DEVELOPMENT AND APPLICATION OF A PHOTOMETRIC METHOD IN QUALITY EVALUATION OF BENZIMIDAZOLE ANTHELMINTHICS IN NAIROBI CITY COUNTY

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF PHARMACY IN PHARMACEUTICAL ANALYSIS OF THE UNIVERSITY OF NAIROBI

JOHNSON KIBIRA MURAGE

B. Pharm. (Nairobi)

U59/6793/2017

Department of Pharmaceutical Chemistry

School of Pharmacy

UNIVERSITY OF NAIROBI

June 2020

DECLARATION

I, Johnson Kibira Murage, declare that this thesis is my original work and, to the best of my knowledge, has not been submitted elsewhere for examination, award of a degree or publication.

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Dr. Johnson Kibira Murage (B. Pharm)

U59/6793/2017

This research thesis has been submitted for examination with our approval as University Supervisors.

Sign……. Date……..

Dr. Beatrice Amugune Ph.D.

Senior lecturer

Department of Pharmaceutical Chemistry

University of Nairobi.

Sign……. Date……..

Dr. Peter M. Njogu Ph.D.

Lecturer

Department of Pharmaceutical Chemistry

University of Nairobi.

Sign……. Date……

Dr. Stanley N. Ndwigah Ph.D.

Senior lecturer

Department of Pharmaceutical Chemistry

University of Nairobi

UNIVERSITY OF NAIROBI

Declaration of Originality

Name of Student: Johnson Kibira Murage

Registration Number: U59/6793/2017

College: Health Sciences

Faculty/School/Institute: School of Pharmacy

Department: Pharmaceutical Chemistry

Course Name: Master of Pharmacy in Pharmaceutical Analysis

Title of the work

Development and application of a photometric method in quality evaluation of benzimidazole anthelminthics in Nairobi City County

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DEDICATION

I dedicate this research thesis to my wife, Maureen Atieno, and my children, Diana Mumbi and William Irungu for their encouragement and patience. They provided the strength that kept me moving on.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Per cent
˚C	Degrees Celsius
µl	Microliter
µm	Micrometre
BP	British Pharmacopoeia
C18	A chromatographic stationary phase consisting of a hydrocarbon chain with 18 carbon atoms
cm	Centimetre
CV	Coefficient of variation
DALYs	Disability adjusted life years
DARU	Drug Analysis and Research Unit
EU	European Union
FDA	Food and Drug Administration
GABA	Gamma-amino butyric acid
GIT	Gastrointestinal tract
GSK	GlaxoSmithKline
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HPLC-MS	High Performance Liquid Chromatography-Mass Spectrometry
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use.
MDGs	Millennium development goals
mg	Milligram
ml	Millilitre
mm	Millimetre
MRL	Maximum Residue Limit
NTDs	Neglected Tropical Diseases
ODS	Octadecylsilane
PPB	Pharmacy and Poisons Board
RSD	Relative standard deviation
SD	Standard deviation
STH	Soil transmitted helminths
TB	Tuberculosis

USA	United States of America
USP	United States Pharmacopeia
UV	Ultra Violet
WHO	World Health Organization
YLD	Years lost to disability
YLL	Life years lost through early death

ABSTRACT

Introduction: Neglected Tropical Diseases (NTDs) are a group of communicable diseases which are prevalent in the tropics affecting more than one billion people. Helminthiases are classified among NTDs. Treatment and prevention of these infections is very costly to developing economies. The communities most afflicted are poor and have limited access to essential resources for their livelihood. Poor quality drugs for NTDs may lead to death or prolonged treatment without achieving the desired results. The limited resources used in purchasing poor quality drugs will therefore be wasted instead of being put to good use.

Study Objectives: The general objective of the study was to determine the quality of benzimidazole anthelminthics in the Kenyan market using a simple, rapid and inexpensive spectroscopic method. More specifically, the study aimed to explore the utility of an ultra-violet (UV) spectroscopic method in the determination of the quality of benzimidazole anthelminthics in the Kenyan market.

Method: The adaptability of a method reported in the literature by Agrawal *et al* for the analysis of albendazole was investigated for the analysis of albendazole and mebendazole. In the method development phase, two solvents were investigated; 0.1M hydrochloric acid (HCl) with 0.05% sodium lauryl sulphate and 0.1M methanolic HCl. Two wavelengths for detection were also investigated; 233 and 294 nm. The working concentration (12 µg/ml) was adapted from the method used by Agrawal *et al*. The method was validated for precision, accuracy, specificity, linearity and range as per the International Council for Harmonization (ICH) guidelines.

The developed method was then used in the assay of commercial products available in pharmaceutical wholesale outlets in Nairobi.

Results and discussion: The suitable solvent of analysis for the analytes was found to be 0.1M methanolic HCl. The wavelength of analysis was set at 294 nm. The range was over which linearity was established was way beyond the 80 to 120% of the working concentration specified by the ICH. Upon validation, the method was found to have good linearity (R^2 = 0.9988 for both albendazole and mebendazole). The method exhibited good precision with the same day coefficient of variation (CV) of 0.184 and 0.579% and the intermediate CV of 0.230 and 0.162% for mebendazole and albendazole respectively against the ICH limit of 2%. This meant that the developed method was precise for the analytes.

Out of 32 commercial samples analysed, five (15.6%) did not comply with compendial specifications. Of great concern is that three (9.4%) of the non-compliant samples were low-cost generic products. These are the products which are popular with the majority members of the society who have a low income. Intra-brand batch variation was also observed. Out of three batches of product A002T analysed, one did not comply with compendial specifications. It was also observed that both batches of product M001S, the suspension of an innovator product, did not comply with compedial specifications.

Utility of results: A major limitation in the analysis of benzimidazole anthelminthics is the lack of reliable, simple, rapid and low-cost methods of analysis. The developed method serves to fill this gap. It can be used in the analysis of raw materials and finished products. It can also be used in the establishment of the quality of products prior to registration. The method will prove very useful in post market surveillance of quality of benzimidazole anthelminthics.

CHAPTER ONE: INTRODUCTION

1.1 Neglected tropical diseases

Neglected tropical diseases (NTDs) are a group of communicable diseases (Table 1.1) which are prevalent mostly in the tropics affecting more than one billion people. Treatment and prevention of these infections is very costly especially to developing economies (WHO, 2010). The global burden of NTDs, as calculated using the disability adjusted life years (DALYs), is huge. The DALYs is calculated as the sum total of life years lost through early death (YLL) plus the years lost to disability (YLD). The global burden of diseases (GBD) due to NTDs is estimated as 56.6, malaria at 46.5 while tuberculosis (TB) stands at 34.7 DALYs (Fenwick, 2012) which illustrates the significance of NTDs to global ill health.

Table 1.1: Infective agents for some neglected tropical diseases

Infective agents	Neglected Tropical Diseases
Bacteria	Buruli ulcer, Hansen's disease (Leprosy), Trachoma, Endemic treponematoses (Yaws).
Protozoa	Chaga's disease, Sleeping sickness (Human African Trypanosomiasis), Leishmaniasis.
Viruses	Dengue, Chikungunya, Rabies.
Helminths	Guinea-worm disease (Dracunculiasis), Ascariasis, Trichuriasis, Hookworm, Echinococcosis, Food-borne trematodiases, Lymphatic filariases, Taeniasis/Cysticercosis.
Fungi	Mycetoma, Deep mycoses including Chromoblastomycosis.
Others	Ectoparasites including Scabies, Snake-bite envenomation.

Approximately 800 million people worldwide are infected with Ascaris, 600 million have Trichuris worms while 600 million have one or another species of hookworm. Hookworms usually infect adults as well as children while other worms are usually found in children.

Neglected Tropical Diseases greatly impacted on the achievement of millennium development goals (MDGs). For instance maternal health (MDG5) could not be improved or child mortality reduced when one of the major causes of poor birth outcomes was anaemia caused by the parasitic infections carried by millions of women of child-bearing age in rural areas of

developing nations (Fenwick, 2012; Qian and Zhou, 2016). The NTDs continue to pose a challenge to the achievement of sustainable development goals (SDGs). Elimination of extreme poverty involves the expansion of the reach to crucial interventions and technologies from high- to low-income economies. This should of essence include the assurance of quality of drugs (Sachs, 2012). It is therefore important to ensure that medicines that are availed for the treatment of NTDs meet high quality criteria for human use.

1.2 Helminthiasis

Helminths are the commonest infectious agents in developing countries. Their global disease burden exceeds better known conditions, such as malaria and tuberculosis. Being NTDs, very little research by commercial pharmaceutical companies is conducted towards the development of new anthelminthics. Yet due to their occurrence among the world's poorest communities, helminths afflict a huge population.

There are two major phyla of helminths, nematodes and platyhelminthes. The nematodes (round worms) include the major intestinal worms (also known as the soil-transmitted helminths – STH) and the filarial worms that cause lymphatic filariases and onchocerciasis. The Platyhelminthes (flat worms) include flukes (trematodes) and tape worm (cestodes) (Hotez et al., 2008).

Ascariasis, trichuriasis and hookworms are the three main STHs. The gastrointestinal tract (GIT) of a child living in poverty in a developing country is likely to be parasitized with at least one and in many cases all the three STHs. This results in impaired physical, intellectual and cognitive development of the child. Table 1.2 shows the estimated global prevalence of common helminth infections.

Table 1.2: Common soil-transmitted helminths and their estimated global prevalence

Helminth	Disease	Estimated global prevalence (millions)
Ascaris lumbricoides	Common roundworm infection	807-1221
Trichuris trichuria	Whipworm infection	604-790
Necator americanus and *Acylostoma duodenale*	Hookworm infection	570-750
Strongyloides stercoralis	Threadworm infection	30-100
Enterobius vermicularis	Pinworm infection	4-25% of children
Toxocara canis and *Toxocara cati*	Visceral and ocular larva migrans	1.5-70% of children

Adopted from (Bethony et al., 2006).

1.3 Chemotherapy of helminthic infections

Anthelminthics are the mainstay of chemotherapy of parasitic infections in human and veterinary animals. Because of the status of helminthiases as NTDs, there is a very small number of therapeutic agents available for their treatment (Table 1.3). Indeed, most of the drugs that are available for the treatment of helminthic infections in humans were first developed as veterinary medicines (Holden-Dye and Walker, 2007). Anthelminthics are classified on the basis of their chemical structure and mode of action. The various classes are described in the sections that follow.

Table 1.3: Helminths and drugs for their treatment

Infection	Drugs
Schistosomiasis	Antimonials, Metrifonate, Oxamniquine, Praziquantel
Cestodiasis	Niclosamide, Benzimidazoles, Praziquantel
Fascioliasis	Praziquantel, Closantel and Halogenated Salicylamides
Intestinal round worms	Piperazine, benzimidazoles, Morantel, Pyrantel, Levamisole, Avermectins and Milbemycins, Closantel and Halogenated Salicylamides, Emodepsides

1.3.1 Piperazine

This drug was first used as an anthelminthic in the 1950s. It is a weak gamma-amino butyric acid (GABA) agonist that causes flaccid reversible paralysis of body wall muscle of the helminth. This facilitates the expulsion of the nematode (Holden-Dye and Walker, 2007). Though piperazine is not effective against filariases, diethyl carbamazine is a more effective anthelminthic and the drug of choice for filariasis and loiasis (Hawking, 1979).

1.3.2 Benzimidazoles

Benzimidazoles are the subject of this study. They are discussed in detail in Section 1.5. Benzimidazoles act by inhibiting the dimerization of α- and β-tubulin to form protozoal microtubules. This inhibition is lethal to the helminth.

1.3.3 Levamisole, morantel and pyrantel

These anthelminthics are nicotinic receptor agonists. They elicit spastic muscle paralysis due to prolonged activation of excitatory nicotinic acetylcholine receptors on body wall muscle of the helminths.

