PREPARATION AND EVALUATION OF DOXYCYCLINE HYDROCHLORIDE AND BROMHEXINE HYDROCHLORIDE DOSAGE FORMS FOR PIGEONS

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"For God does not create a longing or a hope without having a fulfilling reality ready for them."

-Isak Dinesen-

To my parents,
with love.

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ABSTRACT

THE PREPARATION AND EVALUATION OF DOXYCYCLINE HYDROCHLORIDE AND BROMHEXINE HYDROCHLORIDE DOSAGE FORMS FOR PIGEONS

Objective: To prepare and evaluate three different dosage forms, containing doxycycline hydrochloride (HCl) and bromhexine hydrochloride (HCl) respectively and in combination, for the treatment of respiratory diseases in pigeons. **Background:** Birds have held a place in man's affection since the ancient Egyptians and Romans kept birds. Europeans have successfully bred birds, especially smaller birds and pigeons, for centuries. Only in recent years, however, have science and medicine been applied to aviculture and pet care. Pigeon racing is one of the sports not well known to the general public. These sportsmen invest a great deal to ensure that their pigeons are disease free. During racing they are exposed to infectious agents in the racing baskets and bring these pathogens back to the racing flock. If you ask any experienced flier what health problem he fears most for his pigeons during the racing season, he will probably say respiratory infection. Respiratory diseases are very common in pigeons. They are the major cause of poor performance and pigeon loss during the racing season. Doxycyline HCl, a broad-spectrum antibiotic, is the world-wide veterinary therapeutic agent of choice for the treatment of *Chlamydia,* a principle cause of respiratory infection. Doxycyline HCl has several advantages: greater activity, providing effective blood levels for up to 20 hours after a single dose compared to 4 hours for older tetracyclines; causes less disruption to the normal bowel bacteria; has less detrimental effect on the immune system; and is less affected by calcium and other minerals. Bromhexine HCl is an expectorant drug, promoting bronchial secretion and having mucolytic properties. It is commonly used in combination with antibiotics such as doxycycline HCl for the treatment of respiratory infections in the pigeon loft. Because avian medicine has not been commercialised as much as those for human use, it has left fanciers experimenting with dosage forms and strengths resulting in severe consequences. There is a great need for sophisticated medication developed specifically for the pigeon market. **Methods:** This study investigated the formulation of a direct compressed tablet and a water-soluble powder containing doxycycline HCl and bromhexine HCl respectively and in combination. The formulation and evaluation of the stability of an ophthalmic solution, containing doxycycline HCl was also investigated. Initial test were done on all three formulations. The tablets were inspected visually and tested for uniformity of mass, hardness, friability,

disintegration, assay and dissolution. The water-soluble powder was tested for its pH, constitution time, assay, moisture content and visual properties. An "in use" assay was also done on the doxycycline HCl powder. Three containers (stainless steel, glass and plastic) were used and the powder was dissolved in tap water (5 mg/ml). Samples were taken from every container after 0, 6, 12 and 24 hours and analysed. The results obtained were compared to the same powder but with no citric acid in the formulation. The same containers and time intervals were used for the comparing powder. The ophthalmic solution's appearance, pH, density, viscosity, assay, particulate matter and preservative efficacy were tested. The formulations were stored at three different temperatures and humidities for three months. The above mentioned tests were repeated after every month. An HPLC method for the simultaneous determination of doxycycline HCl and bromhexine HCl was developed and validated. **Results and discussion:** Based on the different test results generated over the twelve weeks of stability evaluation of the products that were developed in this study, doxycycline HCl and bromhexine HCl, respectively and in combination, seemed to have been relatively stable. The final tablets, water-soluble powders and ophthalmic solution formulations remained stable. The "in use" assay of the powder containing citric acid showed no discoloration, precipitation or breakdown when dissolved in water for a period of 24 hours. The powder lacking the citric acid showed discoloration after only 3 hours. This powder showed significant breakdown as well. The containers used for the storage of the tablets and the powders didn't seal tight enough. The moisture uptake was very high resulting in poor disintegration and dissolution times. Therefore the powder and the tablets should be stored in tightly sealed containers with enough silica as drying agent. The containers used for the tablets, powders and ophthalmic solution respectively, seemed not to influence the stability of the formulations negatively. The newly developed and validated HPLC method was used to analyse the stability samples and it proved to be reliable and easy to execute. **Conclusion:** Accelerated stability tests indicated that the formulations remained stable and that no significant breakdown occurred. Complete stability trial studies should however be conducted to claim their stability. The newly developed HPLC method was used over the twelve-week period to analyse accelerated stability samples, and it proved to be reliable and easy to carry out.

UITTREKSEL

DIE VOORBEREIDING EN EVALUERING VAN DOKSISIKLIEN HIDROCHLORIED EN BROOMHEKSIEN HIDROCHLORIED DOSEERVORME VIR DUIWE

Oogmerke: Die voorbereiding en evaluering van drie verskillende doseervorme, wat doksisiklien hidrochloried (HCl) en broomheksien hidrochloried (HCl) onderskeidelik en in kombinasie bevat, vir die behandeling van respiratoriese siektes in duiwe. **Agtergrond:** Die mens het nog altyd 'n toegeneentheid jeens voëls gehad sedert die antieke Egiptenare en Romeine voëls aangehou het. Die Europeërs het al vir eeue lank voëls, veral kleiner voëls en duiwe, suksesvol geteel. Wetenskap en geneesmiddels is egter eers onlangs op voëlteelt en troeteldier versorging toegepas. Duif wedvlugte is een van die sportsoorte wat nie alom bekend is aan die algemene publiek nie. Duif entoesiaste investeer baie geld om te verseker dat hulle duiwe vry van siekte is. Duiwe word tydens wedrenne in wedrenmandjies blootgestel aan 'n aantal infektiewe parasiete, waarvandaan patogene oorgedra word na die kudde by die huis. Indien jy enige duif entoesias sou vra watter gesondheidsprobleem hy die meeste vrees gedurende die wedvlugseisoen sal sy antwoord waarskynklik respiratoriese infeksie wees. Respiratoriese siektes kom algemeen onder duiwe voor. Dit is die hoof oorsaak van swak vertoning en duif sterftes gedurende die wedvlug seisoen. Doksisiklien HCl, 'n breë-spektrum antibiotikum, is die wêreldwye veeartsenykundige terapeutiese geneesmiddel van keuse vir die behandeling van *Chlamydia*, die vernaamste oorsaak van respiratoriese infeksie. Doksisiklien het verskeie voordele: groter aktiwiteit, voorsien effektiewe bloedvlakke vir tot 20 uur na 'n enkele dosering in vergelyking met 4 ure vir ouer tetrasikliene; veroorsaak minder ontwrigting van die normale ingewandsbakterieë; die nadelige effek op die imuunsisteem is minder; en dit word minder beïnvloed deur kalsium en ander minerale. Bromheksien HCl is 'n hoesmiddel, wat brongiale sekresie bevorder en mukolitiese eienskappe besit. Dit word algemeen in kombinasie met antibiotikums soos doksisiklien HCl gebruik in die behandeling van respiratoriese infeksies van die duiwehok. Voëlmedikasie is nie so ontwikkel en verfyn soos menslike medikasie nie. Gevolglik eksperimenteer entoesiaste met doseervorme en dosisse wat vir menslike gebruik ontwikkel is, met soms nadelige en fatale gevolge. Die ontwikkeling van medisyne wat spesifieke werking toon in die duiwemark is daarom van groot belang. **Metodes:** Hierdie studie het die formulering van 'n direk saampersbare tablet en 'n wateroplosbare poeier wat doksisiklien HCl en bromheksien HCl onderskeidelik en in kombinasie bevat, ondersoek. Die formulering en evaluering van die stabiliteit van 'n

oftalmiese oplossing wat doksisiklien HCl bevat is ook ondersoek. Aanvangstoetse is gedoen op al drie formulerings. Die tablette is visueel ondersoek en getoets vir hul eenvormigheid van massa, hardheid, brosheid, disintegrasie, gehaltebepaling en dissolusie. Die wateroplosbare poeier is getoets vir pH, oplossingstyd, gehaltebepaling, voggehalte en visuele eienskappe. Op die doksisiklien HCl poeier was 'n "in gebruik" gehaltebepaling ook gedoen. Daar is gebruik gemaak van drie houers (vlekvrye staal, glas en plastiek) om die poeier in kraanwater op te los (5 mg/ml). Monsters is na 0, 6, 12 en 24 uur uit elke houer onttrek en geanaliseer. Die resultate verkry is vergelyk met dieselfde poeier, maar sonder die sitroensuur in die formulering. Dieselfde houers en tydintervalle is gebruik vir die vergelykende poeier ook. Die oftalmiese oplossing se voorkoms, pH, digtheid, viskositeit, gehaltebepaling, deeltjiegrootte en preserveer doeltreffendheid is getoets. Die formulerings is by drie verskillende temperature en humiditeite vir drie maande gestoor. Die bogenoemde toetse is herhaal na elke maand. 'n HPLC metode vir die gesamentlike bepaling van doksisiklien HCl en bromheksien HCl is ontwikkel en gevalideer. **Resultate en bespreking:** Geskoei op die verskillende toetsresultate verkry oor die twaalf weke tydperk van stabiliteitstoetse van die produkte ontwikkel in hierdie studie, blyk dit dat doksisklien HCl en bromheksien HCl, onderskeidelik en in kombinasie, relatief stabiel gebly het. Die finale tablette, wateroplosbare poeiers en die oftalmiese oplossing het stabiel gebly. Die "in gebruik" gehaltebepaling van die sitroensuur bevattende opgeloste poeier, het na 'n periode van 24 uur steeds geen kleurverandering, presipitasie of afbraak getoon nie. Kleurverandering was waarneembaar na slegs 3 ure by die poeier sonder die sitroensuur. Hierdie poeier het aansienlike afbraak ook getoon. Die houers wat gebruik is om die tablette en poeiers in te stoor het nie dig genoeg geseël nie. Die vogopname was uitsonderlik hoog en het gelei tot swak disintegrasie en dissolusietye. As gevolg hiervan moet die poeiers en die tablette in diggeseëlde houers gestoor word met genoeg silika as drogingsmiddel. Die houers gebruik vir die stoor van die tablette, poeiers en oftalmiese oplossing onderskeidelik, blyk nie of dit die stabiliteit van die formulerings negatief beïnvloed nie. 'n Nuut ontwikkelde en gevalideerde HPLC metode is gebruik vir die analise van die stabiliteitsmonsters, en het getoon dat die metode maklik bruikbaar en betroubaar was. **Gevolgtrekking:** Versnelde stabiliteitstoetse het aangedui dat die formulerings stabiel gebly het en dat geen noemenswaardige afbraak plaasgevind het nie. Volledige stabiliteitstoetse sal egter uitgevoer moet word om volledige stabiliteit te bewys. Die nuut ontwikkelde HPLC metode was oor 'n tydperk van twaalf weke gebruik tydens die analise van die stabiliteitsmonsters, en het getoon dat die metode betroubaar en maklik uitvoerbaar was.

AIMS AND OBJECTIVES

Over the last ten years, world-wide avian veterinary knowledge has undergone a quantum leap forward. Following closely have been improved diagnostic capabilities and an ever-increasing range of effective medications. As only healthy birds can become fit and only fit birds can win, the successful management of the birds' health is just another challenging aspect in the overall preparation of the birds for successful racing. For a racing loft to be successful, it must have good and healthy pigeons in a good loft under good management.

It is important to remember that the racing pigeon is naturally a fairly robust bird and, as a species, disease in it is relatively uncommon provided the basics of hygiene and management are met. However, in even the best-managed lofts, because of the very nature of pigeon racing where birds from many different lofts are in intimate contact, disease will occasionally occur. The important diseases during the rigours of the race program and during breeding are canker, the parasitic diseases, respiratory infection, bacterial infections such as *Salmonella* and *E. coli*, fungal infections such as thrush and *Aspergillus*, and viral infections.

If you ask any experienced flier what health problem he fears most, then if it is the breeding season he will probably say canker, but if it is the race season he will probably say respiratory infection. Respiratory diseases are very common in pigeons. They are the major cause of poor performance and pigeon loss during the race season. Clinical respiratory infection in pigeons is the end result of the interplay of a number of factors but, in particular, the type of infective organism and the vulnerability of the birds to infection are important. The respiratory system can be infected by *Chlamydia, Mycoplasma*, bacteria, fungi, viruses and mites.

Doxycyline HCl, a broad-spectrum antibiotic, is the world-wide veterinary therapeutic agent of choice for the treatment of *Chlamydia,* a principal cause of respiratory infection. Doxycyline HCl has several advantages: greater activity, providing effective blood levels for up to 20 hours after a single dose compared to 4 hours for older tetracyclines; causes less disruption to the normal bowel bacteria; has less detrimental effect on the immune system; and is less affected by calcium and other minerals. Not only does it have activity against *Chlamydia*, but also against *Mycoplasma* and a range of bacteria. The only disadvantage is that in areas with

hard water, medicated water will become brown/pink. This can also happen with exposure to sunlight and indicates that the drug has lost its effectiveness due to the oxidation of the drug. The discoloration of the water leads to a decrease in the water uptake of the pigeons thus resulting in an insufficient uptake of doxycycline HCl for the successful therapy against the micro organism.
Bromhexine HCl is an expectorant drug and is commonly used in combination with antibiotics, such as doxycycline HCl, in the treatment of respiratory infections of the pigeon loft.

The main objectives of this study therefore are:

- The development of a tablet formulation containing doxycycline HCl for direct compression tabletting to be used orally in pigeons.
- The development of a tablet formulation containing bromhexine HCl for direct compression tabletting to be used orally in pigeons.
- The development of a tablet formulation containing both doxycycline HCl and bromhexine for direct compression tabletting to be used orally in pigeons.
- The development of a water-soluble powder containing doxycycline HCl and citric acid, and to investigate the effect that citric acid has on the stability and solubility of doxycycline HCl in tap water.
- The development of a water-soluble powder containing bromhexine HCl, to be used as drinking-water medication of pigeons.
- The development of a water-soluble powder containing doxycycline HCl, bromhexine HCl and citric acid, to be used as drinking-water medication of pigeons.
- The development of an ophthalmic solution containing doxycycline HCl as the active substance.
- The evaluation of the stability of the formulations under accelerated conditions for 3 months.
- To develop and validate a HPLC method, to be used for the simultaneous analysis of doxycycline HCl and bromhexine HCl, using the above mentioned formulations.
- The evaluation of results and drawing of conclusions.

CHAPTER 1

AVIAN DISEASES AND TREATMENT

1.1 AVIAN DISEASES

Birds have held a place in man's affection since the ancient Egyptians and Romans kept birds. Europeans have successfully bred bird, especially smaller birds and pigeons, for centuries. Only in recent years, however, have science and medicine been applied to aviculture and pet care (Clubb, 2000:2). Avian medicine is, at the moment, a particularly interesting area, as the level of knowledge, new drugs and diagnostic tests available advance every year.

Infectious disease and other problems do not come from nowhere. In the past, the emphasis has been on the treatment of the individual sick bird, but the fancier must think why it has become sick and how to stop further birds developing the same disease (Walker, 2000:4). One must always ensure the health and safety of the flock over that of the individual bird (Wissman, 1999a:1).

1.1.1 DISEASES OF THE PIGEON LOFT

The primary diseases and infectious problems of pigeons are due to various infective organisms, of which the following are the most common:

- Nematodes (*Capillaria, Ascaridia*),
- Cestodes (tape worms i.e. *Cotuginia* and *Raillietina*),
- Coccidosis (*Eimeria columbae*),
- External parasites (lice and mites),
- Motile protozoa (*Trichomonas* spp., *Hexamita*),
- Salmonella (*Typhimurium var copenhagen*),
- Gram-negative enteric bacteria (*E. coli*),
- Chlamydia and secondary infections (Ornithosis-complex),

- Mycoplasma,
- Pox virus,
- Herpes virus, and
- PMV virus (Marshall, 1990:125).

1.1.2 RESPIRATORY DISEASES OF AVIARY BIRDS

The avian respiratory system is unique. It differs from mammals in that birds have no diaphragm, have a syrinx at the end of the trachea instead of vocal cords in the larynx, and have no epiglottis (Frederickson, 1995:1). Pneumatic bones provide a lightweight skeleton and act as additional reservoirs of air to aid in buoyancy, thermoregulation and respiration. Attached to the lungs are air-filled membranes called air sacs which are the primary reservoirs of air and provide most of the bird's buoyancy (East, 2000a:2). With disease in any part of the respiratory system, problems develop. Careful observation of the nares, choanal slit and trachea, as well as posture and body swellings can tell you a lot about respiratory health (Frederickson, 1995:1).

Diseases of the respiratory tract are very often mixed infections. Outbreaks of the disease result from the combined effects of pathogens and factors within the loft environment that reduce the birds' resistance to infection (River, 2003:2). The respiratory system can be infected by *Chlamydia, Mycoplasma*, bacteria, fungi, viruses and mites (Walker, 2000:36). Upper respiratory tract infections will present with sneezing, nasal discharge, inflamed eyes, and if it also involves the sinuses, there will be swelling of the head around the eyes. These infections can vary from relatively mild conditions that owners will frequently characterise as "colds" to very severe problems where the bird has difficulty eating and breathing and requires hospitalisation (Santa, 2000:3).

The control of respiratory disease is two-pronged:

1. Control of any predisposing stress factors – These can take the form of:
    (a) Environmental triggers, e.g. dampness, overcrowding, low hygiene.
    (b) Management triggers, e.g. poor feeding, excessive tossing.
    (c) Concurrent disease, in particular parasitism. This includes wet canker. The combination of either worms or elevated trichomonad levels and respiratory disease is very common.

The fancier must establish a healthy loft environment; otherwise respiratory disease will continually recur, despite medication.

2. Correct use of appropriate drugs to either eradicate or keep the organism level low so that disease does not occur (Walker, 2000:36).

1.1.3 MOST COMMON RESPIRATORY DISEASES OF AVIARY BIRDS

There are many common and important diseases which can affect the respiratory system (air passages, lungs, air sacs) of aviary birds. Due to modern systems of management, usually with high bird densities, these diseases are able to readily spread (Butcher *et al.*, 1999:1). In pigeons, the most common respiratory diseases which can be treated with antibiotics are: catarrh, chlamydiosis, pigeon pox, paramyxovirus infection, mycoplasmosis and avian influenza.

1.1.3.1 CATARRH

This disease is also known as coryza (Chevita, 2004a:1). The door to infection is opened by mycoplasma and viruses, in addition to fungi and trichomonads. These lower the pigeons' resistance and allow pathogenic bacteria - pasteurella, cocci and coli bacteria – to colonise and multiply. It is these secondary pathogens that engender the actual clinical picture of visible and audible catarrh (River, 2003:2).

Symptoms of the disease

Initially the pigeon fancier notices sneezing and an aqueous nasal discharge, which in the acute form of the disease becomes mucopurulent and yellowish brown in colour. This is accompanied by the first signs that the birds' general condition is impaired, namely reduced feed and water intake, cessation of down moulting and a reluctance to fly (Denica, 2004a:1). The wattle and bridge of the nose turn grey and there is scratching of the head and nose. When the beak is opened, stringy mucus can be seen stretching from the retro lingual region to the palate (See figure 1.1). Additional clinical signs are a reddening and swelling of the pharyngeal mucosa (Jedds, 2003:1).

Transmission of the disease

Catarrh is primarily transmitted by direct bird-to-bird contact. This can be from infected birds brought into the flock as well as from birds which recover from the disease which remain carriers of the organism and may shed intermittently

throughout their lives. Within a flock, inhalation of airborne respiratory droplets, and contamination of feed and/or water are common modes of spread (Butcher *et al.*, 1999:4).

Treatment

Water-soluble antibiotics or antibacterials can be used (Butcher *et al.*, 1999:1). Do not give pigeons any feedstuffs containing calcium (grit) during treatment with chlortetracycline, since calcium binds chlortetracycline and thus reduces efficacy (Denica, 2004a:2).

Prevention

Good management and sanitation are the best ways to avoid infectious catarrh. Most outbreaks occur as a result of mixing flocks (Butcher *et al.*, 1999:4). The loft can be cleaned and disinfected with povidone iodine. (Rovira, 2002:6).

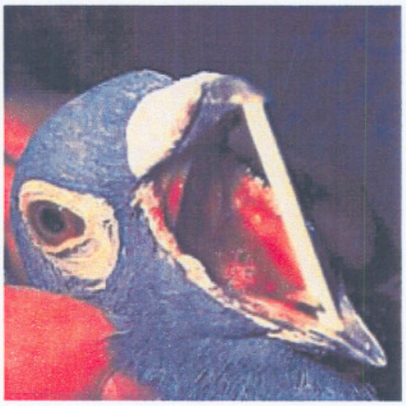


Figure 1.1 Dirty grey deposits in the beak cavity with prolonged catarrhal infection (Chevita, 2004a:3).

1.1.3.2 CHLAMYDIOSIS

The disease was called psittacosis or parrot fever when diagnosed in psittacine (curve-beaked) birds, and called ornithosis when diagnosed in all other birds or in humans. Currently, the term chlamydiosis is used to describe infections in any animal (Butcher *et al.*, 1999:5). Chlamydiosis is considered one of the five most common diseases in aviary birds (Wissman, 1999a:1). It is caused by a Gram negative, coccoid, obligate intracellular bacterium called *Chlamydia psittaci*, which must live and reproduce within the cells of its host (Iowa, 2003:2). Interestingly, there

have been no known outbreaks of chlamydiosis in the wild and it is felt the outbreaks in domestic birds are the result of man-made conditions and procedures which induce stress (Master, 1999/2000:2).

This disease is considered a zoonis, meaning that it is potentially contagious to humans, and in some states, it is a reportable disease (Wissman, 1999a:3). Infection with *C. psittaci* usually occurs when a person inhales the organism, which has been aerosolised from dried faeces or respiratory secretions of infected birds (American, 2004a:4).

a)

b)

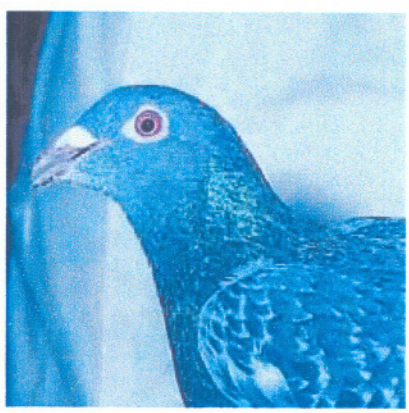


Figure 1.2 (a) Severe unilateral inflammation of the entire eye which has become additionally infected with pus forming pathogens. (b) Breathing with half-open beak: in chlamydiosis this is observed if air sacs and lungs are affected (Chevita, 2004b:3).

Symptoms of the disease

Clinical signs in most birds include nasal-ocular discharge, conjunctivitis, sinusitis, diarrhoea, weakness, loss of body weight, and a reduction in feed consumption (Butcher *et al.*, 1999:5). The one symptom which is suggestive of chlamydiosis rather than other diseases is eye discharge, and any bird with an eye discharge should be considered for chlamydiosis (see figure 1.2) (Owen, 1997:3). Many birds are asymptomatic carriers and appear clinically normal yet infected. Any stress such as transportation, malnutrition, concurrent illness, poor ventilation, overcrowding, and breeding can cause shedding of the organism and clinical disease (Pesek, 1998a:3).

Transmission of the disease

C. psittaci is transmitted frequently by the inhalation of infectious dust, respiratory tract secretions and occasionally by ingestion (Iowa, 2003:4). The organism can be present in large numbers and can remain virulent for several months in dried droppings (Owen, 1997:2). Fomites can also spread chlamydiosis, and biting insects, mites, and lice may be important in mechanical transmission (Iowa, 2003:4). Recovered birds remain carriers and will continue to intermittently shed the infective agent for long periods after clinical signs have subsided (Butcher *et al.*, 1999:5).

Treatment

Without treatment most birds die from this disease (Zweigart, 1999:2). After diagnosis by appropriate test, chlamydiosis is treated by administering doxycycline (Pesek, 2000:3). Treatment for respiratory infection is delayed before racing for as long as possible to allow the birds to develop as strong a natural immunity as possible. However, if, as racing approaches, the birds are showing signs of respiratory infection, medication is given (as shown in figure 1.3). The aim of any treatment is to reduce the *Chlamydia* level in the birds so that more stress is required to cause it to flare up (Walker, 2000:189).

Prevention

C. psittaci is a contagious disease; birds must be quarantined during treatment. While a bird is being treated, the premises should be cleaned and disinfected frequently to eliminate infectious dust (Iowa, 2004:3). No vaccines for chlamydiosis are available. The following recommendations should be followed when treating and caring for birds with confirmed, probable, or suspected cases of chlamydiosis:

- Protect birds from undue stress, poor husbandry and malnutrition. These problems reduce the effectiveness of treatment and promote the development of secondary infections with other bacteria or yeast.
- Observe the birds daily, and weigh them every 3-7 days. If the birds are not maintaining weight, have them re-evaluated by a veterinarian.
- Avoid high dietary concentrations of calcium and other divalent cations because they inhibit the absorption of tetracyclines.
- Isolate birds that are to be treated in clean, uncrowded cages.
- Clean up all spilled food promptly; wash food and water containers daily.
- Provide fresh water and appropriate vitamins daily.
- Continue medication for the full treatment period to avoid relapses (American, 2004a:5)

Before racing:

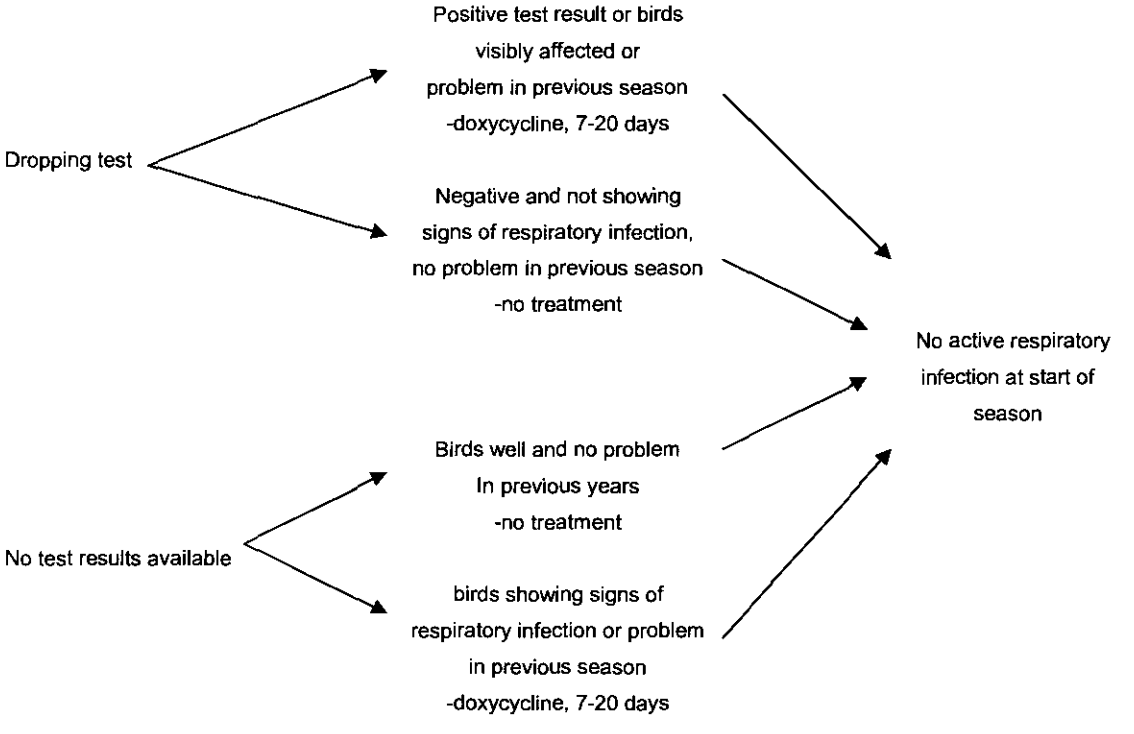


Figure 1.3 A schematic diagram for the treatment of chlamydiosis before the racing season (Walker, 2000:189).

1.1.3.3 PIGEON POX

Pox is a specific virus disease affecting many avian species, and of world-wide distribution (Blount, 1947:325). Increased incidence of pigeon pox is observed in

humid summer and warm winter weather (Chevita, 2004b:1). The disease has become less common as more birds are raised domestically and fewer birds are imported (Santa, 2000:3).

Symptoms of the disease

There are two forms of pigeon pox:

- In the *skin form*, birds develop scabby proliferations (pocks), especially where the outer skin meets the mucosa of the eye and beak region, and additionally on the legs (See figure 1.4a). The virus penetrates the skin through minute lesions (scratches, peck lesions and insect bites). The pocks are clearly differentiated from the unchanged skin, but firmly attached to it (Chevita, 2004b:1). If the pocks are removed before healing is complete, the surface beneath is raw and bleeding. Unthriftiness and retarded growth are typical symptoms of pigeon pox (Butcher *et al.*, 1999:1).
- In the *mucosal form*, firmly attached deposits are formed on the mucosa of the crop and pharyngeal cavity (See figure 1.4b). These can impede feed and water uptake and breathing (Chevita, 2004b:1). This form is the more aggressive form of the disease and may result in the death of the bird due to the swelling and inflammation of the breathing passages (Santa, 2000:2).

Transmission of the disease

Pigeon pox is transmitted by direct contact between infected and susceptible birds or by mosquitoes. Virus-containing scabs also can be sloughed from affected birds and serve as a source of infection. The virus can enter the blood stream through the eye, skin wounds, or respiratory tract. Mosquitoes become infected from feeding on birds with fowl pox in their blood stream. There is some evidence that the mosquito remains infective for life (Butcher *et al.*, 1999:1).

Treatment

As with other viral disease it is not possible to combat pigeon pox itself. In the event of a pox outbreak, emergency vaccination can be carried out on all pigeons that appear healthy in order to prevent the disease from spreading. Visibly affected birds should be excluded from emergency vaccination and removed from the flock. Administration of livimun (improves the pigeon's bodily defence against

contamination with germs) is recommended to stimulate natural resistance, together with chlortetracycline to inhibit secondary bacterial pathogens (Denica, 2004b:2).

Prevention

Pigeon pox outbreaks in poultry confined to houses can be controlled by spraying to kill mosquitoes (Butcher *et al.*, 1999:1). Active immunisation and keeping the birds indoors and isolated can protect them as well (Santa, 2000:3).

a)

b)

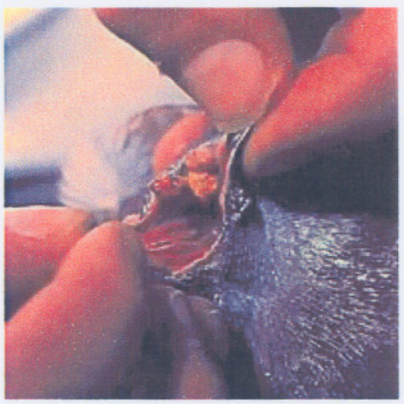


Figure 1.4 (a) Scabby fissured skin proliferations in pox at the beak angle and on the eyelid: secondary bacterial pathogens can settle in the skin fissures and lead to pus formation. (b) Mucosal form of pox (Chevita, 2004b:3).

1.1.3.4 PARAMYXOVIRUS INFECTION

Paramyxovirus (PMV-1) was first recognised as a disease in pigeons in 1975 (East, 2000b:1). PMV-1 is a contagious and fatal viral disease affecting most species of

birds. Clinical signs are extremely variable depending on the strain of virus, species, and age of the bird, concurrent disease and pre-existing immunity (Avian, 2004a:1). PMV-1 is related to another type of Paramyxovirus known as Newcastle Disease. Newcastle Disease is a highly infectious viral disease of poultry and other birds but seldom a disease of pigeons. PMV-1, however, is present in show pigeons, flying breeds and wild pigeons (East, 2000b:1).

Symptoms of the disease

The initial signs of paramyxovirus are: increased water intake combined with reduced feed consumption, emaciation and diarrhoea-like faeces due to a pathogenic increase in fluid excretion (polyuria: puddles containing floating particles of faeces are formed in the loft. (See figure 1.5b) (Chevita, 2004b:1). The gastro-intestinal signs appear first and are followed by the nervous signs. In the current form of the disease, the respiratory and ocular symptoms are practically non-existent. Most pigeons die from this disease (Denica, 2004b:1).

The nervous disorders are very characteristic:

- Tremor of the head
- Torticollis; head inverted (See figure 1.5a)
- Paralysis; of one wing, then both and/or paralysis of the feet
- Disordered balance and flight
- Tottering step, tendency to fall over backwards (River, 2003:3).

Transmission of the disease

The disease is transmitted by direct contact. This may occur in crowded lofts, shipping containers or even by social contact such as territorial aggression between cocks. More importantly, transmission can occur between show cages. Indirect transmission may also take place from contaminated food and water sources. Faecal dust can become airborne and further transmit the disease between lofts and pens. Last, insect vectors, pigeon flies and mosquitoes may infect birds with PMV-1 and thus should be controlled in the loft (East, 2000b:2).

Treatment

There is no specific treatment for paramyxovirus infection (Butcher *et al.*, 1999:2). Supportive care is recommended for the lightly affected and non-affected remaining birds (East, 2000b:2). Antibiotics can be given for 3-5 days to prevent secondary bacterial infections (Butcher *et al*,, 1999:2). The following can be administered

together: Antibiotics, amino acids, vitamins and levamisol (to stimulate the defences) (Rovira, 2002:5).

Prevention

The pigeon fancy, through intermingling of multiple birds from multiple sources during shows and races, predisposes pigeons to contracting PMV-1 and other infectious diseases. Preventing introduction of the virus into the loft is the key to protecting the loft (East, 2000b:5). Prevention programs should include vaccination, good sanitation and implementation of a comprehensive biosecurity program (Buther *et al.*, 1999:2).

a)

b)

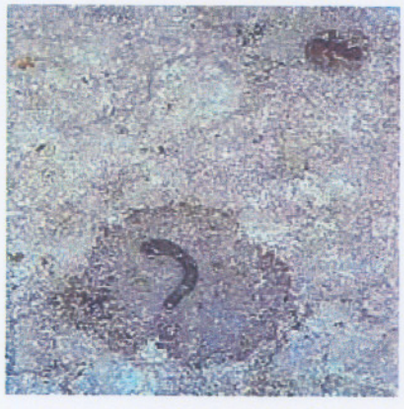


Figure 1.5 (a) Central nervous disorders in pigeons with paramyxovirus: torsion of the head. (b) Faeces in paramyxovirus: formed faecal particles in a water puddle with renal failure (Chevita, 2004b:3).

1.1.3.5 MYCOPLASMOSIS

Mycoplasma is a problem of the race season. It is what is called a primary erosive disease. Many veterinarians agree that *Mycoplasma* by themselves do not cause disease and, in fact, in experiments in which healthy pigeons have been deliberately infected, the birds have not become sick. However, the organisms do superficial injury to the lining of the respiratory system, enabling secondary organisms, notably Chlamydia, bacteria and fungi, to become established. In this way, *Mycoplasma* although not directly affecting health, has a big effect on race performance (Walker, 2000:42). Many pigeons are carriers of the disease, but the disease only appear after the effort of a difficult competition (Rovira, 2002:6).

Symptoms of the disease

Mycoplasma causes primarily a respiratory infection inducing sinusitis, pneumonia and airrsacculitis. The birds show nasal and ocular discharge, swollen paranasal sinuses, tracheal râles, coughing, laboured breathing, a loss of condition and even death, especially if the infection is compounded with secondary infection such as *E. coli.* (Valks & Burch, 2002:1).

Transmission of the disease

Transmission takes place through the faeces, the drinking water, feed, equipment and by droplet infection from pigeon to pigeon (Jedds, 2003:2). Most lofts do have resident *Mycoplasma* strains and new *Mycoplasma* strains can enter the loft through contact with other birds (Walker, 2000:42).

Treatment

Outbreaks of *Mycoplasma* can be controlled with the use of antibiotics (Butcher *et al.*, 1999). The choice of drug is sometimes dependent on the involvement of secondary organisms such as *Chlamydia* and *E. coli.* Baytril® (ciprofloxacin) can be used with care during racing. Other antibiotics such as doxycycline, tiamulin, or tylan are effective (Walker, 2000:43). Administration of these antibiotics can be by feed, water or injection. These are effective in reducing clinical disease. However, birds remain carriers for life (Butcher *et al.*, 1999:6).

Prevention

Mycoplasma can be controlled by eliminating the possible factors that reduce the bird's resistance to infection. Such factors may be: overcrowding in the loft, lack of

cleanliness, latent infections (e.g. ectoparasites, worm infestations, coccidial infection), excessive stress in breeding, deficient feed, poor water supply or stress during the racing season (Jedds, 2003:2). It may be necessary for deep disinfection as well. Preventative treatments can be done in the weeks free of races, and mainly after a hard one (Rovira, 2002:6).

1.1.3.6 AVIAN INFLUENZA

Influenza is caused by an enveloped RNA virus. It is an infectious disease of birds, swine, humans and other animals. Three types of Influenza viruses exist – types A, B and C. Influenza A viruses infects birds and other animals, while B and C infect people (Pesek, 1998b:2). Influenza A viruses have been isolated from humans, from several other mammalian species and a wide variety of avian species, among which, wild aquatic birds represent the natural hosts of influenza viruses (Tollis & Di Trani, 2002:202).

Symptoms of the disease

The signs of illness depend upon the species infected, the age, environmental factors, and virulence of the viral strain (Pesek, 1998b:2). Avian influenza is categorised as mild or highly pathogenic. The mild form produces listlessness, loss of appetite, respiratory distress and diarrhoea. The highly pathogenic form produces facial swelling, blue comb and wattles, and dehydration with respiratory distress (Butcher *et al.*, 1999:3).

Transmission of the disease

The avian influenza virus can remain viable for long periods of time at moderate temperatures and can live indefinitely in frozen material. As a result, the disease can be spread through improper disposal of infected carcasses and manure. Avian influenza can be spread by contaminated shoes, clothing, crates and other equipment. Insects and rodents may mechanically carry the virus from infected to susceptible poultry (Butcher *et al.*, 1999:3). Infected birds can shed the virus via their respiratory system, ocular secretions and faeces. There are no known incidences of vertical transmission. Although direct transmission of avian influenza virus from birds to humans is very rare, it is considered a zoonotic disease, meaning it is capable of being passed from birds and animals to humans. It is also quite possible that humans can infect birds with avian influenza virus, however that has not been documented (Avian, 2004b:1).

Treatment

There is no effective treatment for avian influenza. With the mild form of the disease, good husbandry, proper nutrition, and broad spectrum antibiotics may reduce losses from secondary infections. Recovered flocks continue to shed the virus. Vaccines may only be used with special permit (Butcher *et al.*, 1999:3).

Prevention

Clean and disinfect all surfaces, as well as quarantine all new and infected birds. It is best to keep all free ranging birds away from companion birds, domestic poultry, and fowl (Avian, 2004b:2). A vaccination program used in conjunction with a strict quarantine has been used to control mild forms of the disease. With the more lethal forms, strict quarantine and rapid destruction of all infected flocks remains the only effective method of stopping an avian influenza outbreak (Butcher *et al.*, 1999:3).
A summary of the possible signs for the most common respiratory diseases of poultry is shown in table 1.1.

1.2 ANTIBIOTICS

Antibiotics are drugs that classically are used in the treatment of bacterial diseases (Styles, 1996a:1). Ideally, antibiotic treatment is limited to affected individuals and populations of birds suspected to be subclinical carriers within the aviary. Antibiotics should ideally be chosen according to susceptibility patterns from cultured organisms. If culture and sensitivities are not available as with most viral, fungal, and parasitic diseases, antibiotic choices are based on available literature, if possible, and previous experience (Echols & Speer, 2004:2).

Table 1.1 Possible clinical signs for common respiratory diseases of poultry (Butcher *et al.*, 1999:14)

CLINICAL SIGNS	PIGEON POX	PARAMYXOVIRUS INFECTION	AVIAN INFLUENZA	CATARRH	CHLAMYDIOSIS	MYCOPLASMODIUM
Coughing	×	×	×		×	×
Sneezing	×	×	×	×	×	×
Shaking head		×		×	×	×
Rales (abnormal breathing sound)	×	×	×	×	×	×
Gasping	×	×			×	×
Discharge from eyes	×	×		×	×	×
Nasal discharge		×		×	×	×
Swelling of face and/or wattles		×	×	×	×	×
Bluish-purple discoloration of face		×	×			
Retarded growth	×	×		×	×	×
General diarrhoea		×	×	×	×	×
Green, watery diarrhoea		×			×	
Twisting of head and neck		×				
Warts/scabs	×					
Conjunctivitis		×	×	×	×	
Prostration		×				×

1.2.1 AVIAN ANTIBIOTICS COMMONLY USED

It is important to remember that antibiotics should not be given closer than 2 days before basketing for a race. All of them cause some disruption to the normal bowel bacteria, which then take several days to re-establish (Walker 2000:198). There are multiple subclasses within the class of antibiotics. The subclasses of antibiotics commonly used in avian medicine including some pertinent drugs of each subclass are:

- Subclass: cepahlosporins
  Members: cefotaxime (Claforan); cephalexin (Keflex)
- Subclass: penicillins
  Members: carbenicillin (Geopen); amoxicillin (Amoxi-Drops)
- Subclass: amino glycosides
  Members: amikacin (Amiglyde); gentamicin (Gentocin); tobramycin (Nebcin); spectinomysin (Spectoguard)
- Subclass: quinolones
  Members: enrofloxacin (Baytril); ciprofloxacin (Cipro)
- Subclass: tetracyclines
  Members: doxycycline (Vibramycin); oxytetracycline (multiple trade names)
- Subclass: sulfa drugs
  Members: trimethoprim/sulfamethoxazole (Bactrim); sulfachloropyrizidine (Vetasulid); sulfadiazine/trimehoprim (Ditrim)
- Subclass: protein-synthesis inhibitors
  Members: chloramphenicol (multiple trade names)
- Subclass: macrolide
  Members: erythromycin (Gallimycin); lincomycin (Lincocin); tylosin (Tylan)

Each subclass is used for targeting different bacterial types (Styles, 1996a:1). The aim of antibacterial therapy is to maintain an effective concentration of the drug at the site of infection for as long as possible. An effective concentration may be defined as that which is sufficiently in excess of the minimum inhibitory concentration (MIC) of the drug appropriate for the casual micro-organisms. Effective therapy is thus dependent on the susceptibility of the micro organisms to the drug and the pharmacokinetics which determine its ability to attain and maintain effective concentrations at the infection site (Debuf 1991:58). Table 1.2 is a summary of the most commonly used antibiotics in avian therapy, their description, usage, adverse reactions and dosage.

Table 1.2 Antibiotics used in the treatment of avian diseases (Association, 2000:3)

ANTIBIOTICS	DESCRIPTION	USAGE	ADVERSE REACTIONS	DOSAGE FORM	COMMENTS
Amoxicillin Trihydrate (Amoxil, Amoxi-drops, many others)	A semi synthetic analog of penicillin with a broad range of activity against gram + and gram – bacteria.	Can be used with any bacterial infection showing susceptibility to the drug.	None seen with any frequency.	Via water / tablet.	Its very effective, well absorbed, safe, and well tolerated in the pigeon.
Cephalexin (Keflex, many others)	A member of the cephalosporin group of antibiotics and effective against a broad range of gram + and gram – bacteria.	Bacterial infections shown to be susceptible to cephalexin.	None seen with any frequency.	Via water / tablet	Well tolerated by pigeons and readily accepted in the water. Reported as very affective against streptococcal infections.
Chloramphenicol (Chloromycetin, many others)	A bacteriostatic antibiotic used against a broad range of gram + and gram – bacteria.	Bacterial infections shown to be susceptible to chloramphenicol.	None seen with any frequency.	Intramuscular injection. Ophthalmic ointment or drops are useful for conjunctivitis.	This drug is broken down so quickly by crop flora, adequate blood levels are hard to attain orally.
Doxycycline (Vibramycin)	A bacteriostatic antibiotic with a wide range of activity against gram + and gram – bacteria	Can be used in bacterial infections susceptible to the drug.	None reported as common	Via water/ tablet	Very effective against *Chlamydia*. Remove grit during use as calcium will bind the drug and decrease absorption.
Enrofloxacin (Baytril)	A synthetic chemotherapeutic agent	Can be used with any bacterial infection	Causes increased mortality in the egg when	Via water / tablet	Probably the best drug available for gram –

	from the quinolone class. Has antibacterial activity against a broad spectrum of gram + and gram – bacteria.	showing susceptibility to the drug.	treated during egg formation.		infections of pigeons. It is the only drug shown to prevent recurrence of shedding in most cases of *salmonella* infections.
Erythromycin (Gallimycin)	In the macrolide family of antibiotic. Bacteriostatic and effective against gram + bacteria and mycoplasmas.	Any bacterial infection shown to be susceptible to erythromycin.	None that is common.	Via water/ tablet	Broken down quickly by the crop flora and thus levels found in the water for flock treatment are probably not as effective as bolus doses to individual birds.
Lincomycin (Lincocin)	In the macrolide family of antibiotics and is bacteriostatic against gram + bacteria and mycoplasmas.	Any bacterial infection shown to be susceptible to lincomycin	None that is common	Via water / tablet	Broken down quickly by the crop flora and thus levels found in the water for flock treatment are probably not as effective as bolus doses to individual birds.
Sodium Sulfachlorpyridazine (Vetisulid)	Bacteriostatic against a wide range of gram + and gram – bacteria. It is also coccidiostatic.	Coccidiosis and bacterial infections shown to be susceptible to the drug.	None that is common.	Via water	Vetisulid is very effective against many cases of *E. coli.*
Spectinomysin (Spectoguard)	Bacteriocidal antibiotic that is effective against a broad range of gram +	Any bacterial infection shown to be susceptible to spectinomycin.	None that is common.	Intramuscular injection / via water / tablet	It has been used to some success with enteric infections, but is not

	and gram – bacteria.				effective against systemic infections.
Sulfadiazine/trimethoprim (Ditrim)	A synthetic antibacterial combination product that is bacteriostatic against gram + and gram – bacteria.	Bacterial infections shown to be susceptible to the drug.	None that is common.	Tablet / Injection.	Only available in pill and injectible form for individual bird dosing.
Sulfamethoxazole/Trimethoprim (Bactrim, many others)	A synthetic antibacterial combination product that is bacteriostatic against gram + and gram – bacteria.	Bacterial infections susceptible to the drug.	None that is common.	Via water / tablet.	A good drug in many cases of gram negative bacterial infections.
Tetracyclines, Chlortetracyline (Auereomycin) **Oxytetracycline** (Terramycine)	The tetracylines are bacteriostatic and effective against various gram + and gram – bacteria. Cross resistance is common.	Can be used in bacterial infections shown to be susceptible to tetracylines.	None that is common.	Via water / tablet.	Binds with calcium. Remove grit containing calcium and health grit during use. Very effective in respiratory infections. *Chlamydia* is typically very susceptible to tetracycline drugs.
Tylosin (Tylan, Tylocine)	A macrolide antibiotic that is bacteriostatic against many gram + bacteria and mycoplasma	Bacterial infections shown to be susceptible to tylosin.	None that is common.	Via water / tablet	Very effective against *mycoplasma* and against ornithose complex when combined with tetracyclines.

