after the stability test period, are shown.

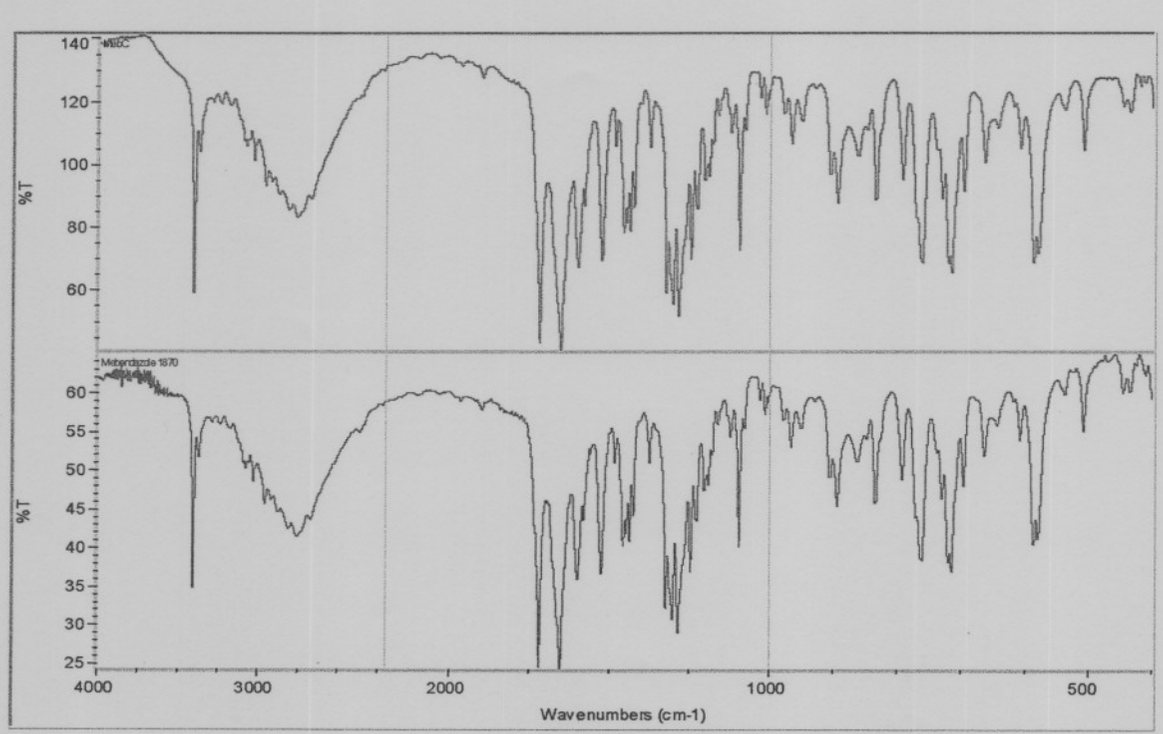


Figure 4.14 Infrared data of mebendazole 1870 initially and after one year.

According to the infrared data in figure 4.14 the –NH and the C=O stretching frequencies remain unchanged after one year. This is a confirmation of the stability of the raw material being used.

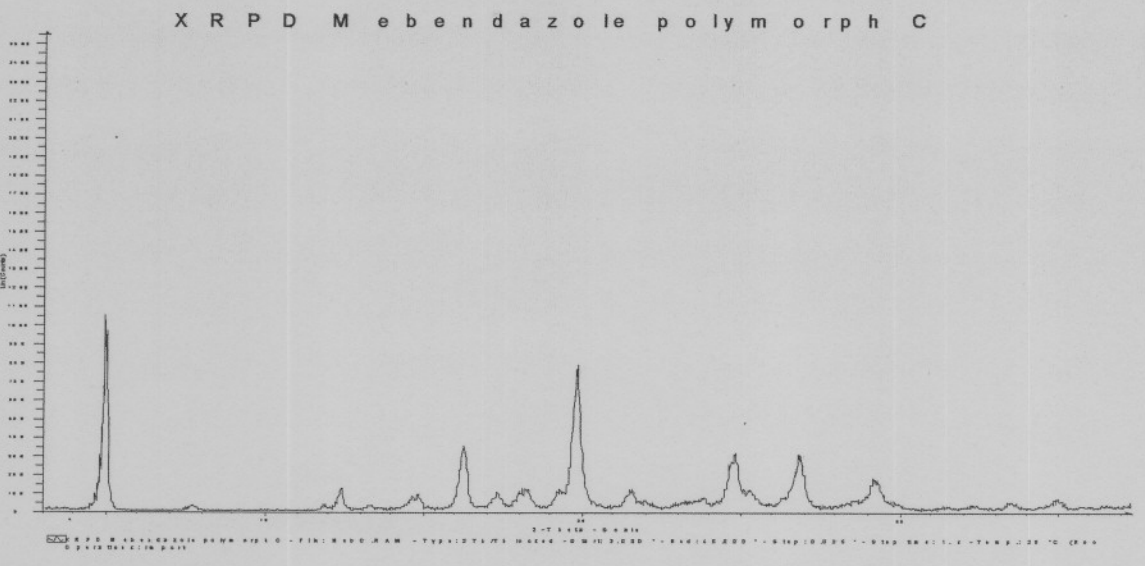


Figure 4.15 X-ray powder diffraction of mebendazole raw material, initially.

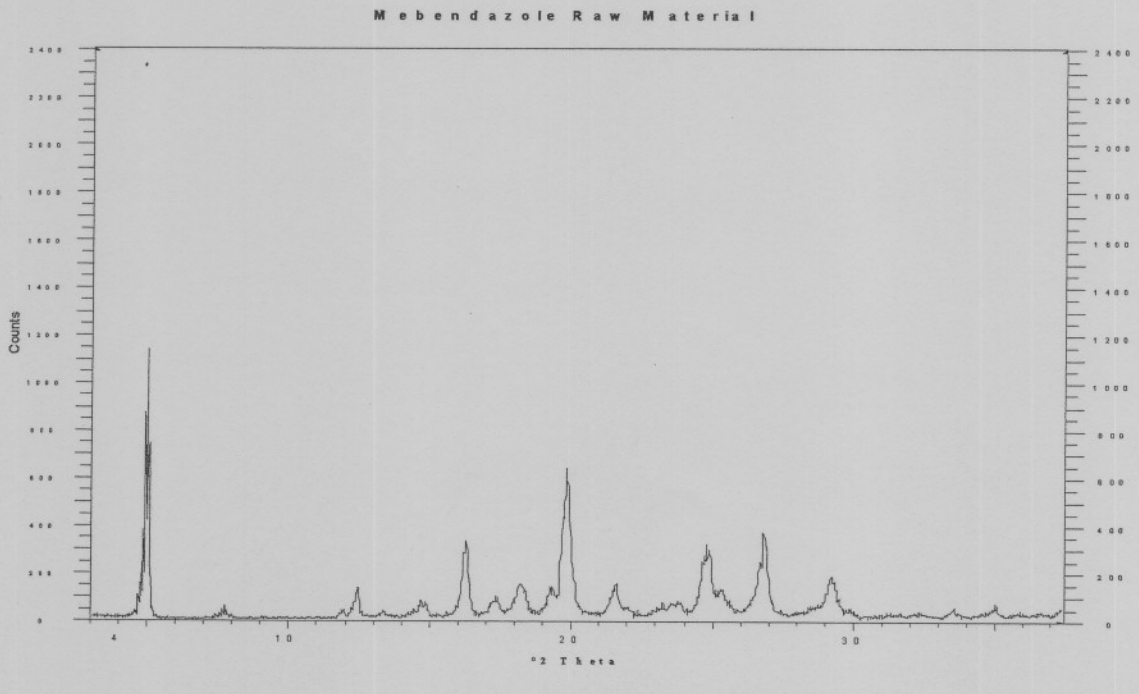


Figure 4.16 X-ray powder diffraction of mebendazole raw material, after one year.

From the XRPD data (figure 4.15 and 4.16) it was clear that mebendazole raw material were stable and unchanged after one year.

4.3.1 Chewable tablets

A tablet enables an accurate dosage of medicament to be administered simply. It is easy to transport in bulk and being carried by the patient. The tablet is a uniform final product with regard to weight and appearance, and is usually more stable than liquid preparations. Tablets can be mass produced simply and quickly and the resultant manufacturing cost is therefore much lower when compared with other dosage forms (Rubenstein, 1988:305). The purpose of this chewable tablet was to formulate a product sweet enough for the multi dosage of children in a deworming program. Palatability is a major compliance issue for infants and young children and acceptable taste is likely to minimise dosing errors resulting from spillage (Simar, 2000:812). The formula of chewable tablet A1 is shown in table 4.2.

Table 4.2 Formula of chewable tablet A1

Excipient/Active	**Function**	**Composition % (w/w)**
Mebendazole	Active	10
Dextrose monohydrate	Sweetening agent	79
Castor sugar	Sweetening agent	9
Lemon flavour	Flavourant	0.5
Yellow colourant	Colour	0.15
Magnesium stearate	Lubricant	1.25
Sodium cyclamate	Sweetening agent	0.1

Method and discussion tablet A1:

All the excipients were weighed and mixed together with a turbula mixer. Approximately 1000 tablets were manufactured at the Department of Pharmaceutics, School of Pharmacy, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, South Africa. The taste and fragrance of this formula was not acceptable. Infrared analysis of the mebendazole raw material used, confirm that polymorph A was used, known for its bad odour and taste.

Formula A2

After confirmation with IR and XRPD that mebendazole polymorph C was used, the formulation processes proceeded, formula A2 (table 4.3).

Table 4.3 Formula of chewable tablet A2

Excipient/Active	**Function**	**Composition %**
Mebendazole	Active	10
Dextrose monohydrate	Sweetening agent	77.72
Castor sugar	Sweetening agent	9.35
Lemon flavour	Flavourant	0.5
Yellow colourant	Colour	0.15
Magnesium stearate	Lubricant	1.28
Sodium cyclamate	Sweetening agent	1

Method and discussion tablet A2:

All the excipients were weighed and mixed together with a turbula mixer.

Approximately 100 tablets were manufactured at the Department of Pharmaceutics, School of Pharmacy, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, South Africa. The taste and fragrance was acceptable. Capping occurred and extra magnesium stearate was added in the next formula (A3).

Formula A3

The magnesium stearate content was increased from 12.8 mg a tablet to 15 mg a tablet, to prevent capping. The formula is given in table 4.4.