1.3.4 Paraherquamide

Paraherquamide A and marcfortine are both oxindole alkaloids, originally isolated from *Penicillium paraherquei* and *Penicillium roqueforti*, respectively. Paraherquamide and its derivative 2-deoxy-paraherquamide, are antagonists of β-nicotinic acetylcholine receptor subtypes. This induces flaccid paralysis in the nematodes thus facilitating their expulsion (Epe and Kaminsky, 2013). The activity of paraherquamide against sheep nematodes has been observed though no commercial preparation is available (Besier, 2007).

1.3.5 Macrocyclic lactones and milbemycins

Ivermectin was introduced as an anthelminthic in the 1980s. It is a semi-synthetic derivative of avermectin which is a large macrocyclic lactone fermentation product of *Streptomyces avermitilis*. The synthesis of ivermectin is achieved through the selective hydrogenation of the C22 – C23 double bond in avermectins 1a and 1b using the Wilkinson's homogeneous hydrogenation catalyst (Campbell *et al*., 1983; Fink, 1988). Other ivermectin analogues developed after its discovery includes moxidectin, milbemycin oxime, doramectin, salamectin, abamectin and eprinomectin.

Ivermectin causes a potent and persistent paralysis of nematode neurones and pharyngeal and body wall musculature. It interacts with a range of ligand-gated ion channels including acetylcholine-gated chloride channels, histamine-gated chloride channels, GABA-gated chloride channels and glycine receptors. Its anthelminthic activity is however attributed to its high affinity for nematode glutamate-gated chloride channels. Ivermectin activates these channels, opening them slowly and irreversibly. This leads to long-lasting hyperpolarisation of the neurone or muscle cell thus blocking further function. Paralysis of the nematode that facilitates expulsion follows (Wolstenholme and Rogers, 2005).

1.3.6 Cyclodepsipeptides

Emodepside is a semi-synthetic derivative of PF1022A, a fermentation product obtained from the fungus, *Mycelia sterilia.* Emodepside is effective against isolates of parasites that are resistant to benzimidazoles, levamisole and ivermectin. Emodepside binds to a presynaptic receptor in nematodes leading to the release of an unknown transmitter which exerts a postsynaptic membrane to cause flaccid paralysis of the pharynx and somatic musculature in nematodes. This facilitates their expulsion (Harder et al., 2005).

1.3.7 Metrifonate

Metrifonate is an antischistosomal drug that is effective against *Schistosoma haematobium.* It is non-enzymatically converted into dichlorvos, an organophosphate cholinesterase inhibitor. Accumulation of acetylcholine in the helminth leads to flaccid paralysis that causes their detachment from the walls of blood vessels. The helminths are hence swept by the blood to the lungs where they are unable to survive (Cioli et al., 1995).

1.3.8 Isoquinolines

This class includes praziquantel and oxamniquine. Praziquantel is the drug of choice for all fluke infections except *Fasciola* species. It interacts with calcium ion channels. It has been shown to induce rapid vacuolisation and disintegration of teguments of parasites. Oxamniquine is used in the treatment of *Schistosoma mansoni* infections. It is an irreversible protein synthesis inhibitor in the trematode leading to the death of the trematode (Lambertucci et al., 1989).

1.3.9 Bithionol

Bithionol was previously used for the treatment of fascioliasis and paragonimiasis. It has however been largely replaced by triclabendazole and praziquantel.

1.4 Potential new drug candidates against food-borne trematodiasis

Certain drugs which are currently used in the treatment of other diseases have shown potential as anthelminthics. These include the artemisinins and synthetic peroxides, mefloquine and tribendimine. Artemisinin derivatives, namely artemether and artesunate have been studied recently in different trematode-rodent models. All liver and intestinal flukes tested were affected by artemisinins in rodent models. Studies have also been carried out in sheep and rabbit models with promising results.

To overcome the chemical, economic and biopharmaceutical shortcomings of synthetic artemisinins, studies have been done on the synthetic peroxide OZ78 with promising results. Structure-activity relationship studies in sheep infected with *Fasciola hepatica* have revealed the potential for the development of clinically useful products (Zhao et al, 2010). Mefloquine

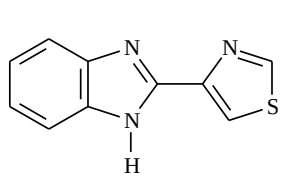
has been reported to show antischistosomal properties against *Schistosoma mansoni* and *S. japonicum* in infected mice (Holden-Dye and Walker, 2007; Keiser and Utzinger, 2008).

1.5 Benzimidazole anthelminthics

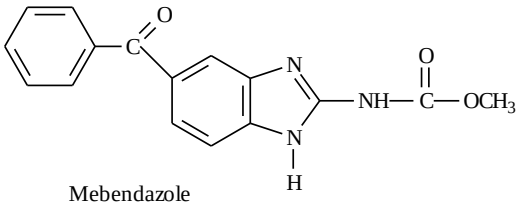
Thiabendazole was the first benzimidazole and highly efficacious broad spectrum anthelminthic to be developed. Since the introduction of thiabendazole, benzimidazoles with improved efficacy and extended spectra of activity have been developed. Parbendazole, cambendazole, mebendazole and oxibendazole were benzimidazole products of research conducted after the introduction of thiabendazole. Other benzimidazoles include fenbendazole, oxfenbendazole, albendazole, triclabendazole and luxabendazole (McKellar and Scott, 1990)..

However, poor gastrointestinal absorption and low water solubility of the earlier benzimidazoles led to the development of probenzimidazoles. Probenzimidazoles are prodrugs that are converted into the respective benzimidazoles *in vivo* upon administration. Netobimin and febantel are examples of probenzimidazoles. Febantel, upon absorption, is converted into fenbendazole (McKellar and Scott, 1990) while Netobimin is converted in the gastrointestinal tract into albendazole. Netobimin is a water-soluble probenzimidazole that can be formulated for parenteral and oral administration in veterinary medicine. Upon oral administration, netobimin undergoes nitro-reduction and cyclization to convert it into albendazole, the active form. This activation is mediated by gastrointestinal microorganisms rather than liver microsomal enzymes. Thus when given parenterally, very low levels of albendazole are detected in the blood. This may suggest that development of netobimin did not solve the pharmacokinetic challenges (poor absorption) of albendazole. Activation of febantel into fenbendazole is thought to occur in the liver. (Lanusse *et al*, 1992).

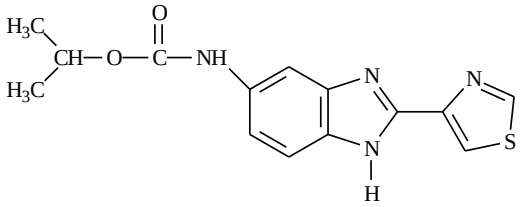
Figure 1.1 shows chemical structures of some benzimidazoles that have been used as anthelminthics while Figure 1.2 is a schematic diagram of the conversion of some probenzimidazoles into their respective benzimidazoles.



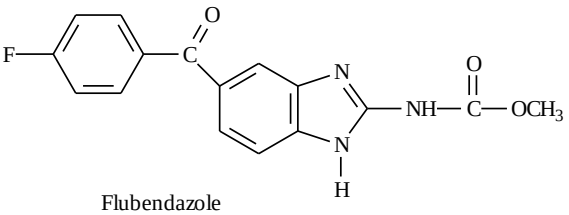
Thiabendazole



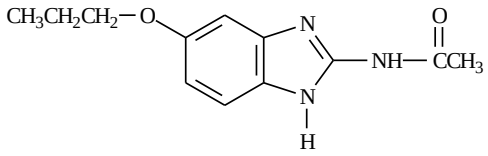
Mebendazole



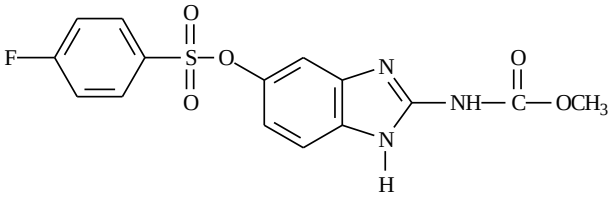
Cambendazole



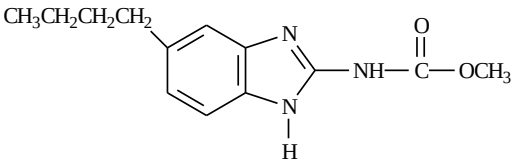
Flubendazole



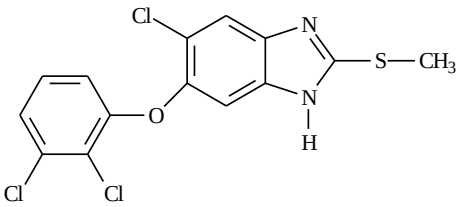
Oxibendazole



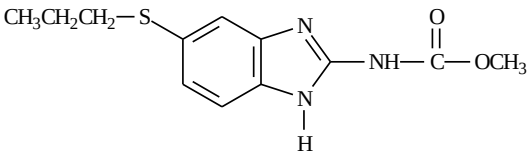
Luxabendazole



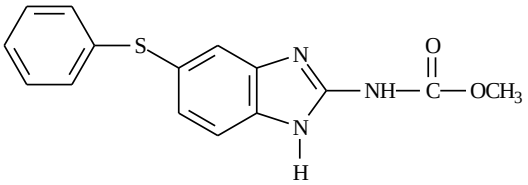
Parbendazole



Triclabendazole



Albendazole



Fenbendazole

Figure 1.1: Examples of some benzimidazoles that have been used as anthelminthics

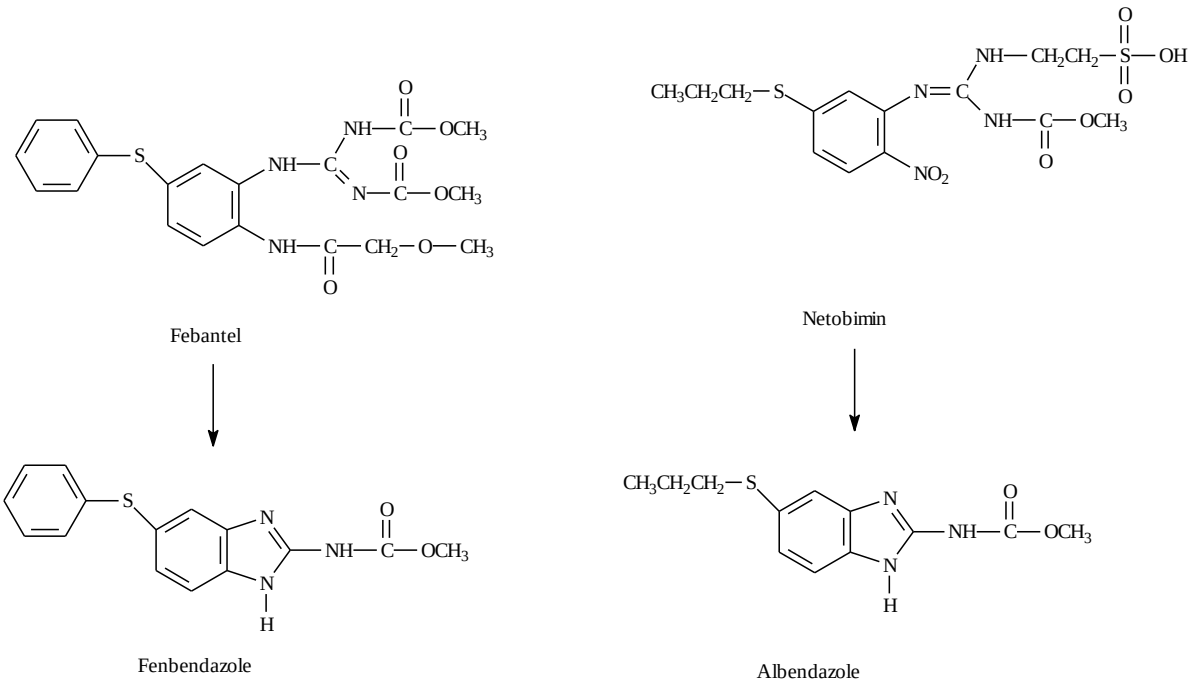


Figure 1.2: Conversion of some probenzimidazoles into the respective benzimidazoles

A survey of various pharmaceutical wholesale outlets revealed that only albendazole and mebendazole are available in the Kenyan market as broad spectrum benzimidazole anthelminthics for human use. The brands identified in the market are as indicated in Tables 1.4 and 1.5.