1.2.2 ANTIBIOTICS AND THEIR MODE OF ACTION

Antibacterial drugs can be broadly divided into two classes: bacteriocidal and bacteriostatic.

Bacteriocidal drugs are designed to kill bacteria when the drugs contact the organisms. These drugs are used in cases of extreme urgency in avian species. The bacteriocidal drugs most commonly used are: penicillins, cephalosporins, quinolones, and aminoglycosides. All these drugs are designed to target mainly Gram-negative bacteria.

Bacteriostatic drugs hold the organisms in stasis or prevent them from multiplying without directly killing them. These drugs are compounds such as the tetracyclines. They are specifically used for *Chlamydia* infections. Due to the unusual nature of the chlamydial organism, only the tetracyclines or tetracycline-like drugs tend to be effective. The bacteriostatic drugs essentially hold the chlamydial organisms in statis until the immune system eliminates them or the bacteria die. These drugs function by interfering with protein synthesis of the bacteria (Styles, 1996b:2).

Antibiotics usually are either broad or narrow in their spectrum of activity. A broad-spectrum antibiotic tends to be active against a broader range of bacteria including both Gram-negative and Gram-positive organisms, while narrow spectrum antibiotics are active against either Gram-positive or Gram-negative organisms (United, 2001:4).

1.2.3 ANTIBIOTIC RESISTANCE

Antibiotic resistance is a global problem that affects both humans and animals. The development of resistance is a consequence of the use of antimicrobials (United, 2001:5). Resistance to antimicrobials existed even before antimicrobials were used.

Resistance depends on different mechanisms and more than one mechanism may operate for the same antimicrobial. Micro organisms resistant to a certain antimicrobial may also be resistant to other antimicrobial that share a mechanism of action or attachment. Such relationships, known as cross-resistance, exist mainly between agents that are closely related chemically, but may also exist between unrelated chemicals. Micro organisms may be resistant to several unrelated

antimicrobials. Use of one such antimicrobial will therefore also select for resistance to the other antimicrobials (American, 2004b:4).

When an animal is treated with an antimicrobial drug, a selective pressure is applied to all bacteria exposed to the drug. Bacteria that are sensitive to the antibiotic are killed or put at a competitive disadvantage, while bacteria that have the ability to resist the antibiotic have an advantage and are able to grow more rapidly than more susceptible bacteria. In addition, bacteria can become resistant when resistance genes are passed from a resistant bacterium to a sensitive one. Thus, antimicrobial agents may increase the prevalence of resistant bacteria among both target pathogens and normal bacterial flora (United, 2001:5).

Broad-spectrum antibiotics lead to the development of resistance in bacteria that are not the ones involved in the infection you are treating. To minimise the development of broad-spectrum resistance, narrow spectrum, and bactericidal antibiotics should be chosen when culture and sensitivity results suggest therapeutic success (American, 2004b:5).

1.3 MUCOLYTICS

Mucolytic agents such as bromhexine reduce mucus viscosity in the tracheobronchial tree and are often prescribed for respiratory diseases. The rationale for their use is that mucus of lower viscosity is more easily expectorated during coughing and is more easily carried up the tracheobroncial tree by the mucociliary clearance mechanism. It is commonly used with antibiotics in the treatment of respiratory diseases where excess tenacious mucus is present (Debuf, 1991:177). When administered the day before basketing for a race, bromhexine insures that the pigeons have a clean throat in the basket (Jedds, 2003:1).

1.4 AVIARY MANAGEMENT

Avian management is best accomplished by the application of the Closed Aviary Concept. The goal of practicing the Closed Aviary Concept is to restrict the introduction of infectious diseases and disruptive factors into the aviary, and to prevent the spread of infectious diseases within the facility. It is much easier and more cost effective to prevent a disease than to try and treat it once it occurs in an aviary (Wissman, 1999b:1).

1.4.1 THE CLOSED AVIARY CONCEPT

The Closed Aviary Concept (CAC) is a system of specific management principles by which aviculturalists maintain their aviaries in a disease-free state. The basis of these principles is to prevent the spread of disease via the most common routes; newly acquired birds, food sources, water sources, litter material and the owner. The CAC is exactly as its name implies – closed. Once the flock is free of disease it is maintained that way (Briggs & Rupiper, 2000:2).

There are five separate areas in the CAC and each should be evaluated separately. These include Quarintine, the Breeding Aviary, the Nursery, Isolation, and the Food and Supply Storage Area.

Quarintine

All new arrivals and those returning from shows, races and other trips to the outside world should be housed in the quarintine area prior to introducing the birds to the breeding flock. This area is the primary defence to protect the collection from infectious diseases (Wissman, 1999b:3). Regardless of the intensity or number of screening tests performed, all entering breeding stock must pass through the quarantine (Model, 2004:4).

The Breeding Aviary

This is the location where the adult breeding birds are housed (Model, 2004:6). The designated breeding aviary will vary from facility to facility, and may be as simple as a room in a home, or as elaborate as entire buildings designed specifically for the birds. Breeding birds should receive a hands-on physical examination at least once a year. It must be remembered that flock health must always take precedence over the individual bird. Protection of the birds from infectious or management-induced disease should always be of primary concern (Wissman, 1999b:3).

The Nursery

This is the location where young are hand-fed and raised (Model, 2004:6). The nursery should be a low traffic area, and should be kept scrupulously clean. Only birds hatched at the facility should be raised in the nursery. Other birds and animals should be strictly forbidden from entering the nursery. Human traffic should also be minimised (Wissman, 1999b:4).

Isolation

Occasionally disease may strike even the closed aviary from unavoidable sources like contaminated feed, water and litter as well as insect and rodent pests. In these cases, affected birds are placed in isolation and must earn their way back into their compartments by passing diagnostic tests. Isolated birds should not be housed with quarantined birds as they are still members of the closed aviary (Briggs & Rupiper, 2000:4).

Storage

The last area in the aviary is the storage area. This is the area within the facility where food and supplies are stored. This area should be placed so that traffic through quarantine and isolation is minimised. Feed should be stored to prevent contamination by vermin, birds and the weather (Wissman, 1999b:4).

In summary, disease can be halted by employing the CAC to any aviary, flock or loft of birds. Emphasis should be placed on establishing a clean, healthy flock and limiting birds' exposure to outside contaminants. The closer the CAC is applied in lofts and aviaries, the lower will be the costs associated with disease control and prevention with drugs (Briggs & Rupiper, 2000:5).

1.5 DOSAGE FORMS IN AVIAN MEDICINE

1.5.1 INTRODUCTION

Selecting the route of drug administration in birds requires careful consideration. Factors to consider when selecting a route include:

- The infection site;
- The severity and type of infection;
- The number of birds to be treated;
- The ability of the owner to complete the treatment;
- The availability and cost of appropriate drug formulations, and
- The frequency of administration.

The following dosage forms and administrations will be discussed in this section: Water medications, feed medications, oral medications, parenteral therapy, topical therapy and nebulisation therapy.

1.5.2 WATER-BASED DRUG ADMINISTRATION

Advantages

Administration of drugs in the drinking water is always preferable as birds will drink when they will not eat (Debuf, 1991:20). It is useful in the therapy of flocks and wild birds because the birds will frequently self-dose during the day. One of the less obvious advantages of the use of water-borne antibiotics in flocks is in the reduction of the spread of disease by water contamination. Pathogens multiply in the oropharynx and are spread by the fecal and oral route. Infected birds may contaminate water supplies by drinking or defecating in a water bowl. Antibiotics in the water supply decrease much of this spread while combating bacteria in their primary ports of entry (Clubb, 1984:358).

Disadvantages

Medications may be rejected due to colour or taste (Clubb, 1984:358). Fluid intake by the birds may also vary due to the climate, to the ease of access or hygiene of drink dispensers, or to the unpalatability of the water due to the drug incorporated (Debuf, 1991:20). Male birds in breeding flocks may consume large amounts of water which may lead to an overdose of the drug. Anorexic birds may not consume adequate amounts of water, and polydipsic birds may increase water consumption, resulting in quite a variation in antibiotic dosage. Some drugs quickly lose their potency in water mixes (Clubb, 1984:358).

1.5.3 FOOD-BASED DRUG ADMINISTRATION

Advantages

Administration of medications in the feed is a more reliable acceptable way of medicating. If the medication can be administered by addition to a favourite food, the chances of acceptance are higher (Clubb, 1984:358). Food-based drug administration is easy, since the birds will self-medicate several times daily. There's no need to capture or handle the bird, whilst food consumption is more consistent than water consumption (Ritchie *et al.*, 1994:439).

Disadvantages

Sick birds consume less food making it difficult to achieve therapeutic concentrations with food-based administration (Ritchie *et al.*, 1994:439). Absorption of the drug may be unpredictable because of binding to feed ingredients (Debuf, 1991:20).

1.5.4 ORAL MEDICATION

Advantages

A precise dose can be administered and dosing is easy. Drugs may be given by gavage, using a metal tube or plastic catheters. This direct method of medication is very reliable, but requires frequent handling (Debuf, 1991:37).

Disadvantages

The frequent handling of the bird may cause stress (Debuf, 1991:37). Rapid administration may result in exhalation of the drug through the nares or in aspiration. Passage of drugs through the choana and nasal passages is very uncomfortable to the bird and contributes to fear and stress during treatments. Some suspensions may be damaging or irritating to nasal mucosa (Clubb, 1984:359).

1.5.5 PARENTERAL THERAPY

1.5.5.1 INTRAMUSCULAR INJECTION

Advantages

An exact dose can be administered, and absorption is rapid (Ritchie *et al.*, 1994:440). Intramuscular injections are given in the posterior part of the pectoral muscles or the quadriceps muscle. Birds have a renal-portal system and some fraction of a medicament may therefore be excreted before reaching the systemic circulation when the drug is given into the leg muscles (Debuf, 1991:37).

Disadvantages

Not all antibiotics can be given by intramuscular injection, and injections may be painful and cause muscle necrosis (Ritchie *et al.*, 1994:440).

1.5.5.2 SUBCUTANEOUS INJECTION

Advantages

An accurate dose and large volumes of the drug can be administered (Ritchie *et al.*, 1994:440). Subcutaneous injections may be given in the groin and over the breast, but are most readily given at the back of the base of the neck (Debuf, 1991:37).

Disadvantages

Full restraint is required. Birds have very thin skin and fluid will often leak out of the injection site. Irritating drugs may cause skin necrosis and ulceration (Ritchie *et al.*, 1994:440).

1.5.5.3 INTRAVENOUS INJECTION

Advantages

An exact dose can be given and therapeutic levels are rapidly achieved (Ritchie *et al.*, 1994:440). Intravenous injections are given into the right jugular or the brachial vein. Intravenous injections are preferred for critically ill birds (Debuf, 1991:37).

Disadvantages

The bird must be fully restrained. Avian veins are fragile and leakage of a drug from the vessel, as well as haematoforma formation, are common (Ritchie *et al.*, 1994:440).

1.5.6 TOPICAL THERAPY

Advantages

Topical preparations of antibiotics and antifungal agents can be useful in treating avian diseases (Clubb, 1984:360).

Disadvantages

Preparations applied topically should be used sparingly. Ointments and creams used in excess are easily spread through preening and may damage plumage, which can lead to loss of body heat (Debuf, 1991:37).

1.5.7 NEBULISATION THERAPY

Advantages

Air sacculitis or deeply seated respiratory infections may be treated by nebulisation (Clubb, 1984: 360).

Disadvantages

At rest, there is little or no air exchanges in much of the respiratory tract. It has been suggested that only 20% of the respiratory tract would be reached by nebulisation (Ritchie *et al.*, 1994:441). The equipment produced for human use may be employed, although the droplet size of the aerosol produced may be too large to penetrate sufficiently into the avian respiratory system (Debuf, 1991:37).

1.6 CONCLUSION

It comes as no surprise that pigeons have become domesticated and used as a source of food, income, sport and entertainment for centuries. Their docile nature, prolific reproductive capacity and minimal housing and nutritional requirements have helped them achieve this popularity. Today, pigeon racing is a popular sport world-wide, and pigeons are trained to race in meets with enormous purse prizes.

Respiratory diseases are one of the most feared health problems for the experienced flier. It is the major cause of poor performance and pigeon loss during the race season. Chlamydiosis is a respiratory disease commonly found in the loft. It is effectively treated with an antibiotic, doxycyline.

Currently available doxycycline preparations lose their potency quickly when mixed with water due to oxidation of the drug. Generally the pigeons are treated at least 20-30 days for effective treatment against the disease, making it very costly for the pigeon owner.

This identified the opportunity of developing a stable, cost-effective, water-based powder, containing doxycyline HCl, and a direct compressed tablet containing doxycyline HCl and bromhexine HCl for individual treatment of the pigeons.

CHAPTER 2
PHYSICOCHEMICAL PROPERTIES OF DOXYCYCLINE HCl AND BROMHEXINE HCl

2.1 INTRODUCTION

Doxycycline HCl is a broad-spectrum tetracycline antibiotic with a wide range of activity against gram + and gram – bacteria. It is considered the most successful agent for the treatment of Chlamydiosis, a respiratory disease in avian species. It is prepared by chemical synthesis, being a semi-synthetic tetracycline derived from oxytetracycline, and is recrystallised from hydrochloric acid and ethanol as the hemihydrate hemialcoholate (Dollery, 1999:D229). Bromhexine HCl is an expectorant drug, promoting bronchial secretion and having mucolytic properties (Bechgaard & Nielsen, 1982:392). It is commonly used in respiratory infections of the pigeon loft.

2.2 DOXYCYCLINE HCl

2.2.1 DESCRIPTION OF DOXYCYLINE HCl

Chemical Name

(4S,4aR,5S,5aR,6R,12aS)-4-dimethyl-amino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide.

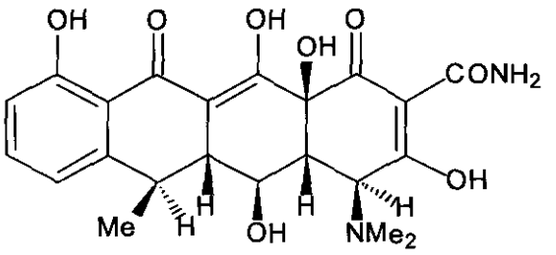
Proprietary Names

Vibramycin®, Ornicure®, Vibra-tabs®, Orni-tab®, Ornimix®.

Non-proprietary Name

Doxycycline hydrochloride

Structural Formula



,HCl, $1/_2$ C_2H_5OH, $1/_2$ H_2O

Empirical Formula

$C_{22}H_{24}N_2O_8$,HCl,$½C_2H_6O$,$½H_2O$

Molecular Weight

512.9 g.

Appearance, Colour and Taste

Doxycycline HCl is a yellow crystalline powder with a bitter taste.

2.2.2 PHYSICOCHEMICAL PROPERTIES

Melting Range

About 200°C with decomposition.

Solubility

Doxycycline HCl is soluble 1 in 3 of water and 1 in 4 of methanol. It is sparingly soluble in ethanol, and practically insoluble in chloroform and in ether. It dissolves in aqueous solutions of alkali hydroxides and carbonates (Lund, 1994:851).

X-Ray Powder Diffraction (XRPD)

The diffraction pattern of doxycycline HCl is illustrated in Figure 2.1, which shows the characteristic XRPD patterns of pure doxycycline HCl.

Infrared Spectroscopy (IR)

The IR absorption spectrum of doxycycline HCl is shown in Figure 2.2.

Thermal Behaviour

The differential scanning calorimetry (DSC) thermogram for doxycycline HCl is illustrated in Figure 2.3, which shows the characteristic melting point at 175.68°C.

Dissociation Constants

pK_a 3.5, 7.7, 9.5 (20°).

Partition Coefficient

Log *P* (octanol/pH 7.5), -0.2

2.2.3 PHARMACOKINETICS

2.2.3.1 Absorption

Doxycycline HCl is readily and almost completely absorbed from the gastrointestinal tract and absorption is not significantly affected by the presence of food in the stomach or duodenum. Mean peak plasma concentrations of 2.6 µg per mL have been reported 2 hours after a 200 mg dose by mouth, falling to 1.45 µg per mL at 24 hours. After intravenous infusion of the same dose peak plasma concentrations are briefly somewhat higher, but become very similar to those after oral administration following equilibration into the tissues (Reynolds, 2002:200).

2.2.3.2 Distribution

From 80 to 95% of doxycycline HCl in the circulation is reported to be bound to plasma proteins. Its biological half-life varies from about 12 to 24 hours. Doxycycline HCl is more lipid-soluble than tetracycline (Reynolds, 2002:200). Tissue distribution is good, with a tissue/serum concentration ratio always greater than 1 except in the gut and lymphoid tissue. There is a strong affinity for renal and lung tissue (Dollery, 1999:D229).

2.2.3.3 Excretion

Doxycycline HCl is eliminated in the urine and in the bile. 20-26% of the active drug is excreted in the urine within 48 h (40% in 2 h) and 20-40% in the faeces over the same period. No significant metabolism occurs (Dollery, 1999:D230).

2.2.4 PHARMACOLOGY

2.2.4.1 Mode of Action

Doxycycline HCl has its main mechanism of action on protein synthesis. It is more lipid soluble than other tetracyclines (except minocycline) and passes directly through the lipid bilayer of the bacterial cell wall. In addition, an energy-dependant active transport system pumps the drug, like all tetracyclines, through the inner cytoplasmic membrane. Once inside the bacterial cell doxycycline HCl inhibits protein synthesis by binding specifically to the 30S ribosomes. The drug appears to prevent access of amino-acyl tRNA to the acceptor site on the mRn-ribosome complex. This prevents the addition of amino acids to the growing peptide chain (Dollery, 1999:D229).

2.2.4.2 Therapeutic Uses

Doxycycline HCl is primarily a bacteriostatic antibiotic. It has similar spectrum of activity to other tetracyclines, but in particular more active against *Staphylococcus aureus* and *Nocardia.* The drug is often active against penicillin-resistant strains of *Staph. aureus* and against strains of those organisms that are resistant to other tetracyclines. Doxycycline HCl is active against most strains of *Escherichia coli, Proteus mirabilis, Klebsiella, Bacteroides fragilis, Haemophilus influenzae, H. duceyi, Actinomyces, Brucella, Vibrio cholerae, Nocardia, Chlamydia, Mycoplasma*, and a wide range of *Rickettsiae.* It is also active against spirochetes such as *Borellia recurrentis, Treponema pallidum, T. pertenue* and *Plasmodium falciparum.* Like all tetracyclines, it will alter the enteric flora (Dollery, 1999:D229).

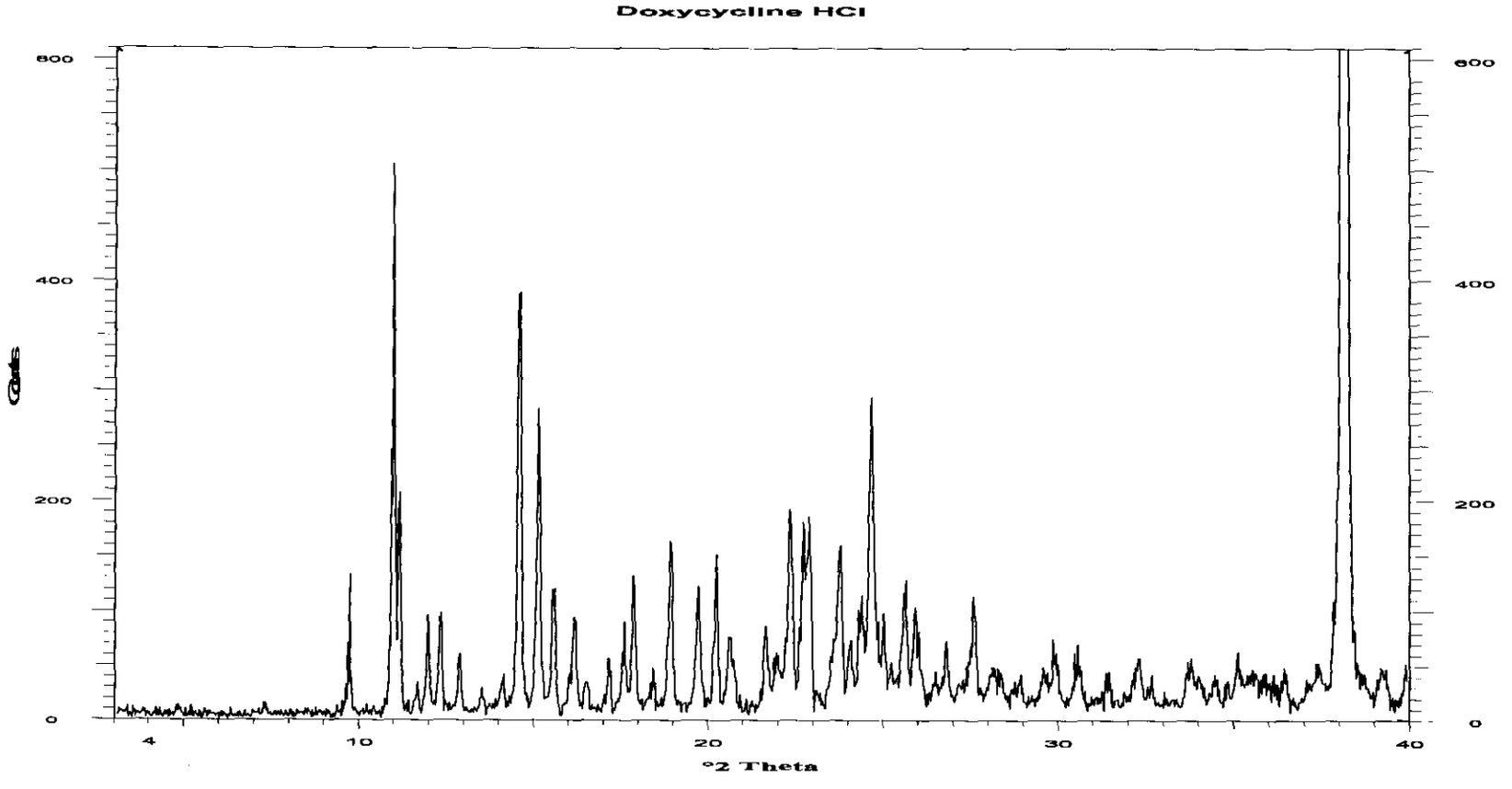


Figure 2.1 XRPD pattern of doxycycline HCl.

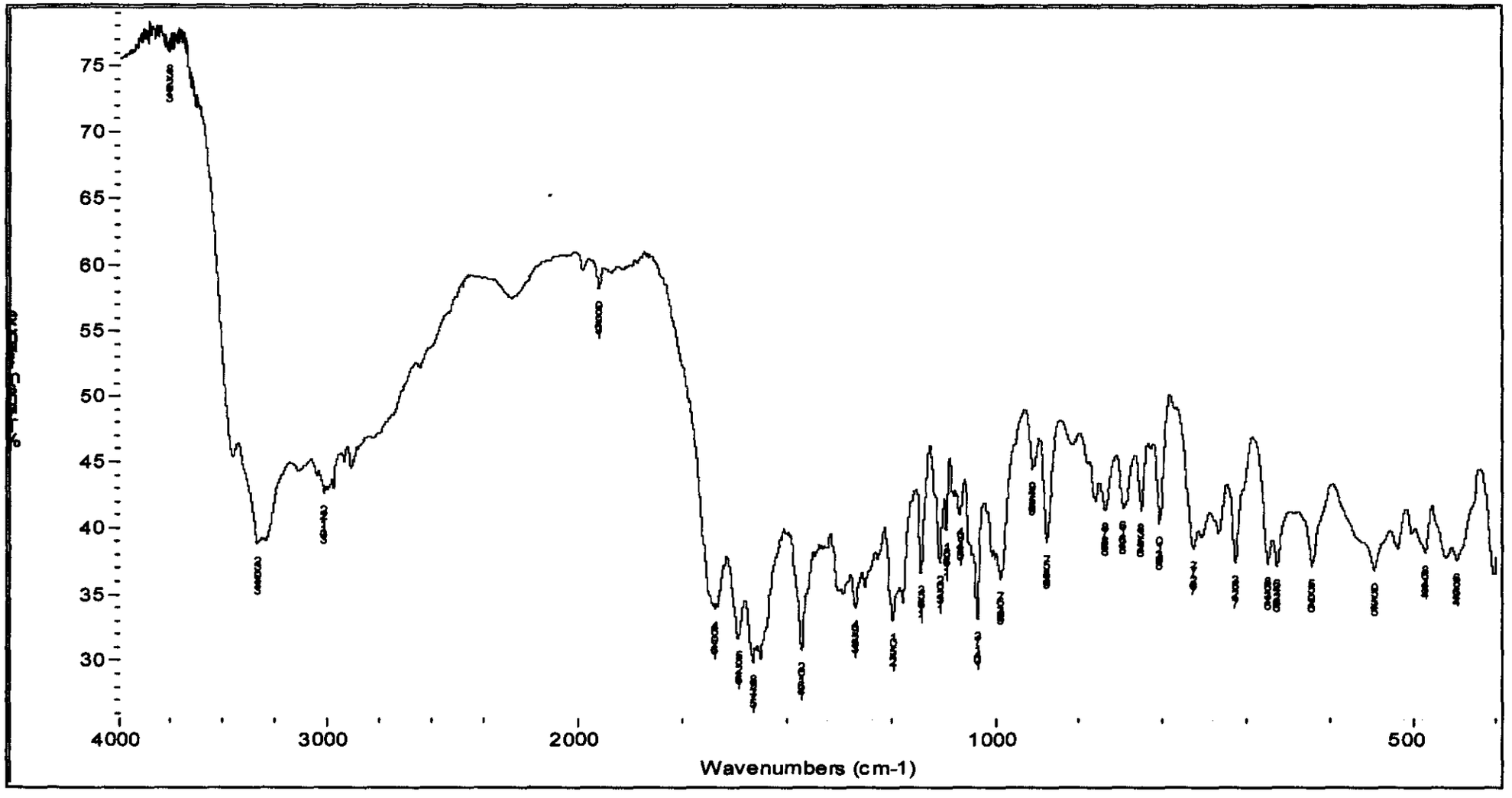


Figure 2.2 Infrared absorption spectrum of doxycycline HCl.

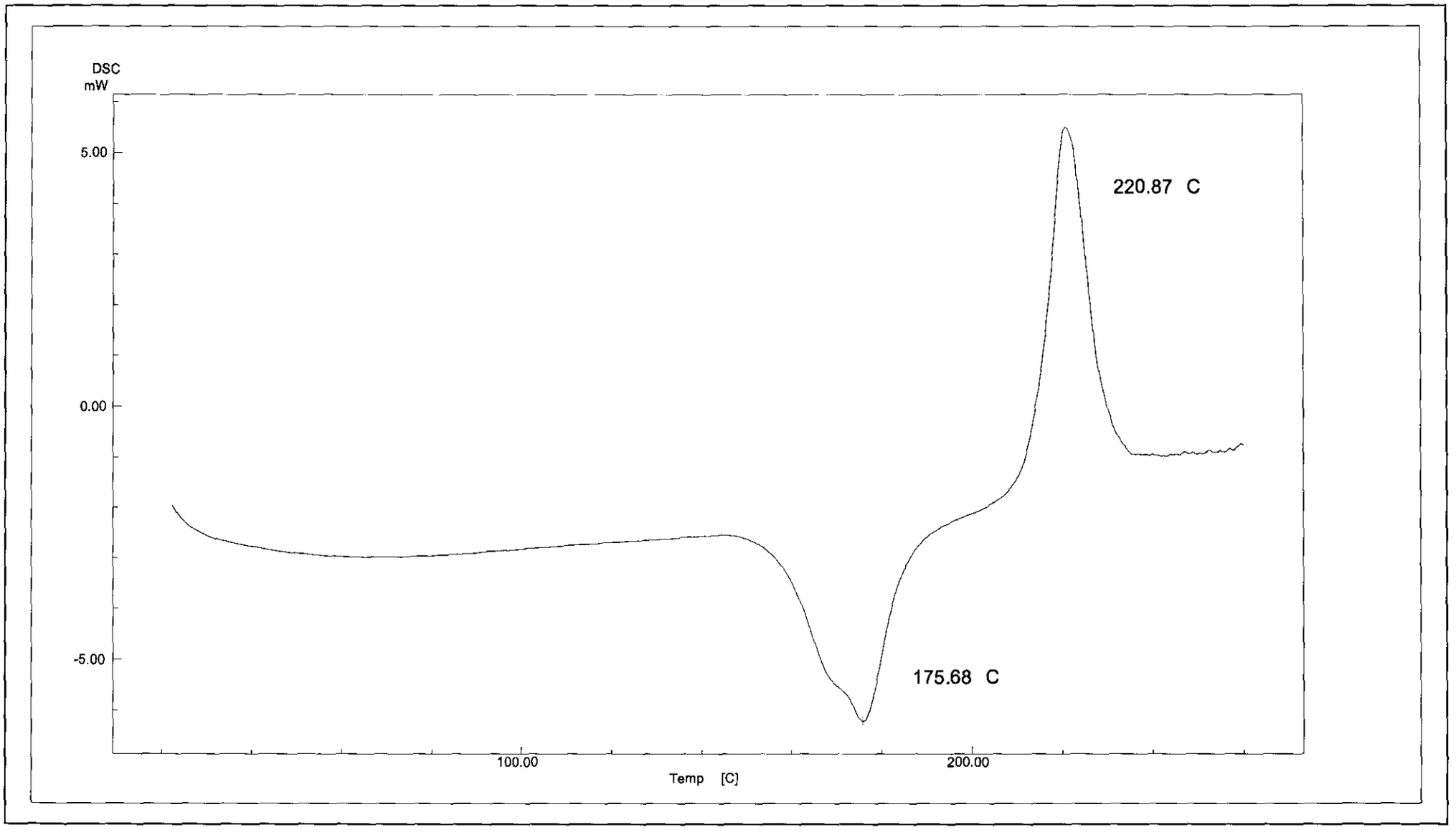


Figure 2.3 DSC thermogram of doxycyline HCl.

2.3 BROMHEXINE HCl

2.3.1 Description of bromhexine HCl

Chemical Name

2-Amino-3,5-dibromobenzyl(cyclohexyl)methylammonium chloride.

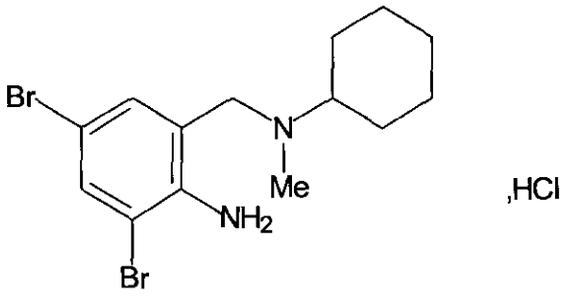
Proprietary Names

Bisolvon®, Broncholin®, Alupen expectorant®, Bisolvomycin®.

Non-proprietary Name

Bromhexine hydrochloride

Chemical Structure



Empirical Formula

$C_{14}H_{21}Br_2ClN_2$

Molecular Weight

412.6 g.

Appearance, Colour and Taste

A white crystalline powder.

2.3.2 PHYSICOCHEMICAL PROPERTIES

Melting Range

About 235°C.

Solubility

Bromhexine HCl is soluble 1 in 250 parts of water, 1 in 100 parts of ethanol, 1 in 300 parts of chloroform and 1 in 50 parts of methanol. It is soluble in glacial acetic acid but practically insoluble in acetone (Clarke, 1986:401).

X-Ray Powder Diffraction

The diffraction pattern of bromhexine HCl is illustrated in Figure 2.4, which shows the characteristic XRPD pattern of pure bromhexine HCl.

Infrared Spectroscopy

The IR absorption spectrum of bromhexine HCl is shown in Figure 2.5.

Thermal Behaviour

The differential scanning calorimety (DSC) thermogram for bromhexine HCl is illustrated in Figure 2.6, which shows the characteristic melting point at 243.52°C.

2.3.3 PHARMACOKINETICS

2.3.3.1 Absorption

Bromhexine HCl is rapidly absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism in the liver: its oral bioavailibilty is stated to be only about 20%. Administration of bromhexine HCl by mouth to healthy subjects produced peak plasma concentrations after about 1 hour (Reynolds, 2002: 1086).

2.3.3.2 Distribution

It is widely distributed to body tissues. Bromhexine HCl is highly bound to plasma proteins. It has a terminal elimination half-life of up to about 12 hours. Bromhexine HCl crosses the blood-brain barrier and small amounts cross the placenta (Reynolds, 2002:1086).

2.3.3.3 Excretion

About 70% of an oral dose is excreted in the urine in 24 hours as metabolites with less than 1% as unchanged drug (Clarke, 1986:401). After an intra-venous dose, about 50% is excreted in urine in 24 hours and, in 5 days, about 80% is excreted in urine and 5% in faeces (Lund, 1994:112).

2.3.4 PHARMACOLOGY

2.3.4.1 Mode of Action

Bromhexine HCl is a mucolytic agent which changes the structure of bronchial secretions by rarefication and fragmentation of the mucopolysaccharide fibres, leading to a reduction in the viscosity of the sputum (Lund, 1994:112).

2.3.4.2 Therapeutic Uses

It is used in the treatment of conditions of the respiratory tract associated with retention of mucous secretions, such as bronchitis and asthma (Lund, 1994:112). Bromhexine HCl has also been used orally and topically in the treatment of dry eye in Sjögren's syndrome but results has been conflicting, and it appears to have no effect on tear secretion in healthy subjects (Reynolds, 2002:1086).

2.4 CONCLUSION

The information in this chapter created an appreciation of the veterinary value of doxycyline HCl as a broad-spectrum antibiotic, and bromhexine HCl as a mucolytic agent, in the treatment of avian diseases.

The effective anti-bacterial activity of doxycycline HCl against *Chlamydia psittaci*, and the reduction of the viscosity of the mucus by bromhexine HCl, makes it an excellent combination for the treatment of respiratory infections in pigeons.

Not only must the physicochemical aspects of drug formulation be considered in veterinary formulation design, but also the difference in species, the route and method of delivery, and the product stability storage requirements.

Water-based powders are one of the easiest routes of administration for treatment of pigeons. An overview of the three different dosage forms (direct compressed tablet, water-based powder and ophthalmic solution) used in this study, for the treatment of Chlamydiosis in pigeons, will be given in the next chapter.

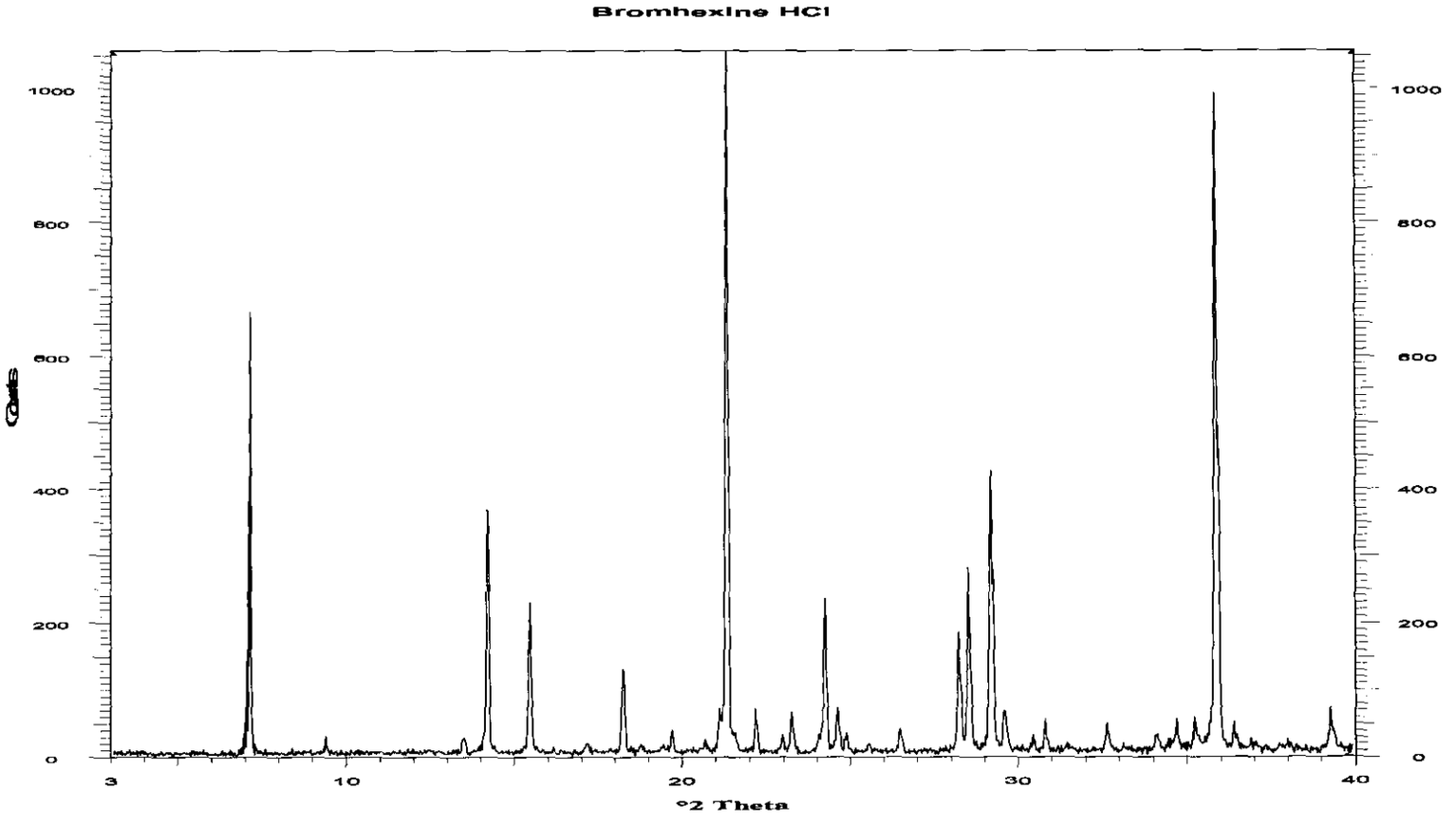


Figure 2.4 XRPD pattern of bromhexine HCl.

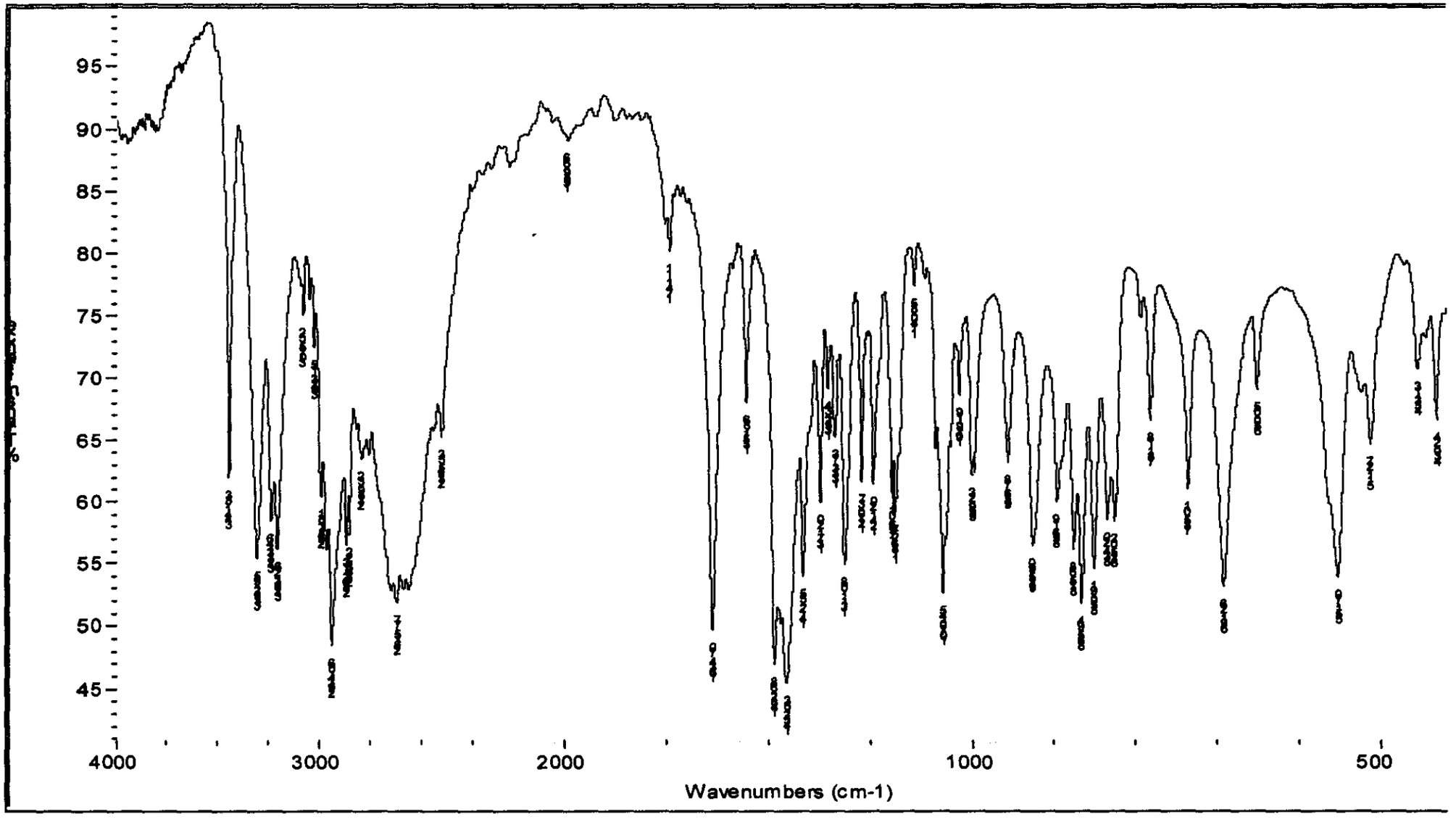


Figure 2.5 Infrared absorption spectrum of bromhexine HCl.

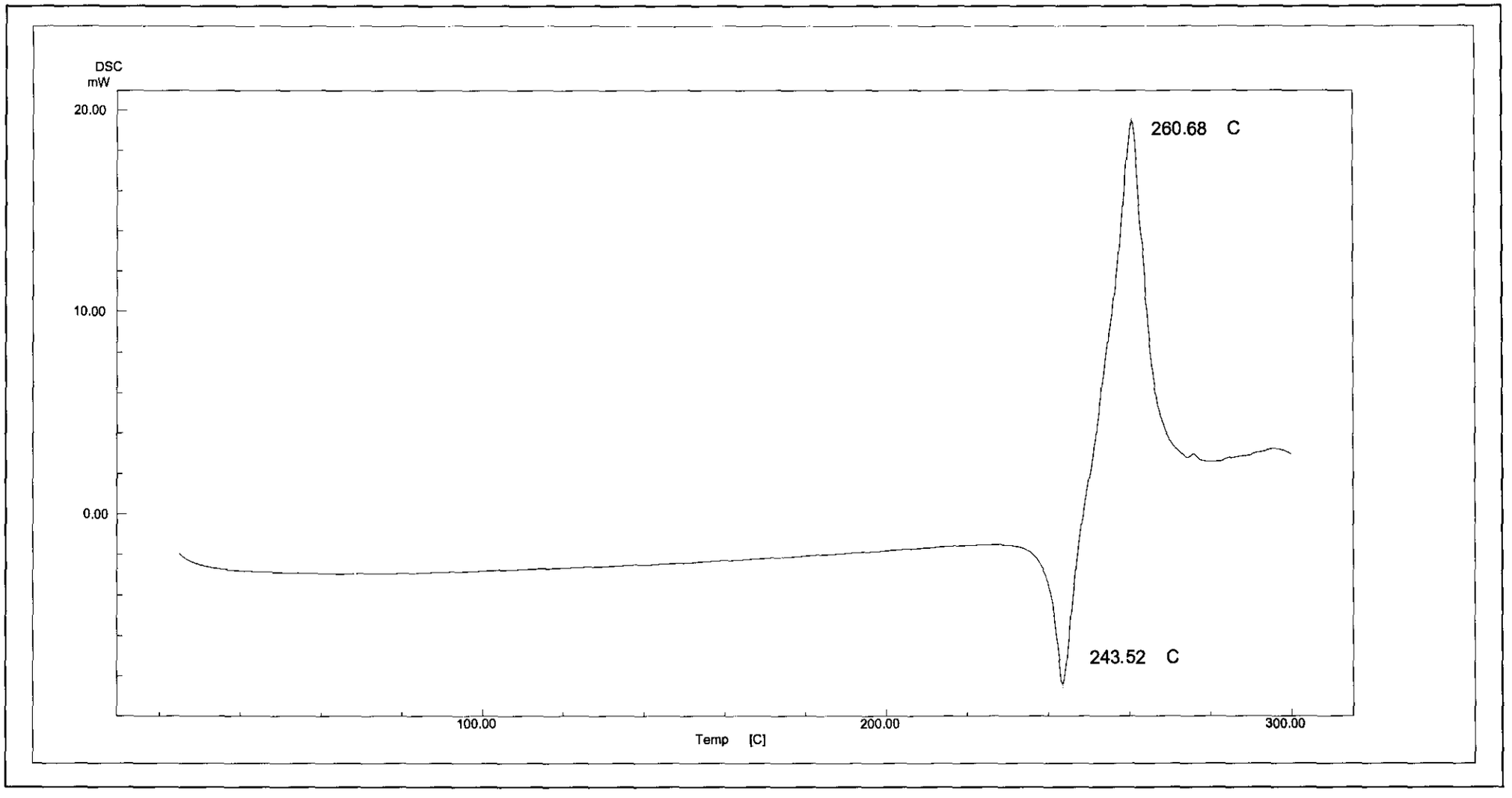


Figure 2.6 DSC thermogram of bromhexine HCl.