Table 4.4 Formula of chewable tablet A3

Excipient/Active	**Function**	**Composition %**
Mebendazole	Active	10
Dextrose monohydrate	Sweetening agent	77.72
Castor sugar	Sweetening agent	9.35
Lemon flavour	Flavourant	0.5
Yellow colourant	Colour	0.15
Magnesium stearate	Lubricant	1.5
Sodium cyclamate	Sweetening agent	1

Method and discussion tablet A3:

All the excipients were weighed and mixed together with a turbula mixer.
Approximately 50 tablets were manufactured at the Department of Pharmaceutics, School of Pharmacy, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, South Africa. Capping still occurred and the tablets stucked to the dies.

Formula A4

The magnesium stearate was increased from 15 mg a tablet to 17.5 mg a tablet. Aerosil was added to the formula to prevent the stickiness of the excipients to the matrix. The formula is given in table 4.5.

Table 4.5 Formula of chewable tablet A4

Excipient/Active	**Function**	**Composition %**
Mebendazole	Active ingredient	10
Dextrose monohydrate	Sweetening agent	78.65
Castor sugar	Sweetening agent	7.75
Lemon flavour	Flavourant	0.5
Yellow colourant	Colour	0.15
Magnesium stearate	Lubricant	1.75
Sodium cyclamate	Sweetening agent	1
Aerosil	Glidant	0.2

Method and discussion tablet A4:

All the excipients for 1500 tablets using formula A4 were weighed. 10.0 g of mebendazole was mixed together with 10 g of dextrose monohydrate. 20 g of mebendazole was added and mixed for 5 minutes with a mixer. 40 g of dextrose was added to this mixture and mixed for 5 minutes. The other excipients were added to this mixture into a large jug. Mix thoroughly for about thirty minutes. Approximately 1500 tablets were manufactured with direct compression, at the Department of Pharmaceutics, School of Pharmacy, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, South Africa. The chewable tablets were packed in 200 ml amber glass bottles, each containing 100 tablets. No capping and stickiness were observed with this formula. This formula was subjected to the accelerated stability test program discussed in chapter 5.

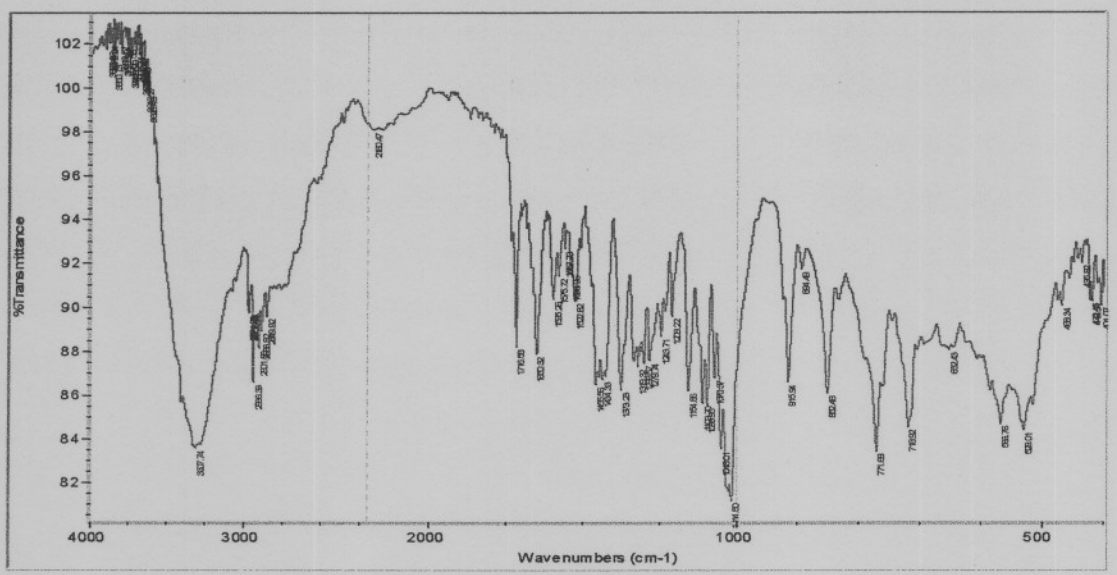


Figure 4.17 Infrared data of mebendazole chewables (formula A4) after 3 months' stability testing.

The infrared spectrum (figure 4.17) of Formula A4 showed after 3 months' stability testing (40°C + 75% RH) that the C=O stretching frequency remained stable at 1716 cm^{-1}. The stretching frequency of the –NH (amide) group shifted from 3404 cm^{-1} to 3307 cm^{-1} probably due to excipient interference, or polymorphic instability.

Formula B1

Possible incompatibilities between mebendazole and dextrose monohydrate, castor sugar and sodium cyclamate led to formula B1 (table 4.6).

Table 4.6 Formula of chewable tablet B1

Excipients/Active	**Function**	**Composition % (w/w)**
Mebendazole	Active ingredient	10
Sorbitol	Sweetener	87.97
Sodium saccharine	Sweetener	0.1
Magnesium stearate	Lubricant	1.28
Lemon flavour	Flavourant	0.5
Eurolake yellow	Colourant	0.15

Method and discussion tablet B1:

All the excipients for 1500 tablets were weighed. 10.0 g of mebendazole was mixed together with 10 g of sorbitol. 20 g of mebendazole was added and mixed thoroughly for 5 minutes. 40 g of sorbitol was added to this mixture and mixed for 5 minutes. The other excipients were added to this mixture into a large jug. Mix thoroughly for about thirty minutes with a conical mixer. Approximately 1500 tablets were manufactured with direct compression, at the Department of Pharmaceutics, School of Pharmacy, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, South Africa. This formulation was not put on stability because of an unpleasant taste and stickiness to the dies.

4.3.2 Gel

Lund, 1994:1117) defines gels as semisolids, being either suspensions of small inorganic particles or large organic molecules interpenetrated with liquid. A drug in a gel form is a convenient means for administration to pets. Dosing a dog or

cat with a liquid can be difficult, and a sudden movement of the animal often results in spillage. A gel contained in a small syringe or tube with a tapered orifice can be ejected into the mouth of the dog with relative ease, and, once in the mouth, will not readily drip out (Blodinger, 1983: 157). A gel should exhibit little viscosity change under the temperature variations of normal use and storage. A well formulated gel can provide accurate dose delivery, the dose being easily individually tailored to the animal's body weight using a dose gun or syringe (Pope & Baggot, 1983:123-132). The aim is to produce a stable, elegant, economic gel suitable for the dosing of animals like dogs, to prevent the spillage of the active mebendazole which could lead to dosing errors. The formula for gel C1 is given in table 4.7.

Table 4.7 Formula of gel C1

Excipients/Active	**Function**	**Composition % (w/v)**
Mebendazole	Active	5
Sodium hydroxide	Alkalising agent	0.15
Citric acid	Acidifying agent	0.1
Potassium sorbate	Preservative	0.1
Carbopol®	Gelling agent	0.5
Polysorbate-80	Nonionic surfactant	1.50
Propylene glycol	Solvent	2.0
Flavor	Flavourant	0.3
Distilled water ad.	Solvent/Vehicle	100

Method and discussion gel C1:

Mix citric acid, potassium sorbate, polysorbate-80 and propylene glycol in 40 ml purified water. Suspend the Carbopol® into this mixture, add the mebendazole. Dilute the sodium hydroxide with 2 ml of water. Add the sodium hydroxide

mixture into the mebendazole mixture. Add the flavourant and make up to 100% with purified water. The gel appearance was not elegant and too viscous.

Formula C2

In this formula the gelling agents were changed in an effort to improve the texture, appearance and viscosity. The formula of gel C2 is given in table 4.8.

Table 4.8 Formula of gel C2

Excipient/Active	**Function**	**Composition % (w/v)**
Mebendazole	Active	5
Hydroxypropylmethyl cellulose	Gelling agent	1.75
Glycerine	Solvent	5
Potassium sorbate	Preservative	0.1
Polysorbate	Nonionic surfuctant	1.5
Citric acid	Acidifying agent	0.1
Propylene glycol	Solvent	2
Distilled water ad.	Solvent/vehicle	100

Method and discussion gel C2:

Wet the hydroxypropylmethyl cellulose with the glycerine. Dissolve the potassium sorbate and citric acid in 80 % of distilled water. Wet the mebendazole with the polysorbate and the propylene glycol. Add the mebendazole to the potassium sorbate mixture and mix. While stirring the mixture, add the glycerine slowly to activate the gelling process. There was too much air entrapped in the gel, but the viscosity and appearance were fine.

Formula C3:

The same formula and methods were used as in table 4.8 to manufacture the final batches. To prevent the incorporation of air in the gel the mixture of glycerine and hydroxypropylmethyl cellulose was stirred very slowly into the mix.

Two liters of gel were manufactured and placed in 20 ml plastic syringes and 150 ml amber plastic bottles respectively. The stability test performed on the gel will be discussed in chapter 5.

In many cases, flavours which were pleasing for some people were rejected by others. With cherry, chocolate, mint, orange and raspberry flavours the most common; flavour problems could be solved (Schumacher, 1969:71-99). In the case of animals like dogs, a meat like flavour will be more suitable.

4.3.3 Suspension

The suspension was formulated to study the stability of mebendazole polymorph C in suspension (table 4.9).

Table 4.9 Formula of suspension

Excipient/Active	**Function**	**Composition % (w/v)**
Mebendazole	Active	2
Xanthan gum	Suspending agent	0.4
Sorbitol 70% solution	Viscosity builder	16
Propylene glycol	Solvent	3.33
Methyl parabene	Preservative	0.18
Propyl parabene	Preservative	0.02
Sodium saccharin	Sweetener	0.045
Citric acid	Acidifying agent	0.4
Sodium hydroxide	Alkalising agent	0.008
Lemon liquid	Flavourant	0.002
Distilled water ad.	Suspending medium	100

Method and discussion:

Dissolve the methyl- and propyl parabens in the propylene glycol and wet the xanthan gum mixture. Mix the sorbitol, sodium saccharin and lemon liquid

together in another beaker, and mix with the xanthan gum mixture. Suspend the mebendazole slowly into the mixture while stirring. Adjust the pH to 6.5 with citric acid or sodium hydroxide. The suspension was placed in 200 ml amber plastic bottles.

Stability data

After IR spectra were recorded, the suspension was placed in an environment of 40°C +75% RH. IR spectra were recorded after 2 weeks and after 4 weeks. In figures 4.18- 4.20 the infrared spectra of polymorph C, the initial and 4 weeks suspensions are shown.