Table 1.4: Some brands of mebendazole available in the Kenyan market

	Brand Name	Manufacturer	Tablets	Oral suspension
1.	Vermox®	Johnson and Johnson	6 × 100 mg 1 × 500 mg	20 mg/ml × 30 ml
2.	Mebendazole®	Regal	6 × 100 mg	N/A
3.	Natoa®	Lab and Allied	6 × 100 mg	20 mg/ml × 30 ml
4.	Vermont®	Dawa Limited	6 × 100 mg	20 mg/ml × 30 ml
5.	Vermisan®	Biodeal	N/A	20 mg/ml × 30 ml
6.	Minyua®	Cosmos	6 × 100 mg	20 mg/ml × 30 ml

N/A – Not available

Table 1.5: Some brands of albendazole available in the Kenyan market

	Brand Name	Manufacturer	Tablets	Oral Suspension
1.	Zentel®	GlaxoSmithKline (GSK)	2 × 200 mg	20 mg/ml × 20 ml
2.	Alben®	Beecham/GSK	1 × 400 mg	40 mg/ml × 10 ml
3.	ABZ®	Indoco	1 × 400 mg	40 mg/ml × 10 ml
4.	Emitel®	Njimia Kenya	1 × 400 mg	40 mg/ml × 10 ml
5.	Wombit®	Biodeal	2 × 200mg	40 mg/ml × 10 ml
6.	Albaxate®	Sphinx	N/A	40 mg/ml × 10 ml
7.	Albasol®	Regal	2 × 200 mg	40 mg/ml × 10 ml
8.	D-Worm®	Dischem	N/A	40 mg/ml × 10 ml
9.	Olworm®	Biopharma	2 × 200 mg	40 mg/ml × 10 ml
10.	Zolex®	Medico	1 × 400 mg	N/A
11.	Tanzol®	Shaliva	1 × 400 mg	40 mg/ml × 10 ml
12.	Benaworm®	Benmed	1 × 400 mg	40 mg/ml × 10 ml
13.	Albalite®	Skylight	N/A	40 mg/ml × 10 ml
14.	Altoa®	Lab and Allied	2 × 200 mg	40 mg/ml × 10 ml

N/A – Not available

1.6 Mode of action of benzimidazole anthelminthics

The biochemical target for benzimidazole anthelminthics is the β-tubulin, a cytoskeletal protein which is a building block of microtubules present in all eukaryotic cells. Microtubules are critical cytoskeletal polymers which are made of repeating α- and β-tubulin dimers. Microtubules are involved in cellular morphology, cell transport, cell motility and cell division (Nogales, 2000).

Benzimidazoles elicit their anthelminthic actions by binding to β-tubulin with a 25-400 fold greater affinity for nematode tubulin than mammalian tubulin. The binding inhibits polymerization of α- and β-tubulin sub-units into microtubules. In rapidly dividing cells, this inhibition leads to cell death. In non-dividing cells, several effects on homeostatic mechanisms is elicited, often leading to non-lethal expulsion of the nematodes (Lacey, 1990). These effects include inhibition of glucose uptake in helminths with a compensatory depletion of glycogen stores, uncoupling of oxidative phosphorylation, depletion of Adenosine triphosphate (ATP) levels and inhibition of fumarate reductase, an essential anaerobic enzyme (Lacey, 1988).

Studies of receptor interactions reveal that a phenylalanine residue at position 200 of the helminth β-tubulin is necessary for binding of benzimidazoles. Hence resistance to benzimidazole anthelminthics has been attributed to a mutation at this position which commonly involves replacement of phenylalanine with tyrosine. Unfortunately, structural modification of the drugs cannot be used to overcome resistance because mammalian β-tubulin has a tyrosine residue at the same position. This complicates the development of new drugs, especially considering that the search of benzimidazoles with improved spectra of activity and efficacy has proved frustrating (Martin et al., 1997; McKellar and Scott, 1990).

1.7 Methods of analysis for benzimidazoles

The British Pharmacopoeia (BP) has described a high performance liquid chromatographic (HPLC) method for the assay of liquid preparations of albendazole for veterinary use. The method uses 1% methanolic sulphuric acid as the solvent for both the sample and the standard API. Gradient elution is used with two mobile phases; 0.015M ammonium dihydrogen orthophosphate and methanol. A stainless steel column of specific dimensions is recommended. It is packed with octadecylsilyl silica gel particles (5 µm). The injection volume is 20 µl and a flow rate of 0.7 ml/min at ambient column temperature. An ultraviolet (UV) detector set at 292 nm is used.

The United States Pharmacopeia (USP) on the other hand has described a HPLC method for the assay of both tablet and liquid preparations of albendazole for human use. For the analysis of oral suspension, the USP recommends the use of monobasic sodium phosphate as the mobile phase buffer. The mobile phase consists of water and methanol in the ratio 2:3. The method recommends the use of standard albendazole USP as an external standard. Detection is accomplished using an UV detector set at 308 nm. The USP recommends a 4 mm x 25 cm column packed with 5 µm particles. The stationary phase is octadecylsilane (ODS, C18). The injection volume is 20 µl and the flow rate set at about 2 ml/minute.

For the assay of albendazole tablets, the USP recommends monobasic ammonium phosphate as the mobile phase buffer. The choice of buffer influences peak shape and resolution (Tindall and Dolan, 2002). The mobile phase consists of water and methanol in the ratio 2:3. The method recommends standard parbendazole USP as an internal standard. Not less than 20 tablets should be used for the assay. Detection is accomplished using a UV detector set at 254 nm. The recommended column is the same as for the suspension. The tailing factor should not be more than 2.0 while the column efficiency should not be less than 1000 theoretical plates

Further the USP describes a UV spectroscopic method for the assay of mebendazole oral suspension. The procedure involves several extraction steps of mebendazole between 96% formic acid and chloroform. Isopropyl alcohol is then used as the solvent in the final step of sample preparation for the extracted drug.

For the assay of mebendazole tablets the USP describes a HPLC method. The mobile phase consists of methanol and 0.05M monobasic potassium phosphate in the ratio 60:40 v/v. The pH is adjusted to 5.5 with either 0.1M phosphoric acid or 0.1M sodium hydroxide.

Other scientists have developed methods for the determination of benzimidazoles. De Ruyck *et al* described a HPLC-mass spectrometry (HPLC-MS) method for the analysis of benzimidazole anthelminthic residues in milk (De Ruyck *et al*, 2002). Specifically, the method was developed to determine levamisole and the benzimidazoles thiabendazole, oxfenbendazole, oxibendazole, albendazole, fenbendazole, triclabendazole and the probenzimidazole febantel in milk. This was necessitated by the fact that in the wet season, the treatment of dairy cows for endoparasites is done. Failure to withdraw the anthelminthics at the right time in milk-producing animals may lead to the presence of anthelminthic residues in milk.

Agrawal *et al* described a simple spectroscopic method for the determination of albendazole in dissolution studies (Agrawal *et al*, 2005). The method can be used for the analysis of albendazole both in the bulk and dosage forms. The solvent used was 0.1N HCl with 0.05%w/v sodium lauryl sulphate (SLS). Absorbance was measured at 229nm. The method was validated as per ICH guidelines.

A stability-indicating HPLC method for the analysis of mebendazole has also been described (Al-Kurdi *et al*, 1999). The solvent used to prepare the analytes was 0.1M methanolic hydrochloric acid. To prepare the degradation product, mebendazole raw material was dissolved in 1M sodium hydroxide solution, heated to boiling under reflux for 30 minutes, cooled and neutralized with 1M nitric acid. The chromatographic separation was carried out using a stainless steel column of specific dimensions from WATERS, packed with 5µm particles. The mobile phase was 0.05M monobasic potassium phosphate: Methanol: Acetonitrile (5:3:2 v/v/v) at a flow rate of 1 ml/min. All measurements were made at room temperature. The injection volume was 20 µl.

Several other analytical methods have been described by other researchers with varied results (Msagati and Nindi, 2001; Santaladchaiyakit and Srijaranai, 2013; Zrnčić *et al*., 2014).

1.8 The study problem and justification

Helminthic infections pose a great challenge to healthcare delivery systems in developing countries (Crompton, 1999; Hotez et al., 2008). In a mass deworming programme in schools in Western Kenya, it was noted that the prevalence of STH before the deworming exercise was 34.8% with *Ascaris lumbricoides* being the most prevalent followed by hookworm infestations and *Trichuris trichuria*. After two rounds of mass deworming, STH prevalence dropped to 19.7% with prevalence decreasing to about 15%, 2% and 5% for *A. lumbricoides*, hookworm and *T. trichuria* respectively (Nikolay et al., 2015). The fact that prevalence did not reduce to zero can be explained by many factors, among them the use of sub-standard anthelminthics. Other factors may include poverty that makes treatment inaccessible (Bethony et al., 2006). For pre-school and school-going children, maternal education can contribute to helminth infection. The mother may not exercise good hygiene or may not be aware of the available treatment options. Consumption of unboiled water and poorly cooked meat are also contributing factors (Wang et al., 2012). Repeated infections, inadequate food intake and Human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS) can also contribute to persistent helminth infections (Oyewole et al., 2002; Wolday et al., 2002). Finally, age, environment (climate and topography), household clustering and drug resistance are also contributing factors (Hotez et al., 2008).

A literature review revealed that no study has ever been conducted to establish whether the benzimidazole anthelminthic products available in the Kenyan market conform to compendial specifications. It is therefore possible that after registration, the quality of the product may gradually deteriorate to sub-compendial specifications. Lack of conformance to compendial specifications would complicate the already heavy disease burden posed by helminthic infections. Limited resources would therefore be spent on treatment with no positive results. Such resources can be redirected towards improving hygiene and the general quality of the healthcare delivery system. It is worth noting that provision of universal health coverage is one among the presidential big four agenda in Kenya in addition to enhancement of manufacturing, provision of affordable housing and enhancement of food and nutrition security (Otinga, 2018). It is therefore necessary to conduct this study and guarantee effective treatment to patients who depend on these drugs as we make a major contribution to national development. The resultant

analytical method can also be used in the assessment of residues of anthelminthics in foods such as in milk and meat.

Many analytical methods are quickly moving towards HPLC. This leaves ultraviolet (UV) spectrophotometers underutilised in many analytical laboratories. Developing a UV spectroscopic analytical method will therefore increase on the utility of this valuable equipment. Spectroscopic methods are also faster than HPLC methods. Spectroscopy requires less skill on the part of the analyst than HPLC. Fewer solvents and other reagents are used in spectroscopy than in HPLC.

1.9 Research question

Can the prevalence of substandard benzimidazole anthelminthics in Nairobi City County be established using a newly developed and validated ultraviolet spectrometric method?

1.10 The study objectives

1.10.1 General objective

The main objective of this study is to determine the quality of benzimidazole anthelminthics in Nairobi City County using a simple, rapid and inexpensive spectroscopic method.

1.10.2 Specific objectives

a. To develop and validate a simple and rapid spectroscopic method in the analysis of benzimidazole anthelminthic drugs.
b. To determine the quality of benzimidazole anthelminthic drugs on the Nairobi City County using the developed method.

CHAPTER TWO: EXPERIMENTAL

2.1 Introduction

Method development is a very important part of pharmaceutical analysis. A developed method helps in the determination of the quality of active pharmaceutical ingredients (APIs) in raw materials and dosage forms. A good analytical method should utilise readily available reagents, solvents and equipment. It should be simple, rapid, precise, reliable, accurate, reproducible, robust and cost effective.