CHAPTER 3

AN OVERVIEW OF TABLETS, POWDERS, OPHTHALMIC SOLUTIONS AND STABILITY

3.1 INTRODUCTION

The different dosage forms available can be viewed as the delivery systems of drugs that are used to introduce a drug into the body of a patient (human or animal) (Kenneth, 1990:1545).

Bioavailability is the amount of intact drug that is found in the systemic circulation after administration (Feldman & Brazeau, 1994:97).

There is a relationship between the bioavailability and the therapeutic response to a drug: an increase in the bioavailability causes an increase in the therapeutic response. It is desirable that the onset of the therapeutic response be as quick as possible. This can be achieved by increasing the rate of absorption of the drug. The uniformity and reproducibility of the therapeutic response are very important. This is generally determined by the rapidness and completeness of drug absorption (Mayersohn, 1990:23).

These factors illustrate that the designed dosage form must improve or complement the therapeutic effectiveness of the specific drug (Kenneth, 1990:1545).

An overview of the three dosage forms used in this study (direct compressed tablet, water-soluble powder and ophthalmic solution) are given in this chapter.

3.2 TABLETS

The tablet is the most frequently prescribed commercial dosage form. This dosage form is stable, elegant, and effective. It provides the patient with a convenient product for handling, identification, and administration (Loyd, 1997:107).

Tablet formulation and design may be described as the process whereby the formulator insures that the correct amount of drug in the right form is delivered at or over the proper time at the proper rate and in the described location, while having its chemical integrity protected to that point (Banker *et al.*, 1980:61).

Tablets as a dosage form have several advantages for both the manufacturer and the patient. The advantages of a tablet for the manufacturer are:

- Its preparation is simple and economic, and
- It is stable and convenient in its packaging when it is transported and dispensed.

The advantages of a tablet for the patient are:

- The dosage is accurate,
- It is compact,
- It is portable,
- It is relatively tasteless, and
- It is relatively easy to administer (Rudnic & Schwartz, 1990:1633).

A validated tablet manufacturing process is one which, when all the operating variables are considered, at any extremes which could ever be encountered in practice, and under the worst possible set of circumstances, will produce products that are within specifications (Banker *et al.*, 1980:62).

The contribution and influence of the active components and nonactive components - both separately and together-must be considered to measure their impact on the pharmacological response of any tablet system. The timing of administration may affect when and how a drug will act and may be crucial in order to reduce gastric irritation; to reduce drug interactions with food, reducing their bioavailability; or enhance the solubility and bioavailability of certain drugs in foods by their administration with foods. Depending on such timing factors, plus the relationship and rationale of fast, intermediate, or slow drug release as well as other release

considerations, a particular design and tablet formulation strategy is often indicated (Banker *et al.*, 1980:62).

3.2.1 Classification of tablets

Tablets are categorised into two main groups, namely tablets manufactured of compression and tablets manufactured by means of moulding (Rudnic & Schwartz, 1990:1633).

Compressed tablets are solid dosage forms prepared by compaction of a formulation containing the drug and certain excipients selected to aid the processing and improve the properties of the product (Sheth *et al.*, 1980:109). Tablets that are categorised as being manufactured by means of compression include, uncoated, sugar coated, film coated, enteric coated, multiple compressed, controlled release, tablets for solution, effervescent, compressed suppositories or inserts, buccal, sublingual and chewable tablets (Rudnic & Schwartz, 1990:1633).

Tablets manufactured by means of moulding are dispensing and hypodermic tablets. These tablets are manufactured from a moist mixture using a triturate mould (Rudnic & Schwartz, 1990:1634).

In this study compressed tablets were made, because of their simplicity and easiness of preparation. The easier it is to manufacture a dosage form, the easier it will be to cut down on manufacturing costs.

3.2.2 Compressed tablets

Compressed tablets are made by compressing a formulation containing a drug or drugs and excipients on a tablet press. Tablet presses operate at production rates ranging from a few to a few thousand tablets per minute. Hence, a tablet formulation must first be prepared in a form suitable for compression on a tablet press. This process is referred to as granulation (Sheth *et al.*, 1980:111).

A granulation must have good flow properties for precise volumetric feeding of the material to the die cavity, compressibility to form the compact and lubricant properties for ejection of the tablet. The methods used for preparing tablet granulations are wet and dry granulation and direct compression. The sequential steps in the

manufacture of tablets by each of these processes are shown in Table 3.1. The choice of methods depends upon a number of factors, the most important being the properties and the dose of the drug. Other factors include the choice of available equipment and relevant practical and regulatory concerns (Sheth *et al.*, 1980:111).

Table 3.1 Steps in the different methods of tablet manufacture (Sheth *et al.*, 1980:112)

Wet granulation	**Dry granulation**	**Direct compression**
1. Milling of drugs and excipients	1. Milling of drugs and excipients	1. Milling of drugs and excipients
2. Mixing of milled powders	2. Mixing of milled powders	2. Mixing of ingredients
3. Preparation of binder solution	3. Compression into large, hard tablets, to make slugs	3. Tablet compression
4. Mixing of binder solution with powder mixture to form wet mass	4. Screening of slugs	
5. Coarse screening of wet mass using 6 to 12 mesh screen	5. Mixing with lubricant and disintegrating agent	
6. Drying of moist granules	6. Tablet compression	
7. Screening of dry granules through 14 to 20 mesh screen		
8. Mixing of screened granules with lubricant and disintegrant		
9. Tablet compression		

3.3.1 Classification of powders

Historically, powders as a solid dosage form have been used as internal and external medications. Internally, they can be taken orally, administered through the nose as snuffs, or blown into a body cavity as an insufflation. Externally, solid powders can be applied to compromised areas of the body. Powders have also been used to make solutions for topical and oral use and for use as douches. These traditional applications and modes of administration of the dosage form are still used today. Additional applications have also been developed; for example, powders containing a bioadhesive material can be applied to a specific body area such that the medication will adhere for a prolonged drug effect (Loyd, 1997:78).

In this study, powders to be taken orally were developed. A powder that dissolves easily in the drinking water of the pigeons is one of the easiest methods for administrating a drug to the flock.

3.3.2 Water-based powders

The addition of medications to drinking water is controversial but is often the only practical means of drug administration. This is the least stressful means of providing medications, especially for those that are palatable (Harrison & Harrison, 1986:328). Antibiotics are commonly administered through the drinking water of pigeons. One very valuable use of water-borne antibiotics in flocks is in the reduction of the spread of disease by water contamination. They will decrease the bacterial contamination of water supplies while combating bacteria in their primary port of entry, the pharyngeal area and the intestinal tract (Harrison & Harrison, 1986:329).

Water-based powders can be used as an adjunct to direct drug administration or in situations where direct medication is impossible (Ritchie *et al.,* 1994:438). Also, if a drug is too bulky to be prepared as a capsule or tablet, it may be suitable for a powder dosage form (Loyd, 1997:78). The active ingredient in the powder must be stable and soluble in water when administered through the drinking water (Ritchie *et al.,* 1994:438).

Powders provide a rapid onset of action since they are readily dispersed, have a large surface area, and generally do not require disintegration but rather just dissolution prior to absorption (Loyd, 1997:79).

3.3.3 Powder properties

Properly prepared, powders will have a uniform, small particle size that has an elegant appearance. Generally, powders are more stable than liquid dosage forms and are rapidly soluble, enabling the drug to be absorbed quickly. The properties of powders are related to the size and surface area of the particles. For example, large particles that are denser tend to settle more rapidly than small particles; particles that are more bulky will settle more slowly. This characteristic must be considered when mixing or storing and shipping, where powders of different particle size may become segregated. Powder dosage forms have a large surface area that is exposed to atmospheric conditions. Thus, powders should be dispensed in tight containers (Loyd, 1997:79).

There are several requirements even for a simple solid drug sold as such:

- ❖ High density for economy in container size,
- ❖ Uniform density, for accuracy of dosing,
- ❖ Good flowability, for ease of filling,
- ❖ Minimum dust, if toxic or irritant,
- ❖ Minimum surface area if prone to oxidation and,
- ● Rapid solution rate, if soluble (Fishburn, 1967:185).

3.3.4 Mixing of powders

Mixing uses a process called geometric dilution; this élans that one starts with the ingredient in the smallest quantity, then adds additional ingredients in order of quantity required by approximately doubling the portion being mixed with each addition (Loyd, 1997:81).

There are two main classes of powders. One type, described as *free flowing powder*, is typified by such systems as coarse dry sand and coarse metal powders. In general, free flowing powders are relatively easy to mix, but the resultant mixture is prone to segregation of the components during subsequent handling procedures. *Cohesive powders* are ones in which the constituent fine particles are aggregated to each other by forces such as electrostatic forces and liquid bridges caused by humidity. Additives are available which can convert a cohesive powder, such as fine cement, to one that flows like water. These agents are known as flow agents or glidants. Glidants added to powdered ingredients in a mix can promote mixing but

can cause problems later in the mixture handling. In general, cohesive powders are more difficult to mix than free flowing powders, but the resultant mixtures are far less susceptible to segregation than free flowing powders (Kaye, 1997:3).

It is of utmost pharmaceutical importance that dosage forms be as uniform as possible. The uniformity of powders is intimately related to the mixing operation, which is part of almost every pharmaceutical manufacturing procedure (Carstensen, 1977:114). Powders are mixed either in active mixing machines or in passive powder mixing systems. An active mixer is one in which there are either internal moving parts to randomise the positions of the ingredients, or air jets to create convection currents and turbulence in the powder container. Ribbon mixers, tumbler mixers, high shear mixing and multimechanism mixers are all examples of active mixers. The term passive mixer is used to describe any mixing equipment in which there are no moving parts and in which the mixing of ingredients is achieved by the use of baffles or by bypass pipes moving parts of a mixture from one portion of a storage container to another. Baffled mixers and gravity in-bin mixing devices are examples of passive mixers (Kaye, 1997:218).

3.4 OPHTHALMIC SOLUTIONS

Ophthalmic formulations range from preparations meant for localised effect on the surface of the eye or preparations that have therapeutic effect on the surface of the eye and on its interior, to preparations that are administered topically for their systemic effects. Conventional ophthalmic dosage forms include solutions, suspensions and ointments. Of these types, solutions are the most commonly used preparation (Reddy & Ganesan, 1996:15). Ophthalmic solutions are sterile, free from foreign particles, and prepared especially for instillation into the eye (Loyd, 1997:219).

3.4.1 Properties of ophthalmic solutions

In preparing ophthalmic solutions, one must consider the general physico-chemical parameters: clarity, tonicity, pH/buffers, and sterility. The addition of excipients such as preservatives, antioxidants, and viscosity enhancers, as well as potential incompatibilities between these excipients and active drugs, should also be considered (Loyd, 1997:220).

Clarity

Ophthalmic solutions must be free form foreign particles. Filtration is generally used to remove these particles and to achieve clarity of the solution (Loyd, 1997:220).

Tonicity

Lacrimal fluid has an isotonicity value equivalent to that of a 0.9% sodium chloride solution. However, the eye can tolerate a value as low as 0.6% and as high as 1.8% sodium chloride equivalency. Some ophthalmic solutions will be hypertonic by virtue of the high concentration required of the drug substance. Others will be hypotonic and will require adjustment to attain the proper tonicity range; sodium chloride, boric acid, and dextrose are commonly used for this purpose (Loyd, 1997:220). A hypotonic solution may cause corneal edema, while hypertonic drops may cause discomfort or irritate the eye upon instillation due to their temporary dehydrating effect on corneal epithelium (Gangrade *et al.*, 1996:380). The ideal tonicity is 300 mOsm/L; however, a range of 200 to 600 mOsm/L is acceptable (Loyd, 1997:220).

pH and buffering

Ophthalmic solutions are ordinarily buffered at the pH of maximum stability for the drugs they contain. The buffers are included to minimise any change in pH that may occur during the storage life of the drug; this change can result from carbon dioxide absorbed from the air or from hydroxyl ions absorbed from a glass container. Changes in pH can affect the solubility and the stability of drugs; consequently, it is important to minimise such fluctuations (Loyd, 1997:220). Three important phenomena related to pH of tear fluid should be considered in the formulation of a drug solution: maintaining physiological pH after applying solution; increasing the drug penetration with change in the degree of drug ionisation; and product stabilisation (Singh & Shah, 1996:34). The buffer system should be designed so that it maintains the pH throughout the expected shelf life of the product, but has a buffer capacity that is low enough that when the ophthalmic solution is dropped into the eye, the buffer system of the tears will rapidly bring the pH of the solution back to that of the tears. Generally a buffer capacity of less than 0.05 is desired; pH in the range of 4 to 8 is considered optimum (Loyd, 1997:222).

Sterility

All ophthalmic formulations must be sterile and must comply with the compendial sterility test requirements which are described in different pharmacopoeias. Multiple dose ophthalmic drug formulations containing aqueous bases of vehicles must also

show preservative effectiveness to ensure the sterility of the product during shelf-life and use conditions (Gangrade *et al.*, 1996:391). Sterility is best achieved through sterile filtration, which involves using a sterile membrane filter of 0.45 µm or 0.2 µm pore size and filtering into a sterile container. Other methods of sterilising ingredients or components of ophthalmics include dry heat; steam under pressure, that is, autoclaving, and gas sterilisation with ethylene oxide (Loyd, 1997:222).

3.4.2 Excipients for ophthalmic solutions

The selection of excipients and their functions depends on the type of topical dosage form being developed. In an ophthalmic solution or suspension, excipients are used for one of the following purposes:

- To adjust the isotonicity or osmolality;
- To adjust the pH;
- To control the viscosity;
- To preserve the formulation;
- To stabilise the solution or suspension.

Sometimes an excipient may serve more than one purpose. For example, bensalkonium chloride can be used both as an anti-microbial as well as a surface active agent. A buffering agent added for pH adjustment can also be used to render the solution isotonic (Gangrade *et al.*, 1996:379).

Tonicity agents

A solution that is instilled into the eye must preferably be isotonic or iso-osmotic with the tears. Ophthalmic solutions or suspensions can be made isotonic by the use of tonicity agents such as sodium chloride, potassium chloride, buffering salts, dextrose and mannitol (Gangrade *et al.*, 1996:380).

Buffering agents

The pH of tears is close to neutral and is regulated by various substances dissolved in the aqueous layer of the tears, including bicarbonate and proteins. The pH also depends on the duration of the eye closure and the diurnal changes owing to the CO_2 evaporation. The pH of an ophthalmic formulation has to be adjusted and suitably buffered for one or more of the following reasons:

- For greater comfort to the eye;
- To make the formulation more stable;

- To increase the solubility of the medicament;
- To enhance the absorption and therapeutic activity of the medicament;
- To achieve maximum preservative efficacy (Gangrade *et al.*, 1996:381).

Two commonly used buffering agents are boric acid and phosphate vehicles. Other buffer systems that are used to prepare ophthalmic formulations in the acidic pH range are acetic acid/sodium acetate and citric acid/sodium citrate buffers (Gangrade *et al.*, 1996:383).

Viscosity enhancers

Viscosity enhancing polymers used in ophthalmic solutions impede the drainage to prolong the retention time of the drug on the surface of the eye for increased bioavailability of the drug (Gangrade *et al.*, 1996:387). Additives that increase viscosity are shown in Table 3.2.

Table 3.2 Viscosity enhancers for ophthalmic preparations (Loyd, 1997:223)

AGENT	USUAL MAXIMUM CONCENTRATION
Hydroxyethylcellulose	0.8%
Hydroxypropyl methylcellulose	1.0%
Methylcellulose	2.0%
Polyvinyl alcohol	1.4%
Polyvinylpyrrolidone	1.7%

With all of these enhancers, however, it is important that the clarity of the solution be maintained (Loyd, 1997:222).

Preservatives

Since most ophthalmic solutions and suspensions are prepared in multi-use containers, they must be preserved. The preservative used must be compatible with the active drug as well as with all the other excipients in the product (Loyd, 1997:222).

The following considerations must be taken into account when selecting a preservative for an ophthalmic formulation:

- Irritation factor/cytotoxicity;
- pH;
- Compatibility with other ingredients;

- ❖ Synergism or antagonism;
- ❖ Processing conditions such as heat;
- ● Packaging.

A list of preservatives used in ophthalmic formulations and their concentration ranges are summarised in Table 3.3 (Gangrade *et al.*, 1996:384).

Table 3.3 Ophthalmic preservatives (Gangrade *et al.*, 1996:385)

PRESERVATIVE	CONCENTRATION RANGE
Bensalkonium chloride	0.004-0.02%
Bensethonium chloride	Up to 0.01%
Chlorhexidine	0.005-0.01%
Chlorobutanol	Up to 0.5%
Methyl paraben	0.03-0.1%
Phenylethyl alcohol	Up to 0.5%
Phenylmercuric acetate	0.002-0.004%
Phenylmercuric nitrate	0.002-0.004%
Propyl paraben	Up to 0.01%
Thimerosal	Up to 0.01%

Surfactants

Surfactants may be added to an ophthalmic solution to solubilise or disperse the drug effectively. One of the most commonly used surface-active agents in ophthalmic formulations is bensalkonium chloride. It is the surfactant of choice because of its anti-microbial activity. Other surfactants used are bensethonium chloride, polysorbate 20, polyoxyl 40 stearate, alkyl aryl polyether alcohol, polyoxypropylene-polyoxyethylenediol, and dioctyl sodium sulfosuccinate (Gangrade *et al.*, 1996:390).

Stabilising agents

If the drug molecule is susceptible to degradation by oxidation, stabilisers such as chelating agents or anti-oxidants are included in an ophthalmic formulation to improve the shelf-life of the product. In general, several anti-oxidant or their combinations have to be tested in a formulation to achieve the best stability possible. Sodium bisulfite and sodium metabisulfite are strong reducing agents and are used as anti-oxidants in ophthalmic formulations containing epinephrine or phenylephrine. Other stabilising agents in use include ascorbic acid, acetylcysteine, 8-hydroxyquinoline, sodium thiosulfate, and antipyrine (Gangrade *et al.*, 1996:390).

3.5 STABILITY OF FORMULATIONS

The term "pharmaceutical stability" implies several things. First of all, it is applied to the chemical stability of a drug substance in a dosage form, and this is the most common interpretation. However, the performance of a drug when given in a particular dosage form depends not only on the content of the drug substance but also on its pharmaceutical properties (dissolution, disintegration, hardness etc.). All of these must, therefore, be a part of the stability program (Carstensen, 1990a:4).

The goal of stability testing, according to the International Conference on Harmonisation (ICH) Expert Working Group (2000:1), is to deterime how the quality of a formulated product will change with time when being exposed to changing environmental conditions of temperature, humidity and light. The information gathered is then used to establish the expiration date of the product and the suitable storage conditions.

Potential incompatibilities, not only between different active ingredients in the same formulation (Fung, 1990:214), but also between the active ingredient and any of the excipients, must be determined during the preformulation studies (Carstensen, 1990b:239). Another factor that needs to be determined during the stability studies is the possible effect of the packaging material on the final product (Hem & Stoll, 1990:748). All of these determinations contribute in ensuring the stability of the active ingredient and of the excipients in the final formulation.

The stability of each different formulation (e.g. for each different concentration of the active and every different packaging used) as intended for the market must be tested. Every aspect of the product including microbiological attributes, must be tested; especially those prone to change during storage. The storage conditions should be as such that the thermal stability and sensitivity to moisture of the product would also be evaluated (ICH Expert Working Group, 2000:7).

Generally according to the ICH Expert Working Group (2000:9), the following conditions apply when evaluating stability. Products should be evaluated for a minimum period of twelve months when long-term stability is tested and for a minimum period of six months when intermediate and accelerated stability are tested. The storage condition for a long-term stability test is 25°C ± 2°C at 60% RH (relative

humidity), for an intermediate stability test it is 30°C ± 2°C at 60% RH ± 5% RH, and for an accelerated stability test it is 40°C ± 2°C at 75% RH ± 5% RH.

When the product requires storage in a refrigerator, the following conditions apply. A minimum evaluation period of 12 months and storage at 5°C ± 3°C for long-term stability, and a minimum evaluation period of 6 months and storage at 25°C ± 2°C at 60% RH ± 5% RH for accelerated stability (International, 2000:11).

Stability evaluations of the products that were developed in this study were done over a period of three months, only to obtain an indication of the possible stability of these products. The accelerated stability conditions were as discussed in Chapters 5-7.

3.6 CONCLUSION

In this chapter the three different dosage forms applicable to this study, i.e. direct compressed tablets, water-based powders and an ophthalmic solution, were discussed. The insights gained will be applied during the development of the planned formulations.

Valuable considerations to be taken into account during the formulation process include that the designed dosage form must improve or complement the therapeutic effectiveness of the specific drug to be administered, the correct excipients and correct combination thereof are very important, the specific final product must possess over specific attributes, and the product must be cost-effective.

Ensuring the stability of each product is also extremely important. This is done by evaluating the stability of each product according to the guidelines as laid down by the ICH Expert Working Group.

CHAPTER 4

PREFORMULATION AND FORMULATION

4.1 INTRODUCTION

Assessment of possible incompatibilities between an active drug substance and different excipients, form an important part of the preformulation stage during the development of dosage forms (Malan, 1997:533). In a preventative role, differential scanning calorimetry (DSC) represents a potentially powerful tool in preformulation, for the screening of candidate excipients (Smith, 1982:560).

4.2 PREFORMULATION: POSSIBLE DRUG-EXCIPIENT INTERACTION

4.2.1 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Differential scanning calorimetry (DSC) is a method, which measures the difference in energy (heat flux or heat flow) between a reference (R) and a sample (S). A typical DSC thermogram is shown in Figure 4.1. The result of a DSC analysis is a thermogram, a plot of $\Delta T = T_S - T_R$ (temperature difference) versus T. The endotherm represents the process in which heat is absorbed, such as solvent loss, phase transitions, or melting. The exotherm represents recrystallisation or chemical reactions where heat is evolved. This method can be used to determine the enthalpies (ΔH) of various processes (Byrn *et al.*, 1999:84).

Thermal methods have been used successfully to study drug-excipient compatibility. In this procedure, drug and excipients are intimately mixed in ratios, varying between 10:1 and 1:10, and each mixture is analysed by DSC. HPLC analysis of the heated samples is used to interpret any changes in the DSC profile of the mixture, and the results are compared with those of the pure components (Byrn *et al.*, 1999:83).

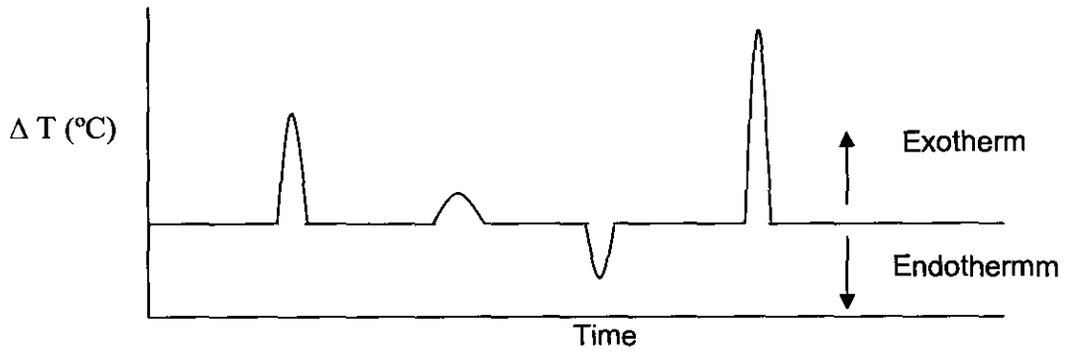


Figure 4.1 Schematic differential scanning calorimetry (DSC) thermogram (Byrn *et al.*, 1999:84).

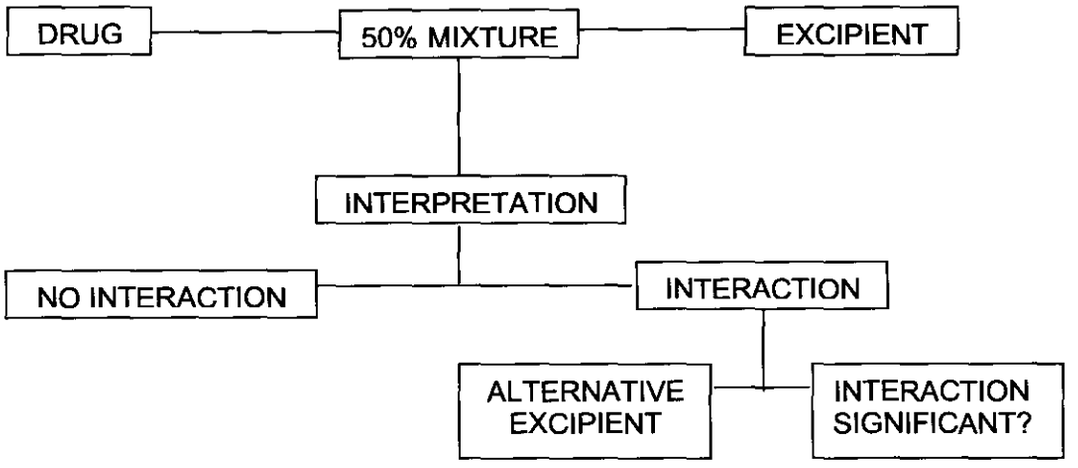
There are a number of factors that may affect the DSC curve, such as purity, heating rate, atmosphere, sampler holder, particle size and sample packing. The shape of the sample holder and whether it is open, totally sealed, or contains a pin prick to vent gases, can also affect a DSC curve. When the experiment is performed in a closed pan, the resulting atmosphere within the sample holder can greatly affect the resulting curve. A tightly sealed sample holder would not allow vapour to escape, thereby changing the behaviour of a desolvation process (Van Dooren, 1983:43).

The stability of a formulation depends, amongst other factors, on the compatibility of the active components with the excipients. It is of importance to detect any possible interactions, since it has been shown that certain interactions can either change the bioavailability or stability of a product. The excipients can affect the solid state stability of a drug in various ways; this may occur directly as a chemical reaction between the drug and the excipients, or mostly directly by sorption of moisture and/or catalysis (Botha & Lötter, 1990:332).

According to Van Dooren (1983:43) differential scanning calorimetry (DSC) is an easy and effective method to determine possible incompatibilities between the active substance and the excipients used in tablets. DSC allows the fast evaluation of possible incompatibilities between the formulation compounds derived from appearance, shift or disappearance of peaks and/or variations in the corresponding ΔH. Thermal analysis however, does not replace the chemical methods for the determination of the concentration of a drug in a dosage form and does not replace stability tests (Botha & Lötter, 1989:415).

4.2.2 TECHNIQUE

The procedure of testing is summarised below (Smith, 1982:560).



DSC requires a very small sample and is a rapid and a convenient means of screening candidate excipients during preformulation. The occurrence of a physical or chemical interaction under the test conditions cannot usually be interpreted as a definite incompatibility in the formulated product. The technique gives an early indication of potential problems, so that excipients can be avoided, or the nature of the interaction be investigated more closely (Smith, 1982:559). If there are no differences between the curves, thermal analysis will suggest that there are no differences between drug and excipient, i.e., there is no physicochemical incompatibility. Problems of interpretation arise when there are differences in these curves. The drug and excipient may still be compatible, as the DSC technique necessarily requires elevated temperatures, and these temperatures might induce reactions that do not occur at normal storage temperatures (Hardy, 1982:556).

4.2.3 INTERPRETATION OF RESULTS

The thermal properties of a mixture are a summation of that of the single substances. An interaction might be judged from DSC, by changes in transition temperature, peak shape and area and transition presence. Almost invariably there is some change in transition temperature, peak shape and area, arising from mixing the components (Smith, 1982:560). Not all changes are negative though. The formation of solid solutions and eutectic mixtures can alter thermograms without these reactions being negative to the formulation.

4.2.4 EXPERIMENTAL PROCEDURES USED

A Shimadzu DSC-50 (Shimadzu, Kyoto, Japan) was used to record the thermograms under the following conditions:

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

The instrument was calibrated using indium as a standard (melting point 156.4°C). The following determinations were carried out:

1. DSC thermograms of the active drug substances and the excipients individually.
2. DSC thermograms of the mixtures of doxycycline HCl, bromhexine HCl and excipients (ration 1:1:1) immediately after mixing.

4.2.5 MATERIALS

Compatibility between doxycycline HCl and bromhexine HCl, with commonly used excipients, was assessed prior to the tablet, powder and ophthalmic solution formulation. Table 4.1 lists the excipients screened for incompatibilities.

Table 4.1 Actives and excipients screened for possible interactions

ACTIVES & EXCIPIENTS	FUNCTION	FORMULATION
Doxycycline HCl	Active	Tablet, powder and ophthalmic solution
Bromhexine HCl	Active	Tablet and powder
Glucose	Diluent	Tablet and powder
Citric Acid	Antioxidant	Powder
Kollidon® 17 PF	Solubilisation and reduction of local toxicity	Ophthalmic solution
Sodium-meta-bisulfite	Antioxidant	Ophthalmic solution
Sodium-formaldehyde-sulfoxylate	Antioxidant	Ophthalmic solution
Kollidon® CL-M	Disintegrant	Tablet
Emcompress®	Direct binder	Tablet
Magnesium stearate	Lubricant	Tablet

4.2.6 RESULTS

Thermograms for doxycycline HCl and bromhexine HCl obtained, were compared to those of active-excipient combinations. Table 4.2 shows the thermal behaviour of the active drug substances and excipients. Table 4.3 illustrates the observed melting points of the physical mixtures. Thermal behaviour results are illustrated in Figures 4.2 - 4.7.

Table 4.2 Melting points of the active drug substances and excipients

ACTIVES & EXCIPIENTS	PEAK MAXIMUM (°C)
Doxycycline HCl	175.7
Bromhexine HCl	243.5
Glucose	83.4, 153.0, 217.8
Citric acid	151.9
Kollidon® 17 PF	119.5
Sodium-meta-bisulfite	201.3
Sodium-formaldehyde-sulfoxylate	100.8
Kollidon® CL-M	90.0
Emcompress®	187.9
Magnesium stearate	115.46

Table 4.3 Observed melting points of physical mixtures

Doxycycline HCl + Bromhexine HCl + excipient	1:1:1			
	Observed melting point			
	Excipient (°C)	Doxycycline-HCl (°C)	Bromhexine-HCl (°C)	Possible interaction
Glucose	84.4/153.5	180.2	--	✓
Citric acid	145.1	183.2	239.9	x
Kollidon® 17 PF	--	179.7	227.5	✓
Sodium metabisulfite	--	182.8	228.3	✓
Sodium fromaldehyde sulfoxylate	104.1	190.2	269.9	✓
Kollidon® CL-M	36.9	159.4	228.3	✓
Emcompress®	189.3	--	275.2	✓
Magnesium stearate	114.3	180.5	266.9	x

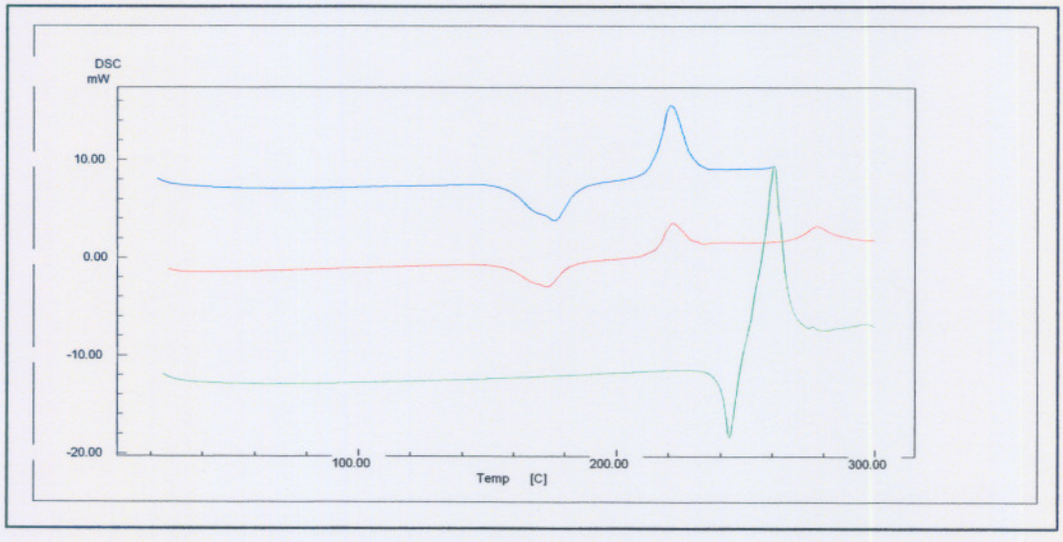


Figure 4.2 DSC analysis results of doxycycline HCl (blue), bromhexine HCl (green), and combination (red).

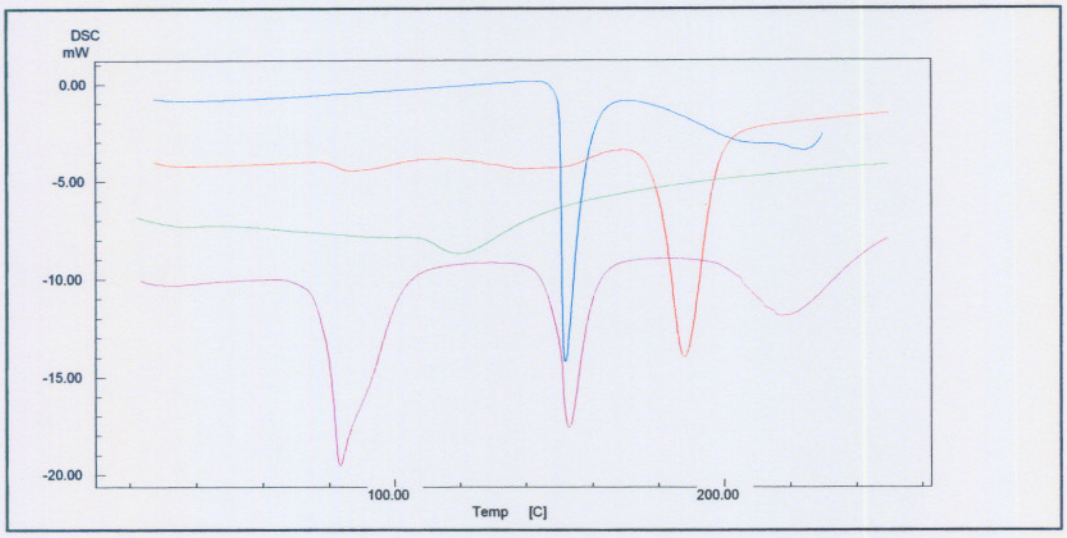


Figure 4.3 DSC analysis results of excipients including citric acid (blue), Emcompress® (red), Kollidon® 17 PF (green), and glucose (pink).

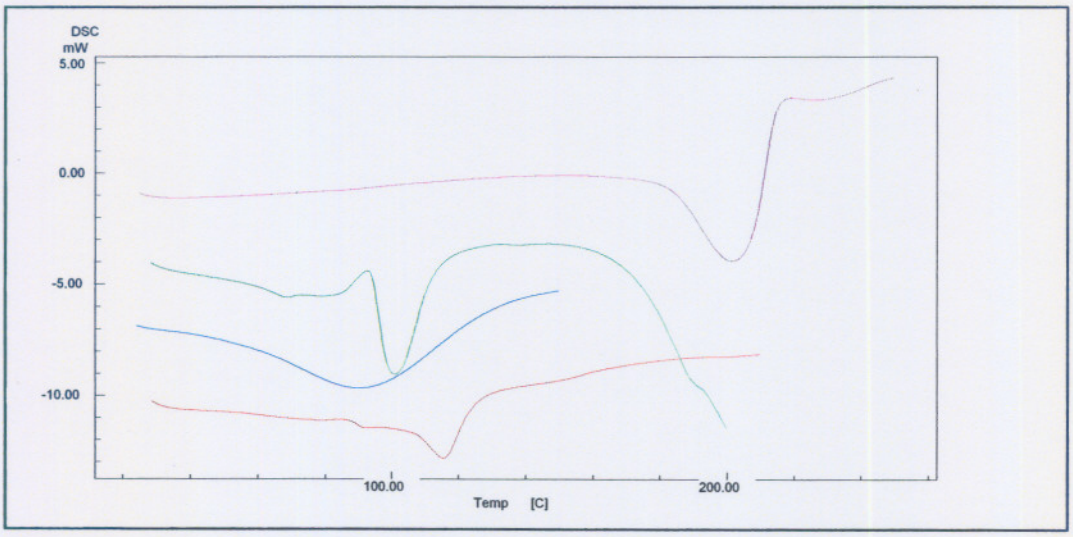


Figure 4.4 DSC analysis results of excipients including sodium metabisulphite (purple), sodium formaldehyde sulfoxylate (green), Kollidon® CL-M (blue), and magnesium stearate (deep red).

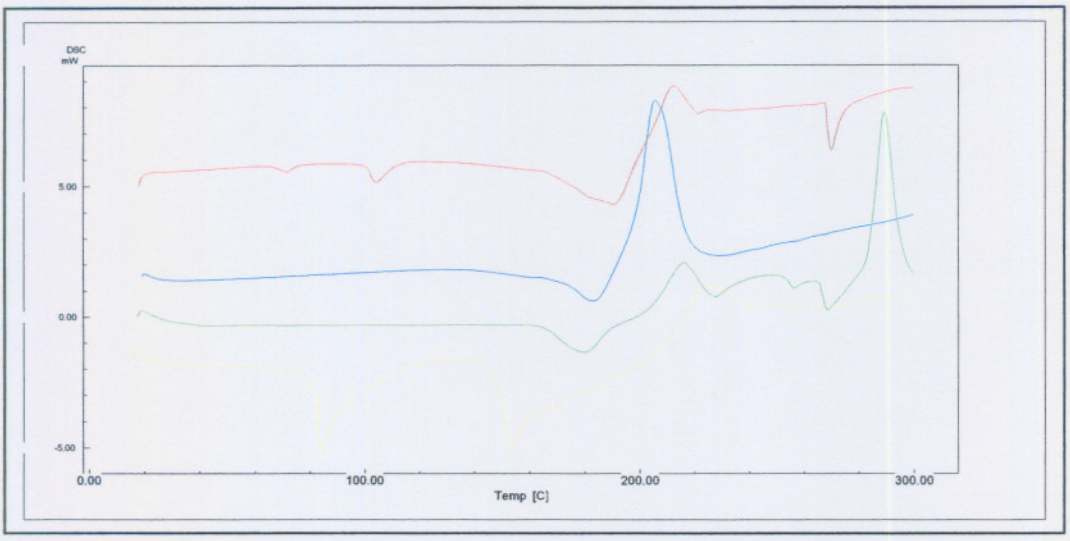


Figure 4.5 DSC analysis results of actives (doxycycline HCl and bromhexine HCl) and excipients including sodium formaldehyde sulfoxylate (deep red), sodium metabisulfite (blue), Kollidon® 17 PF (green) and glucose (yellow).

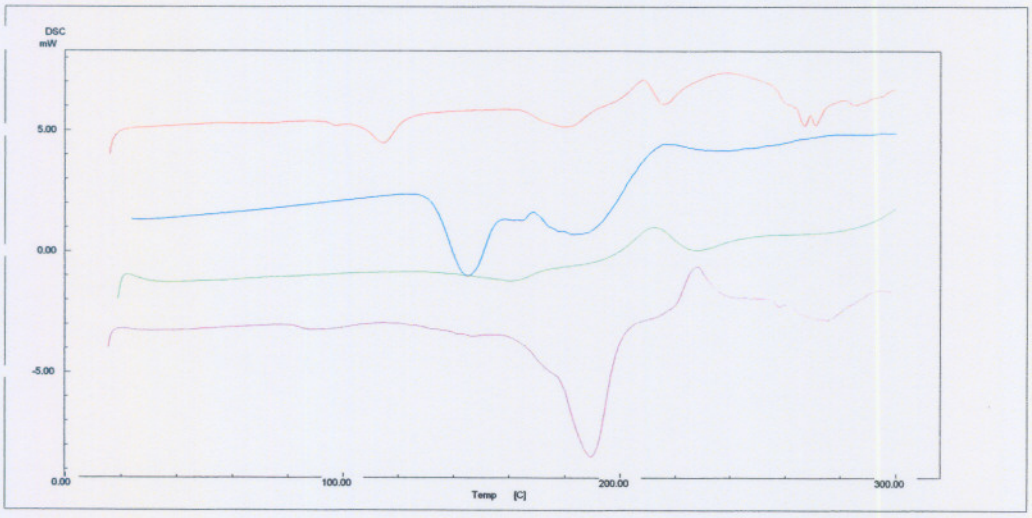


Figure 4.6 DSC analysis results of actives (doxycycline HCl and bromhexine HCl) and excipients including citric acid (blue), Kollidon® CL-M (green), Emcompress® (pink), and magnesium stearate (red).

4.2.7 DISCUSSION

DSC analysis, Figure 4.2, indicated no interaction in the melting behaviour of the 1:1 mixtures of doxycycline HCl and bromhexine HCl. This is an indication of compatibility. Where peak shifting occurred due to possible interaction between the active ingredients and mixtures with the excipients, samples were analysed by HPLC, which revealed that these possible interactions were insignificant and had no influence on the stability of the formulation.

4.3 FORMULATION OF TABLET DOSAGE FORMS CONTAINING DOXYCYCLINE HCl AND BROMHEXINE HCl SEPARATELY AND IN COMBINATION

4.3.1 INTRODUCTION

The development of a direct compressed tablet dosage form, containing doxycycline HCl and bromhexine HCl separately and in combination, was investigated. The same formula was used for al three formulations. A single punch tabletting press and a punch size of 5 mm were used during tabletting. In each case the powder was mixed with a turbula mixer using a glass container sealed with Whatman® laboratory sealing film, unless indicated otherwise. The tablet formulae, with a discussion of each, tried for direct compression tabletting were as indicated in Tables 4.4 to 4.7.

4.3.2 FORMULAE AND DISCUSSIONS

Table 4.4 Tablet formula 1

EXCIPIENT	PURPOSE	QUANTITY PER 150 mg TABLET (mg)
Doxycycline HCl	Active	15.0
Magnesium stearate	Glidant	2.0
Kollidon® CL-M	Disintegrant	6.0
Emcompress®	Diluent/binder	63.5
Glucose	Diluent/taste enhancer	63.5

Glucose was included in the formula not only as a diluent but also as a source of energy for the pigeons. This formula failed in-development friability, weight variation,

disintegration, hardness tests and content uniformity. The powder of the tablet mixture was mixed in a glass bottle without sealing it with Whatman® laboratory sealing film. This was most probably the reason for the failed content uniformity, because of the active clinging to the top and the sides of the screw-on cap of the bottle. The rest of the tests probably failed because of the poor flow characteristics of glucose and the high concentration of it in the formula. Another reason might have been the low concentration of Emcompress® as a binder in the formulation. A new formula had to be developed and the formula in Table 4.5 was designed and tested.

Table 4.5 Tablet formula 2

EXCIPIENT	PURPOSE	QUANTITY PER 150 mg TABLET (mg)
Doxycycline HCl	Active	17.25
Magnesium stearate	Glidant	2.0
Kollidon® CL-M	Disintegrant	6.0
Emcompress®	Diluent/binder	75.0
Glucose	Diluent/taste enhancer	49.75

The amount of Emcompress® in the formula was increased and the amount of glucose decreased. The active was first mixed with the diluent, and after that the glidant and disintegrant were incorporated. The magnesium stearate was put in the mixture at the very end and mixed together for the last 3 minutes. This was done to ensure proper mixing and to enhance the flow properties of the powder. The powder was mixed in a glass bottle sealed off with Whatman® laboratory sealing film. This formula presented the desired outcomes i.e. good content uniformity, less mass variation and good hardness, friability and disintegration. The same formula was therefore used for the other two tablets as well.

Table 4.6 Tablet formula 3

EXCIPIENT	PURPOSE	QUANTITY PER 150 mg TABLET (mg)
Bromhexine HCl	Active	1.0
Magnesium stearate	Glidant	2.0
Kollidon® CL-M	Disintegrant	6.0
Emcompress®	Diluent/binder	85.0
Glucose	Diluent/taste enhancer	56.0

This tablet formula presented the desired outcomes and complied with all the desired tests.

Table 4.7 Tablet formula 4

EXCIPIENT	PURPOSE	QUANTITY PER 150 mg TABLET (mg)
Doxycycline HCl	Active	17.25
Bromhexine HCl	Active	1.00
Magnesium stearate	Glidant	2.00
Kollidon® CL-M	Disintegrant	6.00
Emcompress®	Diluent/binder	75.00
Glucose	Diluent/taste enhancer	48.75

This formula also complied with all the desired tests i.e. hardness, friability, mass uniformity, disintegration and content uniformity.

A large batch of approximately 2000 tablets, for each of the three tablet formulations, was manufactured at the Department of Pharmaceutics, School of Pharmacy, North-West University, Potchefstroom Campus, South Africa. These tablets were packed in plastic containers, containing 300 tablets each, with resealable caps. A silica sachet was included in each container as dessicant.

The feasibility of these final tablet formulations was evaluated by means of accelerated stability studies as discussed in Chapter 5. The results of the accelerated stability evaluation are discussed in Chapter 6.

4.4 FORMULATION OF WATER-SOLUBLE POWDER DOSAGE FORMS CONTAINING DOXYCYCLINE HCl AND BROMHEXINE HCl SEPARATELY AND IN COMBINATION

4.4.1 INTRODUCTION

The development of water-soluble powders, containing doxycycline HCl and bromhexine HCl separately and in combination, were investigated. An antioxidant,

citric acid, was included in the formula to overcome the problem of oxidation of doxycycline HCl when dissolved in water. All the excipients were first sieved before mixing. All powders were mixed in a plastic container, first by hand, then in a V-mixer for half an hour and finally in a cake-mixer for half an hour. The powder formulae were as indicated in Table 4.8 to 4.10.