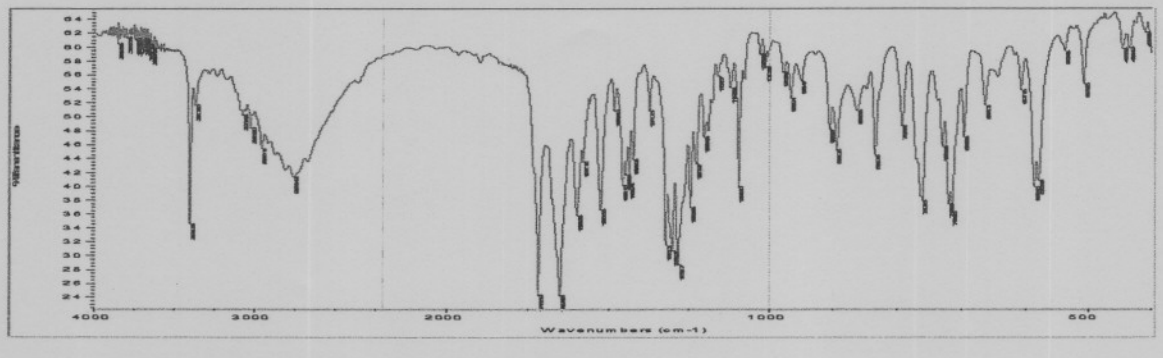


Figure 4.18 Infrared spectra of polymorph C.

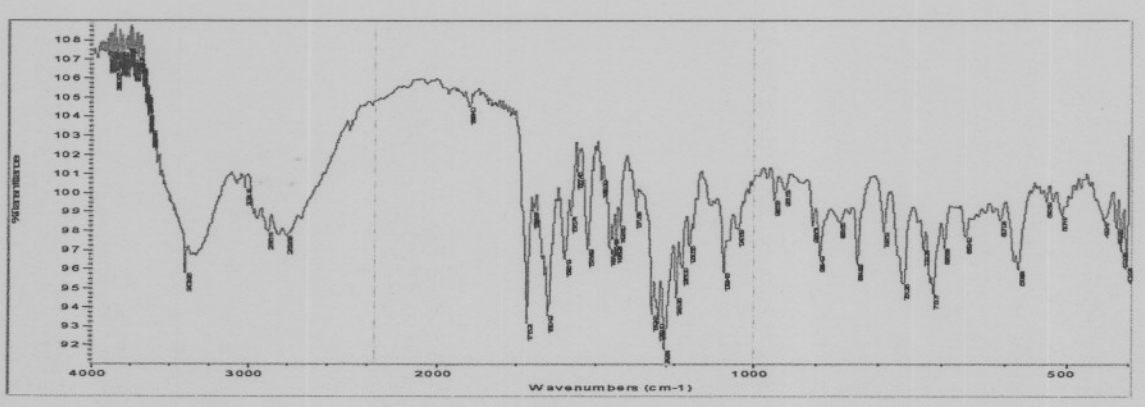


Figure 4.19 Infrared spectra of suspension initial.

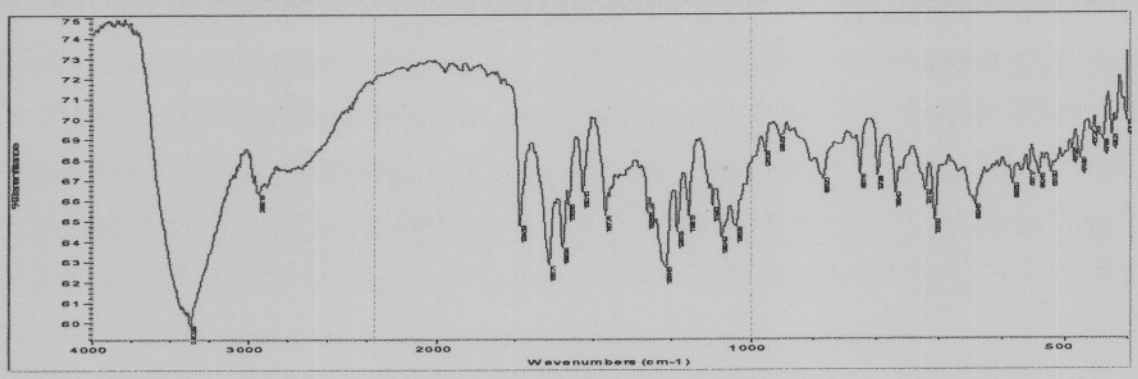


Figure 4.20 Infrared data of the suspension after 4 weeks being exposed to 40°C and 75% relative humidity.

Table 4.10 Stretching frequencies of mebendazole suspension

	-NH (amide) (cm^{-1})	> C=O (carbonyl) (cm^{-1})
Mebendazole	3404.35	1716.69
Suspension (day 1)	3403.96	1716.21
Suspension (day 30)	3370.29	1731.58

The initial spectrum (figure 4.19) of the suspension shows that polymorph C was present. The infrared data of the suspension after 4 weeks, showed that there was a polymorphic transformation from polymorph C to polymorph A. These results pose a question with regards to the stability of mebendazole polymorph C in suspension.

4.4 Mebendazole formulations and stability

Different polymorphs of mebendazole are available on the market; therefore a complete preformulation is necessary before a decision on the use of any mebendazole raw material can be made (Liebenberg *et al.*, 1998:488). According to this study it is not only important to obtain the correct polymorph for the formulation processes but also to make certain throughout the study that there was no change in the polymorphic form. All the formulations tested were subjected to higher stress conditions (40°C + 75 % RH). The suspension

formulated in this study changed from the pharmaceutically favoured form C to the more stable form A, with the lower solubility. The tablet did not show a significant change in infrared frequencies, although the change of the –NH (amide) frequency (from 3404 cm^{-1} to 3307 cm^{-1}) could be indicative of a possible polymorphic transformation. It seems that the polymorphic transformation in the suspension was much quicker than in the case of the tablets. Mebendazole is a thermal and photo-sensitive drug; it's stability can be affected by heat and exposure to light. The extent of photo-decomposition is greater in solution and is higher than thermal decomposition. Additives like sucrose, sodium carboxymethyl cellulose (CMC), povidone (PVP), sodium benzoate, sodium saccharin, keltrol and glycerol were found to decrease the rate of photodecomposition. CMC was the most effective additive (Karim *et al.*, 1996:252). More care should therefore be taken to improve the stability of mebendazole with additives like CMC to prevent photo- and thermal decomposition.

4.5 Conclusion

In this chapter the preformulation and formulation of mebendazole products were discussed. A gel for veterinary use and a chewable tablet for children were successfully formulated and subjected to stability trial studies. The stability results pose a question as to the stability of polymorph C in suspension.

Chapter 5

Stability testing methods and discussion

5.1 Introduction

In this chapter the methods used in the accelerated stability test program are described. The gel and one of the chewable tablet formulas that passed the preformulation and evaluation tests were put on stability. The program will determine the stability of the formulations over a 3 month period. The batches were stored under the following conditions 5°C, 25°C + 60% RH and 40°C + 75% RH. The equipment used for these tests were calibrated according to the requirements of the different Pharmacopoeia. All the equipment is the property of the Research Institute for Industrial Pharmacy (RIIP), Potchefstroomse Universiteit vir Christelike Hoër Onderwys.

5.2 Test methods

5.2.1 Tablets

The stability tests performed on the tablets during the 3 month testing period were the following: assays, dissolution rate, loss on drying, appearance, thickness, diameter, hardness, mass variation and friability.

5.2.1.1 Assay

This test was done with an HPLC method developed and validated at the Research Institute for Industrial Pharmacy. The validation will be discussed in appendix 2.

Chromatographic conditions:

Analytical instrument: HP 1050 series equipped with a HP 1050 quaternary pump, HP 1050 auto sampler, HP 1050 diode array detector and Chemstation Rev. A.06.02 data acquisition and analysis software.

Column:	Phenomenex Luna C18-2, 150 x 4.6 mm, 5 µm
Mobile phase:	A (40:60) mixture of 0.05 *M* monobasic potassium phosphate and methanol. Adjust with phosphoric acid or sodium hydroxide to a pH of 5.5.
Flow rate:	1.0 ml/min
Injection volume:	10 µl
Wavelength of detection:	UV at 247 nm

Sample preparation: Twenty tablets were weighed and powdered. A portion of the powdered tablets, equivalent to 25 mg of mebendazole was weighed in a weighing boat and transferred into a 250 ml volumetric flask. The weighing boat was rinsed with 20 ml of methanol; acetic acid (5 ml) was added to dissolve the mebendazole. The flask was shaked well for a few seconds. Methanol was added almost to volume and then sonicated for ten minutes. After five minutes the solution was finally made up to 250 ml with methanol. This solution was transferred into HPLC vials and analysed.

Standard preparation: A standard solution containing 0.1 mg/ml was prepared and analysed in the same way as the sample preparation, using 25 mg mebendazole.

Calculations:

$$C_{std} = \frac{\text{mass mebendazole weighed (mg) x potency (\%)}}{250 \times 100}$$

$$\% \text{ Mebendazole} = \frac{SAR \times C_{std} \times 250 \times 255 \times 100}{STR \times EqM_{sample} \times 25}$$

SAR = sample peak area

STR = standard peak area

EqM_{sample} = powder weighed (mg) containing equivalent mass of mebendazole

5.2.1.2 Dissolution rate

Dissolution rate is perhaps the most significant property of tablets. The active ingredient must be available and since drugs cannot be absorbed into the blood stream from the solid state, the active ingredient must first dissolve in the gastric or intestinal fluids before absorption can take place (Rubenstein, 1988:306). The dissolution was done according to the USP (USP 25, 2002:1055-1056) under the following conditions:

Dissolution apparatus:	Vanderkamp 600
Dissolution medium:	0.1 N hydrochloric acid + 1% sodium lauryl sulfate
Dissolution volume:	900 ml
Paddle speed:	75 rpm
Volume withdrawn:	5 ml
Sampling time:	30, 60, 90, 120 minutes
Method of analysis:	HPLC

The chewable tablet was crushed with a mortar and pestle into a powder. The powder was transferred into a test tube. Fifty millilitres of dissolution medium were taken from each of the six vessels and then vortexed. The sample was transferred back into the dissolution vessel after being vortexed for two minutes. The samples were analysed against a standard of mebendazole containing 0.11 mg/ml. Not less than 75% (Q) of the labelled amount of mebendazole must be

dissolved in 120 minutes following the method described in the USP (USP 25, 2002:1055).