With many analytical methods moving towards HPLC, spectroscopy seems to be an interesting area for analytical method development. Compared to liquid chromatographs, UV spectrophotometers are much more affordable. Although HPLC is more accurate, precise and reproducible than spectrophotometry, the latter is simpler and faster. When faced with the analysis of many samples, it would be prudent to use a simple spectroscopic method for the analysis and only use HPLC as a confirmatory method of analysis for those samples that do not comply with compendial standards with spectrophotometry. This study involved the development of a simple, rapid, accurate, reproducible, reliable, precise and cost-effective method for the analysis of benzimidazole anthelminthics namely albendazole and mebendazole both as bulk raw material and dosage forms. The developed method was then used to establish the quality of these benzimidazole anthelminthics available on the Kenyan market.

2.2 Method development

2.2.1 Chemicals, reagents and solvents

Methanol of HPLC grade (Finar Ltd, India) was obtained from Chemoquip Ltd Nairobi. Analytical grade concentrated hydrochloric acid and sodium lauryl sulphate were provided by the Drug Analysis and Research Unit (DARU).

2.2.2 Working Standards

Both albendazole and mebendazole working standards were provided by Dawa Pharmaceuticals through DARU.

2.2.3 Instrumentation

All weighing was performed using a Sartorius top loading electronic weighing balance (Sartorius GMBH, Germany). Absorbance was measured using a Genesys 10S UV-Vis Spectrophotometer (ThermoFisher Scientific, China).

A Merck Hitachi HPLC machine (Hitachi Ltd, Tokyo, Japan) kindly availed by the National Quality Control Laboratory (NQCL) was used for the orthogonal HPLC analysis of commercial samples. It was equipped with an L-7100 low pressure quaternary pump; an L-7200 autosampler; an L-7400 variable UV detector set at 308 nm; an L-7350 thermostatic column oven maintained at 40^{o}C and an L-7000 computer interphase. A Varian HPLC column 250 × 4.0 mm LiChrospher 100-5 RP 18 End capped column was used for the analysis.

2.2.4 Determination of a suitable solvent

The first step in the method development was the determination of a solvent suitable for dissolving both APIs. Two solvents were investigated. The first one was 0.1M HCl with 0.05% sodium lauryl sulphate (SLS). This was the solvent used by Agrawal *et al* in the development of a UV spectroscopic method of analysing albendazole for solubility studies (Agrawal et al., 2015). The second solvent was 0.1M methanolic HCl, the solvent used by Al-Kurdi *et al* for the preparation of samples in their HPLC method development for the analysis of mebendazole (Al-Kurdi et al., 1999).

2.2.5 Choice of wavelength of analysis

For the sake of simplicity, it was decided to use a common wavelength suitable for absorbance measurements for both active pharmaceutical ingredients (APIs). To accomplish this, the UV spectra of each API were run independently between 200 and 400 nm before being overlaid. This was accomplished by preparing a solution with a nominal concentration of 12 µg/ml for each API. This was the working concentration used by Agrawal *et al* in their method development (Agrawal *et al*., 2015). First, a stock solution with a concentration of 1 mg/ml was prepared by weighing 50 mg of the respective API into a 50 ml volumetric flask which was dissolved in about 25 ml of 0.1M methanolic HCl and made to volume with the same solvent. Then 0.3 ml of this solution was diluted to 25 ml in a volumetric flask to make the working solution.

2.2.6 Choice of working concentration

In their study, Agrawal *et al* had used 12 µg/ml as their working concentration (Agrawal et al., 2015). This was adopted as the working concentration for both APIs in the study. It fell within

the linear range for both APIs. To prepare a working solution, a 1 mg/ml stock solution of the respective API was prepared by weighing 50 mg of the API into a 50 ml volumetric flask. A minimum amount of 0.1M methanolic HCl was added and the flask shaken to dissolve the API and made to the mark with the same solvent. Then 0.3 ml of this solution was pipetted into a 25 ml volumetric flask and made to volume with 0.1M methanolic HCl.

2.2.7 Method

The method that was taken to the validation stage involved the preparation of a 12 µg/ml working solution of each API and measuring the absorbance at 294 nm.

2.3 Method Validation

2.3.1 Introduction

The objective of validation of an analytical procedure is to ascertain that the method is suitable for its intended purpose. The various attributes of the analytical method, that is, precision, specificity, accuracy, linearity and range, limits of detection and quantitation and robustness are usually investigated as per ICH guidelines (ICH Q2B (R1), 2005).

2.3.2 Linearity and range

The linearity and linearity range of the developed method were determined using linear regression analysis. A 1.0 mg/ml stock solution of the respective API was prepared by weighing 50mg of the respective API into a 50 ml volumetric flask, dissolving with minimum 0.1M methanolic HCl, and the solution made to volume with the same solvent. To prepare the working solutions, aliquots of this solution were transferred into 25 ml volumetric flasks and made to volume with the same solvent to make solutions of 4, 8, 12, 16, 20, 24, 28 32, 36 and 40 µg/ml nominal concentration of the respective API. This represented a range of between 33.3 and 333.3% of the working concentration. The absorbances of these dilutions were measured at 294 nm. The data obtained were then plotted using Microsoft Excel spread sheet and subjected to linear regression analysis.

2.3.3 Precision

Precision of an analytical method seeks to establish the degree of scatter of results of replicate analyses of the same sample from each other. Precision is expressed by the coefficient of variation (CV) of the replicate measurements. The ICH recommends the establishment of precision at three levels; repeatability, intermediate precision and reproducibility (ICH Q2B (R1), 2005). In this study, repeatability and intermediate precision were determined as outlined

in sections 2.3.3.1 and 2.3.3.2 respectively. Reproducibility was not determined because there was no collaborating laboratory in this method development research.

2.3.3.1 Repeatability

About 50 mg of each API were weighed into a 50ml volumetric flask and made to volume with 0.1M methanolic HCl. Then 0.3 ml of this solution was transferred to a 25 ml volumetric flask and made to volume with the solvent. Absorbance of this solution was determined six times at 294 nm. The standard deviation (SD), relative standard deviation (RSD) and CV of this data were then determined.

2.3.3.2 Intermediate precision

For this study, the procedure for the determination of repeatability (section 2.3.3.1) was followed but carried out after 57 days. The ICH requires that intermediate precision should be determined on a different day from repeatability. I therefore carried on with other tests after determining repeatability and on the 57^{th} day, I created time to determine the intermediate precision.

2.3.4 Accuracy

For the developed method, accuracy was established by adding a known amount of the analyte (API) to a solution of a commercial product at 80, 100 and 120% of the working concentration (ICH Q2 (R1), 2005) (Office on Drugs and Crime, 2009). The percentage recovery of the analyte was determined. At each level, the determinations were done in triplicate.

To prepare the samples for analysis, an amount of the commercial drug product equivalent to about 40, 50 and 60 mg of the respective API was weighed into a 50 ml volumetric flask. An amount of the standard that would give a final concentration of about 4 μg/ml of the standard in the final dilution was also weighed into each flask. This was done by weighing about 16.7 mg of the respective API standard into each of the 50 ml volumetric flask containing the commercial product sample. A minimum amount of 0.1M methanolic HCl was added to the flask to dissolve the analyte. The solution was sonicated for five min to facilitate dissolution. It was then made to volume with 0.1M methanolic HCl. This solution was then filtered. Then 0.3 ml of the filtrate was pipetted into a 25 ml volumetric flask and made to volume with the solvent. The absorbance of this solution was measured at 294 nm.

2.3.5 Orthogonal HPLC analysis

2.3.5.1 Introduction

During method development a switch of wavelength was made from 233 nm to 294 nm. This necessitated comparison of the results obtained from analyses at 294 nm with those of a validated method (HPLC) to confirm their reliability and accuracy. The suspension dosage form of product A001 (albendazole) was chosen because it had shown discrepancies with results obtained at 233 nm and those obtained at 294 nm. The HPLC procedure for the analysis of albendazole described in the USP 2018 was used.

2.3.5.2 Preparation of solvents and mobile phase (USP 2018)

Solution A was prepared by mixing methanol and hydrochloric acid in the ratio 99:1. Solution B consisted of a solution of 13.75 g/l monobasic sodium phosphate. The mobile phase was prepared by mixing methanol and solution B in the ratio 60:40.

2.3.5.3 Preparation of standard and test solutions (USP 2018)

To prepare the working standard stock solution, 20 mg of the albendazole working standard were accurately weighed into a 20 ml volumetric flask. About 10 ml of solution A were added and the flask shaken for sample to dissolve. The solution was made to volume with solution A to give a solution with a 1 mg/ml nominal concentration. To prepare the working standard solution, 5 ml of the stock solution were pipetted into a 50 ml volumetric flask and made to volume with the mobile phase. The solutions were prepared in duplicate.

To prepare test stock solutions, the density of the suspension was determined. An amount of the suspension equivalent to 25 mg albendazole was accurately weighed into a 25 ml volumetric flask. About 10 ml of solution A were added and the flask shaken to dissolve. The solution was sonicated for 5 min to facilitate dissolution and made to volume with solution A. The solution was then filtered. To prepare the working test solution, 5 ml of the filtrate were pipetted into a 50 ml volumetric flask and made to volume with the mobile phase. The solutions were prepared in triplicate.

2.3.5.4 The chromatographic analysis (USP 2018)

The injection volume was set at 20µl and the flow rate at 1 ml/min. To determine the run time, one of the standards was injected manually. The albendazole peak eluted between 11 and 12 min. No other peak eluted even after a run of 25 min. The run time was therefore set at 15 min.

The system suitability solution was standard I. The system was programmed to inject the system suitability solution six times continuously and run the chromatograms of the standards

and test solutions four times each. A 4 × 250 mm column (see section 2.2.3) and a UV detector set at 308 nm were used.

2.3.6 Specificity

Specificity of an analytical procedure is its capability to analyse the compound of interest (analyte) in the presence of other components that may be present. These include degradation products, related compounds and excipients (ICH Q2B (R1), 2005). It therefore defines the degree of interference by these components in the analytical process. The process of testing for accuracy (section 2.3.4) involves the analysis of the API in the presence of these components. This therefore helped in the determination of the specificity of the analytical method. The method was as follows: To prepare the samples for analysis, an amount of the commercial drug product equivalent to about 40, 50 and 60 mg of the respective API was weighed into a 50 ml volumetric flask. An amount of the standard that would give a final concentration of about 4 μg/ml of the standard in the final dilution was also weighed into each flask. This was done by weighing about 16.7 mg of the respective API standard into each of the 50 ml volumetric flask containing the commercial product sample. A minimum amount of 0.1M methanolic HCl was added to the flask to dissolve the analyte. The solution was sonicated for five min to facilitate dissolution. It was then made to volume with 0.1M methanolic HCl. This solution was then filtered. Then 0.3 ml of the filtrate was pipetted into a 25 ml volumetric flask and made to volume with the solvent. The absorbance of this solution was measured at 294 nm.

2.4 Analysis of commercial samples

2.4.1 Introduction

The primary objective of this study was to develop a simple, rapid, reliable and cost-effective photometric method for the analysis of benzimidazole anthelminthics available in the Kenyan market. This would help regulatory authorities in monitoring the quality of these drugs and help solve, in part, the prevalence of helminth infections in the Kenyan population. Out of the several benzimidazoles that have been used as anthelminthics, preliminary surveys only came across albendazole and mebendazole brands in the market. A simple photometric method of analysis would allow for the analysis of many samples.

2.4.2 Acquisition of samples

Most of the samples (specific product batches) were acquired from wholesalers in the Central Business District (CBD) of Nairobi City County and a few wholesalers located in the outskirts of the city. Though this was not encountered, expiry date would have been used as the basis of

selection in the event that two batches were encountered, with the shorter expiry batch being selected. An attempt was made at comparing what was available from wholesale outlets with what was available in the retail outlets. It was established that the retail outlets were indeed getting their stocks from the wholesalers.