4.4.2 FORMULAE AND DISCUSSION

Table 4.8 Powder formula 1

EXCIPIENT	PURPOSE	QUANTITY PER 5 g SACHET (g)
Doxycycline HCl	Active	0.288
Citric Acid	Antioxidant/taste enhancer	1.150
Glucose	Diluent/taste enhancer	q.s. to 5 g

The glucose was incorporated in the formula not only as diluent and to enhance the taste of the powder but also as a source of energy for the pigeons. The citric acid showed good compatibility with the active and the solution stayed stable without any discolouration for up to 24 hours. This formula was compared with the same formula but lacking the citric acid, where discolouration occurred after only 3 hours. The citric acid showed good antioxidant qualities in this formulation. It is also suggested that the citric acid might improve the doxycycline HCl absorption form the gastrointestinal tract in pigeons. Citric acid could also improve the palatability of the doxycycline HCl solution with an increase in the water uptake (Santos *et al.*, 1997:1347). The powder was easily dissolved in water. The same formula was used for the other two powders as shown in Table 4.9 and 4.10.

Table 4.9 Powder formula 2

EXCIPIENT	PURPOSE	QUANTITY PER 5 g SACHET (g)
Doxycycline HCl	Active	0.288
Bromhexine HCl	Active	0.017
Citric Acid	Antioxidant/taste enhancer	1.150
Glucose	Diluent/taste enhancer	q.s. to 5 g

Because the powder is dissolved in the drinking water of the pigeons and used as an oral dosage form, it is important that the taste of the water be as appealing as possible. The glucose and the citric acid in the formula are used to enhance the taste. The citric acid showed good compatibilities with the bromhexine HCl as well and didn't influence its stability in this formulation. This powder showed good solubility qualities in water.

Table 4.10 Powder formula 3

EXCIPIENT	PURPOSE	QUANTITY PER 5 g SACHET (g)
Bromhexine HCl	Active	0.1
Glucose	Diluent/taste enhancer	q.s. to 5 g

Because bromhexine HCl shows no oxidation when dissolved in water, there was no need to include citric acid in this powder formula. The powder was easily dissolved in water and the glucose and bromhexine HCl was compatible in this formulation.

A large batch of 1 kg of each powder was made, and the powder was stored in plastic containers with resealable caps each containing 150 g of powder.

The feasibility of these powder formulations was evaluated by means of stability studies as discussed in Chapter 5. The results of the accelerated stability evaluation are discussed in Chapter 7.

4.5 FORMULATION OF AN OPHTHALMIC SOLUTION CONTAINING DOXYCYLINE HCl

4.5.1 INTRODUCTION

Eye discharge, leading to inflammation of the eye, is one of the severe symptoms of chlamydiosis in pigeons. This led to the development of an ophthalmic solution containing doxycycline HCl for administration directly into the eye. Because of the oxidation of doxycycline HCl an antioxidant had to be included in the formula to prevent the discolouration of the solution. The different formulae that were investigated are shown in Tables 4.11 to 4.14.

4.5.2 FORMULAE AND DISCUSSION

Table 4.11 Ophthalmic solution formula 1

EXCIPIENT	PURPOSE	QUANTITY PER 100 ml SOLUTION (g)
Doxycycline HCl	Active	6.29
Magnesium oxide	Source of Mg ions	1.00
Kollidon®17 PF	Co-solvent/reduction of local toxicity	10.00
Sodium formaldehyde sulfoxylate	Antioxidant	1.00
Concentrated hydrochloric acid	pH stabiliser	1.64 ml
Water	Solvent	q.s. to 100 ml

Method:

The Kollidon® 17 PF was mixed with water. The solution was heated to about 50°C and the sodium formaldehyde sulfoxylate was added and dissolved with stirring. The magnesium oxide was then slurried with the solution. The doxycycline HCl was slowly added with stirring and the pH adjusted with concentrated hydrochloric acid. The resultant solution was allowed to cool to room temperature and the pH further adjusted to about 5.2 with concentrated hydrochloric acid. The solution was then brought up to volume with water.

This solution had a pH of 5.67 after adjusting it with a further 0.2 ml concentrated hydrochloric acid. Sodium formaldehyde sulfoxylate was incorporated as the antioxidant. This solution had a dark yellow-brown colour with a low clarity. Some sedimentation was observed at the bottom of the solution. The solution had a strong, unpleasant odour which was probably the cause of the sodium formaldehyde sulfoxylate. The formula in Table 4.12 was developed to improve the clarity and the odour of the solution and to obtain no sedimentation.

Table 4.12 Ophthalmic solution formula 2

EXCIPIENT	PURPOSE	QUANTITY PER 100 ml SOLUTION (g)
Doxycyline HCl	Active	6.29
Magnesium oxide	Source of Mg ions	1.00
Kollidon® 17 PF	Co-solvent/reduction of local toxicity	10.00
Sodium metabisulfite	Antioxidant	0.10
Concentrated hydrochloric acid	pH stabiliser	1.64 ml
Water	Solvent	q.s. to 100 ml

Method:

The Kollidon® 17 PF was mixed with water. The solution was heated to about 50°C and the sodium metabisulfite was added and dissolved with stirring. The magnesium oxide was then slurried with the solution. The doxycycline HCl was slowly added with stirring and the pH adjusted with concentrated hydrochloric acid. The resultant solution was allowed to cool to room temperature and the pH further adjusted to about 5.2 with concentrated hydrochloric acid. The solution was then made up to volume with water.

This solution had an end pH of 4.95. The solution had an adequate odour but the clarity had not been improved. After letting it stand overnight, the solution just got thicker and the clarity decreased. Formula 3 was developed to try and improve the clarity.

A large batch of 2 litres of the ophthalmic solution was made. The solution was then poured into 15 ml sterilised amber glass dropper bottles under aseptic conditions in a clean air environment. The Kollidon® 17 PF was included to reduce the local toxicity of the solution and to perform the following functions:

- Film formation;
- Thickening;
- Prolonged retention of the active substance in the eye;
- Increase in bioavailability;
- Lubrication;
- Solubilisation the active substance, and
- Reduction of eye irritation caused by the active substance (BASF, 2003:127).

The results of the accelerated stability evaluation are discussed in Chapter 8.

4.6 CONCLUSION

In this chapter the development of the three formulations, a tablet for direct compression, a water-based powder and an ophthalmic solution, was discussed.

The formulation of three stable, cost effective tablet dosage forms, three easily dissolved water-soluble powders, without showing oxidation and an easily used ophthalmic solution were successfully developed. Batches of 2000 tablets each, 1 kg of each powder and 2 litres of the ophthalmic solution was manufactured and tested during accelerated stability programs.

CHAPTER 5

STABILITY TESTS AND METHODS

5.1 INTRODUCTION

During the formulation of the different dosage forms certain preformulation tests were performed on the developed formulations to determine whether they will be stable during the accelerated stability test program.

In this chapter the stability-indicating tests and test methods used during the stability programmes of each dosage form to evaluate the newly formulated tablet, the water-based powder and the ophthalmic solution, are discussed. Since the accelerated stability test programme was intended to identify the potential feasibility of the new formulations developed in this study, it was important that the test methods used, would generate accurate and thus reliable test results. Therefore only validated and/or proven methods were used.

All the equipment used was calibrated for their accuracy according to the requirement at the Research Institute for Industrial Pharmacy (RIIP), North-West University, Potchefstroom Campus, South Africa.

5.2 STABILITY TESTS AND TEST METHODS

5.2.1 TABLETS

The tests done on the tablets during stability included assay, content uniformity, dissolution rate, moisture content (Karl Fischer), appearance, hardness, thickness, diameter, mass uniformity, friability and disintegration.

5.2.1.1 Assay

This test was done by means of a high performance liquid chromatographic (HPLC) method for the simultaneous determination of doxycycline HCl and bromhexine HCl. This method was developed and validated by myself, Marga le Roux in co-operation with Dr J. L. du Preez at the Research Institute for Industrial Pharmacy. The validation results are given in Chapter 9.

The chromatographic conditions were as follows:

- Analytical instrument: HP 1090 series equipped with a quaterny gradient pump, autosampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software.
- Column: Column L1, USP 24, 2000, p1925 (Luna C18(2), 150 x 4.6 mm, 5 µm column, 100 Å pores, 21.5% carbon load, end capped, Phenomenex, Torrance, CA) was used. A Lichroshper 100 RP-18, 5µm was used as guard column.
- Mobile phase: 0.005 M octane-sulphonic acid Na-salt in deionised water: acetronitrile. (60 : 40) (v/v). pH adjusted to 3.5 with H_3PO_4.
- Flow rate: 1.0 ml/min
- Injection volume: 10 µl
- Detection: UV at 254 nm

Twenty tablets were weighed, powdered, assayed and evaluated. A standard solution containing 172.5 µg/ml doxycycline HCl and a solution containing 10 µg/ml bromhexine HCl was prepared and analysed. A portion of the powdered tablets equivalent to 17.25 mg of doxycycline HCl for the doxycycline HCl tablets and equivalent to 1 mg of bromhexine HCl for the bromhexine HCl tablets was accurately weighed and transferred into a 100ml volumetric flask and made up to volume with deionised water. These solutions were transferred into HPLC vials and analysed. The following calculations were used to evaluate the results obtained.

For doxycycline HCl:

$$C_{std} = \frac{\text{Mass doxycycline HCl weighed x potency}}{100 \times 100}$$

where mass doxycycline HCl weighed = mg

potency = %

$$\% \text{ Doxycycline of label claim} = \frac{SAR \times 100 \times C_{std} \times 100}{STR \times EqM_{sample}}$$

where SAR = sample peak area

STR = standard peak area

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

For bromhexine HCl:

$$C_{std} = \frac{\text{mass bromhexine HCl weighed} \times \text{potency}}{100 \times 100 \times 20}$$

where mass bromhexine HCl weighed = mg

potency = %

$$\% \text{ bromhexine HCl of label claim} = \frac{SAR \times 100 \times C_{std} \times 100}{STR \times EqM_{sample}}$$

where SAR = sample peak area

STR = standard peak area

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

5.2.1.2 Content uniformity

Content uniformity was done to determine whether the active substances were mixed properly and to determine whether the individual contents are within the set limits. The test specifications for the tablets are that each individual tablet's content must be between 85% and 115% of the average content. The test was done by means of HPLC, according to the above described assay method. The content uniformity of ten tablets was determined individually, and evaluated by using the British Phamacopoeia (British, 2000:A231) as a guideline.

5.2.1.3 Dissolution rate

The dissolution was done according to the USP (United, 2000:1378,1942,1943) and the dissolution conditions were as follow:

- Dissolution apparatus: VanKel 7000 apparatus 2
- Dissolution medium: Water

- Dissolution volume: 500 ml
- Paddle speed: 50 rpm
- Volume withdrawn: 5 ml
- Sampling time: 15, 30, 45, 60, 90 minutes
- Method of analysis: HPLC
- Sample: 1 tablet (doxycycline HCl)
  10 tablets (bromhexine HCl tablets)

Standard preparation:

For doxycycline HCl:

Weigh 34.5 mg doxycycline HCl accurately and transfer to a 100 ml volumetric flask. Dissolve in 100 ml deionised water. Sonicate for 15 minutes. Cool to room temperature and fill up to volume. Dilute 5.0 ml to 50 ml with dissolution medium. Filter through a 0.45 µm filter, transfer to an HPLC vial and analyse.

For bromhexine HCl:

Because of the low concentration of the bromhexine HCl in the tablets, 10 tablets were used for the dissolution. The standard solution was made accordingly. Weigh 20 mg bromhexine HCl accurately and transfer to a 100 ml volumetric flask. Dissolve in 100 ml deionised water. Sonicate for 20 minutes. Cool to room temperature and fill up to volume. Dilute 5.0 ml to 50 ml with dissolution medium. Filter through a 0.45 µm filter, transfer to an HPLC vial and analyse.

The following calculations were used to evaluate the results obtained:

For doxycycline HCl and bromhexine HCl:

$$C_{std} = \frac{\text{mass of active weighed x potency}}{100 \times 10 \times 100}$$

where mass of active weighed = mg

potency = %

$$\%\ \text{active of label claim} = \frac{SAR \times 500 \times C_{std} \times 100}{STR \times \text{strength}}$$

where SAR = sample peak area

STR = standard peak area

Strength = theorectical mass of active substance in each tablet (mg)

5.2.1.4 Moisture Content

The tablets were analysed, using a Mettler DL 18 Karl Fischer titrator (Metrohm Ltd., Herisau, Switzerland), for water content (United, 2000:2004). The tablets were analysed according to an in-house method of the RIIP (2001:SOPMST01).

5.2.1.5 Physical tests

The appearance of the tablets was evaluated visually and recorded accordingly.

The hardness, thickness and the diameter of the tablets were determined using a PharmaTest PTB-311 (Pharma Test, Hainburg, Germany) tablet hardness tester. Twenty tablets were used. The tablets were dusted, using a clean, soft brush, and measured individually according to an in-house method of the RIIP (2001:SOPHRD02).

The method used to determine the uniformity of mass is described in the British Parmacopoeia (British, 2000:A231). The uniformity of tablet mass was recorded by weighing twenty tablets individually, after being dusted. The percentage deviation was calculated.

The method used to determine the friability of the tablets is described in the United States Pharmacopoeia (United, 2003:2439). The total mass of twenty tablets was determined and thereafter placed in the rotating drum of a PharmaTest PTF-E (Pharma Test, Hainburg, Germany) friabilator. The friabilator was operated at 25 ± 1 rpm for 100 rotations (4 minutes). The sample was then removed and dusted again. Only the tablets that were not broken, cracked or chipped were taken into consideration. Any tablets that were not intact were recorded. The sample was weighed to the nearest milligram if none of the tablets were cracked, split or broken.

The USP describes the method and the apparatus used to determine the disintegration of tablets (United, 2000:1941). A PharmaTest PTZ (Pharma Test, Hainburg, Germany) tablet and capsule disintegration tester was used. One tablet was placed in each of the six tubes (without discs) per test. The immersion fluid consisted of 600 ml of water and it was kept at 37°C ± 2°C. The time limit within which all of the six tablets had to disintegrate was set at fifteen minutes.

5.2.2 POWDERS

The tests done on the powders during stability included, assay, an "in use" assay, appearance of the powder and the constituted solution, moisture content (Karl Fischer), pH of the constituted solution and the constitution time.

5.2.2.1 Assay

The HPLC method and the chromatographic conditions used in this test were the same as described for the tablets in 2.1.1.

Standard solutions containing 172.5 µg/ml doxycycline HCl and 10 µg/ml bromhexine HCl in each case were prepared and analysed. The samples were prepared as follow: accurately weigh 300 mg of the doxycycline HCl powder and combination powder and 50 mg of the bromhexine HCl powder, and transfer each powder to a 100 ml volumetric flask. Dissolve in deionised water and sonicate for 10 minutes. Cool to room temperature and fill up to volume with deionised water. Filter through a 0.45 µm filter, transfer to a HPLC vial and analyse.

The same calculations as described in tablets 2.1.1 were used to determine the amount of active substances recovered.

An "in use" assay was also done on the doxycycline HCl powder. Three containers (stainless steel, glass and plastic) were used and the powder was dissolved in tap water (5 mg/ml). Samples were taken from every container after 0, 6, 12 and 24 hours and analysed. The results obtained were compared to the same powder but with no citric acid in the formulation. The same containers and time intervals were used for the comparing powder. The HPLC method, chromatographic conditions and calculations used in this test were the same as described for the tablets in 2.1.1.

5.2.2.2 Moisture Content

Each powder was analysed using a Mettler DL 18 Karl Fischer titrator (Metrohm Ltd., Herisau, Switzerland) for water content (United, 2000:2004). This was done according to an in-house method of the RIIP (2001:SOPMST01).

5.2.2.3 Physical tests

The powder and the constituted solution were examined visually to determine whether any colour changes occurred.

The pH of the constituted solution was determined by the USP (USP 24, 2000:1977). The equipment was a calibrated Mettler Toledo MP220 pH meter (Mettler Toledo, Schwerzerbach) and the measurements were made at 25°C ± 2°C.

The constituted time was measured with a stopwatch. The time it took for the powder to totally dissolve or disintegrate was taken.

5.2.3 OPHTHALMIC SOLUTION

The tests done on the ophthalmic solution during stability were assay, appearance, pH, density, viscosity, particulate matter and preservative efficacy.

5.2.3.1 Assay

The same HPLC method and chromatographic conditions described in tablets 2.1.1 were used to analyse the ophthalmic solution.

A standard solution containing 172.4 µg/ml doxycycline HCl was prepared and analyse. The sample was prepared by weighing 5.5 drops (about 330 mg) of the ophthalmic solution into a 100 ml volumetric flask. Deionised water was added and the solution was sonicated for 5 minutes, left to cool to room temperature, and then made up to volume with deionised water. The sample was filtered through a 0.45 µm filter and transferred to a HPLC vial and analysed.

The same calculations as described in tablets 2.1.1 was used to determine the amount of doxycycline HCl recovered.

5.2.3.2 Preservative efficacy and sterility

Preservative efficacy and sterility tests were done on this formulation according to USP (United, 2000:1809, 1818) by the Wits Health Consortium (Pty) Ltd trading as Adcibact.

5.2.3.3 Physical tests

Appearance and particulate matter were evaluated visually and recorded accordingly. The sample was swirled gently to ensure that no air bubbles were introduced and observed for about five seconds using a viewing station. All the visual observations were recorded.

The pH was determined as described by the USP (United, 2000:1977). The equipment used was a calibrated Mettler Toledo MP220 pH meter (Mettler Toledo, Schwerzerbach) and the measurements were made as 25°C ± 2°C.

The USP describes the method used for the measurement of viscosity (United, 2000:2002). The temperature at which these measurements were made was 25°C ± 0.1°C. A small sample adapter attached to a programmable Brookfield Model DVII+ rotational viscometer (Brookfield, Stoughton, USA), of the RVT type, with spindle number SC4-18, was used.

An in-house method of the RIIP (2001:SOPDEN01) was used to determine the relative density of the ophthalmic solution. A sufficient volume of the sample was injected into an Anton Paar DMA 38 density meter (Anton Paar, Austria, Europe) that was used to measure the relative density, until all the air in the instrument was eliminated. This measurement was made at 25°C ± 2°C.

5.3 STABILITY PROGRAM

Seven products were formulated during this study. Three direct compressed tablets, each with the same formulae but with different actives were developed. The first contained doxycycline HCl as an active (*tablet A*), the second contained bromhexine HCl as active (*tablet B*) and the third was a combination tablet containing both bromhexine HCl and doxycycline HCl (*tablet C*) as actives. Three water-soluble powders were developed. The first powder contained doxycycline HCl as an active

(*powder A)* and the second powder bromhexine HCl as active (*powder B*). The third water-based powder contained both doxycycline HCl and bromhexine HCl in combination as actives (*powder C*). The final product that was formulated was an antibiotic ophthalmic solution containing doxycycline HCl as active.

The trial batches of these seven formulations were stored at three different storage temperatures for the duration of the three month stability testing period.

5.3.1 Concentrations

Table 5.1 shows the different formulations and the concentration of the active(s) in each formulation.

Table 5.1 The concentration of active(s) in the different formulations

FORMULATION	ACTIVE	CONCENTRATION
Tablet A	Doxycycline HCl	17.25 mg/tab
Tablet B	Bromhexine HCl	1.0 mg/tab
Tablet C	Doxycycline HCl	17.25 mg/tab
	Bromhexine HCl	1.0 mg/tab
Powder A	Doxycycline HCl	5.75 % (m/m)
Powder B	Bromhexine HCl	2 % (m/m)
Powder C	Doxycycline HCl	5.75 % (m/m)
	Bromhexine HCl	0.3 % (m/m)
Ophthalmic solution	Doxycycline HCl	6.29 % (m/v)

5.3.2 Storage temperatures

The objective of this study was to determine the stability of a product as a function of time. Controlled 5°C, 25°C, and 40°C storage facilities were used during the stability period. In Table 5.2 it is shown which formulations were stored at the different temperatures and humidity.

Table 5.2 The storage temperatures of the different formulations

	Tablet A	***Tablet B***	***Tablet C***	***Powder A***	***Powder B***	***Powder C***	**Ophthalmic solution**
5°C							✓
25°C+60%RH	✓	✓	✓	✓	✓	✓	✓
40°C+75%RH	✓	✓	✓	✓	✓	✓	✓

5.4 CONCLUSION

The above mentioned stability tests were done on calibrated equipment and validated methods were used to obtain accurate and reliable results during the evaluation because the stability of the formulations will be based on these results obtained.

The results of the tests as described in this chapter are given and represented graphically in Chapter 6 to Chapter 9. The outcome of the different formulations will be discussed separately in these chapters.

CHAPTER 6

STABILITY STUDY TEST RESULTS: TABLETS

6.1 INTRODUCTION

The accelerated stability test conditions and methods used are as described in 5.2.1. The results obtained over the three month period served only as an indication of the stability of the three formulated tablet dosage forms.

Three different tablets were formulated as described in 5.3. *Tablet A* contained doxycycline HCl as active component, *tablet B* contained bromhexine HCl as active component and *tablet C* was a combination of the two active components.

The containers used for the tablets were plastic securitainers each containing 300 tablets and a drying agent. Samples of the formulated tablets were stored at 25°C ± 2°C at 60% RH (relative humidity) (25 RH) as well as 40°C ± 2°C at 75% RH (40 RH) for a period of three months. Stability tests were performed initially and then monthly for a further three months at these storage conditions.

The test results of the three tablet formulations, *tablet A*, *tablet B* and *tablet C*, generated during the stability evaluation, are summarised and discussed in this chapter. The raw data is included in Appendix 3 for reference.

6.2 ASSAY

The HPLC assay results of both the 25 RH and the 40 RH tablet samples of *tablets A, B and C* are summarised and represented graphically in figure 6.1, figure 6.2, figure 6.3 and figure 6.4 respectively.

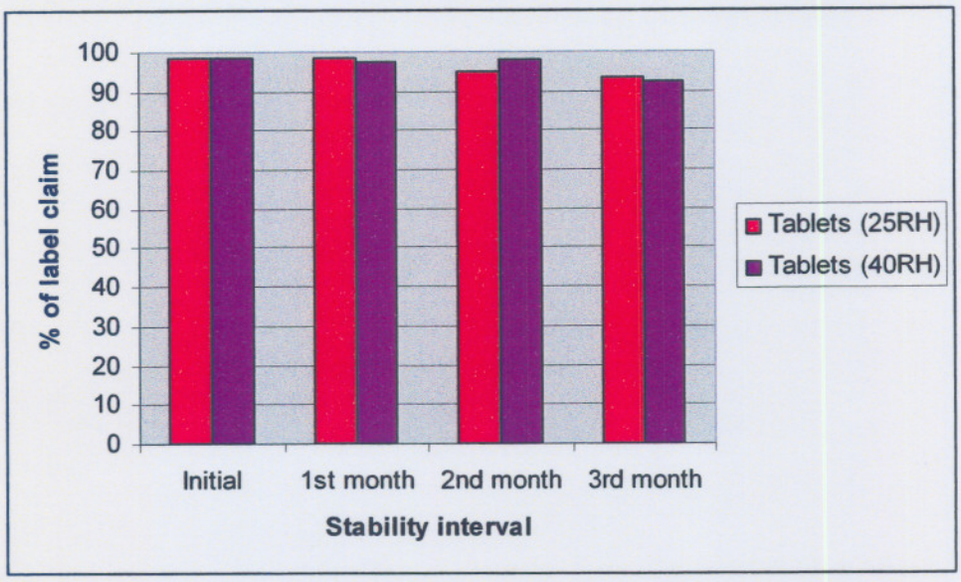


Figure 6.1 HPLC assay results of *tablet A.*

Discussion of assay results of *tablet A*

The assay results of the doxycycline HCl indicated that no significant breakdown occurred during the three month stability period. This indicates that the doxycyline HCl remained relatively stable in this formulation at these storage conditions. The slight decrease of active substance of the tablets subjected to 40 RH observed after 3 months may be due to the oxidation of the doxycycline HCl. However, these assay results remained within the specified limits as described by the USP (United, 2000:1378).

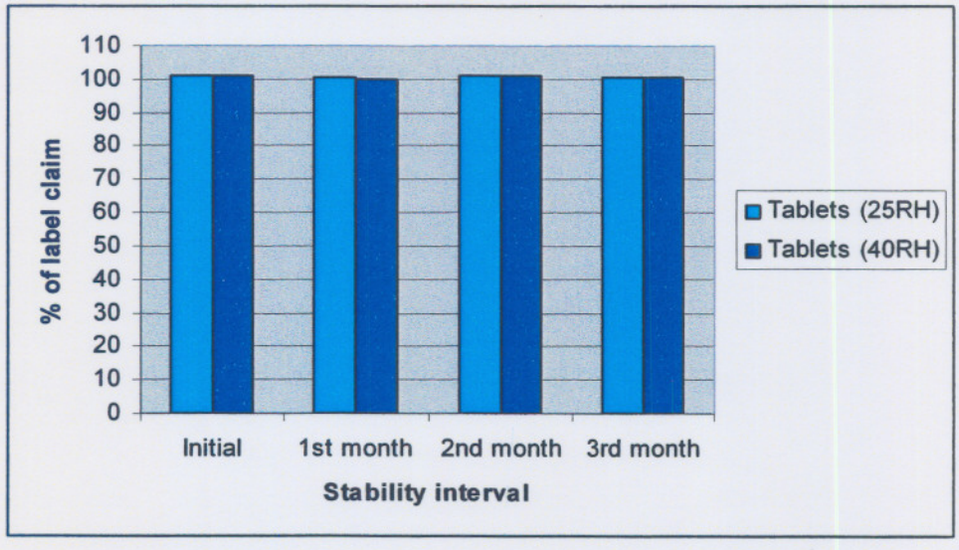


Figure 6.2 HPLC assay results of *tablet B.*

Discussion of assay results of *tablet B*

No significant differences between the tablets stored at the various temperatures were observed, indicating that the bromhexine HCl remained stable at these storage conditions. The results remained within the specified limits according to the USP (United, 2000: 1378).

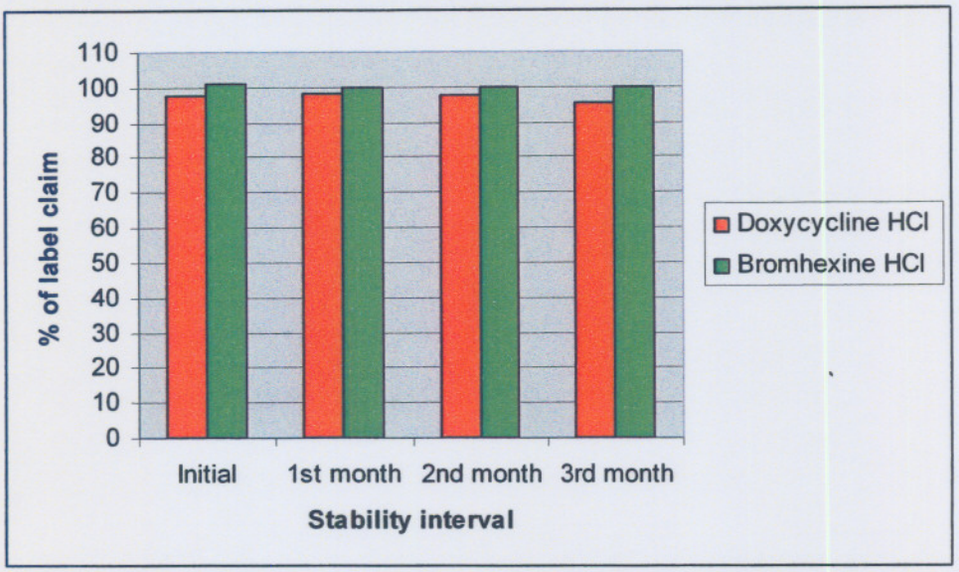


Figure 6.3 HPLC assay results of *tablet C* subjected to 25ºC + 60% RH.

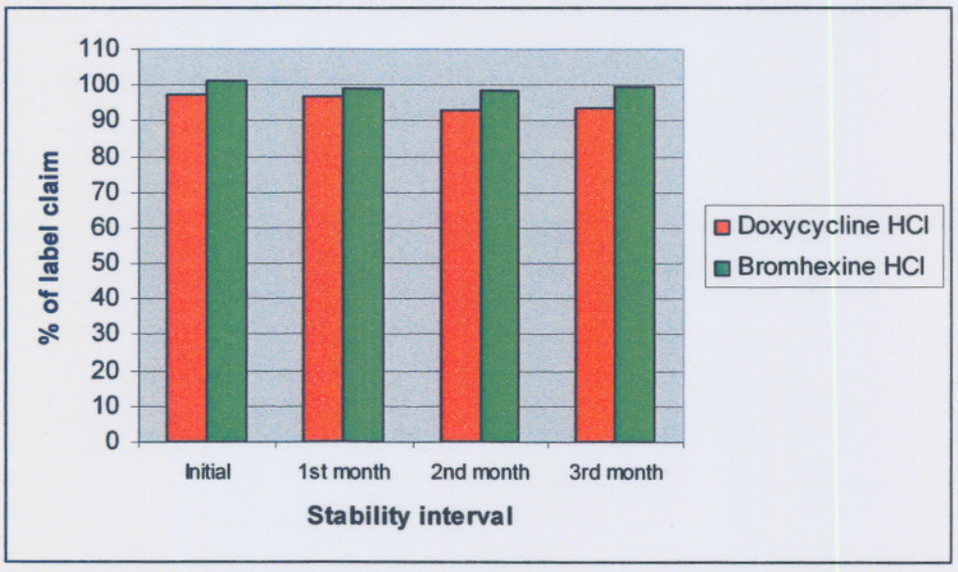


Figure 6.4 HPLC assay results of tablet C subjected to 40ºC + 75% RH.

Discussion of assay results of *tablet C*

There were no significant differences in the HPLC assay results of the two times bromhexine HCl remained stable in this formulation and showed no incompatibilities with each other. The oxidation of the doxycycline HCl at high temperatures might be the reason for the slightly gradual decreased values of doxycycline HCl at 40 RH. These assay results remained within the specified limits as described by the USP (United, 2000:1378).

6.3 CONTENT UNIFORMITY

All three tablet samples (*A, B* and *C*) complied with the test specifications and the content uniformity was between 85% and 115% of the average content. The uniformity results of *tablets A, B* and *C,* are given in tables 6.1 – 6.3 respectively. (See Appendix 3 for the content uniformity results obtained).

Table 6.1 Tablet content uniformity results of *tablet A*

CONTENT UNIFORMITY RESULTS	
TABLET	**DOXYCYCLINE HCl (%)**
1	98.9
2	98.1
3	97.9
4	99.8
5	107.3
6	99.5
7	106.3
8	101.0
9	95.1
10	99.2
Ave	100.3
Sdev	0.6
RSD	3.6

Table 6.2 Tablet content uniformity results of *tablet B*

CONTENT UNIFORMITY RESULTS	
TABLET	**BROMHEXINE HCl (%)**
1	96.6
2	101.2
3	99.1
4	102.6
5	99.8
6	94.3
7	94.4
8	97.0
9	99.8
10	102.0
Ave	98.7
Sdev	0.03
RSD	2.9

Table 6.3 Tablet content uniformity results of *tablet C*

CONTENT UNIFORMITY RESULTS		
TABLET	**DOXYCYCLINE HCl (%)**	**BROMHEXINE HCl (%)**
1	97.8	95.6
2	95.1	97.9
3	98.3	93.8
4	96.4	99.7
5	95.0	95.5
6	100.7	105.2
7	99.8	92.4
8	98.5	100.5
9	100.8	97.9
10	101.2	106.8
Ave	98.4	98.5
Sdev	0.4	0.04
RSD	2.2	4.5

6.4 DISSOLUTION RATE

According to the ICH (International, 1999:11) disintegration may be substituted for dissolution for rapidly dissolving (dissolution >80% in 15 minutes at pH 1.2, 4.0, and 6.8) products containing drugs which are highly soluble throughout the physiological range. Because of the small concentration bromhexine HCl in the tablets (1 mg/tablet) it would have been expedient to substitute the dissolution with the disintegration. Therefore the dissolution rates of the tablets containing bromhexine HCl were determined in all three mediums (pH 1.2, 4.0 and 6.8 buffers). 10 tablets per vessel were used for the dissolutions. The results are given in figure 6.5.

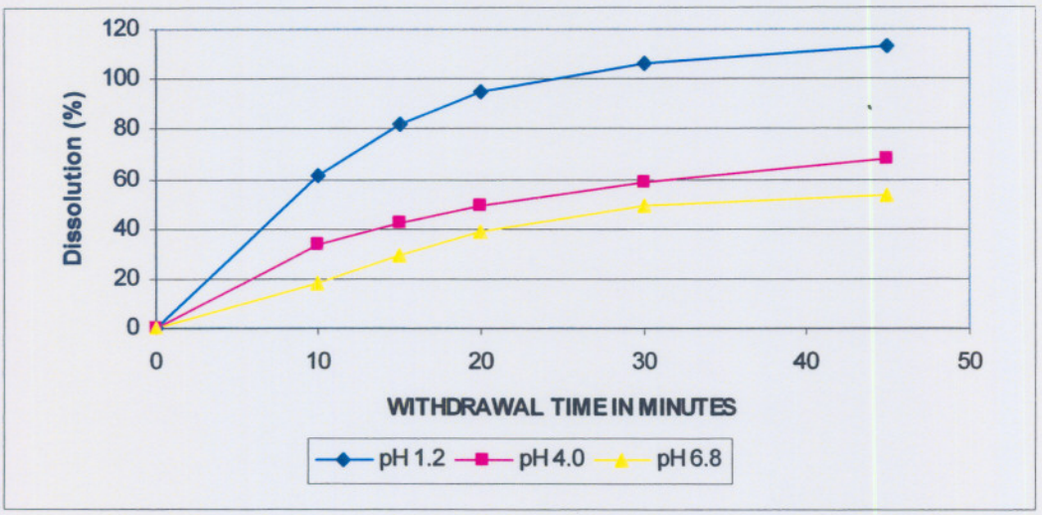


Figure 6.5 Average dissolution rates showing the percentage bromhexine HCl released at different time intervals, in three different mediums.

The release concentrations of the bromhexine HCl after 15 minutes in the pH 1.2, 4.0 and 6.8 buffers were 81.67%, 41.98% and 29.20% respectively. The results obtained wasn't within the limits set by the ICH (International, 1999:11), therefore the dissolution could not be substituted with the disintegration.

The dissolution rates of six tablets from each batch (25 RH and 40 RH) at each of the stability test intervals were determined for *tablet A* and *tablet C* (for the percentage doxycyline HCl recovered). Because of the very low concentration of bromhexine HCl (1 mg/tab) in the tablet formulations, 60 tablets (10 per vessel) of each batch of *tablet B* and *tablet C* (for the percentage bromhexine HCl recovered) were used for the dissolutions. The average dissolution rates for the different tablet formulations

are indicated in figures 6.5 – 6.8 (See appendix 3 for the HPLC dissolution test results of doxycycline HCl and bromhexine HCl).

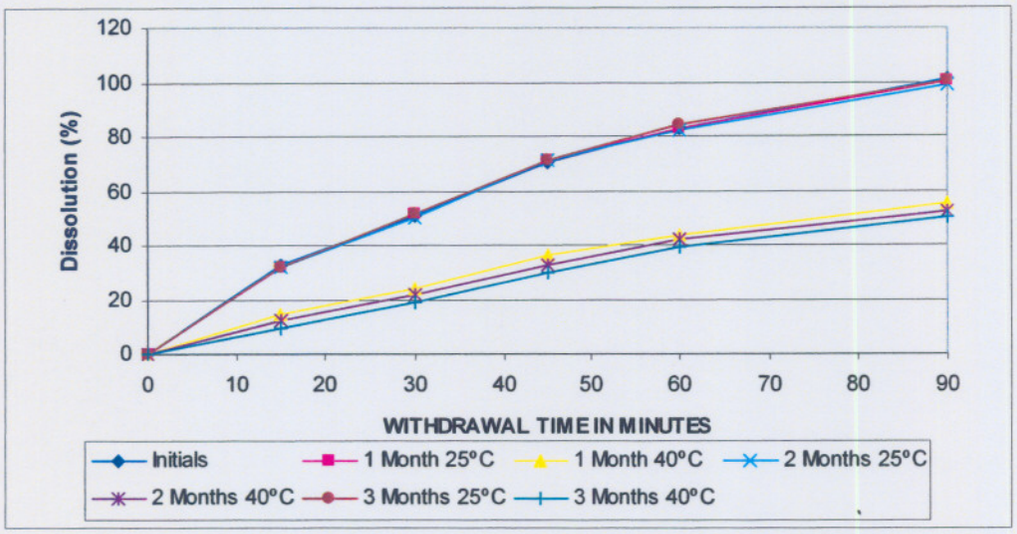


Figure 6.6 Average dissolution rates of *tablet A* showing the percentage of doxycycline HCl released at different time intervals.

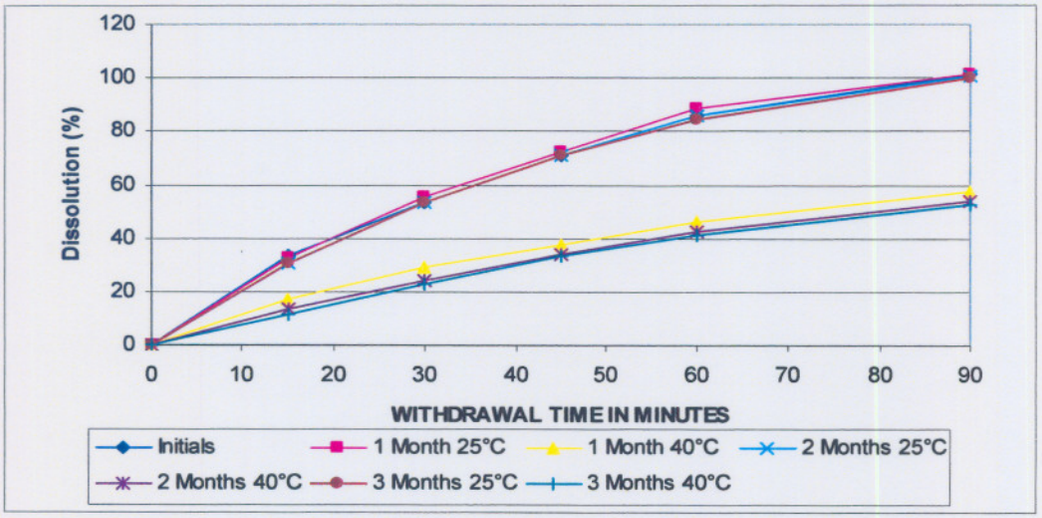


Figure 6.7 Average dissolution rates of *tablet B* showing the percentage of bromhexine HCl released at different time intervals.

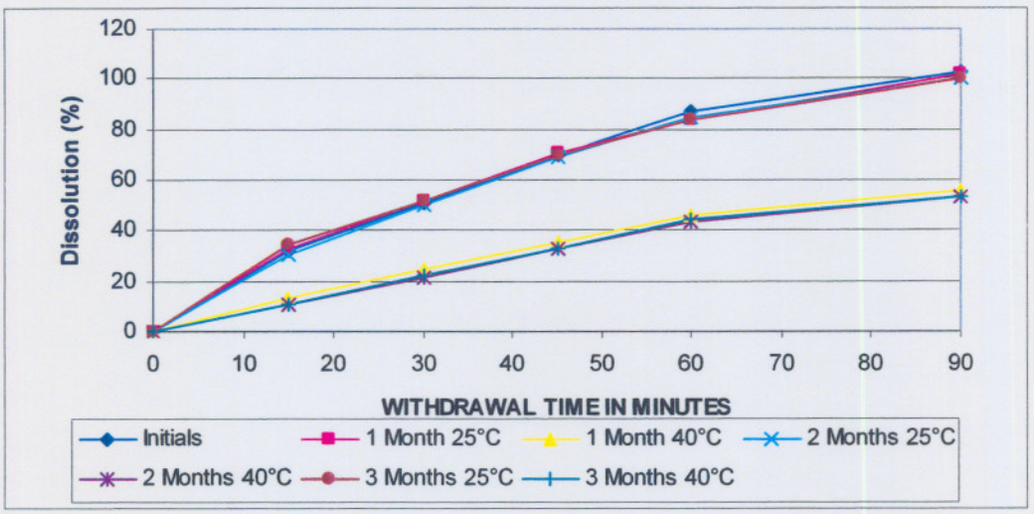


Figure 6.8 Average dissolution rates of *tablet C* showing the percentage of doxycycline HCl released at different time intervals.

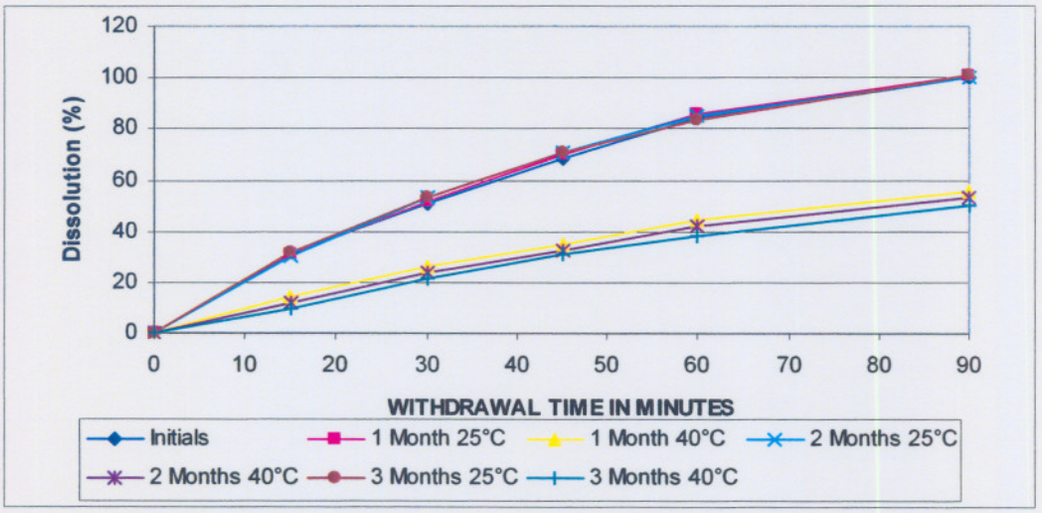


Figure 6.9 Average dissolution rates of *tablet C* showing the percentage of bromhexine HCl released at different time intervals.

6.4.1 Discussion of dissolution results

Dissolution tests were performed using the method as described in 5.2.1.3. The dissolution rates of all three tablets, subjected to 25 RH seemed to be similar, indicating that the release of the doxycycline HCl and the bromhexine HCl from the different tablets remained relatively constant. The maximum release concentration for both of the actives were 95 - 100% for the duration of the stability program. Both the glucose and the disintegrant (Kollidon® CL-M) in the tablets have a high affinity

for water. Maximum disintegration is hindered because of the high uptake of water by the glucose. This is probably the reason for the slow release of the actives. The tablets that were stored at 40 RH showed a characteristic low release profile at the 0 – 90 minutes period, for the duration of the stability program. No disintegration took place, even after the duration of the dissolution (90 minutes). This is probably due to the high concentration of moisture that the tablets were subjected to. The disintegrant used in this formulation (Kollidon® CL-M) already swelled under the 40 RH conditions, because of the moisture uptake. Therefore no disintegration took place during the dissolution. The moisture forms a sorbed layer around the tablets. More drying agents and a tightly sealed glass container can be used to overcome this problem.

6.5 MOISTURE CONTENT

The moisture content was determined using a Mettler DL18 Karl Fischer titrator. The results are given in figures 6.9 – 6.11.

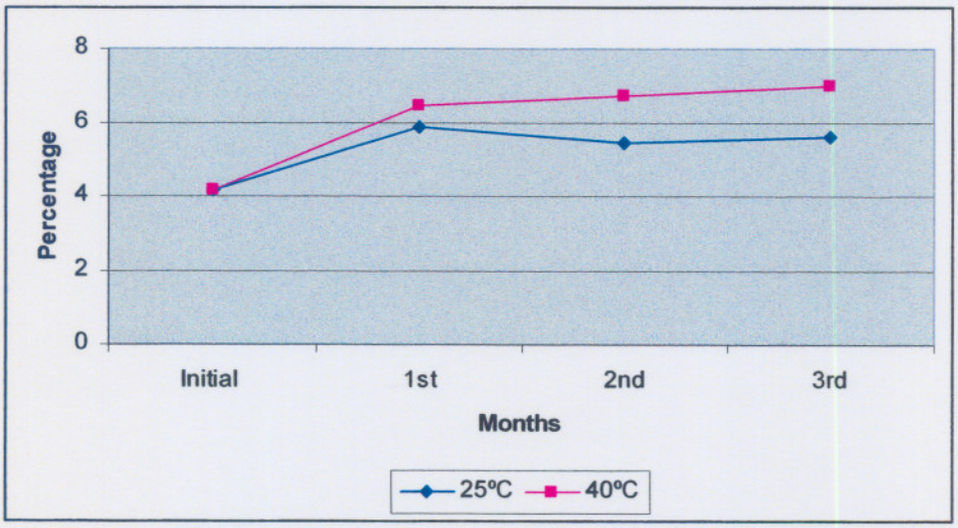


Figure 6.10 Graphic illustration of the moisture content of *tablet A*.

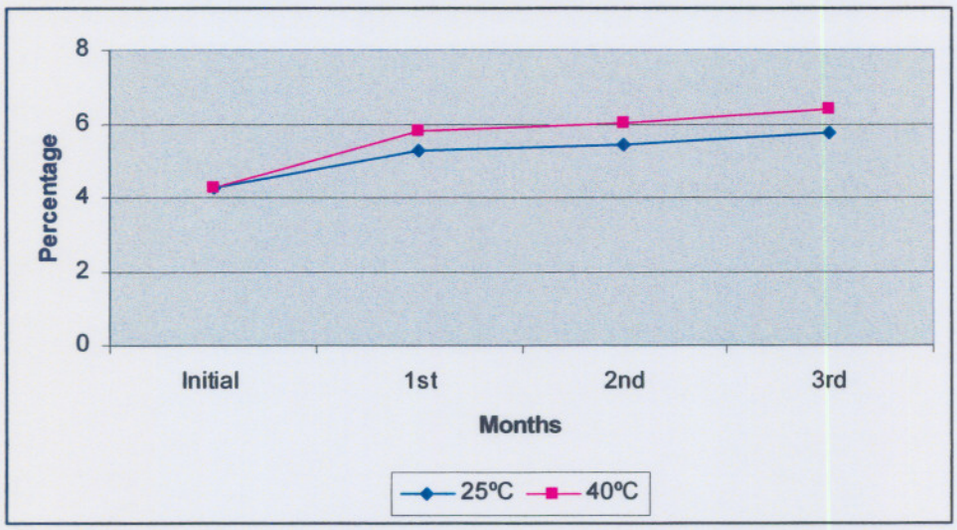


Figure 6.11 Graphic illustration of the moisture content of *tablet B*.