Calculations:

$$C_{std} = \frac{\text{mass mebendazole weighed (mg) x potency (\%)}}{200 \times 100}$$

$$\% \text{ mebendazole released} = \frac{SAR \times 900 \times C_{std} \times 100 \times ATM}{STR \times \text{strength} \times M_{sample}}$$

SAR = sample of peak area
STR = standard peak area
ATM = average tablet mass (g)
M_{sample} = tablet mass (g)
Strength = theoretical mass of active in each tablet

5.2.1.3 Loss on drying

Twenty tablets were crushed with a mortar and pestle into a fine powder. Approximately 1 g was accurately weighed into a dry and clean beaker. The empty beaker was weighed beforehand. The powder was then dried in a vacuum oven for three hours at a temperature of 105°C. After three hours the beaker was placed in a dessicator to cool to room temperature. The beaker was weighed again.

Calculation:

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

5.2.1.4 Appearance

The physical appearance was evaluated by visual examination according to an in-house method of the RIIP (2002:SOPVEX01). Colour, shape, odour, surface and texture were some of the appearances evaluated.

5.2.1.5 Thickness, diameter and hardness

The thickness, diameter and hardness of twenty tablets were determined by using the Pharma Test PTB-311 hardness tester. According to an in-house method of the RIIP (2001:SOPHRD02).

5.2.1.6 Uniformity of mass

Twenty tablets were dusted and then weighed individually with a Sartorius balance using a method described by the British Pharmacopoeia (BP 2000 vol 2, A 231).

5.2.1.7 Friability

Twenty tablets were weighed with a Sartorius balance and then placed in the rotating drum of the Pharma Test PTF-E friabilator. The rotating time was 4 minutes and the speed 25 rpm. The evaluation was done according to a method described by an in-house method of the RIIP (2002:SOPFRB01). Only the tablets not broken were taken into consideration for calculations of the % friability. The amount of broken tablets was recorded.

5.2.2 Gel

The following stability tests were done on the mebendazole gel: assays, pH, density, viscosity, appearance and preservative efficacy.

5.2.2.1 Assays

This test was done with an HPLC method developed and validated at the Research Institute for Industrial Pharmacy.

Chromatographic conditions:

Analytical instrument: HP 1050 series equipped with a HP 1050 quaterny pump, HP 1050 auto sampler, HP 1050 diode array detector and Chemstation Rev. A.06.02 data acquisition and analysis software.

Column:	Phenomenex Luna C18-2, 150 x 4.6 mm, 5 µm
Mobile phase:	A (40:60) mixture of 0.05 *M* monobasic potassium phosphate and methanol adjust with phosphoric acid or sodium hydroxide to a pH of 5.5.
Flow rate:	1.0 ml/min
Injection volume:	10 µl
Wavelength of detection:	UV at 247 nm

Sample preparation: An amount of gel equivalent to 25 mg mebendazole was weighed into a 250 ml volumetric flask. Twenty millilitres of methanol and five millilitres of acetic acid were added to dissolve the mebendazole. The flask was shaken for a few seconds. Methanol was added almost to volume and then sonicated for ten minutes. After the solution reached room temperature it was finally made up to 250 ml with methanol. This solution was transferred into HPLC vials and analysed.

Standard preparation: A standard solution containing 0.1 mg/ml mebendazole and 0.02 mg/ml potassium sorbate were prepared. Twenty five milligram potassium sorbate was weighed and dissolved into a 500 ml volumetric flask with purified water. Ten millilitres of the potassium sorbate solution was added to the

250 ml flask with the mebendazole. The same procedure was used, as in the sample preparation, to make up the final solution.

Calculations:

$$C_{std} = \frac{\text{mass mebendazole weighed (mg) x potency (\%)}}{250 \times 100}$$

(C_{std} was calculated for mebendazole and potassium sorbate)

$$\% \text{ Mebendazole} = \frac{SAR \times C_{std} \times 250 \times 0.5 \times 100}{STR \times EqM_{sample} \times 25}$$

SAR = sample peak area

STR = standard peak area

EqM_{sample} powder weighed (mg) containing equivalent mass of mebendazole

$$\% \text{ Potassium sorbate} = \frac{SAR \times C_{std} \times 250 \times 0.5 \times 100}{STR \times EqM_{sample} \times 25}$$

SAR = sample peak area

STR = standard peak area

EqM_{sample} powder weighed (mg) containing equivalent mass of potassium sorbate

5.2.2.2 Appearance

The physical appearance was evaluated by visual examination according to an in-house method of the RIIP (2002:SOPVEX01). Physical appearance, colour, odour, taste, clarity, phase separation, consistency and foreign particles were some of the parameters evaluated.

5.2.2.3 Density

An empty flask was weighed with a Sartorius balance. Water was added to the flask to volume and it was weighed. The flask was emptied and dried in an oven. Mebendazole gel was put into the flask to volume and weighed. The relative density was calculated.

Calculation:

$$\text{Relative density} = \frac{A - B}{C - B}$$

A = Flask with the gel (g)

B = Empty container (g)

C = Flask with water (g)

5.2.2.4 pH

The pH was determined according to an in-house method of the RIIP (2002:SOPpH01) with a Metler Toledo MP220 pH meter. The measurements were taken at room temperature.

5.2.2.5 Preservative effectiveness

These are tests applied to the formulated medicine in its final container to determine whether it is adequately protected against microbial spoilage. These tests are used because it is not normally possible to predict how the activity of a preservative chemical will be influenced by the active ingredients, excipients and the container itself (Aulton, 1988:502). The Wits Health Consortium (Pty) Ltd. trading as ADCIBACT did the preservative efficacy tests. The effectiveness was done according to a method described in the USP (USP 25, 2002).

5.2.2.6 Viscosity

Viscosity tests were done with a small sample adapter attached to a manual Brookfield Model DV-II + viscometer, with spindle number LVT 4. The viscosity was determined with an in-house method of the RIIP (2002:SOPVIS05B).

5.3 Drug and product stability

Wherever possible, commercial pharmaceutical products should have a shelf life of 5 years. The potency should not fall below 90% at the recommended storage conditions and the product should still look and perform as it did when first manufactured. Drug degradation occurs by four main processes: hydrolysis, oxidation, photolysis and trace metal catalysis. Thermal effects are superimposed on all four chemical processes. A greater available free energy leads to a more rapid reaction and typically a 10°C increase in temperature produces a 2 to 5 fold increase in decay. The most likely cause of drug instability is hydrolysis. Obviously water plays a dominant role but in many cases it is implicated passively, particularly in solid dosage forms. The degradation of most drugs is catalysed by extremes of pH, i.e. high (H_3O^+) and (OH^-). Most drugs are stable between pH 4 and 8. Weakly acidic and basic drugs are most soluble when ionised and it is then that instability is most likely since the species are charged. Solvolysis, oxidation, chelating agents, photolysis and hygroscopicity are some of the other stress conditions responsible for decay (Rubenstein, 1988:243 – 246).

5.4 Conclusion

Instabilities in modern formulations are often detectable only after considerable storage periods under normal conditions. To assess the stability of a formulated product it is usual to expose it to high stress conditions to enhance its deterioration and therefore reduce the time required for testing. This enables more data to be gathered in a shorter time which, in return, will allow unsatisfactory formulations to be eliminated early within a study and will reduce the time for a successful product to reach the market. Extrapolations to 'normal' storage conditions must be made with care and the formulator must be sure that such extrapolations are valid. Common high stresses or challenges are factors like temperature, humidity and light. An increase in temperature causes an increase in the rate of chemical reaction. The products are therefore stored at temperatures greater than room temperature. Storage of products in atmospheres of high humidity will accelerate decomposition that result from hydrolysis (Aulton, 1988:124).

Chapter 6

Stability test results and discussion

6.1 Stability test program

Only the formulations that passed the evaluation and preformulation tests were entered into the accelerated stability test program, i.e. the mebendazole gel and chewable tablets formula A4 and C4 respectively. The other formulations failed due to reasons discussed in chapter 4. The methods for stability testing were described in chapter 5. Half of the chewable tablets were stored at 25°C with a relative humidity of 60% and the other half in conditions of 40°C with a relative humidity of 75%. The same was done with the mebendazole gel except an extra sample was stored at 5°C. The stability study was done over a period of three months. Initial tests were done prior to the 3 month testing, and then again on month one, two and three. Preservative efficacy tests were done initially and again after three months. Table 6.1 and 6.2 illustrate the stability program followed for the tablets and gel respectively.

Table 6.1 Stability tests conducted on chewable tablets

Stability tests on tablets	**Initial**	**1 Month**	**2 Months**	**3 Months**
	Ambient	25°C/60%RH & 40°C/75%RH	25°C/60%RH & 40°C/75%RH	25°C/60%RH & 40°C/75%RH
Appearance	X	X	X	X
Assay	X	X	X	X
Dissolution	X	X	X	X
Friability	X	X	X	X
Hardness	X	X	X	X
Mass uniformity	X	X	X	X
Moisture content	X	X	X	X

Table 6.2 Stability tests conducted on mebendazole gel

Stability tests on gel	Initial	1 Month	2 Months	3 Months
	Ambient	5°C, 25°C/60%RH & 40°C/75%RH	25°C/60%RH & 40°C/75%RH	25°C/60%RH & 40°C/75%RH
Appearance	X	X	X	X
Assays	X	X	X	X
Density	X	X	X	X
pH	X	X	X	X
Preservative efficacy	X			X
Viscosity	X	X	X	X

6.2 Tablets

6.2.1 Assay

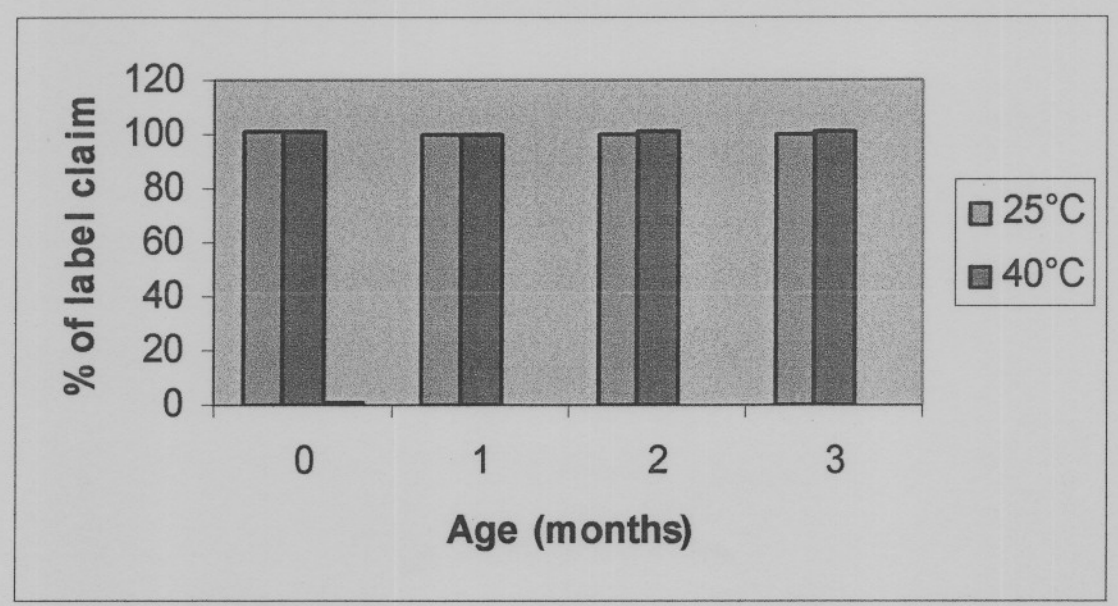


Figure 6.1 HPLC assay results of the mebendazole tablets.