2.4.3 Preparation of the calibration curve

The linear plots used in the determination of linearity and linearity range (section 2.3.2) were also used as the calibration curves for both APIs.

2.4.4 Sample preparation

2.4.4.1 Tablets

Twenty tablets were accurately weighed and crushed to a fine powder. An amount of the powder equivalent to 50 mg of the respective API was accurately weighed into a 50 ml volumetric flask. About 25 ml of 0.1M methanolic HCl was added and the flask shaken to dissolve the API. The solution was ultrasonicated for 5 min, made to volume with the same solvent and the solution filtered. Then 0.3 ml of the filtrate was pipetted into a 25 ml volumetric flask and made to volume with the same solvent. The absorbance of this solution was measured at 294 nm. The samples were prepared in triplicate.

2.4.4.2 Suspension

Twenty millilitres of the suspension was used for the analysis. The density of the suspension was determined using a 10 ml density bottle. An amount of the suspension equivalent to 50 mg of the respective API was accurately weighed into a 50 ml volumetric flask. About 25 ml of 0.1M methanolic HCl was added and the flask shaken to dissolve. The solution was ultrasonicated for 5 min, made to volume with the same solvent and the solution filtered. Then 0.3 ml of the filtrate was pipetted into a 25 ml volumetric flask and made to volume with the same solvent. The absorbance of this solution was measured at 294 nm. The samples were prepared in triplicate.

CHAPTER THREE: RESULTS AND DISCUSSION

3.1 Method development

3.1.1 Choice of solvent

After testing API solubility in the two solvents namely 0.1M HCl with 0.05% Sodium Lauryl Sulphate and 0.1M methanolic HCl, both albendazole and mebendazole were found to have reliable solubility in 0.1M methanolic HCl. This solvent was therefore used for the remaining steps in the development of the analytical method.

3.1.2 Choice of wavelength of analysis

The spectra for both APIs are provided in Figures 3.1 and 3.2. For both APIs a peak of maximum absorption was observed between 230 and 236 nm. It was decided to settle on 233 nm as it was the wavelength of maximum absorption for mebendazole while albendazole showed reliable absorption as it was close to its wavelength of maximum absorption (231 nm). The linearity and range for both APIs were studied at this wavelength. The results are presented in Figure 3.3 and 3.4.

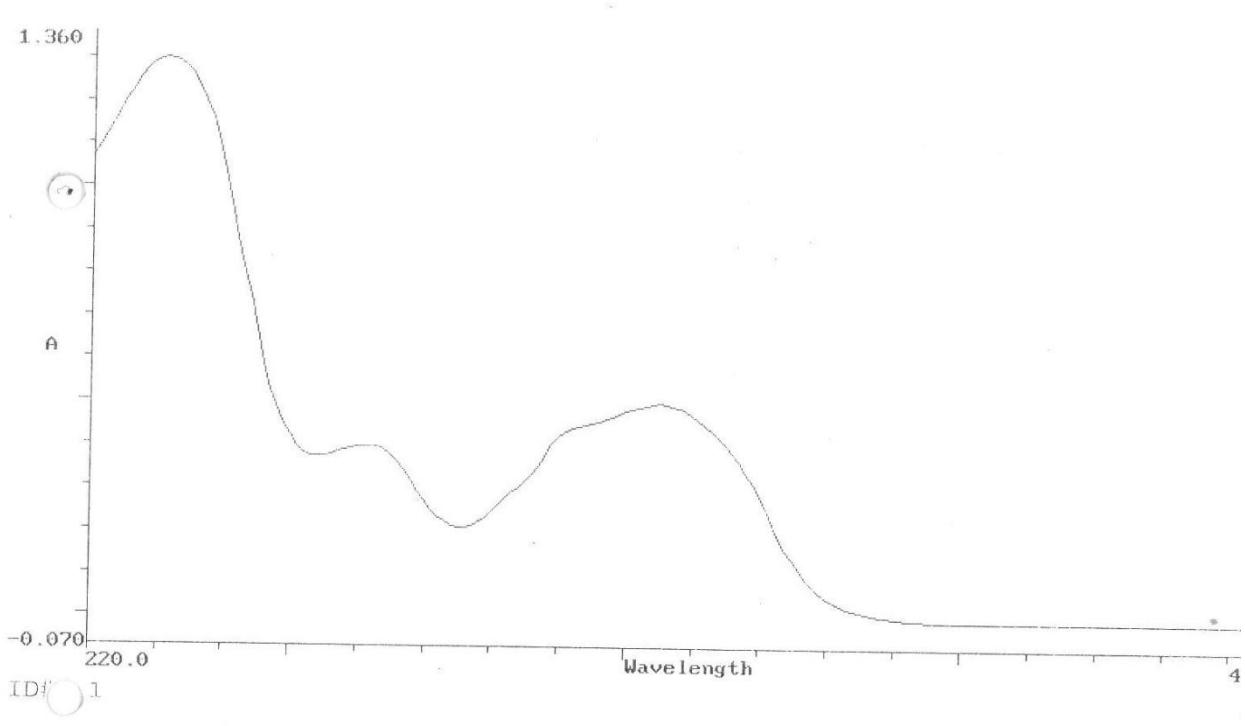


Figure 3.1: Ultraviolet absorption spectrum for albendazole in 0.1M methanolic HCl

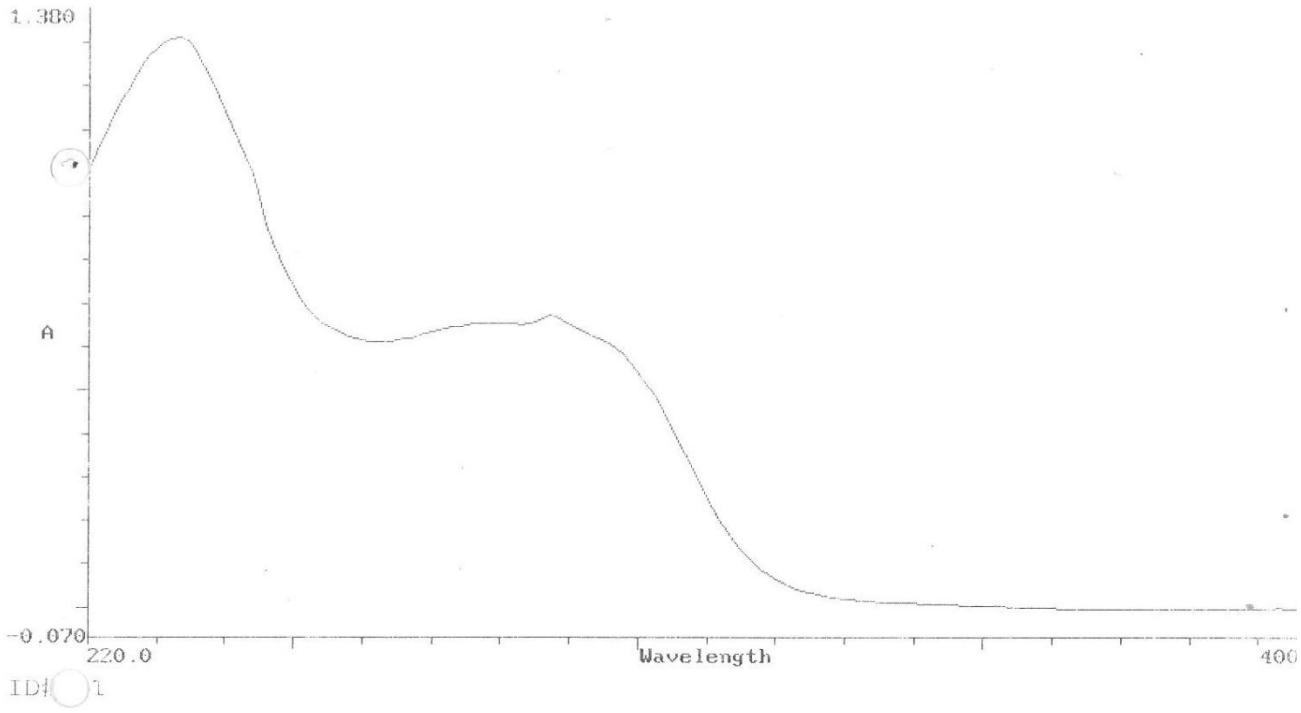


Figure 3.2: Ultraviolet absorption spectrum for mebendazole in 0.1M methanolic HCl