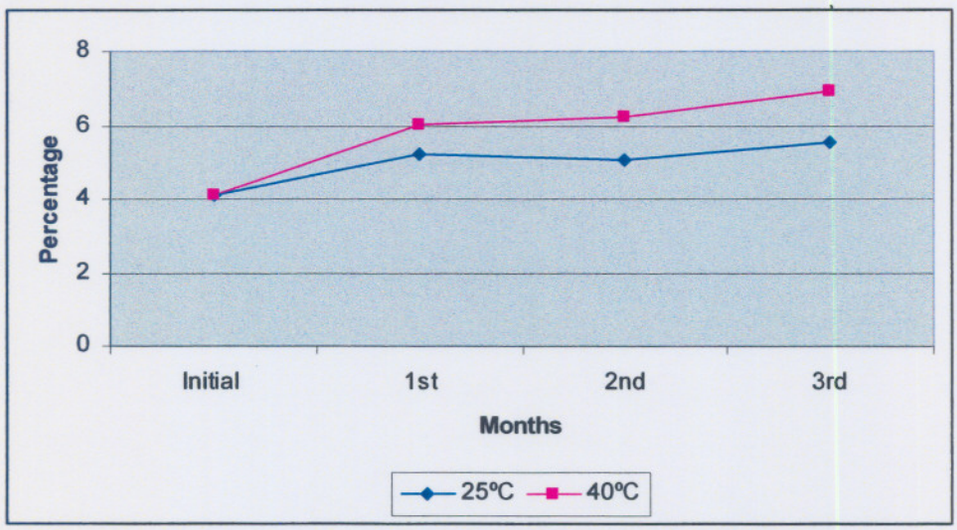


Figure 6.12 Graphic illustration of the moisture content of *tablet C*.

According to the above pictures, the tablets absorbed more moisture at 40 RH than at 25 RH, during the stability program (See appendix 3 for the moisture content results).

6.6 PHYSICAL TESTS

The physical appearance of the tablets was described as round, biconvex, flat-edged, smooth surface texture tablets. *Tablets A* and *C* had a light yellow colour, whereas *tablet B* was white in colour. The tablets stored at 25 RH stayed unchanged for the first two months of the stability program. After the third month the colour of

the tablets changed to a darker yellow and an off-white colour respectively. The tablets stored at 40 RH changed colour after the second month. After the third month the tablets showed dark brown colours around the edges and the colour changed to a mottled grey colour.

Tablets A and *C* showed an insignificant decrease in hardness and remained between 36 N and 55 N (Initial hardness of 51 N and 48 N respectively). *Tablet B* showed an increase in hardness during the stability program and the hardness remained between 73 N and 100 N (Initial hardness of 77 N). The formulation of *tablet B* contained 7% more binder, which could describe the increase in hardness when compared to *tablets A* and *C*. The diameter and thickness of the tablets remained unchanged for the duration of the stability program. The tablets subjected to 40 RH showed an insignificant increase in thickness and diameter, probably caused by moisture uptake of the tablets under these conditions (the hardness, diameter and thickness results are given in appendix 3).

The maximum percentage of weight variation allowed for these tablets is 7.5%. Only one tablet in the initial batch of *tablet C* was outside the set limits. This deviation was insignificant, and the uniformity results were within the limits (See appendix 3 for tablet mass uniformity results).

The friability of the tablets was constant during the stability study and was less than 1% (See appendix 3 for tablet friability results).

The disintegration times for the tablets stored at 25 RH remained between 0 and 9 minutes during the stability period and were within the limit of 15 minutes. The tablets subjected to 40 RH were not within the limits of 15 minutes. Even after 20 minutes no disintegration was observed. The same problem was observed with the dissolutions (see 6.2.3). It was probably caused by the high moisture uptake of the tablets that cause the disintegrant used (Kollidon® CL-M) to swell under these conditions. A tight-sealing container as well as drying agents may overcome this problem.

6.7 CONCLUSION

According to the above mentioned stability tests the formulas developed for the tablets containing doxycycline HCl and bromhexine HCl respectively and in

combination, remained stable over the accelerated three month testing period. This was only an indication of the stability of the tablet and to claim stability, a complete stability trial study is required. To overcome the problems identified with the dissolution and disintegration, tightly sealed glass amber bottles should be used. Because of the high moisture uptake by the tablets, only one drying agent is not enough, more than one drying agent per bottle is a necessity. The decreased release of the active substances from the tablets, as indicated by the assay and dissolution results, occurred only at the extreme temperatures where the moisture concentration is very high. At normal storage conditions, tablets would not be exposed to these conditions.

The HPLC assay results in combination with the dissolution rate profiles showed that there was no interaction between doxycycline HCl and bromhexine HCl and the excipients used in the formulations.

CHAPTER 7

STABILITY STUDY TEST RESULTS: POWDERS

7.1 INTRODUCTION

The accelerated stability test conditions and methods used are as described in 5.2.2. The results obtained over the three month period served only as an indication of the stability of the three formulated water-based powder dosage forms.

Three different water-based powders were formulated as described in 5.3. *Powder A* contained doxycycline HCl as active component, *powder B* contained bromhexine HCl as active component and *powder C* was a combination of the two active components.

The containers used for these powders were plastic containers containing about 150 g powder each. Samples of the formulated powders were stored at 25°C ± 2°C at 60% RH (25 RH) as well as 40°C ± 2°C at 75% RH (40 RH) for a period of three months. Stability tests were performed initially and then monthly for a further three months at these storage conditions.

The test results of the three powder formulations, *powder A, powder B* and *powder C*, generated during the stability evaluation, are summarised and discussed in this chapter. The raw data is included in Appendix 3 for reference.

7.2 ASSAY

The HPLC assay results of both the 25 RH and the 40 RH powder batches of *powders A, B and C* are summarised and represented graphically in figure 7.1, figure 7.2, figure 7.3 and figure 7.4 respectively (See Appendix 3 for the assay results).

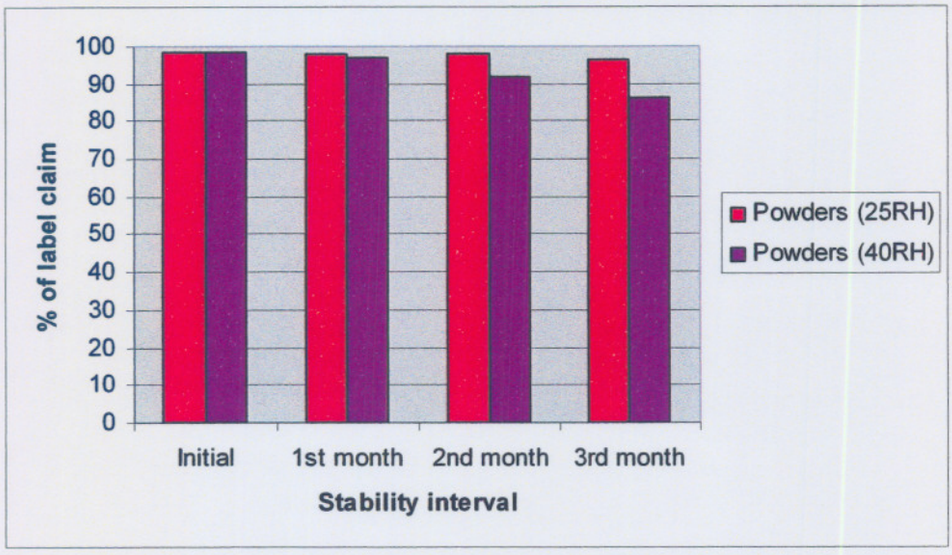


Figure 7.1 HPLC assay results of *powder A*.

Discussion of assay results of *powder A*

The assay results of the doxycycline HCl indicated that no significant breakdown occurred during the three month stability period for the powders subjected to 25 RH. This indicates that the doxycyline HCl remained relatively stable in this formulation at these storage conditions. The results of the powders being subjected to 40 RH conditions, however, indicated a gradual decrease of active substance during the stability test period, and possible breakdown that occurred.

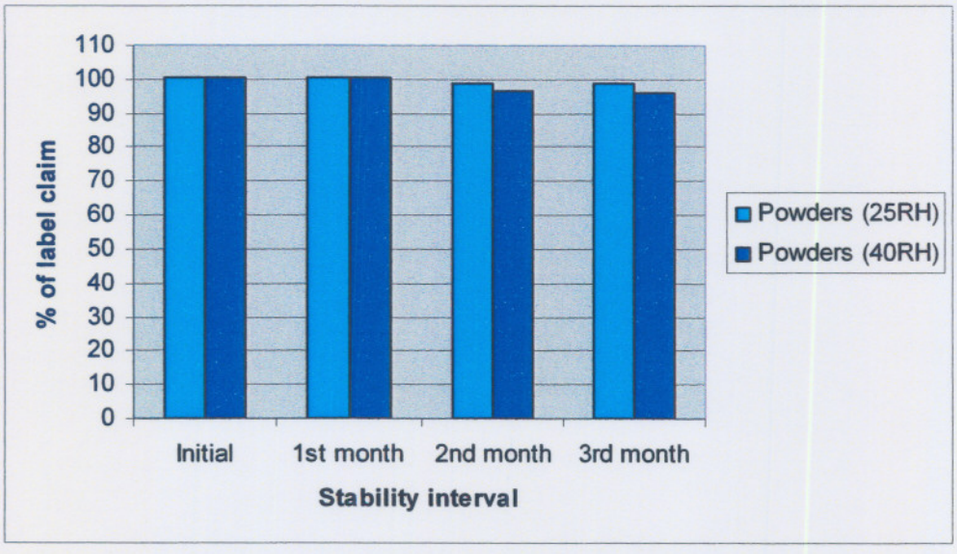


Figure 7.2 HPLC assay results of *powder B*.

Discussion of the assay results of *powder B*

There were no significant differences in the HPLC assay results of the 25 RH samples and the 40 RH samples, indicating that the bromhexine HCl remained relatively stable in this formulation at these storage conditions. These assay results remained within the specified limits of 85% to 115% of the labelled amount.

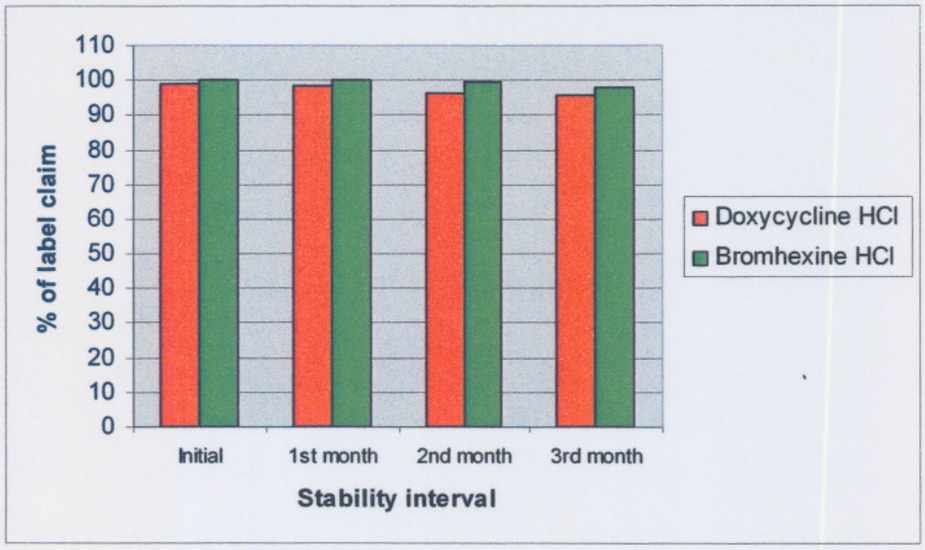


Figure 7.3 HPLC assay results of *powder C* subjected to 25°C + 60% RH.

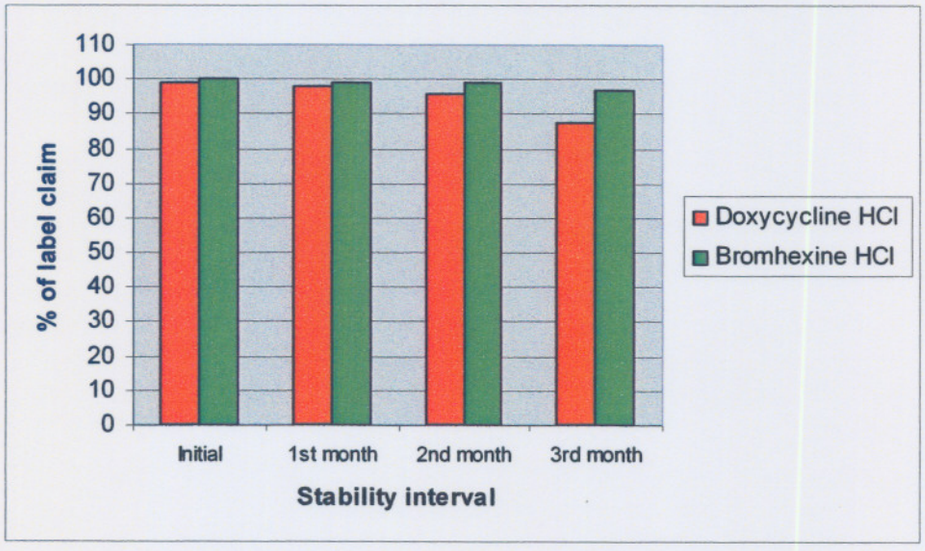


Figure 7.4 HPLC assay results of *powder C* subjected to 40°C + 75% RH.

Discussion of assay results of *powder C*

There were no significant differences in the HPLC assay results of the samples subjected to 25 RH or 40 RH. This indicates that both the doxycycline HCl and the bromhexine HCl remained stable in this formulation and showed no incompatibilities with each other. The oxidation of the doxycycline HCl at high temperatures might be the reason for the slightly gradual decreased values of doxycycline HCl at 40 RH.

7.3 "IN USE" ASSAY

An "in use" assay was done on *powder A* according to the method as described in 5.2.2.1. This was done to determine the effect of the different containers used for the water of the pigeons, on the stability of the doxycycline HCl in the powder. *Powder A* was also compared to a powder with no citric acid (*Powder X*) in the formulation to determine the effectiveness of the citric acid on the stability of the doxycycline HCl. The results are given in figure 7.5 – 7.6 (See Appendix 3 for the "in use" assay results).

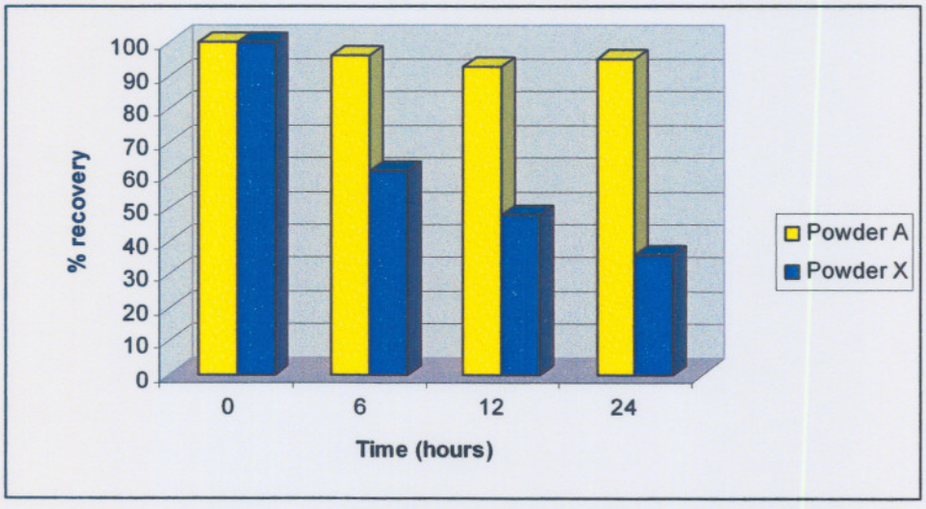


Figure 7.5 "In use" HPLC assay results of the dissolved powders taken from a glass container.

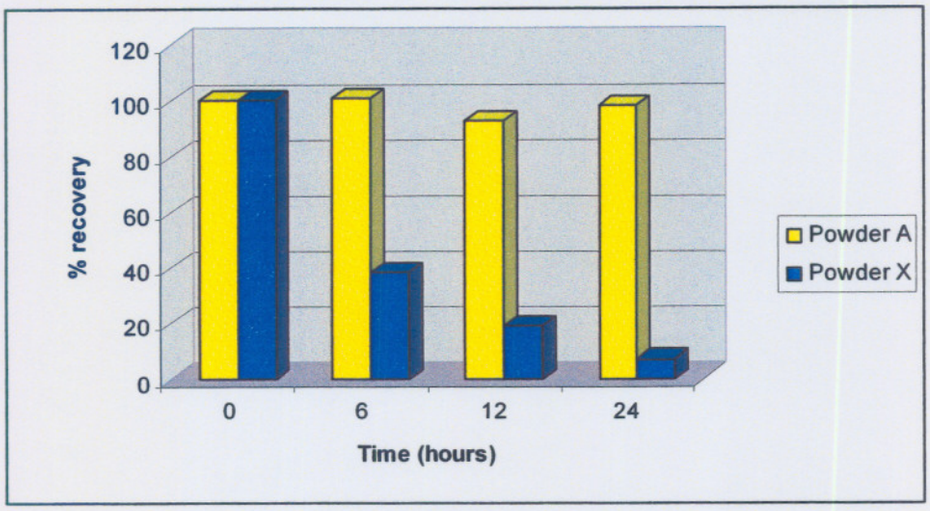


Figure 7.6 "In use" HPLC assay results of the dissolved powders taken from a plastic container.

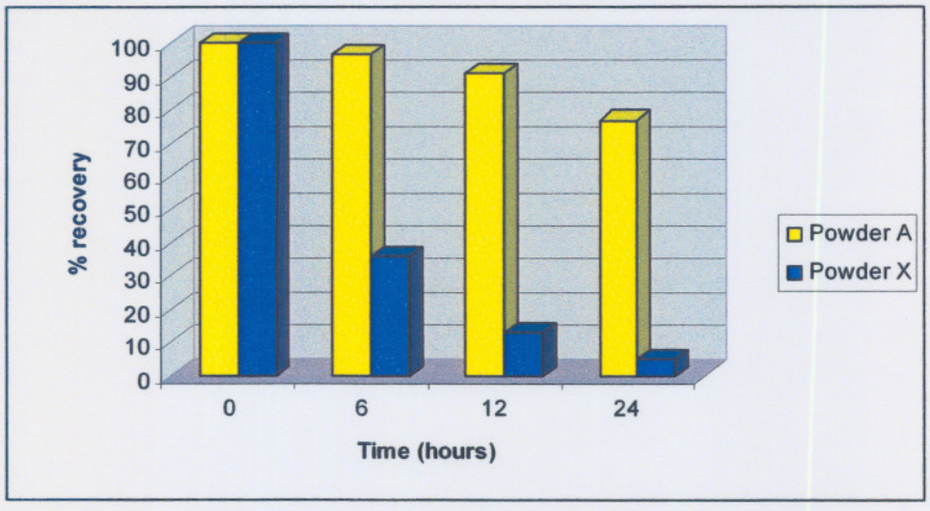


Figure 7.7 "In use" HPLC assay results of the dissolved powders taken form a stainless steel container.

7.3.1 Discussion of the "in use" assay results

In all three of the containers the remarkable difference between *powder A* and *powder X* can be observed. The doxycycline HCl without the citric acid (*powder X*) showed significant breakdown and discolouration in all three containers (See figures 7.9 to 7.11). This is one of the problems associated with the doxycycline HCl preparation currently on the market, because as soon as the water changes its

colour, the pigeons refuse to drink the water. Although the decrease in percentage recovery of the sample taken from the glass container was much less than that taken of the plastic and stainless steel containers, recovery was still only 36% after 24 hours. This comparison between the two powders shows clearly that the citric acid in the formulation inhibits the oxidation of the doxycycline HCl and helps to keep it stable and prevent discolouration of the solution. All of these samples in all three different containers were exposed to the sun and still no remarkable breakdown occurred where the citric acid was present. The doxycycline HCl added to the tap water without citric acid precipated. Citric acid could also improve the palatability of the doxycycline HCl solution with an increase in the water uptake (Santos *et al.*, 1997:1347). The stainless steel container showed a gradual decrease of the active substance of *powder A*. This might be due to the chelating reaction of the doxycycline HCl with the stainless steel, leading to the oxidation of the doxycycline HCl. Even so, the percentage recovery was still 77% after 24 hours compared to the 5% of *powder X*. When considering the results obtained, the best container to use for the water of the pigeons, especially when medicating them, will be a glass container. Figure 7.8 to 7.11 shows the discolouration and precipitation of *powder X* in comparison with *powder A*.

Figure 7.8 *Powder A* (left) and *powder X* (right) at 0 hours.

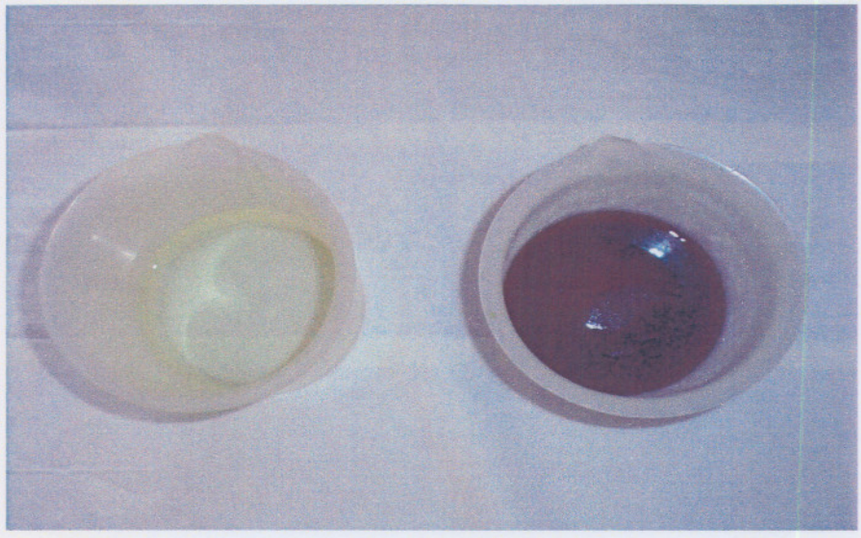


Figure 7.9 *Powder A* (left) and *powder X* (right) after 24 hours in a plastic container.

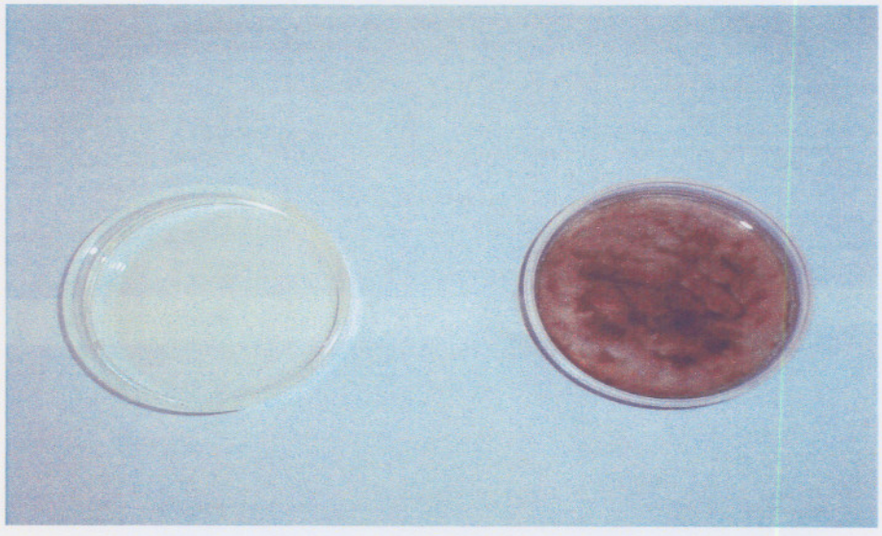


Figure 7.10 *Powder A* (left) and *powder X* (right) after 24 hours in a glass container.

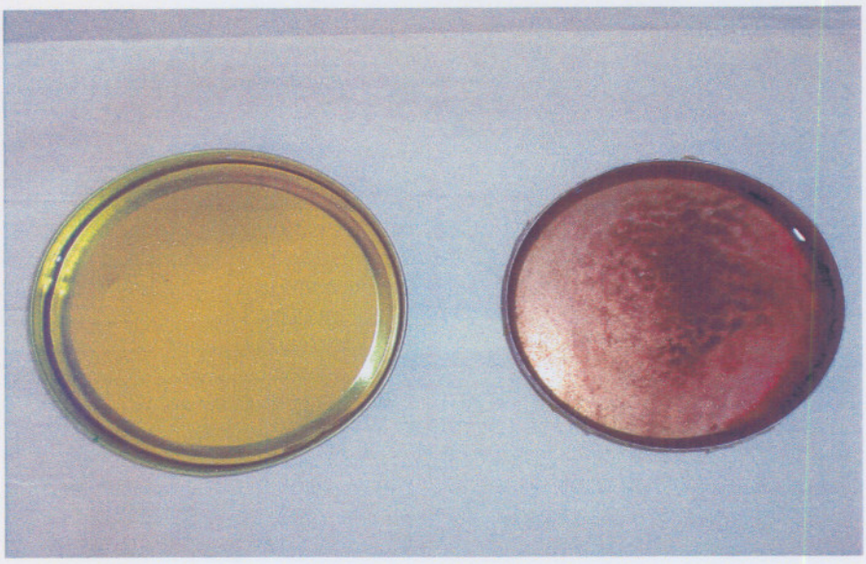


Figure 7.11 *Powder A* (left) and *powder X* (right) after 24 hours in a stainless steel container.

7.4 MOISTURE CONTENT

The moisture content was determined using a Mettler DL18 Karl Fischer titrator. The results are given in figure 7.12 – 7.14.

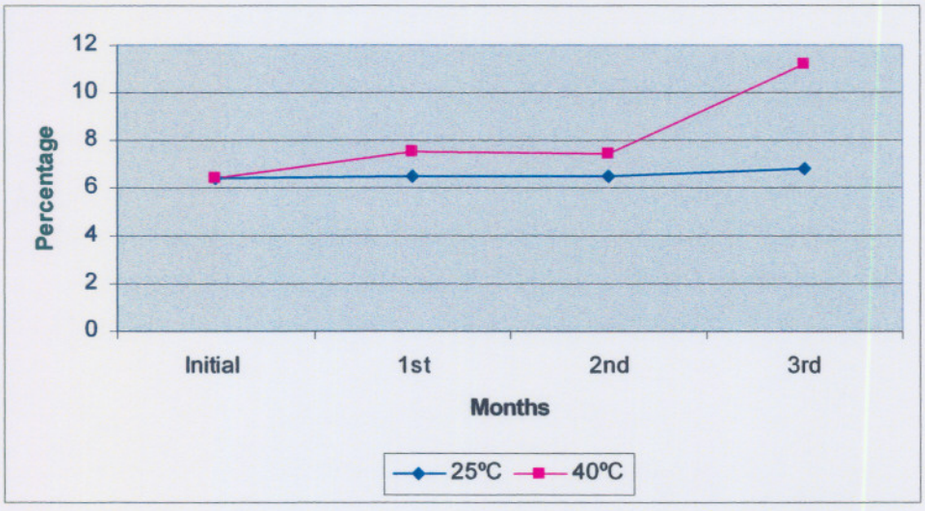


Figure 7.12 Graphic illustration of the moisture content of *powder A*.

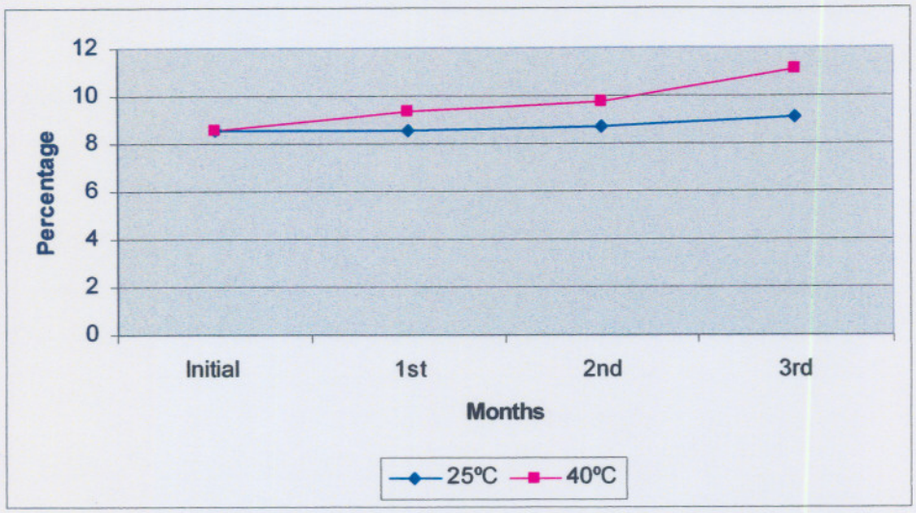


Figure 7.13 Graphic illustration of the moisture content of *powder B.*

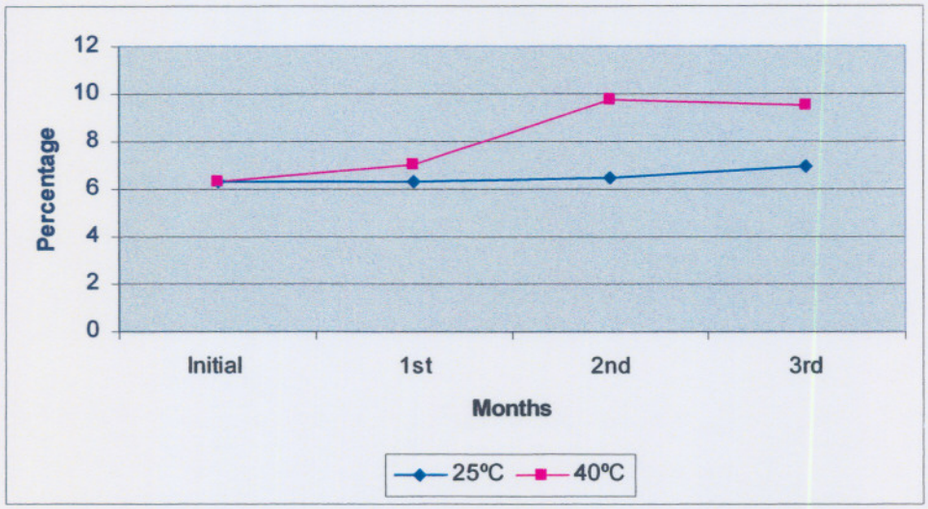


Figure 7.14 Graphic illustration of the moisture content of *powder C.*

7.4.1 Discussion of the moisture content results

The powders subjected to the 25 RH conditions moisture content stayed relatively stable. The powders stored at 40 RH showed a definitive increase in moisture uptake. A dramatic increase of moisture content for *powder A* subjected to the 40 RH conditions was observed between month 2 and 3 (see figure 7.12). The hygroscopic nature of the glucose used as diluent is probably one of the reasons for

The "in use" assays showed the role of the citric acid in this formulation. The solution was stable for over 24 hours and showed no discolouration. This powder was given to a pigeon farmer and racer, Mr W.A. Coetzee, who found the results astonishing. No discolouration appeared for up to 8 days. This is excellent compared to the doxycycline HCl preparations currently on the market, which shows discolouration after only 1 hour. A report written by him is given in Appendix 4.

The HPLC assay results showed that there was no interaction between doxycycline HCl and bromhexine HCl and the excipients used in the formulations.

CHAPTER 8

STABILITY STUDY TEST RESULTS: OPHTHALMIC SOLUTION

8.1 INTRODUCTION

The accelerated stability test conditions and methods used are as described in 5.2.3. The results obtained over the three month period served only as an indication of the stability of the ophthalmic solution.

The ophthalmic solution was stored in sterilised, amber glass dropper bottles. The samples were stored at 5°C ± 3°C (5 F), 25°C ± 2°C at 60% RH (25 RH) as well as 40°C ± 2°C at 75% RH (40 RH). Samples stored at 5°C ± 3°C were only evaluated after one month of stability. The other samples stored at 25 RH and 40 RH were evaluated initially and then monthly for three months except for the preservative efficacy tests that were done initially and after the three month period.

The tests results of the ophthalmic solution, containing doxycycline HCl as active component, are summarised and discussed in this chapter. The raw data is included in Appendix 3 for reference.

8.2 ASSAY

The HPLC method used for the determination of the doxycycline HCl are as described in 5.2.3.1. The HPLC assay results of the 5 F, 25 RH and 40 RH batches of the ophthalmic solution are summarised and presented graphically in figure 8.1.

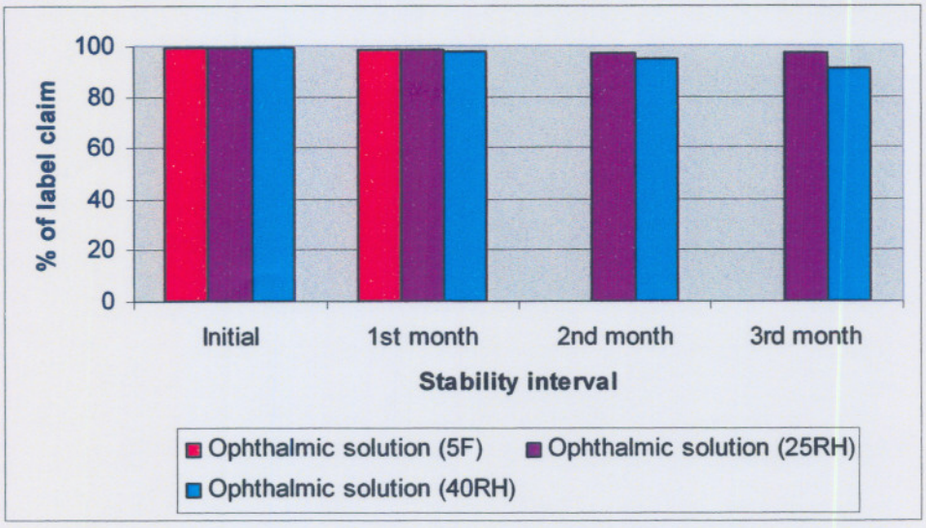


Figure 8.1 HPLC assay results of the ophthalmic solution.

8.2.1 Discussion of the assay results

All the assay results complied for doxycycline HCl which was within the specified limits of 85% to 115% of the labelled amount. The ophthalmic solution stored at 40 RH showed a trivial decrease in percentage of label claim which can be explained as slight degradation of the doxycycline HCl due to the high temperatures it was subjected to. The results indicated that the doxycycline HCl remained relatively stable in this formulation under these storage conditions.

8.3 APPEARANCE AND PARTICULATE MATTER

Initially the physical appearance of the ophthalmic solution was described as a clear, sticky, yellow solution, free form particulate matter. The stopper of the container sealed tightly and was intact.

Very small, white particles appeared after the ophthalmic solution was stored at 40 RH for two months. The samples stored at 5 F for one month and 25 RH for three months showed no change in appearance or particulate matter. The samples stored at 40 RH showed an increase in colour from yellow to a darker yellow to almost dark brown after three months. After three months dark particles appeared on the top of the waterline and on the dropper of the ophthalmic solution stored at 40 RH. Instability may be caused by the amber glass, which contains metal ions; instead a clear glass bottle with external coating can be used.

8.4 pH AND RELATIVE DENSITY

The initial pH of the ophthalmic solution was 5.21. The pH of the 5 F batch increased slightly after one month to 5.37. The pH of the 25 RH batch remained between 5.19 and 5.70 and that of the 40 RH batch between 5.24 and 5.52. It is important that the pH of the ophthalmic solution stays relatively stable because of the sensitivity of the eye. The pH of an ophthalmic solution is considered optimum in the range 4 to 8 (Loyd, 1997:222). The pH results are represented graphically in figure 8.2.

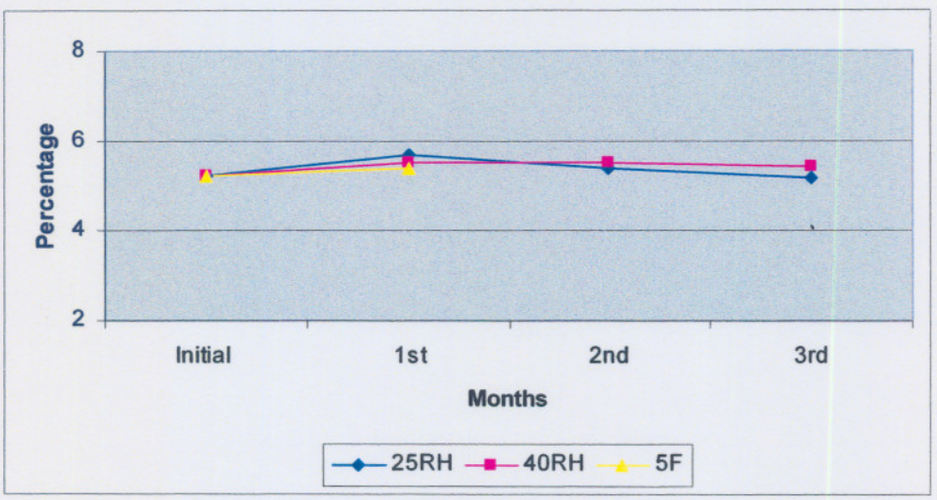


Figure 8.2 Graphic illustration of the pH of the ophthalmic solution.

The relative density of both the 25 RH and the 40 RH batches remained relatively constant with values between 1.0563 g/ml and 1.0579 g/ml for the entire stability testing period.

8.5 VISCOSITY

The viscosities of all three batches were determined in duplicate at 60 rpm. The average initial viscosity of the ophthalmic solution was 5.87 cP and the viscosity of the 5F batch stayed unchanged after one month. The average viscosities of the 25RH batch remained between 5.33 and 5.87 cP and that of the 40RH batch remained between 5.31and 5.87 cP.

It is clear that the viscosity of the ophthalmic solution was not affected by the stability conditions.

8.6 PRESERVATIVE EFFICACY

Preservative efficacy testing was done by Wits Health Consortium (Pty) Ltd. on all three batches of the ophthalmic solution initially and after three months. All the samples complied with the requirements of USP 25 and USP 26 and the specified microbial limits.

8.7 CONCLUSION

The HPLC assay test results indicated that there were no significant interaction between the doxycycline HCl and the excipients used in this formulation. These results also indicated that the doxycycline HCl did not degrade significantly during the three month exposure to the storage conditions.

The colour of the ophthalmic solution intensified after three months at 40 RH. This is probably due to the tendency of Kollidon® 17 PF to discolour when kept at temperatures above 25°C. The yellow or yellow-brown colour is formed as a result of oxidation (BASF, 2003:47). This is an indication that the ophthalmic solution should not be stored above 25°C.

The pH of the ophthalmic solution was acceptable with an acidic pH between 5 and 6. The pH stayed relatively constant throughout the stability testing period.

The relative density and viscosity remained constant and no significant changes were observed which proves that the stability conditions hand no effect on these parameters.

Preservative efficacy test results indicated that Kollidon® 17 PF acted very effectively as a preservative in the ophthalmic solution.

It seemed that this specific ophthalmic solution formula containing doxycycline HCl remained relatively stable over the three month stability trial period.

The three months of stability testing only served to give an indication of the stability of the ophthalmic solution. A complete stability trial study is required to claim stability.

CHAPTER 9

DEVELOPMENT OF A METHOD FOR THE SIMULTANEOUS DETERMINATION OF DOXYCYCLINE HCl AND BROMHEXINE HCl

9.1 ORIGIN OF METHOD

This chapter describes the method that was developed and validated as part of this study.

9.2 CHROMATOGRAPHIC CONDITIONS

- **Analytical instrument:** HP 1090 series equipped with a quaterny gradient pump, autosampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software.
- **Column:** Column L1, USP 24, 2000, p1925 (Luna C18(2), 150 x 4.6 mm, 5 µm column, 100 Å pores, 21.5% carbon load, end capped, Phenomenex, Torrance, CA) was used. A Lichroshper 100 RP-18, 5µm was used as guard column.
- **Mobile phase:** 0.005 M octane-sulphonic acid Na-salt in deionised water: Acetronitrile. (60 : 40) (v/v). pH adjusted to 3.5 with H_3PO_4.
- **Flow rate:** 1.0 ml/min
- **Injection volume:** 10 µl
- **Detection:** UV at 254 nm
- **Retention time:** Doxycycline HCl ± 2.9 minutes
  Bromhexine HCl ± 9.8 minutes
- **Stop time:** 12 minutes

9.3 SAMPLE PREPARATION

1. Accurately weigh twenty tablets and grind into a fine powder.
2. Weigh an amount of powder equal to the mass of one tablet accurately.
3. Transfer the powder into a 100 ml volumetric flask.
4. Add 20 ml of methanol and 50 ml of deionised water.
5. Sonicate for 15 minutes.
6. Leave to cool and fill to volume with deionised water.
7. Filter through a 0.45 µm filter, transfer into an HPLC vial and inject into the HPLC.

9.4 STANDARD PREPARATION

1. Weigh approximately 20 mg bromhexine HCl accurately.
2. Transfer into a 100 ml volumetric flask and dissolve in 20 ml methanol and 50 ml deionised water.
3. Sonicate for 15 minutes.
4. Leave to cool and fill up to volume with deionised water.
5. Weigh approximately 17.25 mg doxycycline HCl accurately.
6. Transfer into a 100 ml volumetric flask and transfer exactly 5 ml of the bromhexine solution into the volumetric flask as well.
7. Dissolve in 50 ml deionised water.
8. Sonicate for 5 minutes.
9. Leave to cool and fill up to volume with deionised water.
10. Filter through a 0.45 µm filter, transfer into an HPLC vial and inject into the HPLC.

9.5 CALCULATIONS

The tablets contained 17.25 mg doxycycline HCl and 1 mg bromhexine HCl. The prepared sample therefore contained 172.5 µg/ml of doxycycline HCl and 10 µg/ml of bromhexine HCl. The following calculations were used to evaluate the results obtained.

For doxycycline HCl:

$$C_{std} = \frac{\text{Mass doxycycline HCl weighed x potency}}{100 \times 100}$$

where mass doxycycline HCl weighed = mg

potency = %

$$\% \text{ Doxycycline of label claim} = \frac{SAR \times 100 \times C_{std} \times 100}{STR \times EqM_{sample}}$$

where SAR = sample peak area

STR = standard peak area

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

For bromhexine HCl:

$$C_{std} = \frac{\text{mass bromhexine HCl weighed x potency}}{100 \times 100 \times 20}$$

where mass bromhexine HCl weighed = mg

potency = %

$$\% \text{ bromhexine HCl of label claim} = \frac{SAR \times 100 \times C_{std} \times 100}{STR \times EqM_{sample}}$$

where SAR = sample peak area

STR = standard peak area

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

9.6 SYSTEM SUITABILITY PARAMETERS

The following parameters were used:

- Analyse six replicate injections of a standard solution
- Calculate the relative standard deviation of the peak areas obtained for the active ingredients.
- Calculate the number of theoretical plates, using the 5-sigma method.
- Calculate the USP tailing factor.

The system would be suitable to perform the analysis if the following criteria were met:

- RSD not more than 1.0 %,
- Number of theoretical plates more than 35000 per column for both analytes,
- USP tailing factor not more than 1.5.

9.7 VALIDATION TEST PROCEDURE AND ACCEPTANCE CRITERIA

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose (International, 1996:2).

9.7.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present (International, 1996:5).

Method

1. Prepare a sample from a placebo of the tablet that does not contain the substance being tested.
2. Diluted a standard solution 1:1 with:
    - Water
    - 0.1 M hydrochloric acid
    - 0.1 M sodium hydroxide
    - 10% hydrogen peroxide.
3. Store these solutions overnight in closed test tubes at 40°C to allow degrading.
4. Inject the samples into the chromatograph with a stop time of 30 minutes.
5. Examine the chromatograms to determine whether any additional peaks were formed.

Acceptance criteria

1. The placebo should not contain any peaks that will interfere with the determination of the actives.

2. Extra peaks formed in the stressed samples should be discernible from those of the active components.

9.7.2 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample (International, 1996:6).

Method

Preparation of standards:

1. Prepare a standard solution as described under standard preparation.
2. Inject variable volumes into the chromatograph to obtain standards from 60-120% of the expected sample concentration.

Accepted criteria

Linear regression analysis should yield a regression coefficient (R^2) of ≥ 0.99.

9.7.3 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found (International, 1996:5).

Method

1. Measure in triplicate 80% (120 mg), 100% (150 mg) and 120% (180 mg) of the placebo into 100 ml volumetric flasks.
2. Spike these samples with concentrations of 80%, 100% and 120% of the expected sample concentrations.
3. Inject into the chromatograph in duplicate.

Accepted criteria

Recovery must be between 98% and 102%.

9.7.4 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained

from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility (International, 1996:5).

9.7.4.1 Intra-day precision (repeatability)

Method

1. Measure approximately 120 mg, 150 mg and 180 mg (in triplicate) of tablet powder each into a 100 ml volumetric flask and fill to volume with deionised water.
2. Inject into the chromatograph in duplicate.

9.7.4.2 Inter-day precision

Method

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

Accepted criteria

Repeatability must be better than 2% (n=9).

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

9.7.5 Ruggedness

9.7.5.1 Stability of sample solutions

Method

1. Prepare a sample as described under sample preparation in the method.
2. Inject the sample into the chromatograph.
3. Leave the sample in the autosampler tray and re-analyse over a period of 24 hours to determine the stability of the sample.

Acceptance criteria

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

9.7.5.2 System repeatability

Method

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

Acceptance criteria

The peak area and retention times should have an RSD of $\leq$ 2%.

9.7.5.3 Robustness

Method

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

Acceptance criteria

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

9.7.6 System suitability (system and method performance characteristics)

Method

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

Acceptance criteria

The USP tailing factor must be less than 1.5. The number of theoretical plates must be more than 35 000 per column for both analytes, and the RSD of 6 injections should not be more than 2%.