The loss on drying results are given in appendix 1, tables 8.4 and 8.5 and in figure 6.4. From the results in figure 6.4 it is clear that the moisture content was constant over the three month period, i.e. between 6.9% and 8.7%.
A slight decrease in moisture content was observed for both temperature groups. After 2 months there was a decrease of ± 1.5% in the average moisture content. Unfortunately the results did not comply with the limits of 0.5% of the USP (USP 25, 2002: 1054). The tablets should be stored under silica gel.

6.2.4 Friability

The friability data are given in appendix 1, tables 8.6 and 8.7. The friability results for both temperature groups were within the limits of 2% according to the in-house method of the RIIP (2002:SOPFRB01).

6.2.5 Uniformity of mass and variation percentage

The weight variation data are given in appendix 1, tables 8.9 and 8.11. The weight variation for both storage conditions was below the limit of 5% deviation for tablets heavier than 250 mg. Not more than two of the individual tablet masses deviate more than 5% from the average tablet mass. No tablet mass deviate more from the average than 10%. The highest deviation was after 2 months in the 40°C/75%RH group with a deviation of 1.9%.

6.2.6 Hardness

The hardness results are given in appendix 1, tables 8.12 and 8.13. The initial hardness of the mebendazole tablets was measured at a value of ± 180 N. After a month the measured value was ± 230 N. The increase in hardness could be partially due to the loss of moisture.

6.3.3 pH

The pH results are given in appendix 1, table 8.16. The pH was consistent over the three month period with values varying between 3.78 and 4.99. After one month the pH values increased from 4.24 to 4.99.

6.3.4 Density

The results of the relative density are given in appendix 1 table 8.17. The density results were consistent for the entire three month stability test period.

6.3.5 Preservative efficacy

In appendix 1, table 8.19 the results from the preservative tests are included. All the samples tested complied with the requirements of the USP 25.

6.3.6 Viscosity

The viscosity results are given in appendix 1, table 8.18. The viscosity's of all the samples at the various storage conditions were determined at a rotating speed of 60 rpm. The initial viscosity of the gel was 2839 cP. The viscosity after one month in the 5°C climate room increased from 2839 cP to 4409 cP and the viscosity in the 25°C/60%RH and 40°C/75%RH rooms increased from 2839 cP to 5009 cP and 4479 cP respectively. Thereafter the values stayed constant for month two and three. The increase in viscosity could be due to the loss of air, which was included during the formulation process.

6.4 Conclusion

With the exception of the moisture content, the chewable mebendazole tablets and gel passed the accelerated stability tests. To overcome the moisture content problem, the tablets could be stored under silica gel. Mebendazole tablets, when kept at 25°C and 60% relative humidity and 40°C and 75% relative humidity remained stable over the three month period tested, because the mebendazole content ranged from 100.9 to 100.1% and 100.5 to 101.1% respectively. The assay results for the mebendazole gel was stable for the three months tested, at 5°C, 25°C/60%RH and 40°C/75%RH. It seemed that the potassium sorbate content decreased from ± 100% (initial) to ± 87% after three months in the 40°C/75%RH temperature room. Despite the lower values the potassium sorbate was still effective as preservative. The dissolution results for both temperature groups were within the USP specifications and there was no significant dissolution variation between the three months and storage conditions of the accelerated stability test program. The appearance, hardness, friability and mass variation of the tablets were excellent. From the accelerated stability tests it can be concluded that both products are stable and that there were no significant breakdown of the active ingredient, mebendazole.

Chapter 7

Summary and conclusion

Parasitic helminths affect almost everyone around the world; they are never beneficial and can sometimes produce severe and even fatal diseases. The prevention, controlling and treatment of parasitic infections are of great importance to all human beings. The controlling and correct treatment of parasitic worms with anthelmintics like menbendazole could be valuable in this difficult task.

Mebendazole polymorph C is apparently pharmaceutically favoured. It is therefore important to make sure that polymorph C is used in mebendazole formulations. Mebendazole is poorly soluble in water which benefits the action against gastro-intestinal helminths. However, the low solubility leads to low bioavailability for systemic diseases like hydatid. Further studies to improve the solubility of mebendazole to get better bioavailibilty against systemic diseases should be investigated.

Manufacturers should take care when buying mebendazole raw material. This is important since all three polymorphic forms of mebendazole are available on the market. Care should also be taken when using IR spectral data alone to identify mebendazole polymorphic forms since a mixture of these forms could exhibit an IR spectrum comparable to either of the components of the mixture. XRPD and DSC analysis should be used in combination with IR analysis to identify the polymorphic form.

Preformulation studies were done to determine any possible incompatibilities between excipients used in the formulations. A gel for veterinary use and a chewable tablet for children were formulated and subjected to stability trial studies.

With the exception of the moisture content, the chewable mebendazole tablets and gel passed the accelerated stability tests. Mebendazole tablets were kept in rooms with 25°C and 60% relative humidity and 40°C and 75% relative humidity. It remained stable over the three month stability period. The mebendazole gel were kept in rooms with an environment of 5°C, 25°C and 60% relative humidity and 40°C and 75% relative humidity. The mebendazole in the gel remained stable over the three month stability period. It seemed that the potassium sorbate content decreased from ± 100% (initial) to ± 87% after the three months stability period. The preservative efficacy results showed that the potassium sorbate was still effective as preservative. From the accelerated stability test results it can be concluded that both the tablets and the gel are stable and that there were no significant breakdown of the mebendazole.

The tasty mebendazole tablets and gel could help in the controlling and treatment of parasitic helminthes in children and dogs; two of the main sources of parasites. The flavoured tablets present an attractive chewable alternative for children who have not yet learned to wash tablets down with water.

What distinguishes this study is that the first successful chewable tablets have been developed. The significance of such a formulation lies in the contribution that is made towards children in developing countries, where water is often inadequate for human consumption.

Chapter 8

Appendix 1

Accelerated stability test results

8.1 Tablets

Table 8.1 HPLC assay results of tablets

Age	% of label claim	
	25°C/60%RH	40°C/75%RH
Initial	100.9	100.9
1 month	100.4	100.5
2 months	100.5	101.1
3 months	100.1	100.9

Table 8.2 Dissolution rate results (%) of tablets (25°C/60%RH)

Tablet	Initial			
	30 minutes	60 minutes	90 minutes	120 minutes
1	94.73	96.43	97.37	97.90
2	93.54	95.24	96.19	96.88
3	95.74	97.10	97.82	98.60
4	93.93	95.51	98.98	99.72
5	93.83	95.59	98.64	99.82
6	95.36	96.79	96.47	101.09
Average	94.52	96.11	97.58	99.00
	1 Month			
Tablet	30 minutes	60 minutes	90 minutes	120 minutes
1	90.66	92.03	95.43	97.14
2	91.61	93.75	95.49	96.28
3	93.48	95.29	97.21	97.87
4	91.33	92.98	94.20	94.65
5	92.09	93.65	94.83	95.51
6	94.22	96.03	97.53	97.67
Average	92.23	93.95	95.78	96.52
	2 Months			
Tablet	30 minutes	60 minutes	90 minutes	120 minutes
1	94.88	98.17	99.78	100.14
2	91.36	96.44	98.12	99.38
3	94.55	97.65	98.50	99.38
4	91.38	92.74	94.60	97.82
5	94.06	95.43	97.48	99.81
6	94.53	97.50	98.32	98.74
Average	93.46	96.32	97.80	99.21
	3 Months			
Tablet	30 minutes	60 minutes	90 minutes	120 minutes
1	91.63	93.90	94.56	96.86
2	93.31	94.32	96.06	97.62
3	92.75	95.65	96.25	99.03
4	91.12	93.30	93.81	97.64
5	90.87	92.56	93.90	95.76
6	92.70	95.32	97.94	101.13
Average	92.06	94.17	95.42	98.01

Table 8.3 Dissolution rate results % of tablets (40°C/75%RH)

Tablet	Initial			
	30 minutes	60 minutes	90 minutes	120 minutes
1	94.73	96.43	97.37	97.90
2	93.54	95.24	96.19	96.88
3	95.74	97.10	97.82	98.60
4	93.93	95.51	98.98	99.72
5	93.83	95.59	98.64	99.82
6	95.36	96.79	96.47	101.09
Average	94.52	96.11	97.58	99.00
	1 Month			
Tablet	30 minutes	60 minutes	90 minutes	120 minutes
1	91.45	93.68	94.30	94.25
2	92.26	94.66	95.23	95.82
3	91.86	94.91	95.83	96.45
4	96.23	98.15	99.19	99.85
5	92.12	95.32	96.51	97.21
6	96.42	99.15	99.88	100.86
Average	93.39	95.98	96.82	97.41
	2 Months			
Tablet	30 minutes	60 minutes	90 minutes	120 minutes
1	94.72	96.05	97.71	97.98
2	92.52	95.10	97.74	98.34
3	93.16	95.18	96.73	98.19
4	93.14	95.83	98.87	99.58
5	93.65	95.79	97.81	98.23
6	93.29	96.03	97.23	99.11
Average	93.41	95.66	97.68	98.57
	3 Months			
Tablet	30 minutes	60 minutes	90 minutes	120 minutes
1	89.81	92.25	92.80	94.34
2	92.02	92.89	93.27	96.33
3	92.28	95.79	96.45	98.97
4	91.41	94.68	95.20	98.15
5	93.81	93.99	97.12	98.60
6	91.11	93.75	96.15	97.93
Average	91.74	93.86	95.16	97.39