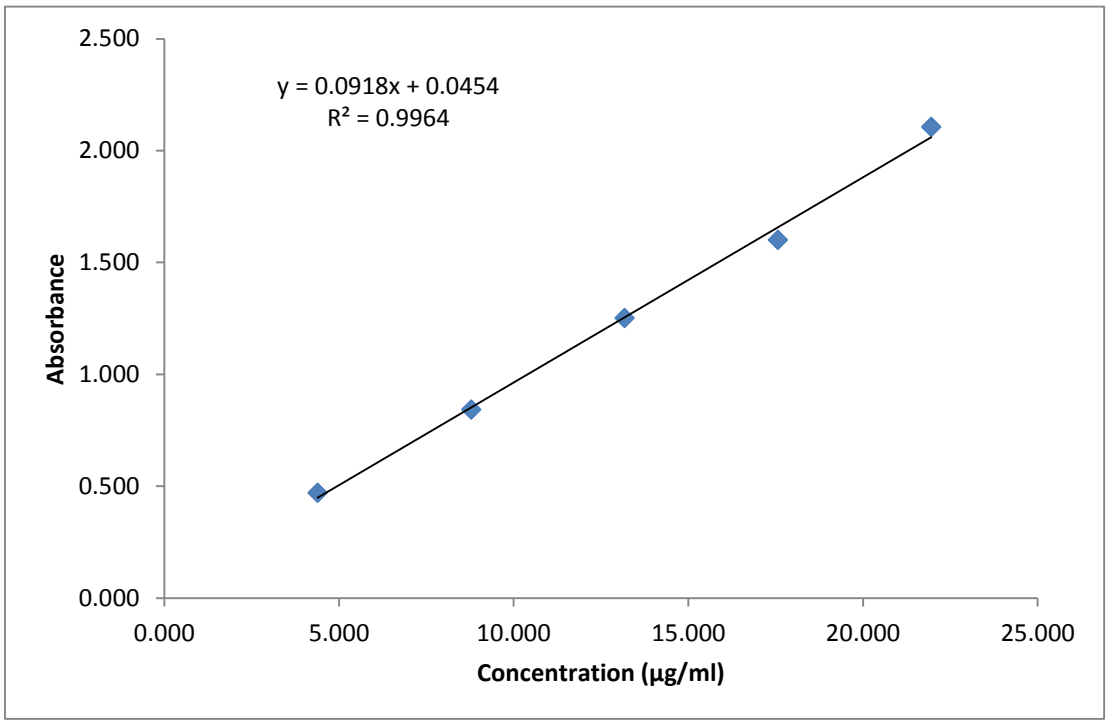


Figure 3.3: Linearity and range plot for albendazole at 233 nm

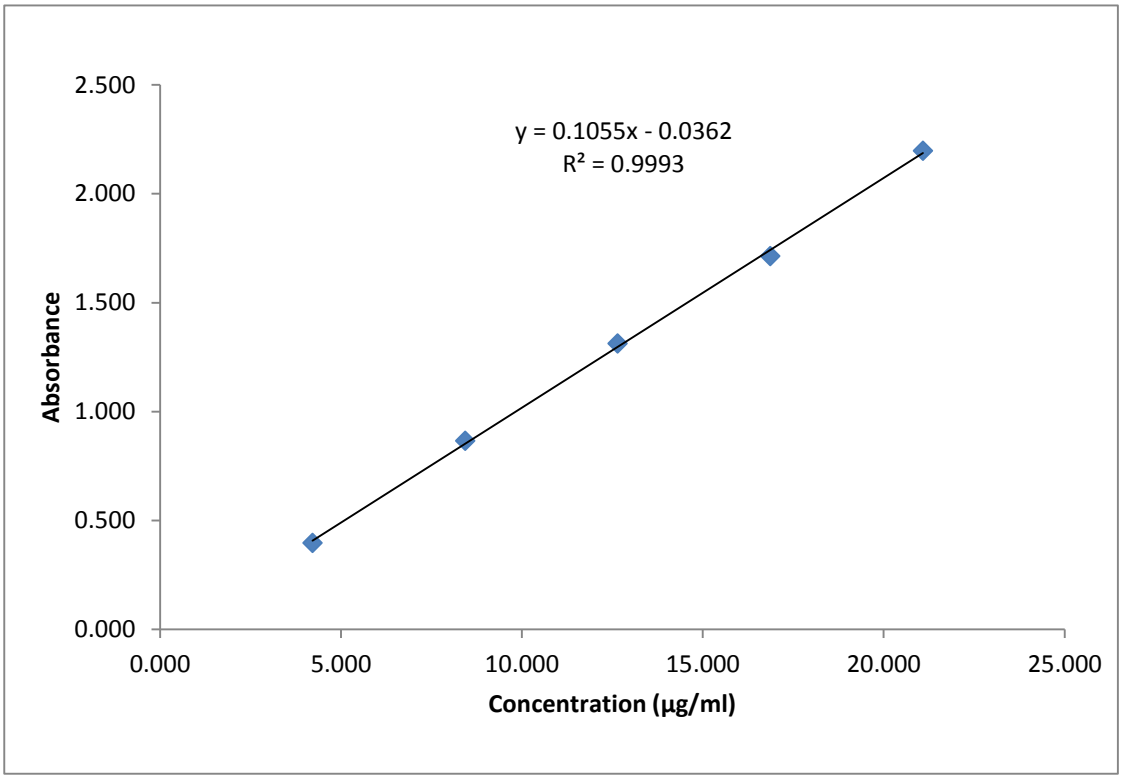


Figure 3.4: Linearity and range plot for mebendazole at 233 nm

The range studied was between 4 and 20 µl/ml (33.3 to166.7% of the working concentration). This was well beyond the 80 to 120% range recommended by ICH. Both APIs showed good linearity with a coefficient of determination, R^2, of 0.9964 and 0.9993 for albendazole and mebendazole respectively.

This wavelength also showed good precision with a coefficient of variation (CV) of 0.108 and 0.137% for albendazole and mebendazole respectively

At this point, it was decided to apply the method in the analysis of 10 commercial products to test for matrix interferences. Since spectroscopic methods are not separatory, it may be possible for matrix interference to occur from excipients, related substances and degradation products with chromophores that absorb at the analytical wavelength. Matrix interference has been known to lead analysts to result to derivative UV spectroscopy (Rojas and Ojeda, 2009; Wang and Asgharnejad, 2000). This requires highly skilled analysts. The equipment is also more expensive hence adding to the cost of analysis. It was not possible to establish with certainty all the excipients used by various manufacturers. Considering that some products were white while some were coloured, it was suspected that different manufacturers use different excipients in their products. The results of these analyses are as shown in Table 3.1.

Table 3.1: Results of analyses of commercial products at 233 nm

Product	API	Dosage Form	Batch	Average (%)	Comment*
A001T	Albendazole	Tablets	1	102.6	Complies
A001S	Albendazole	Suspension	1	130.3	Does not comply
A002S	Albendazole	Suspension	1	105.7	Complies
A003S	Albendazole	Suspension	1	99.9	Complies
A004S	Albendazole	Suspension	1	101.2	Complies
M001T	Mebendazole	Tablets	1	106.7	Complies
M001S	Mebendazole	Suspension	2	116.0	Does not comply
M002T	Mebendazole	Suspension	1	103.1	Complies
M002S	Mebendazole	Suspension	1	118.1	Does not Comply

*USP 2018 specification for content (not less than 90.0% and not more than 110.0% of the label claim).

These results indicate that some products (A001S, M001S and M002S) had overages of the respective APIs by USP standards. Based on this it was decided to repeat the analysis at a different wavelength. After overlaying the spectra for a second time, 294 nm was selected as a suitable wavelength. A repeat analysis of products A001S and M001S whose content fell out of the range specified by the USP at 233 nm was performed at 294 nm. The results of the analysis of product A001S fell within the range specified by the USP while the results of product M001S were still out of range. This further suggested the possibility of interference at 233 nm for product A001S. An orthogonal HPLC analysis performed later (see sections 2.3.5 and 3.2.4) agreed with the results of analysis at 294 nm. This wavelength was therefore adopted for further development of the analytical method.

3.1.3 Choice of working concentration

Based on the work of Agrawal et al, a working concentration of 12 μg/ml was settled on. This concentration fell within the linear range for both APIs.

3.2 Method Validation

3.2.1 Linearity and Range

Figures 3.5 and 3.6 indicate the results obtained.

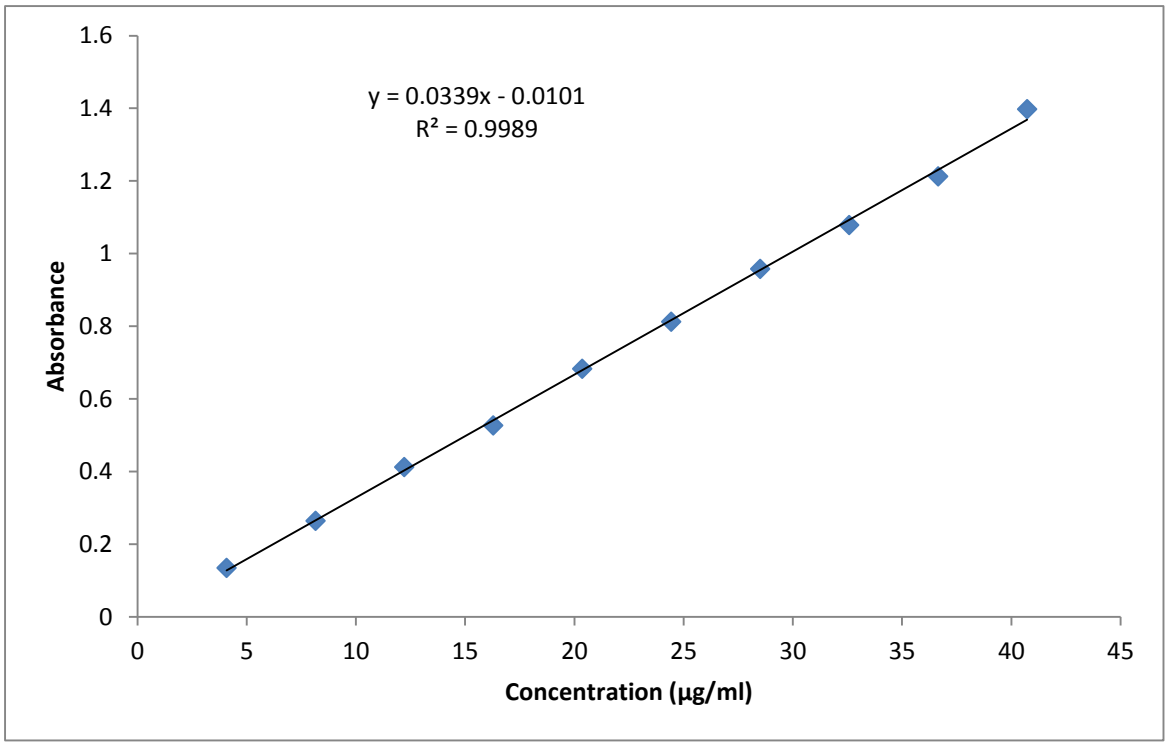


Figure 3.5: Plot of absorbance against concentration for albendazole at 294 nm

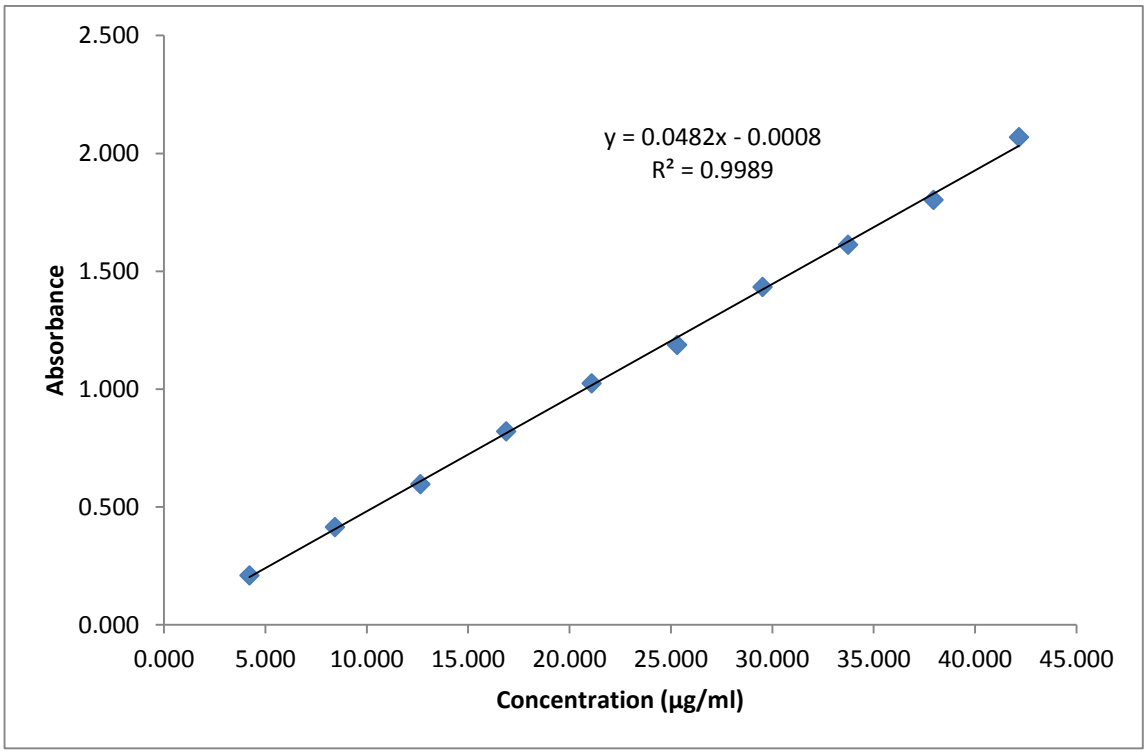


Figure 3.6: Plot of absorbance against concentration for mebendazole at 294 nm

Both APIs exhibited good linearity with a coefficient of determination, R^2, of 0.9989 as shown in the respective figures.

3.2.2 Precision

3.2.2.1 Repeatability

Table 3.2 presents the results of the repeatability studies

Table 3.2: Repeatability studies for the developed method

API	SD	RSD	CV (%)
Albendazole	0.000447	0.001084	0.184
Mebendazole	0.00379	0.00579	0.579

Since the CV should be less than 2%, the results indicated that the developed method exhibited good repeatability as the data for both APIs was within the acceptable range.

3.2.2.2 Intermediate precision

The results of intermediate precision studies are presented in table 3.3

Table 3.3: Intermediate precision studies for the developed method

API	SD	RSD	CV (%)
Albendazole	0.001	0.00230	0.230
Mebendazole	0.001	0.00162	0.162

The ICH recommends that the CV should be less than 2%. The results obtained were within the ICH limits showing that the developed methods exhibited good intermediate precision.

3.2.3 Accuracy

As explained in section 2.3.4, the amounts of drug product and standard in the tables below were dissolved in 50 ml of 0.1M methanolic HCl. Aliquots of 0.3 ml of this stock solution were diluted to 25 ml to make the working solutions. Absorbance of this working solution was measured at 294 nm.

The data for the determination of accuracy is presented in the Tables 3.4, 3.5, 3.6, 3.7, 3.8 and 3.9.