9.8 SUMMARY OF VALIDATION RESULTS

The validation of the method for the simultaneous determination of doxycycline HCl and bromhexine HCl in tablets yielded the following results, as summarised in Table 9.1.

Table 9.1 Summary of validation results

Test	Result
Specificity	Complies
Range	Doxycycline HCl: 17.3 - 207 µg/ml Bromhexine HCl: 6 – 12 µg/ml
Linearity	Doxycycline HCl: R^2 = 0.999 Bromhexine HCl: R^2 = 0.992
Accuracy	Doxycycline HCl: 101.1% Bromhexine HCl: 100.8%
Precision	Doxycycline HCl: RSD < 3% Bromhexine HCl: RSD < 3%
Ruggedness	Complies
Robustness	Complies

9.9 VALIDATION RESULTS

9.9.1 Specificity

The result of a sample prepared from the placebo is shown in Figure 9.1. Figures 9.2 and 9.3 are chromatograms obtained for a standard solution and a tablet sample, respectively. Figures 9.4 – 9.7 are chromatograms of samples that have been stressed for 24 hours as described under section 9.7.1. Figures 9.8 and 9.9 represent the peak purity tests for both doxycycline HCl and bromhexine HCl.

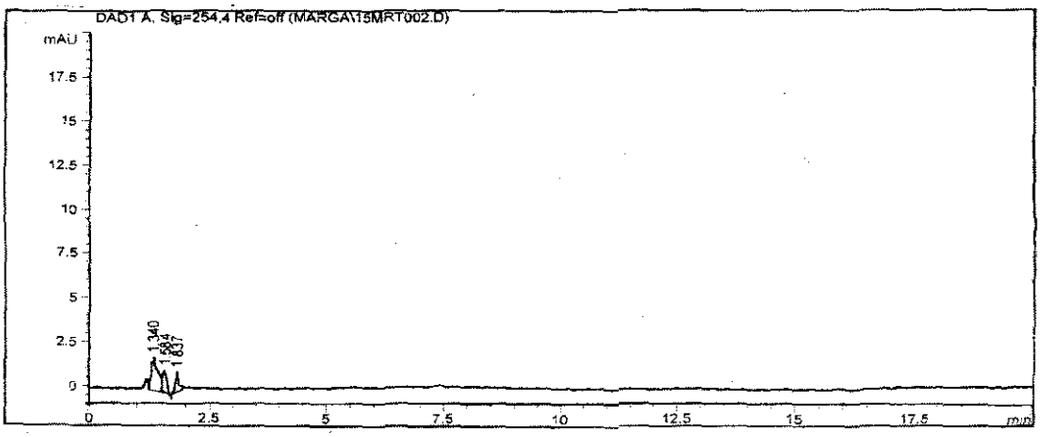


Figure 9.1 Chromatogram of placebo tablet.

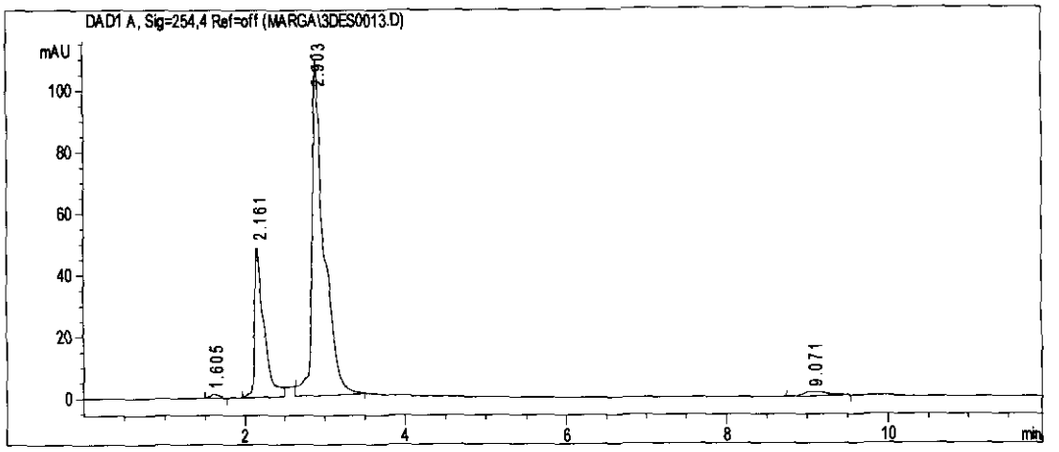


Figure 9.2 Chromatogram of standard solution.

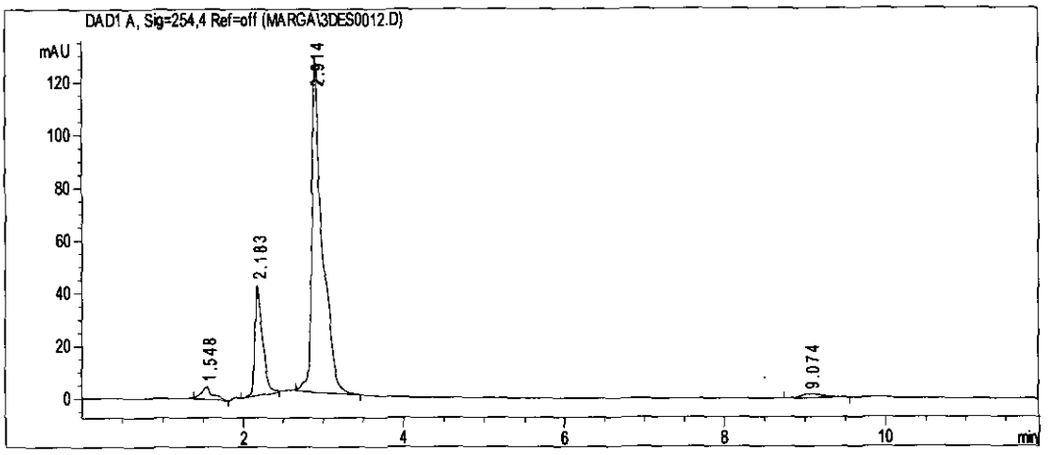


Figure 9.3 Chromatogram of tablet sample.

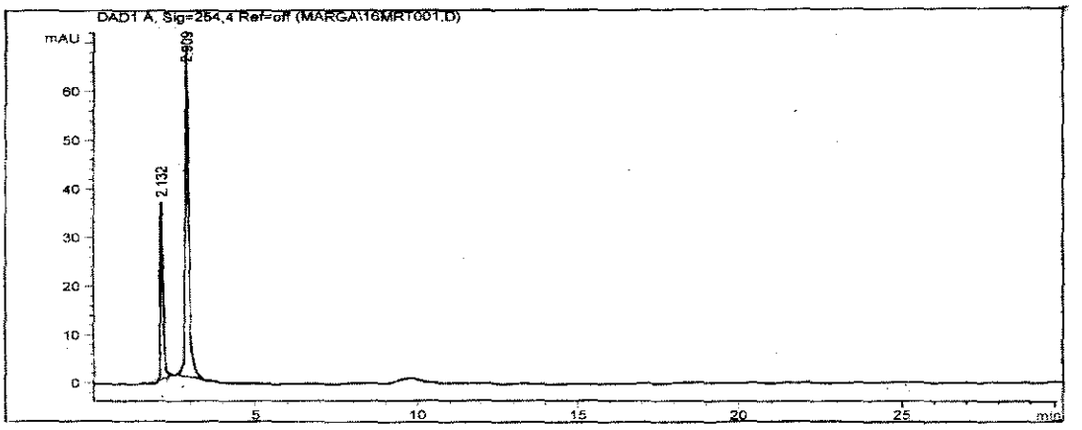


Figure 9.4 Chromatogram of a sample stressed in water at 40°C for 24 hours.

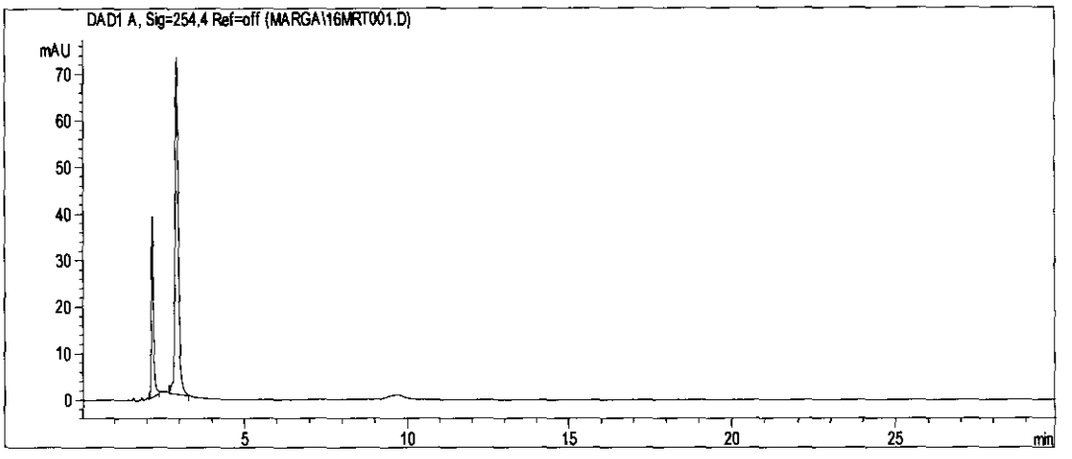


Figure 9.5 Chromatogram of sample stressed in 0.1 M hydrochloric acid at 40°C for 24 hours.

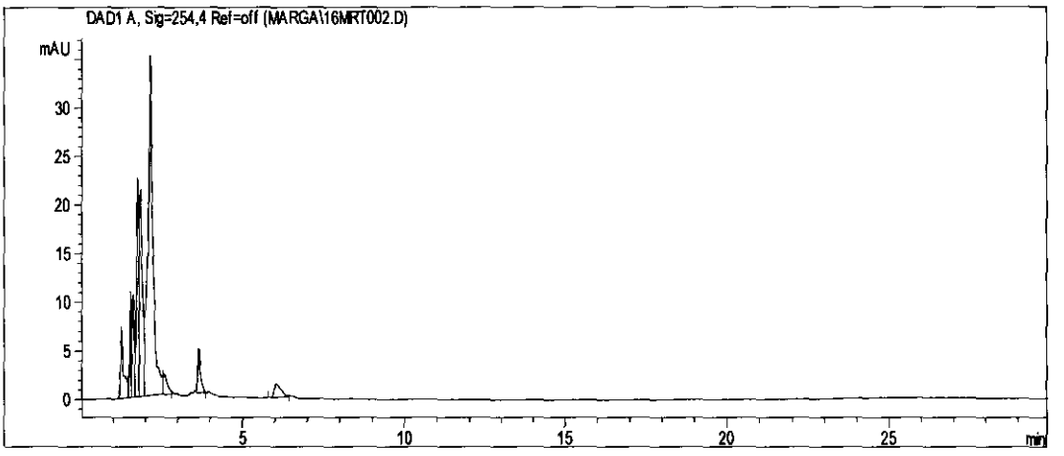


Figure 9.6 Chromatogram of sample stressed in 0.1 M sodium hydroxide at 40°C for 24 hours.

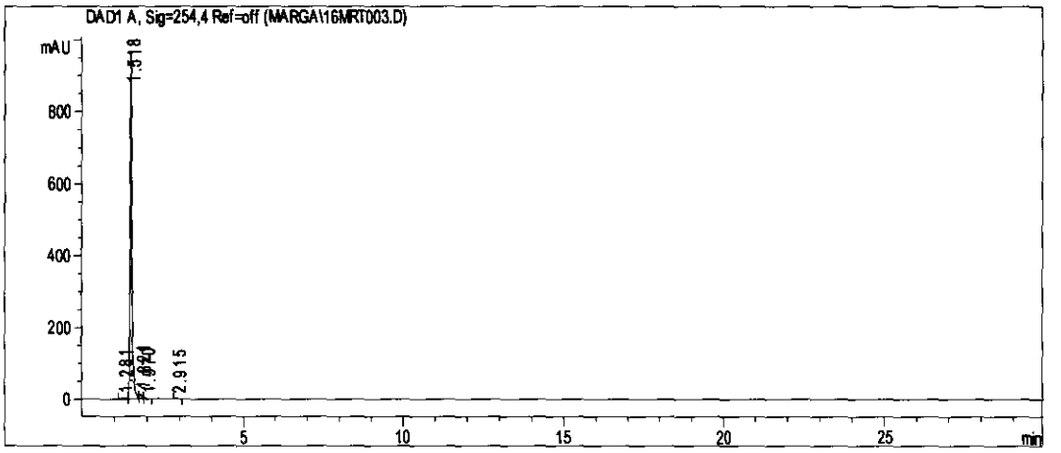


Figure 9.7 Chromatogram of sample stressed in 10% hydrogen peroxide at 40°C for 24 hours.

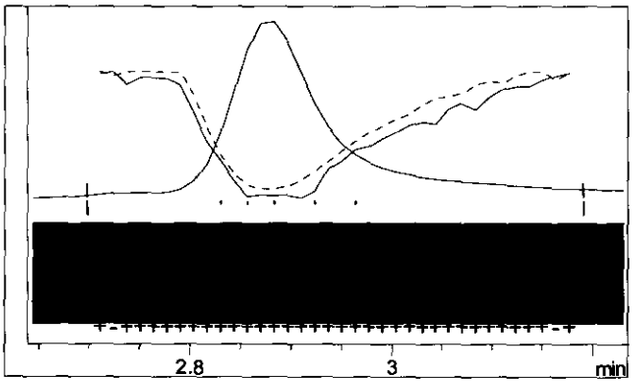


Figure 9.8 Peak purity test of doxycycline HCl.

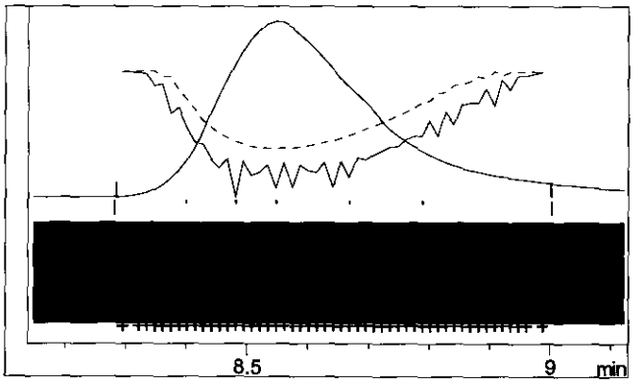


Figure 9.9 Peak purity test of bromhexine HCl.

Conclusion

None of the ingredients in the placebo interfered with the analyte peaks. Peak purity testing of the peaks, after forced degradation in water, showed that the peaks remained pure, thus proving that the method was stability-indicating.

9.9.2 Linearity and range of doxycycline HCl and bromhexine HCl

Tables 9.2 – 9.3 summarise the linearity results of doxycycline HCl and bromhexine HCl, respectively.

Table 9.2 Peak area and concentration found for doxycycline HCl

Doxycycline HCl				
Injection volume (µl)	**Conc (µg/ml)**	**Peak area**		
		Area 1	**Area 2**	**Mean**
10	17.3	63	64	64
10	51.8	279	284	282
10	86.3	492	494	493
10	103.5	592	590	591
10	120.8	700	702	701
10	138.0	800	805	802
10	155.0	900	905	903
10	172.5	1020	1028	1024
11	189.8	1130	1132	1131
12	207.0	1249	1246	1247

Table 9.3 Regression parameters of doxycycline HCl

Regression parameters			
R Squared	0.9996	**Lower 95%**	**Upper 95%**
Intercept	-44.4087	-56.4751	-32.3423
Slope	6.1863	6.0981	6.2745

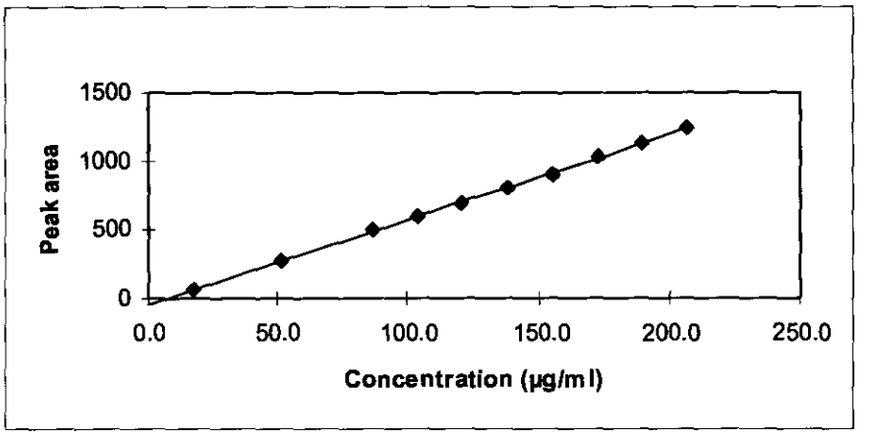


Figure 9.10 Linear regression curve for doxycycline HCl.

Table 9.4 Peak area and concentration found for bromhexine HCl

Bromhexine HCl				
Injection volume (µl)	Conc (µg/ml)	Peak area		
		Area 1	Area 2	Mean
10	6.0	47	46	47
10	7.0	52	54	53
10	8.0	58	58	58
10	9.0	64	65	65
10	10.0	70	69	70
11	11.0	76	76	76
12	12.0	83	81	82

Table 9.5 Regression parameters of bromhexine HCl

Regression parameters			
R Squared	0.9992	Lower 95%	Upper 95%
Intercept	10.2016	4.4758	15.9273
Slope	6.0502	5.4291	6.6712

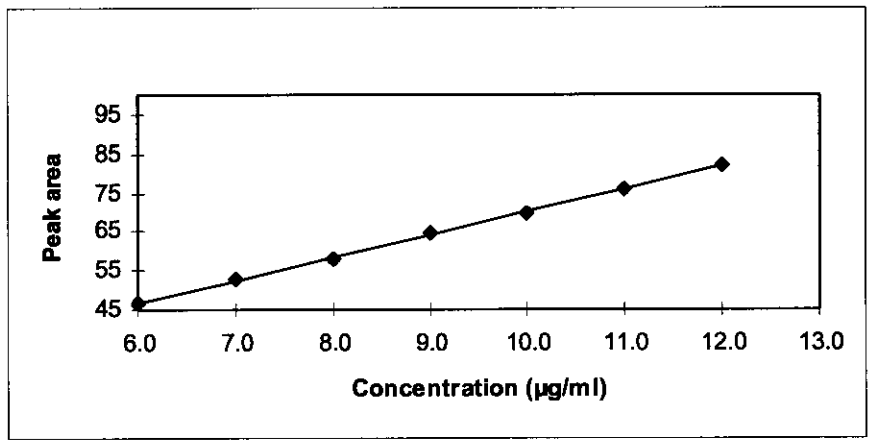


Figure 9.11 Linear regression curve for bromhexine HCl.

Conclusion

This method is linear over the concentration range of 17.3 – 207.0 µg/ml for doxycycline HCl, and 6.0 – 12.0 µg/ml for bromhexine HCl. The method met the acceptance criteria and was suitable for single point calibration.

9.9.3 Accuracy

Table 9.6 Percentage doxycycline HCl recovered

Doxycycline HCl			
Conc Spiked (µg/ml)	**Peak area**	**Conc found (µg/ml)**	**Recovered (%)**
138.0	816	136.2	98.7
138.0	824	137.5	99.6
138.0	813	135.8	98.4
172.5	1042	173.9	100.8
172.5	1046	174.7	101.3
172.5	1049	175.2	101.6
207.0	1275	212.8	102.8
207.0	1280	213.7	103.2
207.0	1281	213.8	103.3

Table 9.7 Confidence intervals for doxycycline HCl

Statistical analysis	
Mean	101.1
% RSD	1.8
SD	1.7
95% Confidence intervals	
Lower limit	99.6
Upper limit	102.5
Confidence level	1.4
Estimated median	101.3

Table 9.8 Percentage bromhexine HCl recovered

Bromhexine HCl			
Conc Spiked (µg/ml)	**Peak area**	**Conc found (µg/ml)**	**Recovered (%)**
8.0	50	8.0	99.8
8.0	51	8.0	100.2
8.0	50	7.9	99.3
10.0	65	10.3	103.5
10.0	63	9.9	99.2
10.0	64	10.2	101.8
12.0	77	12.2	101.8
12.0	77	12.2	101.6
12.0	76	12.1	100.5

Table 9.9 Confidence intervals for bromhexine HCl

Statistical analysis	
Mean	100.8
% RSD	1.3
SD	1.3
95% Confidence intervals	
Lower limit	99.8
Upper limit	101.9
Confidence level	1.1
Estimated median	100.5

Conclusion

Over the concentration of 138 – 207 µg/ml for doxycycline HCl, and 8 – 12 µg/ml for bromhexine HCl, the method yielded and accuracy of 101.1% and 100.8% respectively.

9.9.4 Precision

9.9.4.1 Intra-day precision

Table 9.10 Intra-day precision results for doxycycline HCl

Doxycycline HCl			
Mass (mg)	**Peak area**	**Conc found (µg/ml)**	**Recovered (%)**
121.0	815	168.5	97.7
120.5	813	168.9	97.9
120.7	815	169.0	98.0
150.3	1048	174.6	101.2
150.4	1049	174.5	101.2
150.8	1050	174.3	101.0
180.2	1279	177.6	102.9
180.5	1281	177.7	103.0
180.5	1280	177.6	103.0
		Mean	100.66
		SD	2.12
		% RSD	2.10

Table 9.11 Intra-day precision results for bromhexine HCl

Bromhexine HCl			
Mass (mg)	**Peak area**	**Conc found (µg/ml)**	**Recovered (%)**
120.4	49.8	10.2	102.0
120.4	49.9	10.2	102.2
120.6	49.3	10.1	100.9
150.2	60.9	10.0	100.0
150.4	61.0	10.0	100.1
150.0	61.8	10.2	101.6
180.5	73.8	10.1	100.9
180.5	75.7	10.3	103.4
180.3	74.4	10.2	101.7
		Mean	101.42
		SD	1.03
		% RSD	1.01

9.9.4.2 Inter-day precision

Table 9.12 Inter-day precision results for doxycycline HCl

	Day 1	**Day 2**	**Day 3**	**Inter day**
	101.2	101.3	99.4	
	101.2	101.8	100.7	
	101.0	101.0	99.7	
Mean	101.15	101.37	99.96	100.83
SD	0.08	0.35	0.57	0.62
% RSD	0.07	0.35	0.57	0.61

Table 9.12.1 ANOVA: Single factor for doxycycline HCl

Summary				
Groups	**Count**	**Sum**	**Average**	**Variance**
Day 1	3	303.438	101.146	0.009
Day 2	3	304.115	101.372	0.185
Day 3	3	299.882	99.961	0.487

Source of variation	**SS**	**df**	**MS**	**F**	**P-value**
Inter day	3.446	2.0	1.723	7.593	0.023
Intra day	1.362	6.0	0.227	--	--
Total	4.808	8.0	--	--	--

SS = Sum of squares

df = Degrees of freedom

MS = Mean squares

F = F ratio

Table 9.13 Inter-day precision results for bromhexine HCl

	Day 1	**Day 2**	**Day 3**	**Inter day**
	100.0	101.2	100.9	
	100.1	102.8	101.9	
	101.6	101.5	99.5	
Mean	100.55	101.85	100.75	101.05
SD	0.76	0.70	0.96	0.57
% RSD	0.76	0.69	0.96	0.57

Table 9.13.1 ANOVA: Single factor for bromhexine HCl

Summary				
Groups	**Count**	**Sum**	**Average**	**Variance**
Day 1	3	301.645	100.548	0.868
Day 2	3	305.564	101.855	0.744
Day 3	3	302.265	100.755	1.391

Source of variation	**SS**	**df**	**MS**	**F**	**P-value**
Inter day	2.958	2.0	1.479	1.478	0.301
Intra day	6.006	6.0	1.001	--	--
Total	8.964	8.0	--	--	--

SS = Sum of squares

df = Degrees of freedom

MS = Mean squares

F = F ratio

Conclusion

Precision was agreeable with RSD values of less than 4%. There were no significant differences between the "inter day" and "intra day" variances of doxycycline HCl and bromhexine HCl.

9.9.5 Ruggedness

9.9.5.1 Stability of sample solutions

Table 9.14 Stability results of doxycycline HCl and bromhexine HCl

Time (hours)	Doxycycline HCl		Bromhexine HCl	
	Peak area	% Remaining	Peak area	% Remaining
0	852.2	100.0	74.9	100.0
2	856.7	100.5	79.4	106.0
4	862.2	101.2	75.4	100.7
6	864.4	101.4	77.0	102.9
8	873.4	102.5	74.8	99.9
10	877.0	102.9	76.0	101.5
12	879.1	103.2	76.3	102.0
14	883.6	103.7	77.1	102.9
18	886.6	104.0	76.9	102.7
20	883.1	103.6	76.7	102.5
24	883.0	103.6	77.2	103.0
Mean	872.84	102.42	76.52	102.20
SD	11.46	1.34	1.22	1.63
% RSD	1.31	1.31	1.60	1.60

Conclusion

No breakdown was observed over a 24-hour period.

9.9.5.2 System repeatability

Table 9.15 System repeatability for doxycycline HCl and bromhexine HCl

	Doxycycline HCl		Bromhexine HCl	
	Area	**Retention time (min)**	**Area**	**Retention time (min)**
	1068	2.907	71	9.790
	1069	2.909	70	9.832
	1071	2.911	71	9.816
	1070	2.913	70	9.885
	1074	2.916	71	9.868
	1077	2.918	73	9.937
Mean	1072	2.912	71	9.855
SD	3.05	0.00	1.20	0.05
% RSD	0.28	0.13	1.70	0.49

Conclusion

System performance was acceptable with RSD values of 0.28% and 1.70% for peak area and RSD values of 0.13% and 0.49% for retention times of doxycycline HCl and bromhexine HCl, respectively.

9.9.5.3 Robustness

Deliberate changes were made to the chromatographic conditions to determine the robustness of the method.

The following changes were made:

Mobile phase:	Concentrations of 8-12% acetonitrile is still suitable
Injection volume:	8-12 µl
Flow rate:	0.8-1.2 ml/min
Detection wavelength:	252-256 nm

Column:	Luna C18-2 column, 150 x 4.6 nm, 5µm, 100 Å pores, 17.8% carbon load, endcapped, Phenomenex, Torrance, CA,
	and Lichrospher 100-5 RP-18ec cartridge column, 125 x 4.0 mm, 5µm, 100 Å pores, 21.5% carbon load, endcapped, Marchery-Nagel, Düren, Germany were found to be suitable. With the Lichrospher the retention times differed, thus the wavelength programming must be altered.

Conclusion

The method was not affected by any changes made to the analytical conditions and proved to be robust. The retention times were different with the Lichrospher column, but it was still acceptable.

9.10 CHROMATOGRAPHIC PERFORMANCE PARAMETERS

Reference: USP 24, 2000, p.1923.

Retention time (minutes)

Doxycycline HCl: 2.9

Bromhexine HCl: 9.9

Number of theoretical plates (N)/column

Doxycycline HCl: 56421

Bromhexine HCl: 66167

USP tailing factor (T)

Doxycycline HCl: 1.262

Bromhexine HCl: 1.297

Capacity factor (K')

Doxycycline HCl: 0.16

Bromhexine HCl: 2.04

Resolution between peaks (Rr)

Doxycycline HCl and Bromhexine HCl: 17.75

9.11 SYSTEM SUITABILITY PARAMETERS

- ❖ Analyse six replicate injections of a standard solution.
- ❖ Calculate the relative standard deviation of the peak areas obstained for doxycycline HCl and bromhexine HCl.
- ❖ Calculate the number of theoretical plates for the active substances.
- ❖ Calculate the USP tailing factor for the actives

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

- ❖ RSD of 6 injections not more than 2%
- ● Number of theoretical plates more than 35 000 per column for both analytes,
- ❖ USP tailing factor not more than 1.5.

9.12 CONCLUSION

The method performed well and complied with all the acceptance criteria for the simultaneous determination of doxycycline HCl and bromhexine HCl in tablets, for stability testing, quality control and batch release purposes. No interferences were encountered from stressed samples or known related substances, thus the method can be regarded as being stability-indicating.

CHAPTER 10

SUMMARY AND CONCLUSIONS

Over the last ten years, worldwide avian veterinary knowledge has undergone a quantum leap forward. Following closely have been improved diagnostic capabilities and an ever-increasing range of effective medications. As only healthy birds can become fit and only fit birds can win, the successful management of the bird's health is just another challenging aspect in the overall preparation of the birds successful racing. For a racing loft to be successful, it must have healthy pigeons in a good loft under good management.

If you ask any experienced flier what health problem he fears most for the pigeons, then if it is the breeding season he will probably say canker, but if it is the race season he will probably say respiratory infection. Respiratory diseases are very common in pigeons. They are the major cause of poor performance and pigeon loss during the race season. The respiratory system can be infected by *Chlamydia*, *Mycoplasma*, bacteria, fungi, viruses and mites.

Doxycycline HCl is a broad-spectrum tetracycline antibiotic with a wide range of activity against gram + and gram – bacteria. It is considered the most successful veterinary therapeutic agent for the treatment of *Chlamydia*, a principle cause of respiratory infection in avian species. Bromhexine HCl is an expectorant drug, promoting bronchial secretion and having mucolytic properties. It is commonly used in combination with antibiotics, such as doxycycline HCl, in the treatment of respiratory infections of the pigeon loft.

A complete literature study on the basis for selection of the dosage forms and on the two active substances, doxycycine HCl and bromhexine HCl, and their mode of action were done in the first two chapters. The effective anti-bacterial activity of doxycycline HCl

against *Chlamydia psittaci*, and the reduction of the viscosity of the mucus by bromhexine HCl, makes it an excellent combination for the treatment of respiratory infections in pigeons.

Based on the objectives of this study, the three different dosage forms developed, i.e. tablets, water-soluble powder and an ophthalmic solution, were discussed in Chapter 3. The insights gained were applied during the development of the planned formulations.

Valuable considerations that had to be taken into account during the formulation process included:

- The designed dosage form had to improve or complement the therapeutic effectiveness of the specific drug;
- The correct excipients and the correct combination thereof were very important;
- The specific final products each had to have specific attributes, as discussed in Chapter 3;
- The product had to be cost-effective, and
- The products had to show potential for being stable.

Chapter 4 dealt with the development of formulae for tablets for direct compression, water-soluble powders and an ophthalmic solution. The tablets and the powders contained doxycycline HCl and bromhexine HCl respectively and in combination as the active ingredients, while the ophthalmic solution only contained doxycycline HCl as active ingredient. Although preformulation results indicated possible incompatibilities with certain excipients, HPLC results showed no significant incompatibilities between the actives and the specific excipients.

Chapter 5 continued with the tests and methods used during the accelerated stability testing as well as the stability program that was used.

Validated and/or proven methods must be used in order to generate reliable and accurate test results during the stability testing of the products. This is very important since decisions concerning the stability of a developed formulation will be based on these results.

A suitable HPLC method for the simultaneous analysis of doxycycline HCl and bromhexine HCl, using the tablets, was developed and validated. The validation report of this method is described in Chapter 9.

In Chapter 6, 7 and 8 the results obtained from the tablets, water-soluble powder and the ophthalmic solution are discussed respectively. All the results generated during the stability study are included in Appendix 3 for reference.

Based on the different test results generated over the twelve weeks of stability evaluation of the products that were developed in this study, doxycycline HCl and bromhexine HCl, respectively and in combination, seemed to have been relatively stable. The final tablets, water-soluble powders and ophthalmic solution formulations remained stable.

The containers used for the storage of the tablets and the powders didn't seal tight enough. The moisture uptake was very high resulting in poor disintegration and dissolution times. Therefore the powder and the tablets should be stored in tightly sealed containers with enough silica as drying agent. The containers used for the tablets, powders and ophthalmic solution respectively, seemed not to influence the stability of the formulations negatively.

Twelve weeks of stability testing only served to give and indication of the stability of the developed formulae. However, a complete stability trial study of each of the three developed products will be required to claim their stability.

All of the objectives for this study were met on completion of the research.

Conference contributions, an article originating form this study and a report from a pigeon farmer and racer; Mr W.A Coetzee is included in Appendix 1, 2 and 4 respectively.

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APPENDIX

APPENDIX 1 Publication

APPENDIX 2 Conference contributions

APPENDIX 3 Data sheets

APPENDIX 4 Report written by Mr W.A Coetzee

APPENDIX 1

Development and validation of a stability indicating HPLC assay method for the simultaneous determination of doxycycline HCl and bromhexine HCl, in tablets

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Abstract

An HPLC method for the simultaneous determination of doxycycline HCl and bromhexine HCl in tablets was developed and validated. A Luna C18(2), 150 x 4.6 mm, 5 µm column, a Lichrospher 100 RP-18, 5 µm as guard column and a mobile phase of 0.005 M octane-sulphoric acid Na-salt in deionised water : Acetonitrile (60 : 40), at a flow rate of 1 ml/min were used. The injection volume was 10 µl, with UV detection at 245 nm at ambient temperature. The retention times for doxycycline HCl and bromhexine HCl were ± 2.9 and ± 9.8 minutes, respectively. The method was linear over a range of 17.3-207 µg/ml for doxycycline HCl, and 6-12 µg/ml for bromhexine HCl, with an accuracy of 101.1% for doxycycline HCl, and 100.8% for bromhexine HCl, and a precision of 2.10% and 1.01% for doxycycline HCl and bromhexine HCl respectively. Forced degradation and peak purity analysis were used to prove that the method was stability-indicating.

Keywords: Method validation, HPLC, doxycycline HCl, bromhexine HCl.

Introduction

Doxycycline HCl [(4S,4aR,5S,5aR,6R,12aS)-4-dimethyl-amino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide] is a broad-spectrum tetracycline antibiotic with a wide range of activity against gram + and gram – bacteria. It is considered the most successful agent for the treatment of chlamydiosis, a respiratory disease in avian species. It is prepared

by chemical synthesis, being a semi-synthetic tetracycline derived from oxytetracycline, and is recrystallized from HCl and ethanol as the hemihydrate hemialcoholate (Dollery, 1999:D229). Doxycycline HCl is readily and almost completely absorbed from the gastrointestinal tract and absorption is not significantly affected by the presence of food in the stomach or duodenum. From 80 to 95% of doxycycline HCl in the circulation is reported to be bound to plasma proteins (Reynolds, 2002:200). Tissue distribution is good, with a tissue/serum concentration ratio always greater than 1 except in the gut and lymphoid tissue. There is a strong affinity for renal and lung tissue. The drug appears to prevent access of amino-acyl tRNA to the acceptor site on the mRn-ribosome complex. This prevents the addition of amino acids to the growing peptide chain (Dollery, 1999:D229). Bromhexine HCl (2-Amino-3,5-dibromobenzyl(cyclohexyl)methylammonium chloride) is an expectorant drug, promoting bronchial secretion and having mucolytic properties (Bechgaard & Nielsen, 1982:392). It is commonly used in combination with antibiotics in respiratory infections. Bromhexine HCl is rapidly absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism in the liver (Reynolds, 2002: 1086). Bromhexine HCl is a mucolytic agent which changes the structure of bronchial secretions by rarefication and fragmentation of the mucopolysaccharide fibres, leading to a reduction in the viscosity of the sputum (Lund, 1994:112). Doxycycline HCl and bromhexine HCl were formulated in combination for the treatment of respiratory diseases in pigeons.

Experimental

All the chemicals used were of HPLC or analytical reagent grade. Deionised water from an Elix 10 and Milli-Q purification system (Millipore, Bedford, MA) was used.

Materials and equipment for HPLC

A Hewlett Packard 1050 series HPLC (HP, Palo Alto, CA) equipped with a HP1050 quanternary gradient pump, HP1050 autosampler, HP1050 diode array detector and Chemstation Rev.A.06.02 data acquisition and analysis software was used, with a Luna C18(2), 150 x 4.6 mm, 5 µm column and a mobile phase of 0.005 M octane-sulphoric acid Na-salt in deionised water : Acetonitrile (60 : 40), at a flow rate of 1 ml/min. The injection volume was 10 µl, with UV detection at 245 nm at ambient temperature. The retention times for doxycycline HCl and bromhexine HCl were ± 2.9 and ± 9.8 minutes, respectively.

Sample preparation

Twenty tablets were accurately weighed and ground into a fine powder. The amount of powder equal to the mass of one tablet was accurately weighed and transferred into a 100 ml volumetric flask. 20 ml of methanol and 50 ml of deionised water were added and the solution sonicated for 15 minutes. These samples were left to cool and were each then filled to volume with deionised water. The samples were each filtered through a 0.45 µm filter, transferred into vials and injected into the chormatograph.

Standard preparation

Standards were prepared by dissolving 20 mg bromhexine HCl accurately into a 100 ml volumetric flask. 20 ml of methanol and 50 ml of deionised water were added and the solution sonicated for 15 minutes. The sample was left to cool and then filled up to volume with deionised water. 17.25 mg doxycycline HCl were accurately weighed and transferred into another 100 ml volumetric flask. 5 ml of the bromhexine HCl solution was then accurately added to this volumetric flask. This was then dissolved in 50 ml deionised water and sonicated for 5 minutes. These samples were left to cool and were each then filled to volume with deionised water to obtain a standard solution containing 172.5 µg/ml doxycycline HCl and 10 µg/ml bromhexine HCl.

Validation

The method was validated according to the 1996 ICH-Q2A guidelines. Linearity and range were established by preparing a standard solution, containing both doxycycline HCl and bromhexine HCl, over ranges of 17.3-207 µg/ml and 6-12 µg/ml, respectively. Accuracy was determined by weighing the appropriate amount of tablet placebo into 100 ml volumetric flasks and then spiking each by adding a standard solution to obtain triplicate samples, containing 80%, 100%, and 120% of the expected sample concentration. Precision was tested by analysing nine samples of the same homogenous suspension on day one (also at 80%, 100 and 120% level), and analysing three samples again on two more days, one by a different analyst on another instrument. Sample stability was assessed by leaving a prepared sample on the autosampler at ambient temperature, and injecting it hourly, over a 24 hour period. Repeatability was determined by injecting the sample solution six times. Specificity was tested by forced degradation. A standard solution containing

doxycycline HCl and bromhexine HCl was diluted to 50% with water, 1M hydrochloric acid, 1 M sodium hydroxide and 5% hydrogen peroxide, and stored at 40°C overnight to degrade before being analysed. Robustness was tested by making deliberate changes to the operation conditions to the operation conditions and by recording the effects.

Results and discussion

The validation results are given in Table 1.

Table 1. Validation results

Test	Result
Specificity	Complies
Range	Doxycycline HCl: 17.3 - 207 µg/ml Bromhexine HCl: 6 – 12 µg/ml
Linearity	Doxycycline HCl: R^2 = 0.999 Bromhexine HCl: R^2 = 0.992
Accuracy	Doxycycline HCl: 101.1% Bromhexine HCl: 100.8%
Precision	Doxycycline HCl: RSD < 3% Bromhexine HCl: RSD < 3%
Intra-day precision	Doxycycline HCl: RSD < 3% Bromhexine HCl: RSD < 3%
Inter-day precision	Doxycycline HCl: RSD < 1% Bromhexine HCl: RSD < 1%
Ruggedness	Complies
Robustness	Complies

Samples analysed after forced degradation showed extra peaks, due to breakdown of the samples, but none of the degradation products interfered with the doxycycline HCl and bromhexine HCl peaks. Diode array peak purity analysis showed that the peaks of doxycycline HCl and bromhexine HCl remained pure. No interference was found from the sample that was prepared from a tablet placebo (Figure 2). Small changes in the flow rate, detection, wavelength, mobile phase composition and

injection volume did not affect the separation. A Lichrospher RP-18ec, 125 x 4 mm, 5 µm (Machery-Nagel), was found equally suitable to perform the analysis, proving that the method is robust.

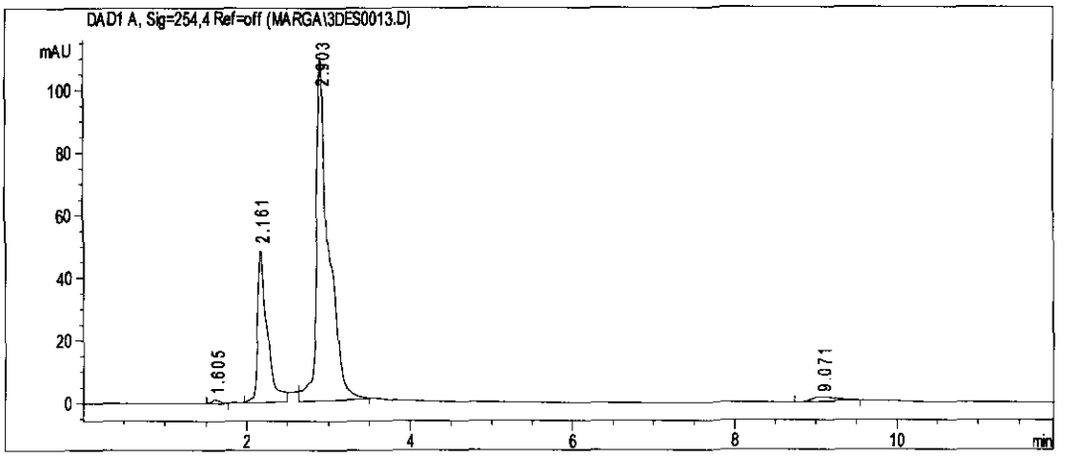

Figure 1 Chromatogram of standard solution.

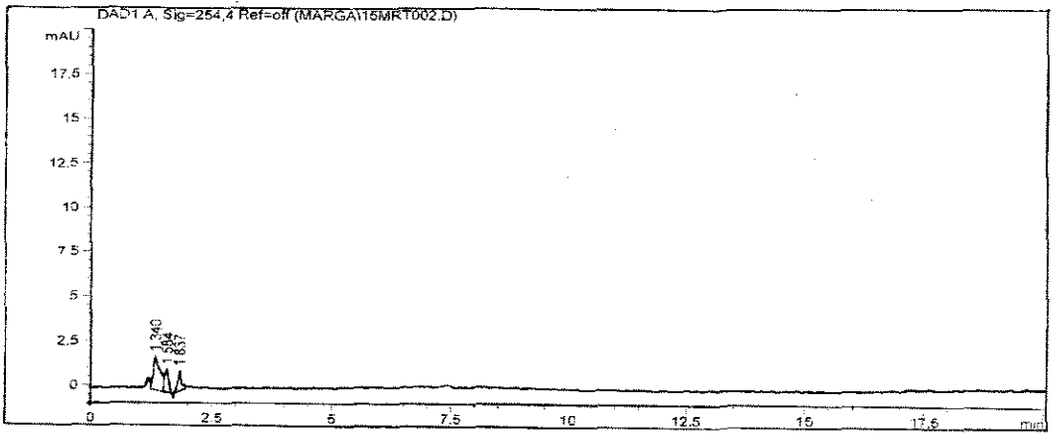

Figure 2 Chromatogram of tablet placebo.

Linearity and range of doxycycline HCl and bromhexine HCl

Table 2 and 3 summarise the linearity results of doxycycline HCl and bromhexine HCl, respectively.

Table 2 Peak area and concentration found for doxycycline HCl

Doxycycline HCl				
Injection volume (µl)	**Conc (µg/ml)**	**Peak area**		
		Area 1	**Area 2**	**Mean**
10	17.3	63	64	64
10	51.8	279	284	282
10	86.3	492	494	493
10	103.5	592	590	591
10	120.8	700	702	701
10	138.0	800	805	802
10	155.0	900	905	903
10	172.5	1020	1028	1024
11	189.8	1130	1132	1131
12	207.0	1249	1246	1247

Table 3 Regression parameters for doxycycline HCl

Regression parameters			
R Squared	0.9996	**Lower 95%**	**Upper 95%**
Intercept	-44.4087	-56.4751	-32.3423
Slope	6.1863	6.0981	6.2745

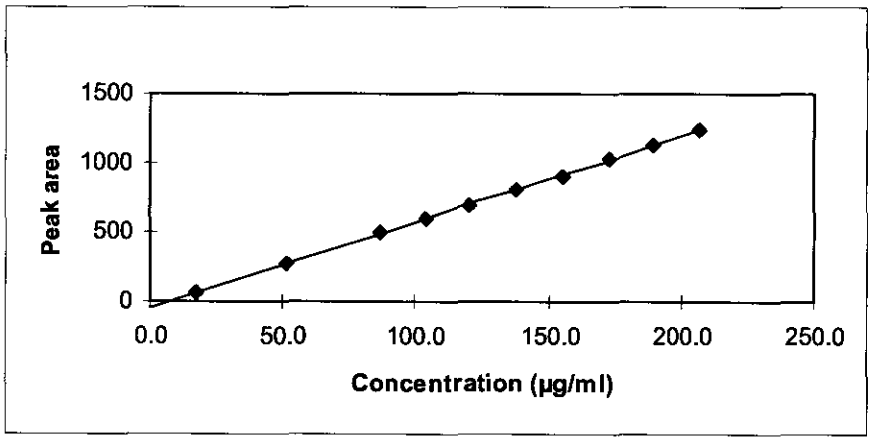

Figure 3 Linear regression curve for doxycycline HCl.

Table 4 Peak area and concentration found for bromhexine HCl

Bromhexine HCl				
Injection volume (µl)	**Conc (µg/ml)**	**Peak area**		
		Area 1	**Area 2**	**Mean**
10	6.0	47	46	47
10	7.0	52	54	53
10	8.0	58	58	58
10	9.0	64	65	65
10	10.0	70	69	70
11	11.0	76	76	76
12	12.0	83	81	82

Table 5 Regression parameters of bromhexine HCl

Regression parameters			
R Squared	0.9992	**Lower 95%**	**Upper 95%**
Intercept	10.2016	4.4758	15.9273
Slope	6.0502	5.4291	6.6712

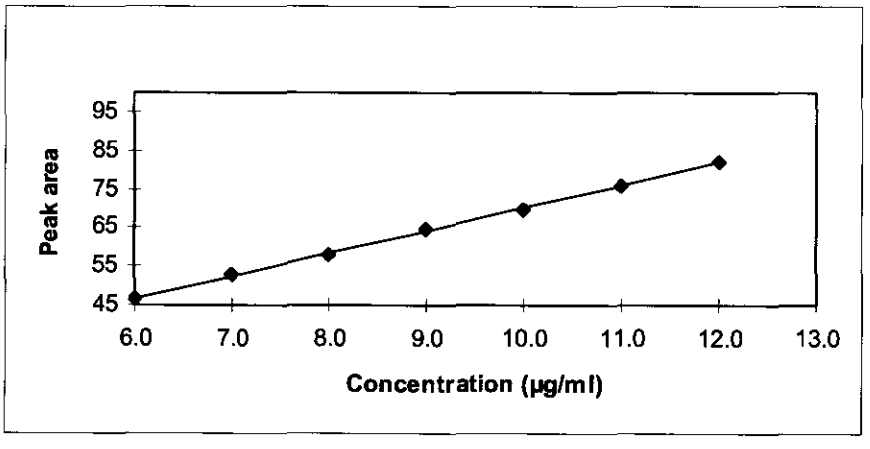

Figure 4 Linear regression curve for bromhexine HCl.