Table 8.4 Loss on drying of tablets (40°C/75%RH)

Initial	Sample 1	Sample 2
Empty container mass (g)	33.25	33.079
Sample mass$_{before}$(g)	1.0038	1.0396
Container + Sample mass$_{after}$ (g)	34.1666	34.0311
Sample mass$_{after}$ (g)	0.9166	0.9521
% Moisture content	8.6834	8.4185
Average % moisture	**8.5509**	
1 Month		
Empty container mass (g)	58.916	45.43
Sample mass$_{before}$(g)	1.0022	1.0524
Container + Sample mass$_{after}$ (g)	59.83	46.395
Sample mass$_{after}$ (g)	0.914	0.965
% Moisture content	8.8006	8.3048
Average % moisture	**8.5527**	
2 Months		
Empty container mass (g)	31.1199	35.3539
Sample mass$_{before}$(g)	1.0058	1.0018
Container + Sample mass$_{after}$ (g)	32.0557	36.2852
Sample mass$_{after}$ (g)	0.9358	0.9313
% Moisture content	6.9596	7.0373
Average % moisture	**6.9985**	
3 Months		
Empty container mass (g)	31.1190	35.3529
Sample mass$_{before}$(g)	1.0023	1.0047
Container + Sample mass$_{after}$ (g)	32.0501	36.2860
Sample mass$_{after}$ (g)	0.9311	0.9331
% Moisture content	7.1075	7.1285
Average % moisture	**7.1180**	

Table 8.5 Loss on drying of tablets (40°C/75%RH)

Initial	Sample 1	Sample 2
Empty container mass (g)	33.25	33.079
Sample mass$_{before}$(g)	1.0038	1.0396
Container + Sample mass$_{after}$ (g)	34.1666	34.0311
Sample mass$_{after}$ (g)	0.9166	0.9521
% Moisture content	8.6834	8.4185
Average % moisture	**8.5509**	
1 Month		
Empty container mass (g)	56.68	53.844
Sample mass$_{before}$(g)	1.0344	1.0211
Container + Sample mass$_{after}$ (g)	57.6240	54.7750
Sample mass$_{after}$ (g)	0.9440	0.9310
% Moisture content	8.7394	8.8238
Average % moisture	**8.7816**	
2 Months		
Empty container mass (g)	33.6393	33.2524
Sample mass$_{before}$(g)	1.0061	1.0465
Container + Sample mass$_{after}$ (g)	34.5758	34.2255
Sample mass$_{after}$ (g)	0.9365	0.9731
% Moisture content	6.9178	7.0139
Average % moisture	**6.9658**	
3 Months		
Empty container mass (g)	33.6387	33.2514
Sample mass$_{before}$(g)	1.0423	1.0214
Container + Sample mass$_{after}$ (g)	34.6049	34.2006
Sample mass$_{after}$ (g)	0.9662	0.9492
% Moisture content	7.2965	7.0689
Average % moisture	**7.1827**	

Table 8.6 Friability results of tablets (25°C/60%RH)

25 RH	Initial	1 Month	2 Months	3 Months
Sample mass$_{before}$ (g)	20.7030	18.6994	20.5986	19.6663
Sample mass$_{after}$ (g)	20.6090	18.6202	20.4151	19.6059
Mass loss (g)	0.0940	0.0792	0.1835	0.0604
% Friability	0.4540	0.4237	0.8908	0.307

Table 8.7 Friability results of tablets (40°C/75%RH)

40 RH	Initial	1 Month	2 Months	3 Months
Sample mass$_{before}$ (g)	20.7030	20.5822	20.5870	20.6223
Sample mass$_{after}$ (g)	20.6090	20.4494	20.4155	20.4899
Mass loss (g)	0.0940	0.1328	0.1715	0.1324
% Friability	0.4540	0.645	0.8330	0.642

Table 8.8 Mass uniformity results of tablets (25°C/60%RH)

	Mass uniformity results			
Tablet	**Initial**	**1 Month**	**2 Months**	**3 Months**
1	1.0150	1.0277	1.0507	1.0141
2	1.0520	1.0038	1.0264	1.0306
3	1.0440	1.0104	1.0339	1.0082
4	1.0590	1.0389	1.0449	1.0318
5	1.0830	1.0334	1.0368	1.0108
6	1.0390	1.0193	1.0322	1.0198
7	1.0420	1.0231	1.0496	1.0077
8	1.0760	1.0562	1.0233	1.0163
9	1.0390	1.0314	1.0152	1.0347
10	1.0780	1.0378	1.0709	1.0273
11	1.0440	1.0215	1.0219	1.0683
12	1.0880	1.0808	1.0508	1.0457
13	1.0160	1.0529	1.0076	1.0171
14	1.0050	1.0215	1.0199	1.0468
15	1.0370	1.0149	1.0445	1.0152
16	1.0280	1.0859	1.0457	1.0231
17	1.0330	1.0343	1.0591	1.0425
18	1.0160	1.0335	1.0669	1.0351
19	1.0360	1.0617	1.0236	1.0712
20	1.0160	1.0166	1.0473	1.0485
Average	**1.0423**	**1.0353**	**1.0386**	**1.03074**

Table 8.9 Weight variation results of tablets (25°C/60%RH)

	Weight variation (%)			
Tablet	**Initial**	**1 Month**	**2 Months**	**3 Months**
1	2.6192	0.7322	1.1689	1.6144
2	0.9306	3.0407	1.1709	0.0136
3	0.1631	2.4032	0.4487	2.1868
4	1.6022	0.3497	0.6105	0.1028
5	3.9048	0.1816	0.1695	1.9345
6	0.3166	1.5435	0.6124	1.0614
7	0.0288	1.1765	1.0630	2.2353
8	3.2332	2.0207	1.4693	1.4009
9	0.3166	0.3748	2.2493	0.3842
10	3.4251	0.2434	3.1139	0.3337
11	0.1631	1.3310	1.6041	3.6440
12	4.3845	4.3969	1.1786	1.4514
13	2.5233	1.7020	2.9811	1.3233
14	3.5786	1.3310	1.7967	1.5581
15	0.5085	1.9685	0.5719	1.5077
16	1.3720	4.8895	06875	0.7412
17	0.8932	0.0947	1.9777	1.1409
18	2.5233	0.1719	2.7288	0.4230
19	0.6044	2.5520	1.4405	3.9253
20	2.5233	1.8043	0.8415	1.7230
Average	**1.7807**	**1.6154**	**1.3942**	**1.4353**

Table 8.10 Mass uniformity results of tablets (40°C/75%RH)

	Mass uniformity results			
Tablet	**Initial**	**1 Month**	**2 Months**	**3 Months**
1	1.0150	1.0173	1.0419	1.0300
2	1.0520	1.0013	1.0954	1.0407
3	1.0440	1.0110	1.0420	1.0133
4	1.0590	1.0500	1.0464	1.0173
5	1.0830	1.0525	1.0443	1.0557
6	1.0390	1.0017	1.0031	1.0068
7	1.0420	1.0171	1.0878	1.0443
8	1.0760	1.0500	1.0257	1.0270
9	1.0390	1.0242	1.0197	1.0484
10	1.0780	1.0205	1.0678	1.0142
11	1.0440	1.0379	1.0181	1.0122
12	1.0880	1.0426	1.0219	1.0379
13	1.0160	1.0213	1.0524	1.0878
14	1.0050	1.0200	1.0112	1.0454
15	1.0370	1.0535	1.0296	1.0019
16	1.0280	1.0538	1.0322	1.0748
17	1.0330	1.0074	1.0170	1.0152
18	1.0160	1.0184	1.0483	1.0337
19	1.0360	1.0157	1.0835	1.0135
20	1.0160	1.0213	1.0514	1.0508
Average	**1.0423**	**1.0269**	**1.0420**	**1.03355**

Table 8.11 Weight variation results of tablets (40°C/75%RH)

	Weight variation (%)			
Tablet	**Initial**	**1 Month**	**2 Months**	**3 Months**
1	2.6192	0.9324	0.0082	0.3430
2	0.9306	2.4906	5.1263	0.6923
3	0.1631	1.5460	0.0014	1.9588
4	1.6022	2.2520	0.4237	1.5718
5	3.9048	2.4954	0.2222	2.1436
6	0.3166	2.4516	3.7318	2.5877
7	0.0288	0.9519	4.3969	1.0406
8	3.2332	2.2520	1.5629	0.6333
9	0.3166	0.2605	2.1387	1.4373
10	3.4251	0.6208	2.4775	1.8717
11	0.1631	1.0736	2.2923	2.0652
12	4.3845	1.5313	1.9276	0.4214
13	2.5233	0.5429	0.9995	5.2494
14	3.5786	0.6695	2.9545	1.1470
15	0.5085	2.5928	1.1886	3.0618
16	1.3720	2.6220	0.9391	3.9916
17	0.8932	1.8965	2.3978	1.7750
18	2.5233	0.8253	0.6061	0.0150
19	0.6044	1.0883	3.9842	1.9394
20	2.5233	0.5429	0.9036	1.6695
Average	**1.7807**	**1.4819**	**1.9141**	**1.7808**

Table 8.12 Hardness results of tablets (25°C/60%RH)

	Hardness (N)			
Tablet	**Initial**	**1 Month**	**2 Months**	**3 Months**
1	165	240.9	258.7	182.5
2	189.6	251.5	201.6	237.1
3	197.8	203.7	169.1	274
4	211.9	234.4	221.8	264.8
5	181.8	249.4	219.4	213.2
6	198.5	231.7	200.6	220.4
7	192.7	237.1	260.4	249.1
8	124.4	248.4	215.6	191.7
9	142.8	172.6	203.3	205.7
10	200.2	289.1	277.1	239.9
11	210.3	254.2	228.3	268.9
12	214.3	277.5	253.9	278.8
13	198.0	220.1	195.8	250.8
14	160.3	234.1	218.7	253.5
15	142.5	244.0	281.9	230.0
16	159.8	276.4	246.4	253.5
17	160.8	218.7	247.7	223.1
18	200.8	250.1	247.0	253.9
19	210.9	257.3	219.0	194.8
20	205.0	238.2	160.6	232.7
Average	**183.37**	**241.47**	**226.345**	**235.92**