Albendazole

Table 3.4: Recovery of albendazole at 80% of working concentration

Sample	Drug Product (g)	Standard (g)	Absorbance
1	0.0862	0.0177	0.471
2	0.0860	0.0178	0.487
3	0.0854	0.0203	0.506

Table 3.5: Recovery of albendazole at100% of the working concentration

Sample	Drug Product (g)	Standard (g)	Absorbance
1	0.1086	0.0174	0.542
2	0.1068	0.0173	0.528
3	0.1077	0.0173	0.553

Table 3.6: Recovery of albendazole at 120% of the working concentration

Sample	Drug Product (g)	Standard (g)	Absorbance
1	0.1283	0.0180	0.640
2	0.1278	0.0179	0.634
3	0.1285	0.0170	0.642

Mebendazole

Table 3.7: Recovery at mebendazole 80% of the working concentration

Sample	Drug Product (g)	Standard (g)	Absorbance
1	0.0611	0.0167	0.714
2	0.0606	0.0183	0.727
3	0.0626	0.0193	0.725

Table 3.8: Recovery of mebendazole at 100% of the working concentration

Sample	Drug Product (g)	Standard (g)	Absorbance
1	0.0756	0.0172	0.818
2	0.0765	0.0209	0.839
3	0.0760	0.0167	0.823

Table 3.9: Recovery of mebendazole at 120% of the working concentration

Sample	Drug Product (g)	Standard (g)	Absorbance
1	0.0910	0.0172	0.901
2	0.0910	0.0169	0.874
3	0.0913	0.0176	0.948

The accuracy of the method through recovery of analyte is summarised in Tables 3.10 and 3.11 for albendazole and mebendazole respectively.

Table 3.10: Recovery of albendazole at 80, 100 and 120% of the working concentration

Recovery Level (%)	80	100	120
Recovery (%)	104.3	100.0	102.3

The average recovery for the three levels was found to be 102.3%.

Table 3.11: Recovery of mebendazole at 80, 100 and 120% of the working concentration

Recovery Level (%)	80	100	120
Recovery (%)	106.6	104.6	101.5

The average recovery for the three levels was found to be 104.2%.

The accuracy of an analytical procedure reflects the closeness of agreement between the value that is accepted as the conventional true value and the value found. The Food and Drug Administration (FDA) of the United States of America (USA) requires that the recovery should be 100 ± 2% at each concentration over the range of 80% to 120% of the working concentration (Shabir, 2003).

Though the results (102.3% and 104.2% recovery for albendazole and mebendazole respectively) were slightly above the upper limit at some concentrations for both APIs, developed method was therefore found to acceptable accuracy.

3.2.4 Orthogonal HPLC assay: Results

Label claim: each 5 ml contains 100mg albendazole

Density of suspension: 1.0201 g/ml

Two solutions of the working standard were prepared. Using each of these standard solutions, chromatograms of each of three test solutions were run. The percentage of the label claim of each test solution was then calculated independently using these chromatograms and the average determined.

The chromatograms from the assay are presented in the Appendix. The results of the assay are presented in table 3.12

Table 3.12: Results of the orthogonal HPLC analysis

Standard	Test replicates	Assay (%)
1	1	109.7
	2	108.6
	3	109.0
2	1	104.7
	2	103.7
	3	104.1

The overall percentage of the label claim for both standards and all injections is 106.6%. This compares well with result obtained by the developed method – 107.3%. This further confirms the accuracy of the developed method.

3.2.3 Specificity

For the developed method, the results of the recovery studies indicate that the method is capable of discriminating the analyte in the presence of the components likely to be present in the commercial products including excipients, related substances and products of degradation. The method was therefore found to be specific for albendazole and mebendazole.

3.3 Analysis of commercial products

3.3.1 Samples analysed

Nine albendazole and two mebendazole brands were found in stock during the period of the study. Five of the albendazole brands were available in both tablet and suspension dosage forms. Two were available as tablets only while two were available as suspensions only. Both mebendazole brands were available in both tablet and suspension dosage forms. For the purpose of this study, the analysed brands were coded to ensure confidentiality in report writing.

3.3.2 Assay results

The results of the assays are summarized in Tables 3.13 and 3.14. Out of 32 samples analysed, five samples (15.6%) did not comply with compendial specifications. From the information gathered in the field, albendazole is the more popular anthelminthic compared to mebendazole. This is because it is administered as a single dose and several low-cost generic brands are available. It is therefore of great concern when a low-cost generic brand fails to conform to compendial specification since these drugs are more affordable and therefore mostly used by a greater percentage of the population. It came as a surprise that the suspension of the innovator product of mebendazole had an overage of the API hence did not conform to compendial specification. This is because the innovator product is usually used as the gold standard when studying the pharmaceutical equivalence of generic products. Also, inter-batch variation was observed with product A002T, a tablet dosage form of albendazole which is a popular anthelminthic. One batch of the product had an overage of the API hence did not comply with compendial specifications.

The percentage of the label claim for all the products was calculated as shown below for sample one of batch one of product A001S.

Label claim: Each 5 ml of the suspension contains albendazole BP 100 mg
Density of the suspension = 1.0212 g/ml
Equation of the calibration curve:
A = 0.0339c – 0.0101 where
A = absorbance and
c = concentration
The weights of the triplicate samples were 2.5579 g, 2.5635 g and 2.5468 g
The absorbances of the triplicate samples were 0.425, 0.410 and 0.446 respectively.
Using sample one:
0.425 = 0.0339c – 0.0101
0.425 + 0.0101 = 0.4351 = 0.0339c
c = 0.4351/0.0339 = 12.8348 µg/ml
This means that 25 ml of the working solution contains
25 × 12.8348 = 320.87 µg = 0.32087 mg
Since the working solution was prepared from 0.3 ml of the stock solution, this translates to
50/0.3 × 0.32087 = 53.4783 mg albendazole in the weighed sample
From the density, the volume of the sample = 2.5579/1.0212 = 2.5048 ml
From the assay, 2.5048 ml of the product contains 53.4783 mg albendazole
Therefore 5 ml of the product contains (5/2.5048) × 53.4783 = 106.7516 mg albendazole
Percentage of the label claim = 106.7516/100 × 100 = 106.8%
Similarly, the percentage of the label claim of samples two and three were 102.8% and 112.4% respectively.
The average percentage of the label claim for batch one was therefore
(106.8 + 102.8 + 112.4)/3 = 107.3%

Table 3.13: Results of analyses of commercial products for albendazole

Product code	Dosage form	Batch	Assay (%)	Comment*
A001S	Suspension	1	107.3	Complies
		2	108.4	Complies
		3	107.3	Complies
A001T	Tablets	1	96.6	Complies
		2	98.6	Complies
		3	100.1	Complies
A002S	Suspension	1	104.1	Complies
		2	105.0	Complies
A002T	Tablets	1	98.3	Complies
		2	145.4	Does not comply
		3	96.9	Complies
A003S	Suspension	1	98.0	Complies
		2	97.1	Complies
		3	96.2	Complies
A003T	Tablets	1	96.8	Complies
		2	99.6	Complies
A004S	Suspension	1	100.2	Complies
A004T	Tablets	1	97.2	Complies
		2	95.9	Complies
A005S	Suspension	1	87.2	Does not comply
A005T	Tablets	1	103.3	Complies
A006T	Tablets	1	93.3	Complies
A007S	Suspension	1	109.0	Complies
A008T	Tablets	1	100.3	Complies
A009S	Suspension	1	7.9	Does not comply

*USP 2018 specification for content (not less than 90.0% and not more than 110.0% of the label claim).

Table 3.14: Results of analyses of commercial products for mebendazole

Product code	Dosage form	Batch	Assay (%)	Comment*
M001S	Suspension	1	112.9	Does not comply
		2	111.5	Does not comply
M001T	Tablets	1	102.5	Complies
		2	105.7	Complies
M002S	Suspension	1	103.4	Complies
		2	100.0	Complies
M002T	Tablets	1	99.6	Complies

*USP 2018 Specification for content (not less than 90.0% and not more than 110.0% of the label claim).

CHAPTER FOUR: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

A UV spectroscopic method for the analysis of benzimidazole anthelminthics albendazole and mebendazole was developed. The suitable solvent for the analysis was found to be 0.1M methanolic hydrochloric acid. A wavelength of 294 nm was found to be suitable with negligible matrix interferences by excipients and other substances that may be present in commercial products like related substances and degradation products. Over a range of 33.3 to 333.3% of the working concentration, the developed method was found to exhibit good linearity for both APIs.

The development and validation of a spectroscopic method for the analysis of benzimidazole anthelminthics available in the Kenyan market provides an alternative method for their analysis. The developed method uses a readily available and cost-effective solvent – HPLC grade methanol. Sample preparation is a simple process. The use of a single wavelength of analysis means that the UV spectrophotometer can be set up by a skilled analyst while the actual measurements are carried out by a less skilled analyst.

The validation of the developed method confirms that the method is linear, precise, sensitive and accurate. Compared to HPLC the method is simple, cost effective, faster and requires less skilled personnel. Whereas it took a whole day to analyse one sample using HPLC, up to four samples could be analysed in one day with the developed method. This is because compared to HPLC, the developed method has a shorter turn-around-time. This proves especially useful when a large number of samples is to be analysed within a limited period of time. The results of the HPLC analysis of A001S batch 3, an albendazole suspension, showed good agreement with the results obtained using the developed method.

4.2 Major findings

Analysis of commercial products revealed the presence of benzimidazole anthelminthic products that do not conform to compendial specifications for assay. This emphasizes the need for a fast, reliable, versatile and cost-effective method of analysis of these products after marketing authorization has been granted.

4.3 Recommendations

The developed method can be applied in the analysis of benzimidazole anthelminthics countrywide and the results compared with those obtained in Nairobi. Bearing in mind that only repeatability and intermediate precision were determined, the method can be adapted by different laboratories to allow for the determination of its reproducibility. Because of its versatility, the method can be adopted routinely by policy makers and implementers. The success of this study should also stimulate research into the possibility of using UV spectrophotometry in the analysis of other anthelminthics that have chromophores in their structures.

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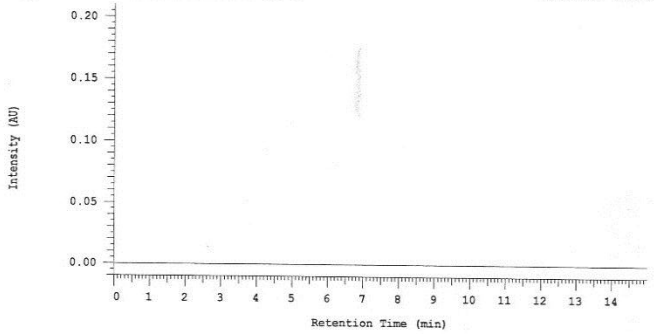
APPENDIX: CHROMATOGRAMS OF THE ORTHOGONAL ANALYSIS

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Mobile Phase Blank
Injection from this vial: 1 of 1

Analyzed: 07-05-19 01:44 PM
Series:0045
Volume: 20.0 ul

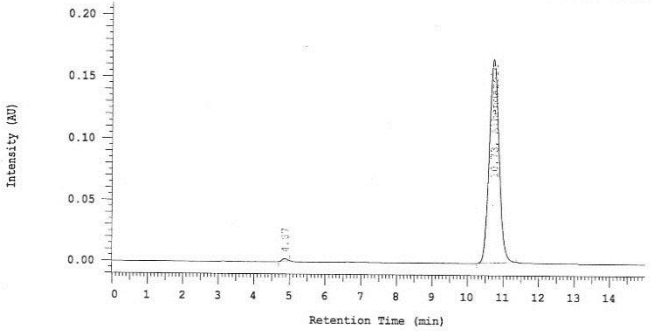


Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole SST Std
Injection from this vial: 1 of 6