Accuracy

Table 6 Percentage doxycycline HCl recovered

Doxycycline HCl			
Conc Spiked (µg/ml)	**Peak area**	**Conc found (µg/ml)**	**Recovered (%)**
138.0	816	136.2	98.7
138.0	824	137.5	99.6
138.0	813	135.8	98.4
172.5	1042	173.9	100.8
172.5	1046	174.7	101.3
172.5	1049	175.2	101.6
207.0	1275	212.8	102.8
207.0	1280	213.7	103.2
207.0	1281	213.8	103.3

Table 7 Confidence intervals for doxycycline HCl

Statistical analysis	
Mean	101.1
% RSD	1.8
SD	1.7
95% Confidence intervals	
Lower limit	99.6
Upper limit	102.5
Confidence level	1.4
Estimated median	101.3

Table 8 Percentage bromhexine HCl recovered

Bromhexine HCl			
Conc Spiked (µg/ml)	**Peak area**	**Conc found (µg/ml)**	**Recovered (%)**
8.0	50	8.0	99.8
8.0	51	8.0	100.2
8.0	50	7.9	99.3
10.0	65	10.3	103.5
10.0	63	9.9	99.2
10.0	64	10.2	101.8
12.0	77	12.2	101.8
12.0	77	12.2	101.6
12.0	76	12.1	100.5

Table 9 Confidence intervals for bromhexine HCl

Statistical analysis	
Mean	100.8
% RSD	1.3
SD	1.3
95% Confidence intervals	
Lower limit	99.8
Upper limit	101.9
Confidence level	1.1
Estimated median	100.5

Intra-day precision

Table 10 Intra-day precision results for doxycycline HCl

Doxycycline HCl			
Mass (mg)	**Peak area**	**Conc found (µg/ml)**	**Recovered (%)**
121.0	815	168.5	97.7
120.5	813	168.9	97.9
120.7	815	169.0	98.0
150.3	1048	174.6	101.2
150.4	1049	174.5	101.2
150.8	1050	174.3	101.0
180.2	1279	177.6	102.9
180.5	1281	177.7	103.0
180.5	1280	177.6	103.0
		Mean	100.66
		SD	2.12
		% RSD	2.10

Table 11 Intra-day precision results for bromhexine HCl

Bromhexine HCl			
Mass (mg)	**Peak area**	**Conc found (µg/ml)**	**Recovered (%)**
120.4	49.8	10.2	102.0
120.4	49.9	10.2	102.2
120.6	49.3	10.1	100.9
150.2	60.9	10.0	100.0
150.4	61.0	10.0	100.1
150.0	61.8	10.2	101.6
180.5	73.8	10.1	100.9
180.5	75.7	10.3	103.4
180.3	74.4	10.2	101.7
		Mean	101.42
		SD	1.03
		% RSD	1.01

Inter-day precision

Table 12 Inter-day precision results for doxycycline HCl

	Day 1	Day 2	Day 3	Inter day
	101.2	101.3	99.4	
	101.2	101.8	100.7	
	101.0	101.0	99.7	
Mean	101.15	101.37	99.96	100.83
SD	0.08	0.35	0.57	0.62
% RSD	0.07	0.35	0.57	0.61

Table 12.1 ANOVA: Single factor for doxycycline HCl

Summary				
Groups	**Count**	**Sum**	**Average**	**Variance**
Day 1	3	303.438	101.146	0.009
Day 2	3	304.115	101.372	0.185
Day 3	3	299.882	99.961	0.487

Source of variation	**SS**	**df**	**MS**	**F**	**P-value**
Inter day	3.446	2.0	1.723	7.593	0.023
Intra day	1.362	6.0	0.227	--	--
Total	4.808	8.0	--	--	--

SS = Sum of squares

df = Degrees of freedom

MS = Mean squares

F = F ratio

Table 13 Inter-day precision results for bromhexine HCl

	Day 1	Day 2	Day 3	Inter day
	100.0	101.2	100.9	
	100.1	102.8	101.9	
	101.6	101.5	99.5	
Mean	100.55	101.85	100.75	101.05
SD	0.76	0.70	0.96	0.57
% RSD	0.76	0.69	0.96	0.57

Table 13.1 ANOVA: Single factor for bromhexine HCl

Summary				
Groups	**Count**	**Sum**	**Average**	**Variance**
Day 1	3	301.645	100.548	0.868
Day 2	3	305.564	101.855	0.744
Day 3	3	302.265	100.755	1.391

Source of variation	**SS**	**df**	**MS**	**F**	**P-value**
Inter day	2.958	2.0	1.479	1.478	0.301
Intra day	6.006	6.0	1.001	--	--
Total	8.964	8.0	--	--	--

SS = Sum of squares

df = Degrees of freedom

MS = Mean squares

F = F ratio

Stability of sample solutions

Table 14 Stability results of doxycycline HCl and bromhexine HCl

Time (hours)	Doxycycline HCl		Bromhexine HCl	
	Peak area	% Remaining	Peak area	% Remaining
0	852.2	100.0	74.9	100.0
2	856.7	100.5	79.4	106.0
4	862.2	101.2	75.4	100.7
6	864.4	101.4	77.0	102.9
8	873.4	102.5	74.8	99.9
10	877.0	102.9	76.0	101.5
12	879.1	103.2	76.3	102.0
14	883.6	103.7	77.1	102.9
18	886.6	104.0	76.9	102.7
20	883.1	103.6	76.7	102.5
24	883.0	103.6	77.2	103.0
Mean	872.84	102.42	76.52	102.20
SD	11.46	1.34	1.22	1.63
% RSD	1.31	1.31	1.60	1.60

System repeatability

Table 15 System repeatability for doxycycline HCl and bromhexine HCl

	Doxycycline HCl		Bromhexine HCl	
	Area	Retention time (min)	Area	Retention time (min)
	1068	2.907	71	9.790
	1069	2.909	70	9.832
	1071	2.911	71	9.816
	1070	2.913	70	9.885
	1074	2.916	71	9.868
	1077	2.918	73	9.937
Mean	1072	2.912	71	9.855
SD	3.05	0.00	1.20	0.05
% RSD	0.28	0.13	1.70	0.49

Conclusion

The method was used over a period of twelve weeks to analyse stability samples placed at accelerated environmental conditions, and it proved to be reliable and easy to execute.

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APPENDIX 2

CONFERENCE CONTRIBUTION

LE ROUX, M., LÖTTER, A.P. & DU PREEZ, J.L. 2004. Preparation and evaluation of doxycycline HCl and bromhexine HCl dosage forms for pigeons. Poster presented at the 25th Annual Congress of the Academy of Pharmaceutical Sciences, September 12-15, Grahamstown, South-Africa.

Page 161 represents the poster.

The Preparation and Evaluation of Doxycycline HCl and Bromhexine HCl Dosage Forms for Pigeons

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PURPOSE

To prepare and evaluate three different dosage forms, each containing doxycycline HCl and bromhexine HCl respectively and in combination, for the treatment of respiratory diseases in pigeons.

BACKGROUND

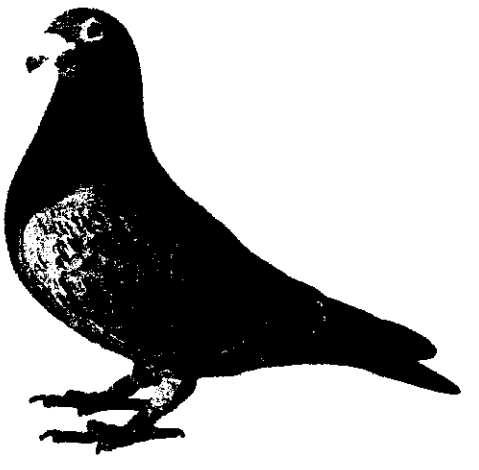
Doxycycline HCl, a broad-spectrum antibiotic, is the world-wide veterinary therapeutic agent of choice for the treatment of *Chlamydia*, a principle cause of respiratory infection in pigeons.

Bromhexine HCl is a mucolytic agent which reduces the mucus viscosity in the tracheobronchial tree and is often prescribed in combination with antibiotics for respiratory diseases in pigeons.

METHODS

A direct-compressed tablet, a water-soluble powder and an ophthalmic solution were successfully developed. The tablet and the powder formulations each contained doxycycline HCl and bromhexine HCl respectively and in combination. The ophthalmic solution contained only doxycycline HCl as active ingredient. The two powder formulations containing doxycycline HCl, contained citric acid as an anti-oxidant thereof. Only the stability test results obtained from the water-soluble powder, containing the doxycycline HCl and bromhexine HCl combination (powder A), are discussed here.

Powder X was prepared from powder A by omitting the citric acid from the formulation (powder X = powder A without citric acid). Both powders A and X were assayed and the test results compared. Solutions of powders A and X in water were each stored in three types of containers; plastic, glass and stainless steel. Further tests that were performed on the water-soluble powder A included pH, constitution time, moisture content, assay, and the visual properties were investigated. An HPLC method for the simultaneous determination of doxycycline HCl and bromhexine HCl was developed and validated.



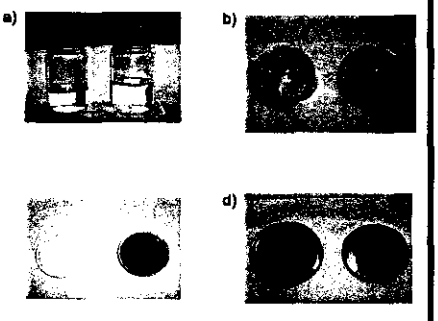


Figure C. (a) Powder A (left) and powder X (right) at 0 hours (b) Powder A (left) and powder X (right) after 24 hours in a plastic container (c) Powder A (left) and powder X (right) after 24 hours in a glass container (d) Powder A (left) and powder X (right) after 24 hours in a stainless steel container.

RESULTS

All the tests done on powder A showed that the powder remained stable over the accelerated three-month test period. As this was only an indication of the stability of the tablet, a complete stability trial study is required to claim stability.

The assay results showed that both the doxycycline HCl and the bromhexine HCl remained stable in this formulation and showed no incompatibilities with each other.

The comparative test outcomes of powders A and X showed remarkable results (Figures A and C). The assay of powder A, containing citric acid, showed no discoloration or precipitation when dissolved in water and left to stand for a period of 24 hours. Powder X, however, showed discoloration after only 3 hours. This powder showed significant breakdown as well.

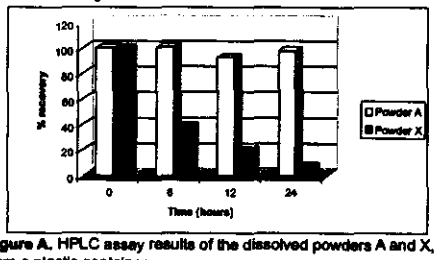


Figure A. HPLC assay results of the dissolved powders A and X, sampled from a plastic container.

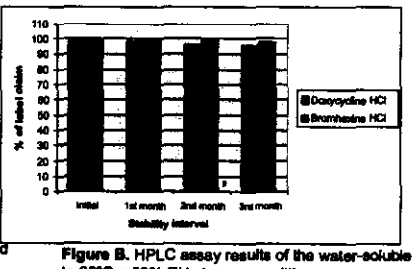


Figure B. HPLC assay results of the water-soluble powder A, subjected to 25°C + 60% RH storage conditions.

CONCLUSION

A stable, cost-effective, water-soluble powder for the treatment of respiratory diseases in pigeons was developed.
It proved to be stable over the accelerated three-month test period. The incorporation of citric acid in the formula showed outstanding results. No discoloration or precipitation occurred even after 24 hours. The newly developed HPLC method was used over a twelve-week period to analyse stability samples, and it proved to be reliable and easy to perform.

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APPENDIX 3

DATA SHEETS

Table 1 HPLC assay results of *tablet A*

DOXYCYCLINE HCl ASSAY RESULTS								
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	mg/tab	% Recovered
Initial	Assay 1	132.74	952.0	954.0	953.0	169.9	16.9	98.2
	Assay 2	132.70	959.0	955.0	957.0	170.1	17.0	98.6
1st Month 25°C	Assay 1	130.48	940.0	942.0	941.0	170.1	17.0	98.6
	Assay 2	130.48	941.0	942.0	941.5	170.2	17.0	98.7
2nd	Assay 1	129.24	896.0	898.0	897.0	163.7	16.4	94.9
	Assay 2	129.28	897.0	897.0	897.0	163.6	16.4	94.9
3rd	Assay 1	129.86	885.0	881.0	883.0	160.4	16.0	93.0
	Assay 2	129.83	886.0	888.0	887.0	161.2	16.1	93.4
1st Month 40°C	Assay 1	128.73	919.0	921.0	920.0	168.6	16.9	97.7
	Assay 2	128.72	915.0	916.0	915.5	167.7	16.8	97.2
2nd	Assay 1	123.57	884.0	885.0	884.5	168.8	16.9	97.9
	Assay 2	123.60	887.0	890.0	889.0	169.5	17.0	98.3
3rd	Assay 1	123.39	816.0	818.0	817.0	156.1	15.6	90.5
	Assay 2	123.35	852.0	852.0	852.0	162.9	16.3	94.4

Table 2 HPLC assay results of *tablet B*

BROMHEXINE HCl ASSAY RESULTS								
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	mg/tab	% Recovered
Initial	Assay 1	118.70	64.0	64.0	64.0	10.1	1.0	101.2
	Assay 2	118.67	63.0	64.0	63.5	10.0	1.0	100.4
1st Month 25°C	Assay 1	120.34	65.0	65.0	65.0	10.1	1.0	101.1
	Assay 2	120.36	64.0	65.0	64.5	10.0	1.0	100.3
2nd	Assay 1	122.31	65.0	66.0	65.5	10.2	1.0	101.5
	Assay 2	122.31	65.0	65.0	65.0	10.1	1.0	100.7
3rd	Assay 1	122.33	67.0	66.0	66.5	10.1	1.0	101.0
	Assay 2	122.36	66.0	66.0	66.0	10.0	1.0	100.2
1st Month 40°C	Assay 1	121.94	65.0	66.0	65.5	10.0	1.0	100.5
	Assay 2	121.93	65.0	65.0	65.0	10.0	1.0	99.7
2nd	Assay 1	120.88	64.0	66.0	65.0	10.2	1.0	101.9
	Assay 2	120.86	65.0	63.0	64.0	10.0	1.0	100.0
3rd	Assay 1	120.97	65.0	65.0	65.0	10.0	1.0	99.9
	Assay 2	121.09	66.0	65.0	66.0	10.0	1.0	100.5

Table 3 HPLC assay results of doxycycline HCl in *tablet C*

DOXYCYCLINE HCl ASSAY RESULTS								
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	mg/tab	% Recovered
Initial	Assay 1	130.41	937.4	938.2	937.8	169.7	17.0	98.4
	Assay 2	130.44	936.2	934.1	935.2	169.2	16.9	98.1
1st Month 25°C	Assay 1	130.44	939.2	938.9	939.1	169.8	17.0	98.4
	Assay 2	130.46	934.4	934.0	934.2	168.9	16.9	97.9
2nd	Assay 1	130.44	926.4	927.0	926.7	167.7	16.8	97.2
	Assay 2	130.46	931.3	931.6	931.5	168.6	16.9	97.7
3rd	Assay 1	129.68	916.4	912.8	914.6	166.5	16.7	96.5
	Assay 2	129.68	886.2	894.7	890.4	162.1	16.2	94.0
1st Month 40°C	Assay 1	129.20	921.2	921.9	921.6	168.2	16.8	97.5
	Assay 2	129.16	912.6	912.9	912.8	166.6	16.7	96.6
2nd	Assay 1	125.68	853.3	852.9	853.2	160.2	16.0	92.9
	Assay 2	125.62	855.1	856.0	855.6	160.8	16.1	93.2
3rd	Assay 1	124.11	854.0	862.0	858.0	163.2	16.3	94.6
	Assay 2	124.15	841.0	835.0	838.0	159.3	15.9	92.3

Table 4 HPLC assay results of bromhexine HCl in *tablet C*

BROMHEXINE HCl ASSAY RESULTS								
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	mg/tab	% Recovered
Initial	Assay 1	130.41	69.0	71.0	70.0	10.1	1.0	101.0
	Assay 2	130.44	69.8	70.1	70.0	10.1	1.0	100.9
1st Month 25°C	Assay 1	130.44	69.5	70.0	69.8	10.0	1.0	100.5
	Assay 2	130.46	68.5	69.0	68.8	9.9	1.0	99.0
2nd	Assay 1	130.44	68.5	68.1	68.3	9.9	1.0	99.1
	Assay 2	130.46	69.3	69.0	69.2	10.0	1.0	100.3
3rd	Assay 1	129.68	69.3	68.7	69.0	10.0	1.0	99.8
	Assay 2	129.68	69.0	69.1	69.1	10.0	1.0	99.9
1st Month 40°C	Assay 1	129.20	68.0	67.5	67.8	9.9	1.0	98.5
	Assay 2	129.16	69.0	68.0	69.0	10.0	1.0	99.6
2nd	Assay 1	125.68	64.4	65.5	65.0	9.8	1.0	97.8
	Assay 2	125.62	65.7	66.3	66.0	9.9	1.0	99.4
3rd	Assay 1	124.11	65.5	66.4	66.0	10.0	1.0	99.7
	Assay 2	124.15	66.1	65.5	66.0	9.9	1.0	99.4

Table 5 Content uniformity results of *tablet A*

DOXYCYCLINE HCl CONTENT UNIFORMITY					
STD_{CONC} 172.3 µg/ml STD_{AREA} 1095.9					
Tablet	**Area 1**	**Area 2**	**Mean area**	**mg/tab**	**% Label claim**
1	1085.3	1083.7	1084.5	17.05	98.85
2	1076.5	1076.4	1076.4	16.92	98.11
3	1075.5	1073.2	1074.4	16.89	97.92
4	1098.4	1091.1	1094.8	17.21	99.78
5	1176.7	1178.8	1177.7	18.52	107.34
6	1090.4	1092.0	1091.2	17.16	99.46
7	1166.6	1166.5	1166.6	18.34	106.33
8	1108.5	1108.6	1108.6	17.43	101.04
9	1043.6	1043.6	1043.6	16.41	95.12
10	1087.7	1088.7	1088.2	17.11	99.19

Table 6 Content uniformity results of *tablet B*

BROMHEXINE HCl CONTENT UNIFORMITY					
		STD_{CONC} 10.0 µg/ml	STD_{AREA} 89.0		
Tablet	**Area 1**	**Area 2**	**Mean area**	**mg/tab**	**% Label claim**
1	85.0	87.9	86.4	1.0	96.6
2	92.1	88.9	90.5	1.0	101.2
3	88.8	88.4	88.6	1.0	99.1
4	91.9	91.7	91.8	1.0	102.6
5	89.1	89.5	89.3	1.0	99.8
6	83.9	84.9	84.4	0.9	94.3
7	84.4	84.5	84.4	0.9	94.4
8	86.6	87.0	86.8	1.0	97.0
9	88.8	89.9	89.3	1.0	99.8
10	91.5	90.9	91.2	1.0	102.0

Table 7 Content uniformity results of doxycycline HCl in *tablet C*

DOXYCYCLINE CONTENT UNIFORMITY					
		STD_{CONC} 172.8 µg/ml		STD_{AREA} 1168.1	
Tablet	**Area 1**	**Area 2**	**Mean area**	**mg/tab**	**% Label claim**
1	1140.9	1138.9	1139.9	16.9	97.8
2	1109.2	1108.6	1108.9	16.4	95.1
3	1144.1	1147.2	1145.7	17.0	98.3
4	1122.7	1125.4	1124.0	16.6	96.4
5	1107.6	1108.4	1108.0	16.4	95.0
6	1174.5	1174.8	1174.6	17.4	100.7
7	1163.5	1162.9	1163.2	17.2	99.8
8	1149.2	1147.8	1148.5	17.0	99.5
9	1175.0	1175.8	1175.4	17.4	100.8
10	1179.9	1180.9	1180.4	17.5	101.2

Table 8 Content uniformity results of bromhexine HCl in *tablet C*

BROMHEXINE CONTENT UNIFORMITY					
		STD_{CONC} 10 µg/ml		STD_{AREA} 79.29	
Tablet	**Area 1**	**Area 2**	**Mean area**	**mg/tab**	**% Label claim**
1	75.7	75.9	75.8	1.0	95.6
2	77.7	77.5	77.6	1.0	97.9
3	74.2	74.6	74.4	0.9	93.8
4	79.2	78.9	79.0	1.0	99.7
5	75.7	75.6	75.7	1.0	95.5
6	83.3	83.4	83.4	1.1	105.2
7	73.4	73.2	73.3	0.9	92.4
8	80.3	79.0	79.7	1.0	100.5
9	78.2	77.0	77.6	1.0	97.9
10	84.5	84.8	84.7	1.1	106.8

Table 9 HPLC dissolution rate results of *tablet A*

	Initial	1st month	2nd month	3rd month
Area STD A	176.0	177.6	180.8	173.6
STD mass (mg)	35.3	35.0	34.7	34.6

INITIAL										
Mass	15 min		30 min		45 min		60 min		90 min	
(mg)	Area	%	Area	%	Area	%	Area	%	Area	%
128.6	58.8	30.8	96.2	50.4	131.6	69.0	156.8	82.2	191.9	100.6
129.5	63.8	33.5	97.4	51.1	135.6	71.1	157.0	82.3	194.0	101.7
131.3	65.7	34.5	99.4	52.1	137.6	72.1	161.1	84.4	192.8	101.0
126.4	62.3	32.7	91.5	48.0	132.7	69.5	156.2	81.9	188.6	98.9
131.0	64.4	33.8	100.0	52.4	137.7	72.2	164.4	86.2	193.5	101.4
128.5	62.6	32.8	98.1	51.4	131.7	69.1	156.2	81.8	193.6	101.5
Average		**33.0**		**50.7**		**70.5**		**83.1**		**100.8**
1st MONTH 25°C										
Mass	15 min		30 min		45 min		60 min		90 min	
(mg)	Area	%	Area	%	Area	%	Area	%	Area	%
134.1	66.0	34.0	102.0	52.6	144.6	74.5	167.1	86.1	198.1	102.1
127.0	60.5	31.2	98.9	51.0	138.7	71.4	157.4	81.1	193.1	99.5
129.3	61.0	31.4	95.6	49.3	138.4	71.3	161.0	83.0	193.9	99.9
129.7	62.2	32.0	96.9	50.0	137.9	71.0	162.7	83.8	197.6	101.8
126.1	59.0	30.4	98.8	50.9	138.0	71.1	157.7	81.2	193.9	99.9
127.1	61.0	31.4	97.7	50.3	135.7	69.9	156.7	80.7	194.7	100.3
Average		**31.7**		**51.8**		**71.5**		**82.6**		**100.6**
1st MONTH 40°C										
Mass	15 min		30 min		45 min		60 min		90 min	
(mg)	Area	%	Area	%	Area	%	Area	%	Area	%
125.1	27.2	14.1	47.9	24.7	69.7	35.9	85.7	44.2	106.4	54.8
128.1	30.7	15.8	46.1	23.8	72.1	37.2	86.0	44.3	106.6	54.9
124.8	28.5	14.7	44.5	22.9	68.7	34.4	84.6	43.6	105.6	54.4
128.5	27.8	14.3	42.4	21.8	70.8	36.5	85.8	44.2	106.6	54.9
125.4	29.1	14.0	44.6	23.0	68.7	35.4	83.9	43.2	106.5	54.8
128.1	29.1	15.0	45.8	23.6	71.2	36.6	85.0	43.8	107.8	55.5
Average		**14.8**		**24.2**		**36.2**		**43.9**		**54.9**
2nd MONTH 25°C										
Mass	15 min		30 min		45 min		60 min		90 min	
(mg)	Area	%	Area	%	Area	%	Area	%	Area	%
129.4	62.6	31.4	100.4	50.4	141.9	71.1	160.8	80.63	195.4	98.0
131.3	65.2	32.7	99.9	50.2	138.2	69.3	159.3	79.9	198.4	99.5
129.0	67.1	33.7	100.7	50.5	142.9	71.7	162.9	81.7	198.4	99.5
127.8	66.5	33.4	101.1	50.7	144.0	72.2	164.8	82.7	198.0	99.3
128.5	61.1	30.6	101.4	50.8	143.2	71.9	167.5	84.0	199.2	99.9
128.7	62.9	31.6	100.1	50.2	144.9	72.7	168.6	84.6	197.4	99.0
Average		**32.2**		**50.3**		**71.5**		**82.2**		**99.2**

Table 9 HPLC dissolution rate results of *tablet A* (continued)

2nd MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
125.1	25.5	12.8	43.4	21.8	66.1	33.2	85.2	42.8	100.2	50.2
126.7	24.3	12.2	44.6	22.4	64.3	32.2	84.6	42.4	102.5	51.4
125.2	25.2	12.6	44.2	22.2	37.4	33.8	84.1	42.2	105.7	53.0
124.5	24.2	12.1	43.6	21.8	65.8	33.0	82.3	41.3	107.6	53.9
124.3	23.5	11.8	41.5	20.8	63.3	31.7	80.3	40.3	104.4	52.3
122.5	25.8	12.9	43.2	21.7	64.2	32.2	85.0	42.6	103.5	51.9
Average		**12.4**		**22.1**		**32.7**		**41.9**		**52.2**
3rd MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
125.6	58.3	30.3	92.3	50.1	138.9	72.3	163.1	84.8	190.5	99.1
130.2	63.9	33.3	101.6	52.9	137.2	71.4	162.2	84.3	192.9	100.3
127.4	61.6	32.1	96.6	50.2	136.6	71.0	158.0	82.2	195.7	101.8
131.3	64.9	33.8	97.2	50.5	135.5	70.5	161.9	84.2	189.7	98.7
128.9	58.1	30.2	98.9	51.5	134.2	69.8	163.7	85.2	194.7	101.2
129.0	61.2	31.8	100.2	52.1	136.0	70.8	160.7	83.6	191.9	99.8
Average		**31.9**		**51.5**		**70.9**		**84.0**		**100.2**
3rd MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
123.7	18.0	9.4	36.3	18.9	60.9	31.7	75.1	39.0	98.2	51.1
121.1	19.4	10.1	37.5	19.5	57.5	29.9	72.8	37.8	99.2	51.6
122.6	18.4	9.6	35.1	18.2	54.6	28.4	75.9	39.5	96.0	49.9
121.8	19.2	9.9	35.9	18.7	59.2	30.8	76.1	39.6	94.0	48.9
122.9	20.1	10.4	36.7	19.1	55.8	29.0	78.7	40.9	93.3	48.5
124.4	17.3	9.0	37.6	19.6	58.8	30.6	74.9	39.0	93.9	48.9
Average		**9.8**		**19.2**		**30.1**		**39.3**		**49.8**

Table 10 HPLC dissolution rate results of *tablet B*

	Initial	1st month	2nd month	3rd month
Area STD A	168.9	168.9	170.1	170.1
STD mass (mg)	20.1	20.1	20.2	20.1

INITIAL										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
118.5	57.0	33.8	88.0	52.1	120.0	70.7	146.0	85.9	173.0	102.2
119.0	56.0	32.9	92.0	54.1	119.0	70.0	146.0	86.3	174.0	102.5
118.7	58.0	34.3	91.0	53.6	120.0	71.0	145.0	85.3	172.0	101.3
118.2	57.0	33.7	90.0	52.9	122.0	71.7	147.0	86.9	172.0	101.5
119.5	58.0	34.3	90.0	52.8	120.0	70.8	147.0	86.6	170.0	100.2
119.0	56.0	33.3	91.0	53.4	120.0	70.7	146.0	86.1	173.0	102.1
Average		**33.7**		**53.1**		**70.8**		**86.2**		**101.6**
1st MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
119.0	54.0	32.1	92.0	54.5	124.0	73.1	149.0	87.8	169.0	100.0
119.0	57.0	33.6	95.0	55.9	122.0	71.8	150.0	88.8	174.0	102.7
118.2	55.0	32.6	94.0	55.5	122.0	72.3	152.0	89.6	175.0	103.3
121.4	55.0	32.5	90.0	52.9	124.0	73.0	148.0	87.7	173.0	102.3
123.5	56.0	33.2	91.0	53.5	122.0	72.1	150.0	88.5	171.0	100.9
120.1	55.0	32.8	95.0	55.9	123.0	72.6	150.0	88.6	171.0	101.1
Average		**32.8**		**55.2**		**72.5**		**88.5**		**101.7**
1st MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
118.0	29.0	17.3	47.0	27.9	63.0	37.4	76.0	44.6	95.0	56.3
120.1	31.0	18.2	51.0	29.9	62.0	36.4	76.0	45.1	99.0	58.3
118.9	28.0	16.6	48.0	28.3	61.0	36.3	81.0	47.7	99.0	58.3
119.5	27.0	16.0	46.0	26.9	65.0	38.1	80.0	47.5	97.0	57.3
119.3	29.0	17.2	46.0	26.9	66.0	39.1	79.0	46.5	96.0	56.6
120.4	29.0	17.4	45.0	26.3	65.0	38.3	79.0	46.7	98.0	57.9
Average		**17.1**		**28.9**		**37.6**		**46.3**		**57.5**
2nd MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
120.6	50.0	29.6	89.0	52.6	121.0	71.1	146.0	85.7	171.0	100.9
119.5	50.0	29.3	92.0	54.0	120.0	70.4	144.0	85.0	169.0	99.4
119.3	53.0	31.3	91.0	53.5	121.0	71.5	147.0	86.4	173.0	101.8
119.0	53.0	31.2	94.0	55.2	121.0	71.0	145.0	85.7	169.0	99.6
120.6	52.0	30.7	93.0	54.5	120.0	70.6	144.0	84.7	173.0	101.8
119.0	50.0	29.7	92.0	53.9	122.0	71.8	146.0	86.0	172.0	101.4
Average		**30.3**		**53.3**		**71.1**		**85.6**		**100.8**

Table 10 HPLC dissolution rate results of *tablet B* (continued)

2nd MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
119.5	20.0	11.9	39.0	23.1	56.0	33.1	74.0	43.3	93.0	54.9
118.5	22.0	12.8	42.0	24.5	60.0	35.1	72.0	42.6	91.0	53.4
118.6	23.0	13.6	45.0	26.4	55.0	32.6	72.0	42.2	92.0	54.0
119.6	24.0	14.1	45.0	26.3	57.0	33.3	70.0	41.5	90.0	53.0
118.4	22.0	13.0	44.0	25.6	62.0	36.8	73.0	42.9	92.0	54.1
120.0	23.0	13.8	43.0	25.1	60.0	35.2	75.0	44.2	93.0	54.8
Average		**13.2**		**23.8**		**34.4**		**42.8**		**54.1**
3rd MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
118.6	52.0	30.4	92.0	53.9	122.0	71.5	143.0	83.7	173.0	101.3
118.5	51.0	29.9	89.0	52.1	123.0	72.0	142.0	83.2	170.0	99.6
119.7	52.0	30.4	90.0	52.7	120.0	70.3	144.0	84.3	171.0	100.1
120.1	53.0	31.0	93.0	54.5	124.0	72.6	146.0	85.5	172.0	100.7
118.4	51.0	29.9	90.0	52.7	121.0	70.9	147.0	86.1	169.0	99.0
118.9	50.0	29.5	91.0	53.3	120.0	70.3	145.0	84.9	171.0	100.1
Average		**30.2**		**53.0**		**71.3**		**84.6**		**100.1**
3rd MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
118.4	18.0	10.5	37.0	21.7	57.0	33.4	73.0	42.7	91.0	53.3
118.0	21.0	12.3	40.0	23.4	56.0	32.8	71.0	41.6	89.0	52.1
118.6	20.0	11.7	43.0	25.2	57.0	33.4	67.0	39.2	90.0	52.7
118.4	19.0	11.1	41.0	24.0	56.0	32.8	71.0	41.6	90.0	52.7
118.1	20.0	11.7	39.0	22.8	56.0	32.8	69.0	40.4	87.0	50.9
118.5	18.0	10.5	41.0	24.0	58.0	34.0	71.0	41.6	88.0	51.5
Average		**11.3**		**22.5**		**33.2**		**41.2**		**52.2**

Table 11 HPLC dissolution rate results of doxycycline HCl in *tablet C*

	Initial	1st month	2nd month	3rd month
Area STD A	205.8	201.3	202.3	202.7
STD mass (mg)	35.3	34.7	34.5	34.6

INITIAL										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
129.7	72.2	32.4	115.2	51.6	155.7	69.8	194.5	87.2	233.3	104.6
129.5	70.8	31.7	113.6	50.9	158.6	71.1	191.3	85.8	229.7	103.0
129.6	71.1	31.9	113.8	51.0	154.4	69.2	193.6	86.8	227.7	102.1
128.2	73.0	32.7	112.6	50.5	157.5	70.6	195.4	87.6	228.0	102.2
126.7	69.1	30.9	108.5	48.7	153.9	69.0	194.7	87.3	226.8	101.7
126.0	73.4	32.9	114.5	51.3	156.8	70.3	193.9	86.9	228.1	102.2
Average		**32.1**		**51.3**		**70.0**		**86.9**		**102.6**
1st MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
129.0	69.8	31.4	113.4	51.1	151.6	68.3	180.7	81.4	220.7	99.4
129.7	75.7	34.1	115.1	51.9	153.7	69.2	182.4	82.2	223.4	100.7
129.4	72.7	32.8	113.4	51.1	154.9	69.8	181.5	81.8	229.2	103.3
129.4	70.5	31.8	110.6	49.8	157.1	70.8	184.5	83.1	225.1	101.4
131.0	74.3	33.5	119.1	53.7	159.0	71.6	190.7	86.0	232.9	104.9
131.2	78.3	35.3	120.3	54.2	160.3	72.2	194.5	87.6	229.4	103.4
Average		**33.1**		**51.5**		**70.3**		**83.7**		**102.2**
1st MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
128.0	28.6	12.9	53.5	24.1	79.3	35.7	101.2	45.6	122.6	55.3
128.1	27.7	12.5	55.1	24.8	78.9	35.5	105.0	47.3	122.3	55.1
129.4	29.9	13.4	61.5	27.7	74.3	33.5	101.0	45.5	132.8	59.8
126.7	30.4	13.7	53.9	24.3	78.7	35.4	101.0	45.5	121.3	54.6
128.7	29.4	13.2	53.8	24.2	81.0	36.5	102.7	43.3	125.4	56.5
128.7	29.8	13.4	51.2	23.1	78.8	35.5	99.6	44.9	122.9	55.3
Average		**13.2**		**24.5**		**35.4**		**45.8**		**56.1**
2nd MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
130.0	68.4	30.5	113.6	50.7	151.4	67.5	188.9	84.2	227.1	101.3
130.4	61.3	27.3	109.9	49.0	155.7	69.4	189.2	84.4	220.0	98.1
128.6	70.0	31.2	108.5	48.4	154.9	69.1	194.9	86.9	226.4	101.1
129.7	73.2	32.6	108.2	48.3	152.9	68.2	189.0	84.3	223.5	99.7
126.8	66.1	29.5	107.3	47.9	154.0	68.7	188.5	84.1	226.2	100.9
130.4	65.1	29.0	109.4	48.8	156.8	69.9	191.7	85.5	225.3	100.5
Average		**30.0**		**49.8**		**68.8**		**84.9**		**100.2**

Table 11 HPLC dissolution rate results of doxycycline HCl in *tablet C* (continued)

2nd MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
125.9	23.6	10.6	48.9	21.8	73.6	32.8	99.7	43.1	112.7	50.2
126.3	22.6	10.1	48.2	21.5	72.9	32.5	98.0	43.7	119.7	53.4
128.6	26.0	11.6	53.6	23.9	78.5	35.0	99.2	44.3	126.2	56.3
126.4	23.6	10.5	49.7	22.2	74.6	33.3	92.9	41.4	121.6	54.2
125.4	24.1	10.8	47.9	21.4	73.7	32.9	96.7	43.1	119.4	53.3
125.4	25.8	11.5	48.2	21.5	73.1	32.6	97.6	43.5	116.6	52.0
Average		**10.8**		**21.7**		**33.2**		**43.2**		**53.2**
3rd MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
130.4	73.4	32.8	118.3	52.8	154.4	68.9	182.9	81.6	222.3	99.2
131.5	77.8	34.8	114.7	51.22	157.6	70.3	189.3	84.5	226.0	100.9
129.4	79.7	35.6	114.4	51.1	156.1	69.7	188.8	84.3	231.0	103.1
129.6	78.1	34.9	119.4	53.3	154.9	69.2	189.9	84.8	220.4	98.4
134.6	82.3	36.7	121.8	54.4	153.8	68.7	185.2	82.7	227.1	101.4
128.4	77.0	34.4	116.5	52.0	156.9	70.0	187.7	83.7	221.1	98.8
Average		**34.9**		**52.0**		**69.5**		**83.6**		**100.3**
3rd MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
124.5	23.9	10.7	51.6	23.0	72.1	32.2	97.1	43.3	123.4	55.1
123.3	22.7	10.1	49.0	21.9	74.6	33.3	102.6	45.8	118.3	52.8
121.6	23.0	10.3	46.9	20.9	72.3	32.3	98.4	43.9	120.7	53.9
122.9	23.2	10.4	48.5	21.7	72.7	32.5	99.6	44.5	116.7	52.1
121.3	25.1	11.2	49.8	22.2	73.9	33.0	100.9	45.0	120.7	53.9
125.2	22.6	10.1	50.8	22.7	73.0	32.6	103.1	46.0	119.3	53.2
Average		**10.5**		**22.5**		**32.6**		**44.8**		**53.5**

Table 12 HPLC dissolution rate results of bromhexine HCl in *tablet C*

	Initial	1st month	2nd month	3rd month
Area STD A	170.6	170.5	172.8	170.5
STD mass (mg)	20.1	20.1	20.1	20.1

INITIAL										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
129.7	54.3	31.8	85.2	49.9	115.7	67.8	144.5	84.7	170.2	99.7
129.5	55.8	32.7	89.6	52.5	115.6	67.7	141.3	82.8	171.7	100.6
129.6	53.1	31.1	87.8	51.5	117.4	68.8	143.6	84.1	170.7	100.0
128.2	54.0	31.6	87.6	51.3	119.5	70.0	145.4	85.2	168.0	98.4
126.7	53.1	31.1	86.5	50.7	118.9	69.7	144.7	84.8	173.8	101.8
126.0	53.4	31.3	87.5	51.3	116.8	68.4	143.9	84.3	172.1	100.8
Average		**31.6**		**51.2**		**68.7**		**84.3**		**100.2**
1st MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
129.0	52.0	30.6	87.0	51.0	122.0	71.2	148.0	86.2	171.0	100.1
129.7	54.0	31.5	90.0	52.4	120.0	69.9	144.0	84.4	175.0	102.1
129.4	50.0	29.3	91.0	53.1	118.0	69.2	148.0	86.3	172.0	100.4
129.4	52.0	30.4	92.0	53.6	120.0	69.9	147.0	86.2	172.0	100.6
131.0	54.0	31.6	89.0	51.8	119.0	69.5	148.0	86.4	174.0	101.6
131.2	53.0	31.2	90.0	52.3	118.0	68.9	146.0	85.3	170.0	99.4
Average		**30.8**		**51.7**		**69.8**		**85.8**		**100.7**
1st MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
128.0	24.0	14.2	45.0	26.4	55.0	32.3	78.0	45.3	94.0	55.1
128.1	26.0	15.1	44.0	25.5	63.0	36.6	76.0	44.6	96.0	55.9
129.4	23.0	13.5	43.0	25.1	62.0	36.5	76.0	44.2	95.0	55.4
126.7	25.0	14.6	43.0	24.9	61.0	35.4	76.0	44.7	95.0	55.5
128.7	23.0	13.5	42.0	24.3	60.0	35.0	78.0	44.5	97.0	56.6
128.7	26.0	15.5	44.0	25.5	61.0	35.6	75.0	43.8	93.0	54.4
Average		**14.4**		**26.0**		**35.2**		**44.7**		**55.5**
2nd MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
130.0	50.0	29.1	90.0	52.2	121.0	69.9	145.0	83.6	170.0	98.5
130.4	53.0	30.6	94.0	54.2	121.0	69.8	146.0	84.7	174.0	100.6
128.6	53.0	30.7	93.0	53.8	122.0	70.8	147.0	84.9	171.0	98.8
129.7	55.0	31.9	93.0	53.6	123.0	70.9	148.0	85.9	174.0	100.7
126.8	52.0	30.2	94.0	54.2	124.0	71.7	147.0	84.9	175.0	101.2
130.4	50.0	29.2	92.0	53.0	125.0	72.3	149.0	86.2	173.0	100.2
Average		**30.3**		**53.2**		**70.9**		**85.1**		**100.0**

Table 12 HPLC dissolution rate results of bromhexine HCl in *tablet C* (continued)

2nd MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
125.87	20.0	11.8	40.0	23.3	55.0	32.1	73.0	42.0	92.0	53.4
126.3	22.0	12.6	42.0	24.1	58.0	33.3	70.0	70.7	91.0	52.5
128.6	21.0	12.2	40.0	23.1	56.0	32.6	72.0	41.5	91.0	52.5
126.4	20.0	11.6	39.0	22.4	57.0	32.7	71.0	41.3	93.0	53.9
125.4	22.0	12.8	42.0	24.1	58.0	33.5	74.0	42.7	92.0	53.2
125.4	20.0	11.8	42.0	24.1	58.0	33.5	73.0	42.2	92.0	53.3
Average		**12.1**		**23.7**		**32.9**		**41.7**		**53.1**
3rd MONTH 25°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
130.4	53.0	31.2	89.0	52.1	121.0	70.6	144.0	83.9	172.0	100.7
131.5	56.0	32.6	92.0	53.6	122.0	71.1	144.0	84.4	173.0	101.0
129.4	55.0	32.2	91.0	53.1	120.0	70.4	143.0	83.4	172.0	100.4
129.6	54.0	31.6	91.0	52.9	121.0	70.5	142.0	83.3	172.0	100.6
134.6	53.0	31.0	93.0	54.1	122.0	71.3	144.0	84.0	174.0	101.6
128.4	55.0	32.4	92.0	53.5	123.0	71.8	143.0	83.6	174.0	101.7
Average		**31.8**		**52.9**		**71.0**		**83.8**		**101.0**
3rd MONTH 40°C										
Mass (mg)	15 min		30 min		45 min		60 min		90 min	
	Area	%	Area	%	Area	%	Area	%	Area	%
124.5	18.0	10.8	38.0	22.4	51.0	29.8	67.0	39.0	85.0	50.0
123.3	16.0	9.3	35.0	20.3	53.0	30.8	64.0	37.8	86.0	50.3
121.6	15.0	8.9	38.0	22.2	57.0	33.5	64.0	37.3	87.0	50.9
122.9	17.0	10.0	37.0	21.5	54.0	31.4	64.0	37.8	86.0	50.5
121.3	16.0	9.4	36.0	20.9	51.0	30.0	66.0	38.6	84.0	49.2
125.2	17.0	10.2	35.0	20.3	52.0	30.4	65.0	38.1	85.0	49.9
Average		**9.8**		**21.4**		**31.0**		**38.1**		**50.1**

Table 13 Moisture content of *tablet A*

	INITIAL	1ST MONTH 25°C	1ST MONTH 40°c	2ND MONTH 25°C	2ND MONTH 40°C	3RD MONTH 25°c	3RD MONTH 40°C
SAMPLE 1							
Weight (g)	0.249	0.250	0.249	0.249	0.251	0.249	0.250
Stirtime (min)	4:03	3:06	5:16	1:58	2:04	2:11	2:39
Result (%)	4.312	5.732	6.476	5.628	6.556	5.524	6.980
SAMPLE 2							
Weight (g)	0.249	0.250	0.250	0.251	0.251	0.250	0.251
Stirtime (min)	2:17	4:36	5:22	2:25	1:47	2:36	2:33
Result (%)	4.044	6.039	6.393	5.251	6.903	5.684	6.960
RESULTS							
n	2	2	2	2	2	2	2
% mean	4.178	5.886	6.435	5.439	6.729	5.604	6.970
% SD	0.189	0.217	0.059	0.188	0.245	0.080	0.014
% RSD	4.529	3.692	0.914	3.461	3.646	1.431	0.203

Table 14 Moisture content of *tablet B*

	INITIAL	1ST MONTH 25°C	1ST MONTH 40°c	2ND MONTH 25°C	2ND MONTH 40°C	3RD MONTH 25°c	3RD MONTH 40°C
SAMPLE 1							
Weight (g)	0.250	0.249	0.250	0.250	0.251	0.250	0.250
Stirtime (min)	1:56	3:09	3:05	2:06	2:45	3:22	2:15
Result (%)	4.184	5.234	5.648	5.563	6.078	5.888	6.414
SAMPLE 2							
Weight (g)	0.250	0.250	0.250	0.251	0.250	0.251	0.250
Stirtime (min)	2:11	3:31	3:38	3:01	2:24	2:24	3:42
Result (%)	4.371	5.329	5.948	5.294	5.981	5.667	6.558
RESULTS							
n	2	2	2	2	2	2	2
% mean	4.277	5.282	5.798	5.429	6.029	5.777	6.486
% SD	0.133	0.067	0.212	0.190	0.048	0.110	0.102
% RSD	3.099	1.272	3.657	3.504	0.802	1.907	1.570