Table 8.13 Hardness results of tablets (40°C/75%RH)

	Hardness (N)			
Tablet	**Initial**	**1 Month**	**2 Months**	**3 Months**
1	165	225.9	204.3	218.3
2	189.6	218.3	286.7	226.5
3	197.8	224.8	283.3	208.1
4	211.9	240.6	256.6	238.8
5	181.8	231.3	224.5	212.2
6	198.5	218.7	209.8	212.2
7	192.7	215.3	241.2	247.4
8	124.4	265.2	235.1	152.4
9	142.8	219.0	221.4	264.5
10	200.2	249.8	284.6	249.8
11	210.3	267.2	237.8	239.9
12	214.3	198.9	255.6	261.7
13	198.0	227.6	272.7	291.8
14	160.3	175.3	254.2	281.2
15	142.5	251.8	253.5	240.9
16	159.8	217.3	275.1	279.2
17	160.8	195.1	236.1	221.1
18	200.8	227.9	229.6	246.7
19	210.9	253.2	281.6	188.3
20	205.0	220.7	276.1	265.2
Average	**183.37**	**227.195**	**250.99**	**237.31**

8.2 Gel

Table 8.14 HPLC assay results of gel at 25°C/60%RH and 40°C/75%RH

Age	% of label claim			
	Mebendazole		Potassium sorbate	
	25 RH	40 RH	25 RH	40 RH
Initial	98.0	98.0	100.56	100.56
1 Months	94.87	97.57	97.23	96.12
2 Months	93.83	101.48	92.31	91.80
3 Months	97.49	94.30	87.48	87.10

Table 8.15 HPLC assay results of gel at 5°C

Age	% of label claim	
	Mebendazole	Potassium sorbate
	5°C	5°C
Initial	98.0	100.56
1 Month	96.85	99.37

Table 8.16 pH results of gel

Age	pH		
	5°C	25°C/60%RH	40°C/75%RH
Initial	4.24	4.24	4.24
1 Month	4.94	4.99	4.97
2 Months		3.82	3.78
3 Months		4.04	4.03

Table 8.17 Relative density results of gel

	Relative density (g/cm^3)		
Age	**5°C**	**25°C/60%RH**	**40°C/75%RH**
Initial	1.0300	1.0030	1.0300
1 Month	1.0111	1.0129	1.0380
2 Months		1.0267	1.0313
3 Months		1.0144	1.0149

Table 8.18 Viscosity results of gel

	Viscosity (cP)		
Age	**5°C**	**25°C/60%RH**	**40°C/75%RH**
Initial	2839	2839	2839
1 Month	4409	5009	4479
2 Months		4659	5318
3 Months		4759	4409

Table 8.19 Preservative efficacy results of gel

Initial					
Test organism	**Initial inoculum**	**Log Unit reduction**			**Specified limits for Category 3 products**
		Day 7	**Day 14**	**Day 28**	
E.coli	6.5×10^5	> 3.0	> 3.0	> 3.0	> 1.0 log reduction at 14 days & no increase at 28 days
P.aeruginosa	1.0×10^6	> 3.0	> 3.0	> 3.0	
S.aureus	8.0×10^5	> 3.0	> 3.0	> 3.0	
A. niger	3.7×10^5	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	3.9×10^5	> 3.0	> 3.0	> 3.0	
25°C/60%RH 3 Months					
Test organism	**Initial inoculum**	**Log Unit reduction**			**Specified limits for Category 3 products**
		Day 7	**Day 14**	**Day 28**	
E.coli	5.0×10^5	> 3.0	> 3.0	> 3.0	> 1.0 log reduction at 14 days & no increase at 28 days
P.aeruginosa	8.5×10^5	> 3.0	> 3.0	> 3.0	
S.aureus	1.0×10^6	> 3.0	> 3.0	> 3.0	
A. niger	2.7×10^5	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	1.5×10^5	2.3	> 3.0	> 3.0	
40°C/75%RH 3 Months					
Test organism	**Initial inoculum**	**Log Unit reduction**			**Specified limits for Category 3 products**
		Day 7	**Day 14**	**Day 28**	
E.coli	5.0×10^5	> 3.0	> 3.0	> 3.0	> 1.0 log reduction at 14 days & no increase at 28 days
P.aeruginosa	8.5×10^5	> 3.0	> 3.0	> 3.0	
S.aureus	1.0×10^6	> 3.0	> 3.0	> 3.0	
A. niger	2.7×10^5	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	1.5×10^5	2.2	> 3.0	> 3.0	

8.3 Content uniformity results

Table 8.20 Content uniformity of mebedazole chewable tablets

Sample positions	Percentage of mebendazole
Top	101.32
Middle	99.65
Bottom	98.97

Chapter 9

APPENDIX 2

Validation of an HPLC assay for the simultaneous determination of potassium sorbate and mebendazole in a gel

9.1 ORIGIN OF METHOD

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

9.2 CHROMATOGRAPHIC CONDITIONS

Analytical instrument: HP1050 series HPLC equipped with a HP1050 quaternary gradient pump, HP1050 auto sampler, HP1050 diode array detector and Chemstation Rev. A.06.02 data acquisition and analysis software.

Column:	USP 25 (2002) packing L1, p1056 (Luna C18-2 column, 150 x 4.6 mm, 5 µm, Phenomenex, Torrance, CA was used)
Mobile phase:	A mixture of methanol and 0.05 M monobasic potassium phosphate (60:40), adjust to a pH of 5.5
Flow rate:	1.0 ml/min
Injection volume:	10 µl
Detection:	UV at 247 nm
Retention time:	Potassium sorbate - ±3.2 minutes Mebendazole - ± 6.2 minutes

9.3 SAMPLE PREPARATION

1. Accurately weigh 0.5 g of gel into a 250 ml volumetric flask.
2. Add 20 ml of methanol and shake.
3. Add 5 ml of acetic acid and shake.
4. Add methanol almost to volume and sonicate for 10 minutes.
5. Allow cooling to room temperature and make up to volume with methanol.
6. Transfer the sample into an auto sampler vial with a Pasteur pipette and analyse.

9.4 STANDARD PREPARATION

1. Weigh approximately 25 mg of mebendazole accurately.
2. Transfer into a 250 ml volumetric flask and rinse weighing boat with 20 ml of methanol.
3. Add 5 ml of acetic acid and shake.
4. Weigh 25 mg of potassium sorbate and transfer into a 500 ml volumetric flask.
5. Fill the flask with water for HPLC and sonicate for 5 minutes.
6. Transfer 10 ml from the 500 ml flask to the 250 ml volumetric flask.
7. Fill the 250 ml flask with methanol almost to volume and sonicate for 10 minutes.
8. Allow cooling to room temperature and make up to volume.
9. Transfer with a Pasteur pipette into an auto sampler vial and analyse.

9.5 CALCULATIONS

9.5.1 Potassium sorbate

The gel concentrate contains 0.1 g of potassium sorbate per 100 ml of gel. Therefore 0.5 ml of gel contains 0.5 mg of potassium sorbate. If 0.5 ml of gel is dissolved in a 250 ml volumetric flask, the final concentration will be 2 µg/ml.

$$\textbf{Cstd}\ (\text{mg/ml}) = \frac{\text{standard mass weighed} \times \text{standard potency} \times 10}{500 \times 250 \times 100}$$

$$\textbf{µg/ml}\ \text{in gel} = \frac{\text{sample area} \times \text{Cstd} \times 1000}{\text{standard area}}$$

$$\textbf{\%}\ \text{in gel} = \frac{\text{µg/ml in gel} \times 100 \times \text{theoretical mass}}{2 \times \text{sample mass}}$$

9.5.2 Mebendazole

The gel contains 5 g of mebendazole per 100 ml of gel. Thus 0.5 ml of gel contains 25 mg of mebendazole. If 0.5 ml of gel is dissolved in 250 ml, the final concentration will be 100µg/ml.

Cstd (mg/ml) = $\frac{\text{standard mass weighed x standard potency}}{250 \times 100}$

µg/ml in gel = $\frac{\text{sample area x C std x 1000}}{\text{standard area}}$

% in gel = $\frac{\text{µg/ml in gel x 100 x theoretical mass}}{2 \times \text{sample mass}}$

9.6 SYSTEM SUITABILITY PARAMETERS

Analyse six replicate injections of a standard solution.
Calculate the relative standard deviation of the peak areas obtained for mebendazole.
Calculate the number of theoretical plates for mebendazole.
Calculate the USP tailing factor for mebendazole.

The system is suitable to perform the analysis if the following criteria are met:
1) RSD not more than 1.0%
2) Number of theoretical plates more then 3500 per column.
3) USP tailing factor not more than 1.5.

9.7 SUMMARY OF VALIDATION RESULTS

The validation of the method for the simultaneous determination of potassium sorbate and mebendazole in gel yielded the following results:

Test	Result
Specificity	Complies
Range	Mebendazole: 50 – 120 µg/ml Potassium sorbate: 0.5 – 1.2 µg/ml
Linearity	Mebendazole: R2 = 0.99998 Potassium sorbate = 0.9997
Accuracy	Mebendazole: 99.9% Potassium sorbate: 99.5%
Precision	Mebendazole: RSD < 2% Potassium sorbate: RSD < 2%
Ruggedness	Complies
Robustness	Complies

CONCLUSION:

The method complied with all the acceptance criteria and should be suitable for the simultaneous determination of potassium sorbate and mebendazole in gel for stability testing, identification and batch release.

9.8 VALIDATION RESULTS

9.8.1 Specificity

The chromatogram of a placebo sample analysed at 247 nm is given in figure 9.1. The chromatogram of a placebo analysed at 247 nm is given in Figure 1. A standard solution was analysed at 247 nm before exposing it to a temperature of 40 °C; this chromatogram is given in Figure 9.2. Standard solutions that were stressed at 40°C in water, 1 M NaOH, 1 M HCl and hydrogen peroxide were analysed at 247 nm. The chromatograms are given in figures 9.3 to 9.6.

The optimum wavelength for the detection of mebendazole and potassium sorbate is 247 nm.

Results and discussion:

The placebo sample exhibited no peaks that will interfere with the detection of both potassium sorbate and mebendazole.

Forced degradation of both potassium sorbate and mebendazole caused the appearance of extra peaks. The residual peaks of both potassium sorbate and mebendazole were examined by means of diode array peak purity testing and were found to be pure. This implies that none of the degradates formed from potassium sorbate and mebendazole interferes with the simultaneous detection of potassium sorbate and mebendazole.