Table 15 Moisture content of *tablet C*

	INITIAL	1ST MONTH 25°C	1ST MONTH 40°c	2ND MONTH 25°C	2ND MONTH 40°C	3RD MONTH 25°c	3RD MONTH 40°C
SAMPLE 1							
Weight (g)	0.250	0.249	0.249	0.250	0.250	0.251	0.251
Stirtime (min)	1:39	3:14	5:57	1:53	2:05	1:35	5:49
Result (%)	4.058	5.286	6.131	4.959	6.236	5.694	7.111
SAMPLE 2							
Weight (g)	0.250	0.249	0.249	0.250	0.251	0.251	0.250
Stirtime (min)	2:43	3:55	5:11	1:53	2:08	1:42	4:30
Result (%)	4.151	5.202	5.920	5.179	6.289	5.354	6.808
RESULTS							
n	2	2	2	2	2	2	2
% mean	4.105	5.244	6.026	5.069	6.263	5.524	6.959
% SD	0.066	0.056	0.149	0.110	0.037	0.169	0.152
% RSD	1.606	0.975	2.474	2.169	0.598	3.077	2.178

Table 16 Uniformity results of *tablet A*

Initial										1st Month 40°C				
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviation form avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	1.11	5.55	3.70	0.1293	1	0.36	5.58	3.74	0.1304	1	0.89	5.63	3.86	0.1296
2	0.73	5.57	3.79	0.1317	2	0.41	5.58	3.76	0.1314	2	1.60	5.64	3.81	0.1264
3	0.42	5.57	3.72	0.1302	3	1.27	5.58	3.74	0.1292	3	0.12	5.64	3.81	0.1283
4	0.49	5.57	3.71	0.1301	4	0.13	5.58	3.74	0.1307	4	2.61	5.64	3.80	0.1251
5	0.11	5.56	3.74	0.1306	5	0.28	5.58	3.75	0.1305	5	0.82	5.64	3.81	0.1274
6	1.04	5.57	3.81	0.1321	6	0.28	5.57	3.71	0.1305	6	0.35	5.62	3.79	0.1280
7	0.96	5.57	3.72	0.1320	7	0.56	5.58	3.73	0.1316	7	0.59	5.63	3.78	0.1277
8	2.03	5.57	3.74	0.1334	8	0.41	5.58	3.74	0.1314	8	0.90	5.61	3.85	0.1273
9	0.12	5.57	3.70	0.1309	9	0.79	5.58	3.77	0.1319	9	1.59	5.64	3.82	0.1305
10	0.04	5.57	3.70	0.1308	10	0.18	5.58	3.75	0.1311	10	1.28	5.62	3.80	0.1301
11	1.49	-	-	0.1288	11	0.36	-	-	0.1304	11	0.27	-	-	0.1288
12	3.71	-	-	0.1356	12	1.56	-	-	0.1329	12	1.21	-	-	0.1269
13	0.20	-	-	0.1310	13	1.27	-	-	0.1292	13	0.82	-	-	0.1274
14	0.34	-	-	0.1303	14	0.58	-	-	0.1301	14	0.27	-	-	0.1288
15	0.42	-	-	0.1302	15	0.56	-	-	0.1316	15	1.20	-	-	0.1300
16	1.33	-	-	0.1290	16	0.33	-	-	0.1313	16	1.98	-	-	0.1310
17	0.96	-	-	0.1320	17	0.66	-	-	0.1300	17	0.51	-	-	0.1278
18	1.49	-	-	0.1288	18	0.66	-	-	0.1300	18	0.04	-	-	0.1284
19	1.18	-	-	0.1292	19	1.02	-	-	0.1322	19	1.28	-	-	0.1301
20	1.41	-	-	0.1289	20	0.03	-	-	0.1309	20	0.81	-	-	0.1295
(X)		5.57	3.37	0.13	(X)		5.58	3.74	0.13	(X)		5.63	3.81	0.13
(SD)		0.01	0.04	0.00	(SD)		0.00	0.02	0.00	(SD)		0.01	0.03	0.00
% RSD		0.12	1.03	1.32	% RSD		0.06	0.44	0.73	% RSD		0.20	0.65	1.18

Table 16 Uniformity results of *tablet A* (continued)

2nd Month 25°C					2nd Month 40°C				
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	0.58	5.56	3.73	0.1306	1	0.72	5.63	3.83	0.1258
2	2.43	5.55	3.84	0.1331	2	1.79	5.62	3.85	0.1272
3	0.35	5.56	3.75	0.1303	3	1.46	5.62	3.83	0.1231
4	1.35	5.55	3.75	0.1281	4	1.64	5.61	3.82	0.1229
5	0.28	5.56	3.73	0.1303	5	0.15	5.62	3.81	0.1247
6	0.48	5.52	3.74	0.1305	6	1.73	5.61	3.88	0.1271
7	0.28	5.55	3.75	0.1295	7	2.11	5.60	3.76	0.1223
8	0.24	5.55	3.76	0.1302	8	0.01	5.62	3.85	0.1249
9	0.61	5.55	3.77	0.1307	9	1.58	5.61	3.88	0.1269
10	1.32	5.56	3.76	0.1316	10	0.90	5.61	3.80	0.1238
11	1.68	-	-	0.1277	11	0.01	-	-	0.1249
12	0.19	-	-	0.1301	12	0.03	-	-	0.1250
13	1.05	-	-	0.1285	13	0.22	-	-	0.1252
14	1.32	-	-	0.1316	14	1.39	-	-	0.1267
15	1.92	-	-	0.1274	15	0.83	-	-	0.1239
16	1.02	-	-	0.1286	16	0.17	-	-	0.1251
17	0.52	-	-	0.1306	17	0.23	-	-	0.1252
18	0.91	-	-	0.1287	18	0.43	-	-	0.1244
19	0.87	-	-	0.1288	19	0.27	-	-	0.1246
20	0.75	-	-	0.1309	20	0.11	-	-	0.1248
(X)		5.55	3.75	0.13	(X)		5.62	3.83	0.13
(SD)		0.01	0.03	0.00	(SD)		0.01	0.04	0.00
% RSD		0.22	0.84	1.11	% RSD		0.15	0.96	1.08

Table 16 Uniformity results of *tablet A* (continued)

3rd Month 25°C					3rd Month 40°C				
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	1.22	5.57	3.76	0.1306	1	0.52	5.61	3.84	0.1236
2	1.65	5.58	3.71	0.1312	2	1.24	5.60	3.75	0.1215
3	1.34	5.57	3.71	0.1273	3	1.70	5.62	3.84	0.1209
4	1.23	5.57	3.72	0.1306	4	0.27	5.60	3.80	0.1226
5	2.30	5.57	3.78	0.1320	5	0.45	5.60	3.79	0.1224
6	1.67	5.55	3.75	0.1269	6	0.03	5.63	3.79	0.1230
7	0.18	5.57	3.71	0.1288	7	1.45	5.60	3.78	0.1212
8	1.15	5.56	3.71	0.1305	8	1.31	5.59	3.78	0.1214
9	2.27	5.57	3.69	0.1261	9	2.66	5.59	3.81	0.1236
10	2.01	5.56	3.70	0.1264	10	0.11	5.61	3.84	0.1231
11	0.97	-	-	0.1278	11	1.49	-	-	0.1248
12	1.02	-	-	0.1303	12	0.58	-	-	0.1237
13	1.05	-	-	0.1277	13	1.38	-	-	0.1247
14	0.35	-	-	0.1286	14	1.24	-	-	0.1215
15	0.71	-	-	0.1297	15	0.63	-	-	0.1222
16	1.51	-	-	0.1310	16	1.86	-	-	0.1253
17	0.34	-	-	0.1286	17	2.43	-	-	0.1200
18	1.35	-	-	0.1273	18	2.35	-	-	0.1259
19	0.83	-	-	0.1301	19	1.05	-	-	0.1217
20	0.08	-	-	0.1289	20	0.80	-	-	0.1240
(X)		5.57	3.72	0.13	(X)		5.61	3.80	0.12
(SD)		0.01	0.03	0.00	(SD)		0.01	0.03	0.00
% RSD		0.15	0.78	1.35	% RSD		0.23	0.80	1.43

Table 17 Uniformity results of *tablet B*

Initial										1st Month 40°C				
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviation form avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	0.15	5.57	3.20	0.1227	1	2.47	5.58	3.23	0.1227	1	1.87	5.61	3.27	0.1219
2	1.88	5.58	3.29	0.1252	2	0.96	5.57	3.26	0.1246	2	1.84	5.62	3.31	0.1265
3	1.15	5.57	3.28	0.1243	3	2.30	5.60	3.24	0.1287	3	0.95	5.63	3.28	0.1254
4	0.09	5.57	3.22	0.1230	4	0.55	5.58	3.29	0.1265	4	0.82	5.62	3.32	0.1232
5	0.32	5.59	3.22	0.1225	5	1.52	5.58	3.17	0.1239	5	1.59	5.63	3.40	0.1262
6	2.04	5.58	3.29	0.1254	6	1.68	5.58	3.18	0.1237	6	0.34	5.60	3.28	0.1238
7	1.39	5.58	3.25	0.1246	7	1.42	5.58	3.28	0.1276	7	1.55	5.60	3.28	0.1223
8	8.40	5.58	3.28	0.1126	8	0.15	5.58	3.23	0.1260	8	1.79	5.62	3.26	0.1220
9	1.05	5.57	3.19	0.1216	9	1.58	5.58	3.27	0.1278	9	2.51	5.61	3.26	0.1211
10	0.40	5.58	3.19	0.1224	10	2.06	5.58	3.29	0.1284	10	1.51	5.64	3.37	0.1261
11	0.64	-	-	0.1221	11	1.42	-	-	0.1276	11	0.90	-	-	0.1231
12	0.58	-	-	0.1236	12	0.87	-	-	0.1269	12	2.88	-	-	0.1278
13	2.51	-	-	0.1198	13	1.44	-	-	0.1240	13	1.43	-	-	0.1260
14	1.29	-	-	0.1213	14	2.31	-	-	0.1229	14	0.10	-	-	0.1241
15	0.17	-	-	0.1231	15	0.39	-	-	0.1263	15	1.79	-	-	0.1220
16	2.04	-	-	0.1254	16	0.01	-	-	0.1258	16	1.47	-	-	0.1224
17	2.94	-	-	0.1265	17	0.96	-	-	0.1246	17	0.90	-	-	0.1231
18	1.47	-	-	0.1247	18	1.74	-	-	0.1280	18	2.32	-	-	0.1271
19	1.80	-	-	0.1251	19	2.15	-	-	0.1231	19	0.02	-	-	0.1242
20	0.80	-	-	0.1219	20	2.03	-	-	0.1271	20	1.51	-	-	0.1261
(X)		5.58	3.24	0.12	(X)		5.58	3.24	0.13	(X)		5.62	3.30	0.12
(SD)		0.01	0.04	0.00	(SD)		0.01	0.04	0.00	(SD)		0.01	0.05	0.00
% RSD		0.12	1.30	2.41	% RSD		0.13	1.32	1.56	% RSD		0.25	1.45	1.63

Table 17 Uniformity results of *tablet B* (continued)

2nd Month 25°C					2nd Month 40°C				
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	0.96	5.57	3.27	0.1253	1	1.34	5.62	3.29	0.1242
2	1.38	5.58	3.31	0.1258	2	1.63	5.62	3.33	0.1246
3	1.06	5.57	3.23	0.1228	3	1.90	5.61	3.34	0.1249
4	1.40	5.59	3.22	0.1224	4	2.99	5.62	3.38	0.1263
5	0.82	5.58	3.26	0.1251	5	0.41	5.62	3.28	0.1221
6	1.34	5.57	3.25	0.1258	6	3.31	5.61	3.21	0.1185
7	3.56	5.57	3.33	0.1258	7	0.56	5.63	3.31	0.1233
8	0.94	5.58	3.24	0.1230	8	0.19	5.61	3.31	0.1228
9	0.40	5.56	3.25	0.1236	9	1.97	5.62	3.35	0.1250
10	0.01	5.57	3.25	0.1241	10	0.48	5.61	3.30	0.1232
11	0.82	-	-	0.1251	11	1.32	-	-	0.1210
12	1.50	-	-	0.1260	12	1.29	-	-	0.1210
13	1.89	-	-	0.1218	13	0.38	-	-	0.1231
14	0.95	-	-	0.1229	14	0.11	-	-	0.1227
15	0.08	-	-	0.1242	15	1.36	-	-	0.1243
16	0.58	-	-	0.1234	16	3.40	-	-	0.1184
17	0.36	-	-	0.1237	17	0.67	-	-	0.1218
18	2.32	-	-	0.1212	18	1.13	-	-	0.1240
19	0.34	-	-	0.1245	19	0.24	-	-	0.1223
20	0.89	-	-	0.1230	20	3.42	-	-	0.1184
(X)		5.57	3.26	0.12	(X)		5.62	3.31	0.12
(SD)		0.01	0.03	0.00	(SD)		0.01	0.05	0.00
% RSD		0.15	1.06	1.38	% RSD		0.12	1.40	1.82

Table 17 Uniformity results of *tablet B* (continued)

3rd Month 25°C					3rd Month 40°C				
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	2.07	5.58	3.28	0.1255	1	0.41	5.63	3.28	0.1205
2	1.74	5.57	3.27	0.1251	2	6.23	5.62	3.28	0.1134
3	0.66	5.57	3.22	0.1221	3	0.35	5.62	3.27	0.1214
4	5.41	5.57	3.13	0.1163	4	0.60	5.64	3.15	0.1202
5	0.18	5.57	3.24	0.1232	5	0.84	5.64	3.30	0.1220
6	1.10	5.58	3.28	0.1243	6	0.26	5.63	3.26	0.1207
7	0.01	5.58	3.24	0.1230	7	2.39	5.64	3.36	0.1239
8	2.58	5.58	3.19	0.1198	8	1.50	5.63	3.24	0.1192
9	0.84	5.58	3.21	0.1219	9	1.17	5.63	3.28	0.1224
10	3.25	5.58	3.33	0.1269	10	3.72	5.61	3.17	0.1165
11	2.30	-	-	0.1201	11	0.40	-	-	0.1205
12	1.41	-	-	0.1247	12	2.86	-	-	0.1244
13	0.57	-	-	0.1223	13	1.03	-	-	0.1222
14	0.87	-	-	0.1240	14	6.72	-	-	0.1291
15	1.66	-	-	0.1250	15	0.96	-	-	0.1221
16	0.88	-	-	0.1240	16	5.69	-	-	0.1279
17	0.33	-	-	0.1234	17	3.10	-	-	0.1172
18	2.07	-	-	0.1204	18	1.19	-	-	0.1187
19	0.29	-	-	0.1226	19	0.50	-	-	0.1120
20	1.21	-	-	0.1244	20	3.39	-	-	0.1169
(X)		5.58	3.24	0.12	(X)		5.63	3.26	0.12
(SD)		0.01	0.06	0.00	(SD)		0.01	0.06	0.00
% RSD		0.09	1.72	1.97	% RSD		0.18	1.87	3.05

Table 18 Uniformity results of *tablet C*

Initial					1st Month 40°C									
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviation form avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	0.92	5.56	3.70	0.1315	1	0.52	5.58	3.70	0.1296	1	0.47	5.64	3.82	0.1285
2	1.15	5.57	3.75	0.1288	2	0.63	5.56	3.80	0.1311	2	3.11	5.62	3.79	0.1251
3	2.15	5.57	3.78	0.1331	3	0.55	5.59	3.72	0.1310	3	1.01	5.62	3.77	0.1278
4	0.15	5.56	3.73	0.1301	4	0.09	5.57	3.73	0.1304	4	1.09	5.62	3.82	0.1277
5	0.23	5.57	3.78	0.1306	5	0.06	5.56	3.73	0.1302	5	0.30	5.63	3.82	0.1295
6	0.08	5.55	3.72	0.1304	6	1.24	5.56	3.75	0.1319	6	0.22	5.63	3.84	0.1294
7	1.76	5.57	3.71	0.1280	7	1.52	5.58	3.72	0.1283	7	1.46	5.64	3.85	0.1310
8	0.00	5.56	3.71	0.1303	8	0.86	5.58	3.71	0.1314	8	0.07	5.61	3.76	0.1292
9	0.69	5.58	3.75	0.1294	9	1.32	5.59	3.70	0.1320	9	0.84	5.63	3.85	0.1302
10	0.07	5.58	3.73	0.1302	10	1.40	5.58	3.77	0.1321	10	0.69	5.63	3.80	0.1300
11	1.23	-	-	0.1319	11	0.37	-	-	0.1298	11	1.39	-	-	0.1309
12	0.84	-	-	0.1292	12	0.45	-	-	0.1297	12	0.86	-	-	0.1280
13	0.85	-	-	0.1314	13	1.09	-	-	0.1317	13	1.54	-	-	0.1311
14	0.07	-	-	0.1302	14	0.48	-	-	0.1309	14	0.78	-	-	0.1281
15	1.45	-	-	0.1284	15	1.90	-	-	0.1278	15	1.23	-	-	0.1307
16	1.00	-	-	0.1316	16	2.06	-	-	0.1276	16	1.15	-	-	0.1306
17	0.92	-	-	0.1315	17	0.52	-	-	0.1296	17	0.63	-	-	0.1283
18	1.38	-	-	0.1285	18	0.94	-	-	0.1315	18	0.09	-	-	0.1290
19	0.77	-	-	0.1313	19	0.60	-	-	0.1295	19	0.16	-	-	0.1289
20	0.61	-	-	0.1295	20	0.60	-	-	0.1295	20	0.70	-	-	0.1282
(X)		5.57	3.74	0.13	(X)		5.58	3.73	0.13	(X)		5.63	3.81	0.13
(SD)		0.01	0.03	0.00	(SD)		0.01	0.03	0.00	(SD)		0.01	0.03	0.00
% RSD		0.17	0.79	1.03	% RSD		0.21	0.86	1.04	% RSD		0.17	0.83	1.15

Table 18 Uniformity results of *tablet C* (continued)

2nd Month 25°C					2nd Month 40°C				
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	0.85	5.55	3.75	0.1285	1	0.28	5.60	3.84	0.1255
2	1.16	5.58	3.69	0.1281	2	0.72	5.61	3.82	0.1267
3	0.10	5.54	3.72	0.1297	3	0.44	5.61	3.78	0.1253
4	0.81	5.56	3.75	0.1307	4	0.73	5.61	3.75	0.1249
5	2.76	5.56	3.68	0.1260	5	1.23	5.61	3.79	0.1274
6	0.42	5.56	3.72	0.1301	6	0.32	5.61	3.79	0.1254
7	1.08	5.55	3.73	0.1310	7	0.93	5.61	3.75	0.1247
8	0.50	5.53	3.73	0.1302	8	2.49	5.61	3.81	0.1227
9	0.19	5.56	3.73	0.1298	9	0.06	5.63	3.91	0.1259
10	0.51	5.55	3.70	0.1289	10	0.74	5.62	3.82	0.1249
11	0.20	-	-	0.1293	11	5.94	-	-	0.1333
12	0.05	-	-	0.1297	12	0.34	-	-	0.1263
13	1.32	-	-	0.1279	13	0.68	-	-	0.1267
14	1.73	-	-	0.1318	14	2.04	-	-	0.1233
15	0.32	-	-	0.1292	15	0.97	-	-	0.1246
16	1.07	-	-	0.1310	16	0.33	-	-	0.1263
17	1.21	-	-	0.1312	17	0.25	-	-	0.1255
18	0.50	-	-	0.1303	18	0.37	-	-	0.1254
19	0.11	-	-	0.1295	19	0.52	-	-	0.1252
20	0.44	-	-	0.1290	20	0.76	-	-	0.1268
(X)		5.55	3.72	0.13	(X)		5.61	3.81	0.13
(SD)		0.01	0.02	0.00	(SD)		0.01	0.05	0.00
% RSD		0.24	0.63	1.03	% RSD		0.14	1.23	1.66

Table 18 Uniformity results of *tablet C* (continued)

3rd Month 25°C					3rd Month 40°C				
Tablet	% Mass Deviation from avg	Diameter (mm)	Thickness (mm)	Mass (g)	Tablet	% Mass Deviaton from avg	Diameter (mm)	Thickness (mm)	Mass (g)
1	0.43	5.56	3.71	0.1302	1	1.19	5.63	3.74	0.1215
2	0.84	5.56	3.72	0.1297	2	0.89	5.59	3.82	0.1233
3	1.60	5.56	3.70	0.1287	3	1.48	5.58	3.82	0.1225
4	0.84	5.56	3.77	0.1319	4	0.90	5.62	3.82	0.1255
5	0.19	5.57	3.77	0.1306	5	0.51	5.63	3.80	0.1237
6	0.53	5.56	3.68	0.1301	6	0.41	5.62	3.82	0.1249
7	0.38	5.56	3.71	0.1313	7	0.54	5.61	3.74	0.1237
8	0.57	5.57	3.73	0.1316	8	0.00	5.62	3.82	0.1244
9	0.05	5.58	3.72	0.1307	9	0.01	5.63	3.80	0.1244
10	0.11	5.57	3.79	0.1309	10	1.40	5.60	3.78	0.1226
11	0.54	-	-	0.1301	11	0.93	-	-	0.1255
12	5.44	-	-	0.1379	12	1.33	-	-	0.1227
13	0.27	-	-	0.1305	13	0.75	-	-	0.1253
14	2.31	-	-	0.1338	14	0.40	-	-	0.1249
15	0.21	-	-	0.1305	15	0.86	-	-	0.1233
16	1.62	-	-	0.1287	16	0.54	-	-	0.1251
17	0.53	-	-	0.1301	17	1.43	-	-	0.1262
18	0.12	-	-	0.1306	18	0.57	-	-	0.1237
19	2.18	-	-	0.1280	19	0.45	-	-	0.1249
20	0.53	-	-	0.1301	20	0.60	-	-	0.1251
(X)		5.57	3.73	0.13	(X)		5.61	3.80	1.12
(SD)		0.01	0.04	0.00	(SD)		0.02	0.03	0.00
% RSD		0.13	0.95	1.59	% RSD		0.31	0.85	0.89

Table 19 Friability results of *tablet A*

Initial		
Storage temperature	25°C + 60% RH	
Total tablet mass weighed	2.604	
Avg tablet mass y	0.130	
Mass a	2.604	
Mass b	2.594	
Mass c (a – b)	0.010	
Total capped	0	
% Friability	0.388	
1st Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.610	2.560
Avg tablet mass y	0.131	0.128
Mass a	2.610	2.560
Mass b	2.595	2.538
Mass c (a – b)	0.015	0.022
Total capped	0	0
% Friability	0.575	0.859
2nd Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.576	2.497
Avg tablet mass y	0.129	0.125
Mass a	2.576	2.497
Mass b	2.561	2.483
Mass c (a – b)	0.015	0.014
Total capped	0	0
% Friability	0.580	0.560
3rd Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.598	2.473
Avg tablet mass y	0.129	0.124
Mass a	2.598	2.473
Mass b	2.581	2.460
Mass c (a – b)	0.017	0.013
Total capped	0	0
% Friability	0.672	0.521

Table 20 Friability results of *tablet B*

Initial		
Storage temperature	25°C + 60% RH	
Total tablet mass weighed	2.475	
Avg tablet mass y	0.124	
Mass a	2.475	
Mass b	2.472	
Mass c (a – b)	0.004	
Total capped	0	
% Friability	0.142	
1st Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.434	2.407
Avg tablet mass y	0.122	0.120
Mass a	2.434	2.407
Mass b	2.427	2.399
Mass c (a – b)	0.007	0.009
Total capped	0	0
% Friability	0.275	0.353
2nd Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.451	2.463
Avg tablet mass y	0.123	0.123
Mass a	2.451	2.463
Mass b	2.447	2.459
Mass c (a – b)	0.004	0.004
Total capped	0	0
% Friability	0.169	0.166
3rd Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.454	2.387
Avg tablet mass y	0.123	0.119
Mass a	2.454	2.387
Mass b	2.449	2.382
Mass c (a – b)	0.004	0.005
Total capped	0	0
% Friability	0.178	0.199

Table 21 Friability results of *tablet C*

Initial		
Storage temperature	25°C + 60% RH	
Total tablet mass weighed	2.605	
Avg tablet mass y	0.130	
Mass a	2.605	
Mass b	2.595	
Mass c (a – b)	0.403	
Total capped	0	
% Friability	0.011	
1st Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.600	2.568
Avg tablet mass y	0.130	0.128
Mass a	2.600	2.568
Mass b	2.580	2.549
Mass c (a – b)	0.019	0.019
Total capped	0	0
% Friability	0.762	0.732
2nd Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.590	2.510
Avg tablet mass y	0.129	0.125
Mass a	2.590	2.510
Mass b	2.572	2.487
Mass c (a – b)	0.018	0.023
Total capped	0	0
% Friability	0.712	0.917
3rd Month		
Storage temperature	25°C + 60% RH	40°C + 75% RH
Total tablet mass weighed	2.594	2.480
Avg tablet mass y	0.130	0.124
Mass a	2.594	2.480
Mass b	2.574	2.468
Mass c (a – b)	0.021	0.011
Total capped	0	0
% Friability	0.801	0.457

Table 22 Hardness results of *tablet A*

Initial		1st Month 25°C		1st Month 40°C		2nd Month 25°C	
Tablet	Hardness	Tablet	Hardness	Tablet	Hardness	Tablet	Hardness
1	52.6	1	49.3	1	36.9	1	39.3
2	49.8	2	53.2	2	39.8	2	36.8
3	49.7	3	48.7	3	38.5	3	37.8
4	47.3	4	47.1	4	36.2	4	38.8
5	48.9	5	52.8	5	43.1	5	43.7
6	54.0	6	53.6	6	43.4	6	44.3
7	51.3	7	52.3	7	42.7	7	44.8
8	50.6	8	53.0	8	43.9	8	41.0
9	55.0	9	47.2	9	42.5	9	39.4
10	55.0	10	49.7	10	36.9	10	39.5
Mean(N)	51.4	Mean(N)	50.7	Mean(N)	40.4	Mean(N)	40.5
% CV	4.9	% CV	4.8	% CV	7.2	% CV	6.6
2nd Month 40°C		3rd Month 25°C		3rd Month 40°C			
Tablet	Hardness	Tablet	Hardness	Tablet	Hardness		
1	37.1	1	41.7	1	38.3		
2	39.6	2	39.5	2	38.6		
3	36.0	3	39.0	3	36.0		
4	39.0	4	40.1	4	36.9		
5	38.8	5	37.2	5	37.3		
6	39.3	6	43.1	6	41.7		
7	40.6	7	41.8	7	39.5		
8	38.1	8	38.4	8	37.7		
9	39.7	9	37.5	9	42.0		
10	36.7	10	39.9	10	37.5		
Mean(N)	38.5	Mean(N)	39.8	Mean(N)	38.5		
% CV	3.6	% CV	4.6	% CV	4.8		

Table 23 Hardness results of *tablet B*

Initial		1st Month 25°C		1st Month 40°C		2nd Month 25°C	
Tablet	Hardness	Tablet	Hardness	Tablet	Hardness	Tablet	Hardness
1	73.3	1	82.7	1	75.1	1	91.2
2	77.0	2	82.3	2	74.1	2	93.6
3	83.2	3	75.5	3	69.7	3	95.0
4	76.4	4	85.8	4	78.2	4	86.8
5	79.1	5	75.5	5	79.7	5	97.0
6	80.0	6	75.2	6	76.5	6	93.6
7	80.0	7	83.7	7	80.3	7	102.9
8	79.4	8	82.7	8	71.1	8	89.9
9	75.7	9	82.3	9	78.6	9	92.9
10	73.2	10	83.7	10	77.6	10	94.0
Mean(N)	77.7	Mean(N)	80.9	Mean(N)	76.1	Mean(N)	93.7
% CV	3.9	% CV	4.6	% CV	4.5	% CV	4.4
2nd Month 40°C		3rd Month 25°C		3rd Month 40°C			
Tablet	Hardness	Tablet	Hardness	Tablet	Hardness		
1	89.5	1	97.0	1	73.1		
2	83.0	2	100.1	2	74.6		
3	87.1	3	95.7	3	79.6		
4	79.3	4	92.3	4	76.4		
5	84.5	5	96.7	5	75.9		
6	83.8	6	91.2	6	76.2		
7	76.2	7	86.8	7	77.9		
8	89.2	8	95.8	8	72.4		
9	87.8	9	95.7	9	73.1		
10	77.6	10	98.7	10	72.1		
Mean(N)	83.8	Mean(N)	95.0	Mean(N)	75.1		
% CV	5.4	% CV	3.9	% CV	3.2		

Table 24 Hardness results of *tablet C*

Initial		1st Month 25°C		1st Month 40°C		2nd Month 25°C	
Tablet	Hardness	Tablet	Hardness	Tablet	Hardness	Tablet	Hardness
1	47.3	1	49.3	1	36.9	1	43.1
2	45.0	2	47.2	2	41.8	2	45.8
3	46.4	3	48.7	3	38.5	3	44.2
4	47.1	4	48.1	4	36.2	4	43.5
5	53.1	5	52.8	5	38.1	5	43.5
6	46.5	6	53.6	6	41.4	6	44.9
7	46.9	7	52.3	7	36.7	7	40.1
8	48.1	8	52.0	8	40.9	8	38.9
9	49.0	9	47.2	9	37.5	9	39.6
10	47.3	10	53.7	10	36.9	10	43.6
Mean(N)	47.7	Mean(N)	50.5	Mean(N)	38.5	Mean(N)	42.7
% CV	4.3	% CV	4.9	% CV	5.2	% CV	5.2
2nd Month 40°C		3rd Month 25°C		3rd Month 40°C			
Tablet	Hardness	Tablet	Hardness	Tablet	Hardness		
1	40.2	1	41.8	1	37.3		
2	39.2	2	36.6	2	41.8		
3	40.7	3	40.3	3	39.2		
4	43.4	4	40.5	4	36.7		
5	41.5	5	36.6	5	38.9		
6	42.4	6	38.0	6	37.7		
7	41.1	7	39.6	7	36.9		
8	43.1	8	36.9	8	36.5		
9	43.7	9	40.3	9	41.7		
10	41.3	10	36.8	10	38.1		
Mean(N)	41.7	Mean(N)	38.7	Mean(N)	38.5		
% CV	3.3	% CV	4.8	% CV	4.8		

Table 25 HPLC assay results of *powder A*

DOXYCYCLINE HCl ASSAY RESULTS							
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	% Recovered
Initial	Assay 1	300.2	1089.0	1083.0	1086.0	170.6	98.9
	Assay 2	300.1	1081.0	1082.0	1081.5	170.0	98.5
1st Month 25°C	Assay 1	300.2	1079.0	1079.0	1079.0	169.6	98.3
	Assay 2	300.1	1076.0	1077.0	1076.5	169.2	98.1
2nd	Assay 1	300.1	1077.0	1079.0	1078.0	169.5	98.2
	Assay 2	300.1	1071.0	1070.0	1070.5	168.2	97.5
3rd	Assay 1	300.0	1065.0	1061.0	1063.0	167.2	96.9
	Assay 2	300.4	1061.0	1057.0	1059.0	166.3	96.4
1st Month 40°C	Assay 1	300.2	1064.0	1066.0	1065.0	167.4	97.0
	Assay 2	300.1	1065.0	1065.0	1065.0	167.4	97.0
2nd	Assay 1	300.0	1005.0	1007.0	1006.0	158.2	91.7
	Assay 2	300.1	1008.0	1007.0	1007.5	158.4	91.8
3rd	Assay 1	300.3	947.0	945.0	946.0	148.6	86.1
	Assay 2	300.8	958.0	950.0	954.0	149.6	86.7

Table 26 HPLC assay results of *powder B*

BROMHEXINE HCl ASSAY RESULTS							
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	% Recovered
Initial	Assay 1	50.9	83.0	81.0	82.0	10.1	100.8
	Assay 2	50.9	81.0	82.0	81.5	10.0	100.2
1st Month 25°C	Assay 1	50.4	81.0	80.0	81.0	10.0	99.5
	Assay 2	50.3	82.0	82.0	82.0	10.2	101.7
2nd	Assay 1	50.7	79.0	80.0	79.5	9.9	99.1
	Assay 2	50.4	78.0	79.0	78.5	9.8	98.3
3rd	Assay 1	50.2	81.0	80.0	80.5	9.9	99.3
	Assay 2	50.4	81.0	81.0	81.0	10.0	99.6
1st Month 40°C	Assay 1	50.4	81.0	81.0	81.0	10.0	100.2
	Assay 2	50.6	82.0	81.0	81.5	10.1	100.5
2nd	Assay 1	50.4	76.0	78.0	77.0	9.6	96.5
	Assay 2	50.4	77.0	75.0	76.0	9.5	95.3
3rd	Assay 1	50.7	78.0	80.0	79.0	9.7	96.6
	Assay 2	50.3	77.0	79.0	78.0	9.6	96.0

Table 27 HPLC assay results of doxycycline HCl in *powder C*

DOXYCYCLINE HCl ASSAY RESULTS							
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	% Recovered
Initial	Assay 1	299.9	1086.2	1086.9	1087.0	171.0	99.1
	Assay 2	300.1	1089.9	1091.0	1090.0	171.5	99.4
1st Month 25°C	Assay 1	300.0	1081.8	1082.0	1082.0	170.1	98.6
	Assay 2	299.9	1080.9	1080.5	1081.0	169.9	98.5
2nd	Assay 1	300.0	1056.9	1057.4	1057.0	166.4	96.4
	Assay 2	300.0	1052.1	1052.4	1052.0	165.6	96.0
3rd	Assay 1	300.1	1059.0	1063.0	1061.0	166.9	96.8
	Assay 2	300.1	1037.0	1037.0	1037.0	163.2	94.6
1st Month 40°C	Assay 1	300.0	1076.2	1076.0	1076.0	169.2	98.1
	Assay 2	300.0	1070.4	1069.7	1070.0	168.2	97.5
2nd	Assay 1	300.1	1049.9	1049.3	1050.0	165.1	95.7
	Assay 2	300.1	1047.1	1048.3	1048.0	164.8	95.6
3rd	Assay 1	300.3	973.0	967.0	970.0	152.5	88.4
	Assay 2	300.5	952.0	948.0	950.0	149.2	86.5

Table 28 HPLC assay results of bromhexine HCl in *powder C*

BROMHEXINE HCl ASSAY RESULTS							
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	% Recovered
Initial	Assay 1	300.0	80.0	79.0	79.5	10.0	99.7
	Assay 2	300.1	81.0	80.0	81.0	10.1	101.0
1st Month 25°C	Assay 1	300.0	81.0	80.0	81.0	10.1	100.8
	Assay 2	299.9	79.5	80.0	80.0	10.0	99.9
2nd	Assay 1	300.0	78.4	79.5	79.0	10.0	99.6
	Assay 2	300.0	79.2	79.0	79.0	10.0	99.8
3rd	Assay 1	300.1	79.9	79.4	80.0	10.0	99.6
	Assay 2	300.1	76.9	76.4	77.0	9.6	95.8
1st Month 40°C	Assay 1	300.0	79.0	78.5	79.0	9.9	98.6
	Assay 2	300.0	80.0	79.5	80.0	10.0	99.9
2nd	Assay 1	300.1	77.4	78.7	78.0	9.8	98.4
	Assay 2	300.1	78.3	79.0	79.0	9.9	99.2
3rd	Assay 1	300.3	77.1	77.0	77.0	9.6	96.3
	Assay 2	300.5	77.9	78.2	78.0	9.7	97.4

Table 29 HPLC "in use" assay results of *powder A* (containing citric acid)

DOXYCYCLINE HCl "IN USE" ASSAY RESULTS			
Container	**Time (hours)**	**Area**	**% Recovered**
Glass container	0	1214	100
	6	1163	96
	12	1131	93
	24	1152	95
Plastic container	0	1214	100
	6	1225	101
	12	1131	93
	24	1189	98
Stainless steel	0	1214	100
	6	1173	97
	12	1109	91
	24	939	77

Table 30 HPLC "in use" assay results of *powder X* (containing no citric acid)

DOXYCYCLINE HCl "IN USE" ASSAY RESULTS			
Container	**Time (hours)**	**Area**	**% Recovered**
Glass container	0	2304	100
	6	1409	61
	12	1095	48
	24	819	36
Plastic container	0	2304	100
	6	877	38
	12	437	19
	24	152	7
Stainless steel	0	2304	100
	6	828	36
	12	295	13
	24	113	5

Table 31 Moisture content of *powder A*

	INITIAL	1ST MONTH 25°C	1ST MONTH 40°c	2ND MONTH 25°C	2ND MONTH 40°C	3RD MONTH 25°c	3RD MONTH 40°C
SAMPLE 1							
Weight (g)	0.249	0.249	0.250	0.249	0.250	0.249	0.246
Stirtime (min)	3:11	3:04	2:42	2:12	2:35	1:38	6:38
Result (%)	6.496	6.487	7.684	6.464	7.543	6.827	10.978
SAMPLE 2							
Weight (g)	0.249	0.249	0.249	0.249	0.250	0.249	0.254
Stirtime (min)	2:59	2:59	2:26	1:59	1:52	1:47	7:01
Result (%)	6.353	6.399	7.326	6.443	7.334	6.741	11.441
RESULTS							
n	2	2	2	2	2	2	2
% mean	6.426	6.443	7.505	6.453	7.438	6.784	11.209
% SD	0.099	0.062	0.253	0.015	0.149	0.043	0.232
% RSD	1.548	0.969	3.367	0.227	1.997	0.639	2.065

Table 32 Moisture content of *powder B*

	INITIAL	1ST MONTH 25°C	1ST MONTH 40°c	2ND MONTH 25°C	2ND MONTH 40°C	3RD MONTH 25°c	3RD MONTH 40°C
SAMPLE 1							
Weight (g)	0.248	0.247	0.251	0.249	0.250	0.249	0.249
Stirtime (min)	3:12	2:44	2:21	2:35	2:24	1:43	2:38
Result (%)	8.714	8.534	9.338	8.770	9.727	9.086	11.097
SAMPLE 2							
Weight (g)	0.251	0.252	0.252	0.249	0.250	0.250	0.252
Stirtime (min)	2:23	1:57	2:21	2:01	2:05	1:18	1:42
Result (%)	8.432	8.529	9.380	8.594	9.792	9.100	11.069
RESULTS							
n	2	2	2	2	2	2	2
% mean	8.573	8.532	9.359	8.682	9.759	9.093	11.083
% SD	0.198	0.003	0.029	0.124	0.046	0.007	0.014
% RSD	2.319	0.037	0.351	1.430	0.526	0.076	0.154

Table 33 Moisture content of *powder C*

	INITIAL	1ST MONTH 25°C	1ST MONTH 40°c	2ND MONTH 25°C	2ND MONTH 40°C	3RD MONTH 25°c	3RD MONTH 40°C
SAMPLE 1							
Weight (g)	0.248	0.247	0.250	0.249	0.249	0.249	0.249
Stirtime (min)	2:51	2:49	2:33	2:35	3:01	1:37	2:55
Result (%)	6.421	6.323	7.176	6.487	9.750	7.134	9.333
SAMPLE 2							
Weight (g)	0.250	0.250	0.250	0.250	0.251	0.249	0.252
Stirtime (min)	2:32	2:43	1:40	2:19	6:01	1:13	1:03
Result (%)	6.275	6.257	6.942	6.461	9.813	6.818	9.637
RESULTS							
n	2	2	2	2	2	2	2
% mean	6.348	6.290	7.059	6.474	9.782	6.976	9.485
% SD	0.104	0.046	0.165	0.019	0.044	0.158	0.152
% RSD	1.631	0.736	2.341	0.289	0.451	2.259	1.600

Table 34 HPLC assay results of doxycycline HCl in the ophthalmic solution

DOXYCYCLINE HCl ASSAY RESULTS							
STABILITY INTERVAL		Powder weighed (mg)	Peak area 1	Peak area 2	Avg area	µg/ml	% Recovered
Initial	Assay 1	332.9	1061.0	1060.0	1061.0	170.3	98.7
	Assay 2	339.9	1087.0	1088.0	1088.0	171.1	99.2
1st Month 5°C	Assay 1	329.7	1050.0	1053.0	1052.0	170.5	98.8
	Assay 2	330.7	1053.0	1055.0	1054.0	170.4	98.8
1st Month 25°C	Assay 1	356.6	1135.0	1137.0	1136.0	170.3	98.7
	Assay 2	348.7	1112.0	1113.0	1113.0	170.6	98.9
2nd	Assay 1	369.1	1150.0	1151.0	1151.0	166.7	96.6
	Assay 2	338.4	1054.0	1057.0	1056.0	166.7	96.7
3rd	Assay 1	320.7	998.0	996.0	997.0	166.1	96.3
	Assay 2	350.1	1100.0	1104.0	1102.0	168.3	97.6
1st Month 40°C	Assay 1	335.9	1062.0	1060.0	1061.0	168.8	97.9
	Assay 2	347.8	1093.0	1093.0	1093.0	168.0	97.4
2nd	Assay 1	339.1	1035.0	1038.0	1037.0	163.4	94.7
	Assay 2	347.9	1061.0	1058.0	1060.0	162.8	94.4
3rd	Assay 1	324.7	950.0	950.0	950.0	156.4	90.7
	Assay 2	348.6	1020.0	1028.0	1024.0	157.0	91.0

Table 35 pH and relative density of the ophthalmic solution

Age	pH	Relative density
Initial	5.21	1.0563
1st Month 5°C	5.37	1.0574
25°C	5.70	1.0577
40°C	5.52	1.0579
2nd Month 25°C	5.37	1.0572
40°C	5.52	1.0575
3rd Month 25°C	5.19	1.0568
40°C	5.24	1.0570

Table 36 Viscosity results of the ophthalmic solution

	Viscosity (cP)		
Age	**5°C**	**25°C**	**40°C**
Initial	5.87	5.87	5.87
1 Month	5.87	5.87	5.87
2 Months	-	5.33	5.87
3 Months	-	5.33	5.31

Table 37 Preservative efficacy results of the ophthalmic solution

Initial				
Test organism	**Initial inoculum**	**Log unit reduction**		
		Day 7	**Day 14**	**Day 28**
E.coli	3.9×10^5	> 3.0	> 3.0	> 3.0
P.aeruginosa	2.8×10^5	> 3.0	> 3.0	> 3.0
S.aureus	4.8×10^5	> 3.0	> 3.0	> 3.0
A.niger	3.0×10^5	2.4	> 3.0	2.8
C.albicans	2.9×10^5	2.2	3.0	3.0
3rd Month 40°C + 75% RH				
Test organism	**Initial inoculum**	**Log unit reduction**		
		Day 7	**Day 14**	**Day 28**
E.coli	8.0×10^5	> 3.0	> 3.0	> 3.0
P.aeruginosa	2.6×10^6	> 3.0	> 3.0	> 3.0
S.aureus	6.5×10^5	> 3.0	> 3.0	> 3.0
A.niger	2.3×10^5	> 3.0	> 3.0	> 3.0
C.albicans	2.8×10^5	> 3.0	> 3.0	> 3.0

APPENDIX 4

REPORT

A report written by pigeon enthusiast, Mr W.A Coetzee is given on page 207 – 208.

Verslag- Doxycyclin

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Ornitose is 'n algemene siekte toestand by wedvlug duiwe en wanneer dit nie onder beheer is nie word wedvlug prestasie geweldig beinvloed asgevolg van asemhalings probleme. Die siekte wanneer dit nie behandel word nie lei tot sekondere siektes. Die meeste duiwe boere behandel gereeld met die volgende produkte wat vrylik op die mark beskikbaar is:

- Doxy-Biotic
- Chlor Tetracyline

Beide die produkte bevat vitamine en daar word ondervind dat dit baie onstabiel is en oksideer binne 30 – 40 minute en is dan heeltemal oneffektief. Die algemene reel is om die duiwe vir ten minste 20 – 30 dae te behandel wat dit baie duur maak. Tekens van Ornitose by duiwe is dat die neus vratte en oogringe grys van kleur word en behandeling word dus aangehou totdat daar waargeneem word dat die vratte en oogringe spierwit van kleur is. Ongelukkig onderdruk die genoemde produkte die siekte toestand en moet meer dikwels darvoor behandel word. Die fyn waarnemer sal klein borreltjies in die oog vloeistof waarneem en dan moer daar dan weer behandel word.

Doxy mengesel (Poeier) – Prof Lotter

Ek het van 5 vooraanstaande wedvlug duiwe boere gedurende die 2003 wedvlug seisoen gebruik gemaak om die poeier mengsel te gebruik en is die volgende waarnemings gedoen;

WA Coetzee
JJ van Tonder
M Klingbill
T Munton
J Prinsloo

1. Geen oksiedasie het plaas gevind nie en kon mengsels meer ekonomies aangewend word.
2. Korter periodes was nodig om baie goeie resutate te verkry aangesien die vratte en oogringe binne 2 – 3 dae spierwit word en geen teken van klein borreltjies kan binne die oog waargeneem word nie
3. Voorkomende behandeling in die wedvlug seisoen was meer suksesvol vanwee die beskikbaarheid van die aktiewe stof
4. Al bogenoemde duiwe boere het onder die eerste 5 in hul onderskeie klubs geeindig

Vanwee trop behandeling gee die meeste duiwe boere water in houers van 1 – 2 liter en is in die meeste gevalle aan direkte sonlig bloot gestel. Die algemene gevoel van al die genoemde persone was die ongelooflike hou – vermoe van die mengsel sonder dat dit geoksideer het.
In my persoonlike geval het ek water tot 8 dae gelaat en net daagliks aangevul en het ek ook gevind dat daar geen oksiedasie plaasgevind het nie.

Doxy mengesel (Oog vloeistof) – Prof Lotter

Ongelukkig was daar 'n bepekte hoeveelheid vloeistof tot my beskikking gestel en het ek alleen die produk op die proef gestel. Wanneer vroeë waarneming van Ornitose moontlik was het ek een druppel per oog aangewend vir twee dae en ongelooflike goeie resultate gekry. Vroeë waarneming is veral belangrik wanneer duiwe van wedvlugte terugkeer en kan die toestand dus gekeer word by individuele duiwe en sodoende verspreiding in die hok beperk.

Afleiding

Omdat Ornitose 'n hoogs aansteeklike siekte toestand by duiwe is en lei tot sekondere infeksies is dit baie belangrik dat die toestand onder goeie beheer moet wees. Dit was baie duidelik dat groot sukses behaal is met die middel en dat dit geweldig koste effektief aangewend kan word. Verdere voordele van die vloeistof is dat duiwe op 'n individuele basis voorberei kan word vir spesiale wedvlugte en kan die doxy – vloeistof in die oog aangewend word en kan ander middels in die water gevoeg word. Dit is dus my mening dat beide die twee produkte 'n groot rol sal speel by wedvlug boere wat goed wil presteer.