9.8.2 Linearity and range

9.8.2.1 Mebendazole

The peak area and concentration found for mebendazole are shown in table 9.1

Table 9.1 Peak area and concentration found for mebendazole

Concentration (µg/ml)	Peak area		
	1	2	Mean
50.3	2599	2602	2601
60.4	3118	3113	3116
70.4	3640	3627	3634
80.5	4143	4146	4145
90.5	4642	4651	4647
100.6	5157	5169	5163
110.7	5687	5689	5688
120.7	6179	6188	6184

Table 9.4 Regression parameters for potassiumsorbate

REGRESSION PARAMETERS			
R Squared	0.9997	**Lower 95%**	**Upper 95%**
Intercept	-0.32	-1.60	0.95
Slope	84.87	83.44	86.31

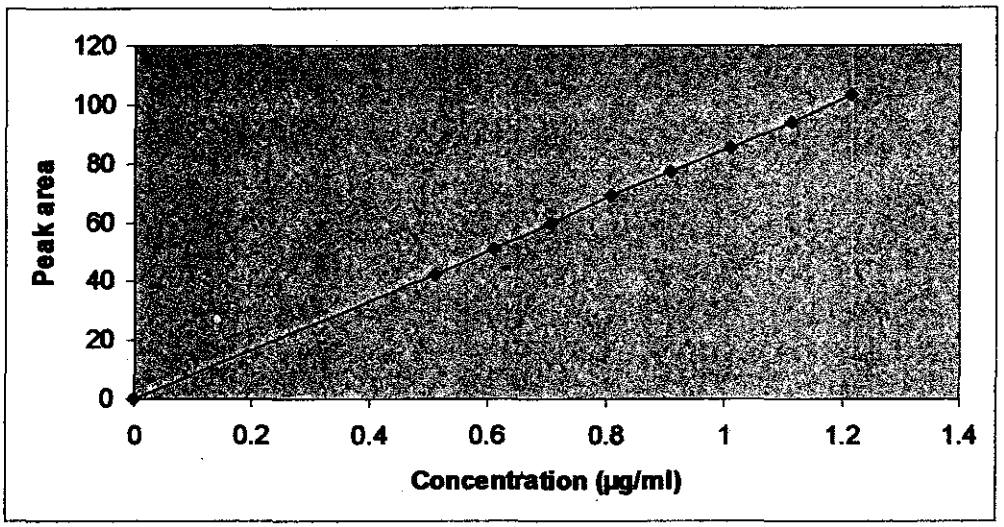


Figure 9.8 Linear regression curve for potassium sorbate.

Conclusion:

The method is linear over the concentration range of 50 – 100 µg/ml for mebendazole and 0.5 – 1.2 µg/ml for potassium sorbate. The method meets the acceptance criteria and is suitable for fixed point calibration.

9.8.3 Accuracy

9.8.3.1 Mebendazole

The percentage mebendazole recovered and the confidence intervals are shown in tables 9.5 and 9.6.

Table 9.5 Percentage mebendazole recovered

Conc. spiked (µg/ml)	Conc. found (µg/ml)	Recovered (%)
79.98	80.3	100.4
79.98	79.8	99.8
79.98	79.9	99.9
99.98	99.8	99.8
99.98	99.9	99.9
99.98	99.7	99.7
119.98	120.4	100.4
119.98	119.6	99.7
119.98	119.5	99.6
Mean		99.9
SD		0.3
% RSD		0.3

Table 9.6 Confidence intervals for mebendazole

95% CONFIDENCE INTERVALS:	
Lower limit	99.7
Upper limit	100.1
Estimated median	99.8

9.8.3.2 Potassium sorbate

The percentage potassium sorbate recovered and the confidence intervals are shown in tables 9.7 and 9.8.

Table 9.7 Percentage potassium sorbate recovered

Conc. spiked (µg/ml)	Conc. found (µg/ml)	Recovered (%)
1.6	1.6	98.0
1.6	1.6	98.7
1.6	1.6	100.4
2	2.0	98.3
2	2.1	102.9
2	2.1	103.8
2.4	2.4	98.2
2.4	2.4	98.4
2.4	2.3	97.1
Mean		99.5
SD		2.2
% RSD		2.2

Table 9.8 Confidence intervals for potassium sorbate

95% CONFIDENCE INTERVALS:	
Lower limit	97.7
Upper limit	101.3
Estimated median	98.4

Conclusion:

Over the concentration of 80 – 120 µg/ml for mebendazole and 1.6 – 2.4 µg/ml for potassium sorbate, the method yielded an accuracy of 99.9% and 99.5% respectively. This is well within the acceptable range.

9.8.4 Precision

9.8.4.1 Intraday precision

The intraday precision results for mebendazole and potassium sorbate are shown in tables 9.9 and 9.10.

9.8.4.1.1 Mebendazole

Table 9.9 Intraday precision results for mebendazole

Concentration found (µg/ml)	Recovered (%)
80.2	100.07
80.1	101.95
75.3	98.09
103	99.87
99.9	100.57
96.2	98.06
105.4	97.68
119	98.62
113.7	95.24
Mean	99.0
SD	1.68
% RSD	1.70

9.8.4.1.2 Potassium sorbate

Table 9.10 Intraday precision results for potassium sorbate

Concentration found (µg/ml)	Recovered (%)
1.6	97.99
1.5	96.05
1.4	85.63
2.0	98.68
2.0	100.26
1.9	97.89
2.2	88.61
2.4	101.42
2.4	99.91
Mean	98.76
SD	1.79
% RSD	1.81

9.8.4.2 Interday precision

The interday precision results for mebendazole and potassium sorbate are shown in tables 9.11 and 9.12.

9.8.4.2.1 Mebendazole

Table 9.11 Interday precision results for mebendazole

	Day 1	Day 2	Day 3
	99.9	98.5	99.0
	100.6	94.3	96.2
	98.1	96.8	97.0
Mean	99.5	96.54	97.37
SD	1.06	1.75	1.17
% RSD	1.06	1.81	1.20

ANOVA: Single factor

SUMMARY				
Groups	**Count**	**Sum**	**Average**	**Variance**
Day 1	3	298.5	99.5	1.7
Day 2	3	289.6	96.5	4.6
Day 3	3	292.1	97.4	2.1

ANOVA					
Source of variation	**SS**	**df**	**MS**	**F**	**P-value**
Inter day	**13.96**	**2**	**6.98**	**2.52**	**0.16**
Intra day	**16.65**	**6**	**2.77**		
Total	**30.61**	**8.0**			

9.8.4.2.2 Potassium sorbate

Table 9.12 Interday precision results for potassium sorbate

	Day 1	Day 2	Day 3
	98.7	103.8	99.2
	100.3	100.6	95.9
	97.9	99.4	97.9
Mean	98.94	101.25	97.64
SD	0.99	1.86	1.35
% RSD	1.0	1.84	1.39

ANOVA: Single factor

SUMMARY				
Groups	**Count**	**Sum**	**Average**	**Variance**
Day 1	3	296.83	98.94	1.46
Day 2	3	303.75	101.25	5.19
Day 3	3	292.93	97.64	2.75

ANOVA					
Source of variation	**SS**	**df**	**MS**	**F**	**P-value**
Inter day	20.02	2	10.01	3.20	0.11
Intra day	18.79	6	3.13		
Total	38.81	8			

SS = sum of squares

df = degrees of freedom

MS = mean squares

F = F ratio

Conclusion:

Precision was satisfactory with RSD's of < 4%. The inter-day and intra-day mean values were not significantly different. The assay should perform well, even when executed by other personnel in a different laboratory.

9.8.5 Ruggedness

9.8.5.1 Stability of sample solutions:

A sample was left on the auto sampler tray and reanalysed over several time intervals to determine the sample stability. The stability results of mebendazole and potassium sorbate are shown in tables 9.13 and 9.14.

9.8.5.1.1 Mebendazole

Table 9.13 Stability results of mebendazole in sample

Time (hours)	Peak area	% remaining
0	5487	100.0
1	5485	100.0
2	5504	100.3
3	5523	100.7
4	5520	100.6
5	5480	99.9
6	5460	99.5
7	5590	101.9
8	5499	100.2
9	5470	99.7
10	5465	99.6
11	5420	98.8
12	5400	98.4
Mean	5485	99.96
SD		0.83
% RSD		0.83

9.8.5.1.2 Potassium sorbate

Table 9.14 Stability results of potassium sorbate in sample

Time (hours)	Peak area	% remaining
0	140	100.0
1	139	99.3
2	140	100
3	142	101.4
4	140	100
5	138	98.6
6	136	97.1
7	136	97.1
8	138	98.6
9	140	100
10	139	99.3
11	140	100
12	139	99.3
Mean	139	99.29
SD		1.16
% RSD		1.16

Conclusion:

No significant breakdown was observed for mebendazole or potassium sorbate over a 12 hour period.

9.8.5.2 System repeatability

A sample was injected six times in order to test the repeatability of the peak area as well as the retention time. The results are shown in tables 9.15 and 9.16.

9.8.5.2.1 Mebendazole

Table 9.15 System repeatability for mebendazole

	Peak area	Retention time
	5487	6.394
	5485	6.372
	5504	6.356
	5523	6.382
	5520	6.287
	5530	6.237
Mean	5508	6.338
SD	17.51	0.06
% RSD	0.32	0.90

Conclusion:

System performance of mebendazole proved well with RSD values of 0.32 % for peak area and 0.90 % for retention time.

9.8.5.2.2 Potassium sorbate

Table 9.16 System repeatability for potassium sorbate

	Peak area	Retention time
	140	3.290
	140	3.284
	140	3.281
	141	3.266
	141	3.255
	141	3.271
Mean	141	3.275
SD	0.50	0.01
% RSD	0.36	0.36

Conclusion:

System performance concerning potassium sorbate proved well with RSD values of 0.36 % for peak area and 0.36% for retention time.

9.8.6 Robustness

Deliberate changes were made to the chromatographic conditions to determine the robustness of the method. The following changes were made and found to be acceptable:

Injection volume:	6 – 12 µl/ml
Flow rate:	0.8 – 1.2 ml/minute
Detection wavelength:	260 – 270 nm
Columns:	Luna C18-2 column, 150 x 4.6 mm, 5 µm, Phenomenex, Torrance, CA
	Waters Nova-Pak C18, 4.6 x 150 mm

Conclusion:

The method is robust and was not affected by the changes in the analytical conditions.

9.9 CONCLUSION

The method complied with all the criteria and should be suitable for the analysis of batch release and stability samples.

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