Analyzed: 07-05-19 02:00 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.87			10080	0.45	0.637	BB
2	10.73	Albendazole	R	1571828	1.00	99.363	BB
				1581908		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.73	Albendazole	3.43	1.12	7423	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.73	Albendazole	7493	---	7506	---	10353.31	16.00

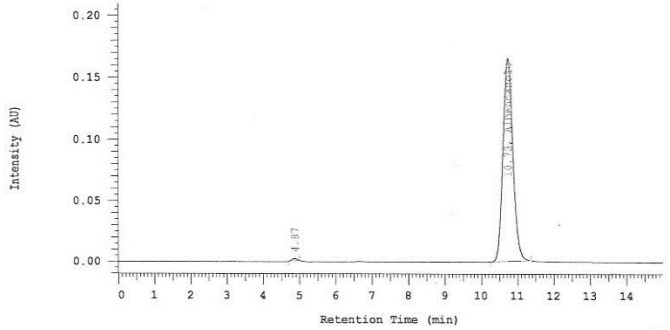
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole SST Std
Injection from this vial: 2 of 6

Analyzed: 07-05-19 02:16 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.87			10119	0.45	0.639	BB
2	10.73	Albendazole	R	1573173	1.00	99.361	BB
				1583292		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.73	Albendazole	3.43	1.12	7421	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.73	Albendazole	7493	---	7506	---	10362.50	16.00

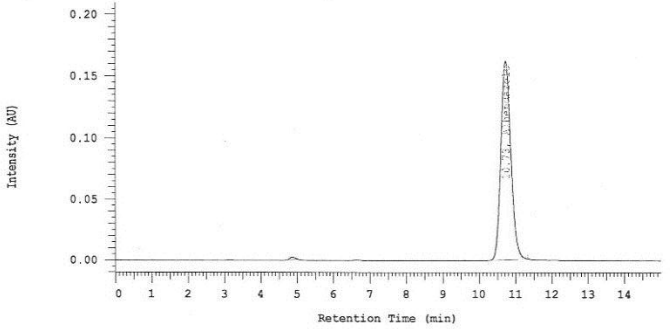
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole SST Std
Injection from this vial: 3 of 6

Analyzed: 07-05-19 02:32 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	10.73	Albendazole	R	1535643	1.00	100.000	BB
				1535643		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.73	Albendazole	3.43	1.13	7437	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.73	Albendazole	7493	---	7506	---	10134.50	16.00

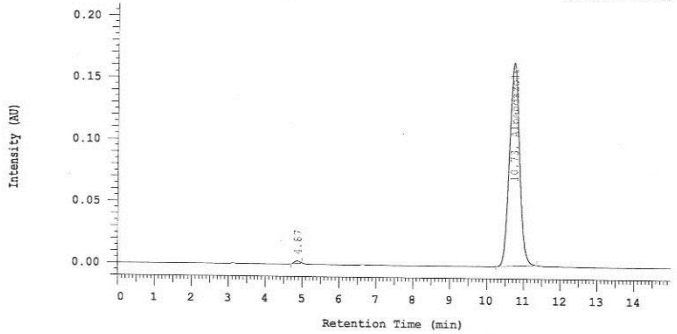
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole SST Std
Injection from this vial: 4 of 6

Analyzed: 07-05-19 02:48 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name	Area	RRT	Area %	BC
1	4.87		10146	0.45	0.646	BB
2	10.73	Albendazole R	1561413	1.00	99.354	BB
			1571559		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.73	Albendazole	3.43	1.12	7424	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.73	Albendazole	7482	---	7495	---	10286.25	16.00

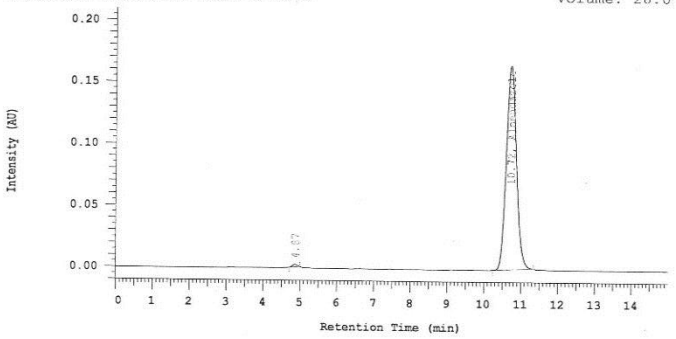
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole SST Std
Injection from this vial: 5 of 6

Analyzed: 07-05-19 03:04 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name	Area	RRT	Area %	BC
1	4.87		10108	0.45	0.640	BB
2	10.72	Albendazole R	1569035	1.00	99.360	BB
			1579143		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.13	7423	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7483	---	7497	---	10337.47	16.00

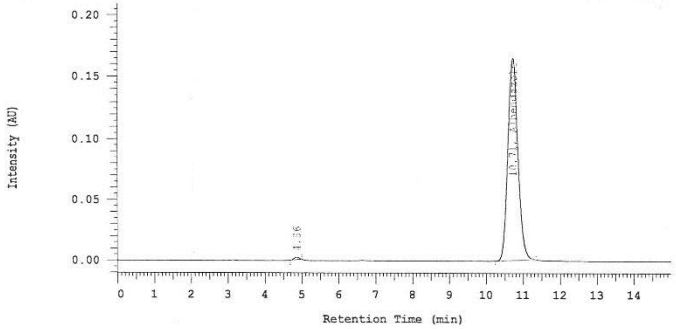
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole SST Std
Injection from this vial: 6 of 6

Analyzed: 07-05-19 03:20 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name	Area	RRT	Area %	BC
1	4.86		10017	0.45	0.635	BB
2	10.71	Albendazole R	1568626	1.00	99.365	BB
			1578643		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.71	Albendazole	3.43	1.13	7410	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.71	Albendazole	7474	---	7488	---	10334.19	16.00

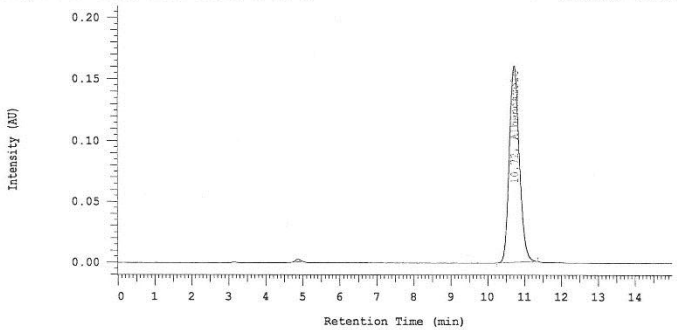
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole Std 1
Injection from this vial: 1 of 1

Analyzed: 07-05-19 03:37 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name	Area	RRT	Area %	BC
1	10.72	Albendazole R	1524696	1.00	100.000	BB
			1524696		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.13	7430	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7494	---	7508	---	10052.78	16.00

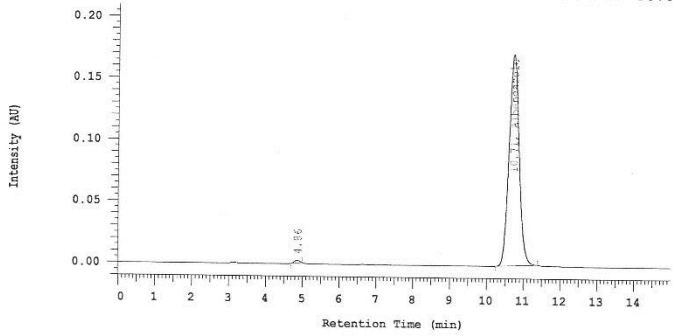
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole Std 2
Injection from this vial: 1 of 1

Analyzed: 07-05-19 03:53 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.86			10505	0.45	0.639	BB
2	10.71	Albendazole	R	1632923	1.00	99.361	BB
				1643428		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.71	Albendazole	3.43	1.13	7408	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.71	Albendazole	7474	---	7488	---	10744.41	16.00

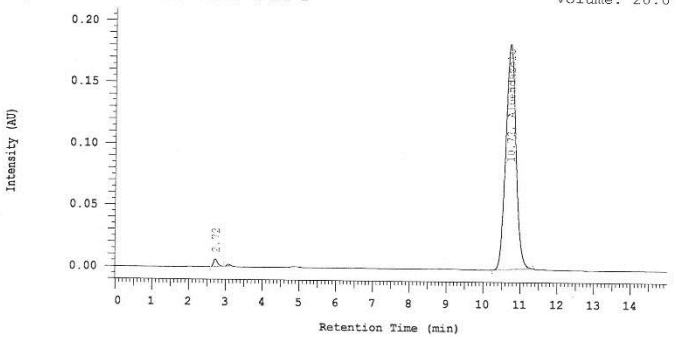
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 1
Injection from this vial: 1 of 1

Analyzed: 07-05-19 04:09 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23842	0.25	1.353	BB
2	10.72	Albendazole	R	1737980	1.00	98.647	BB
				1761822		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7417	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7483	---	7497	---	11456.22	16.00

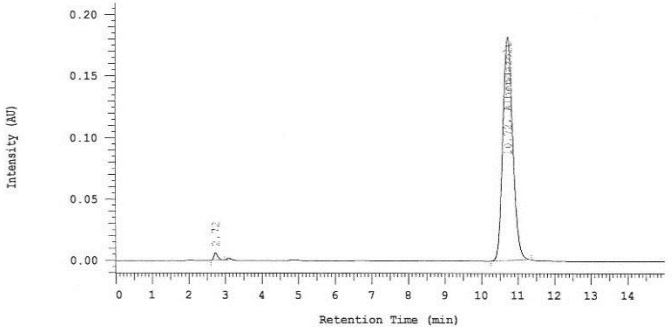
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 2
Injection from this vial: 1 of 1

Analyzed: 07-05-19 04:25 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23687	0.25	1.354	BB
2	10.72	Albendazole	R	1725461	1.00	98.646	BB
				1749148		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7425	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7494	---	7508	---	11378.91	16.00

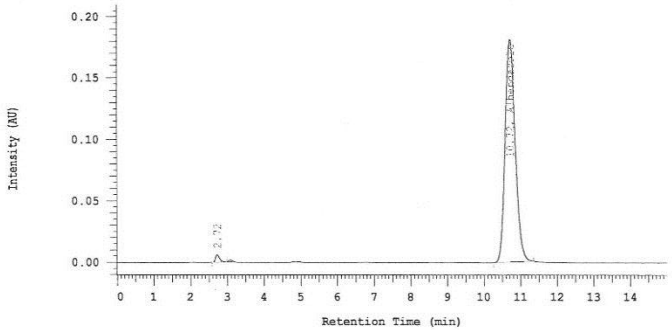
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 3
Injection from this vial: 1 of 1

Analyzed: 07-05-19 04:41 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23489	0.25	1.349	BB
2	10.72	Albendazole	R	1717737	1.00	98.651	BB
				1741226		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7442	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7505	---	7518	---	11339.50	16.00

Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

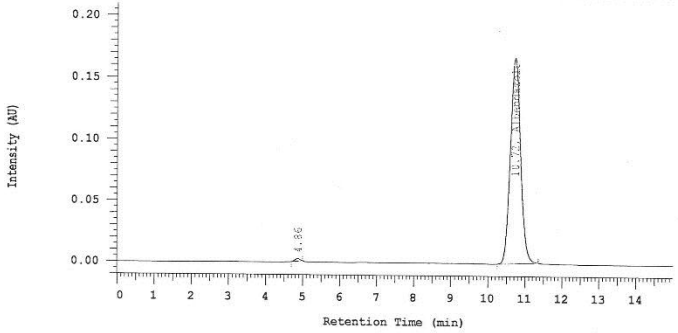
(2)

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole Std 1
Injection from this vial: 1 of 1

Analyzed: 07-05-19 04:57 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.86			10156	0.45	0.635	BB
2	10.72	Albendazole	R	1590384	1.00	99.365	BB
				1600540		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7420	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7473	---	7486	---	10479.69	16.00

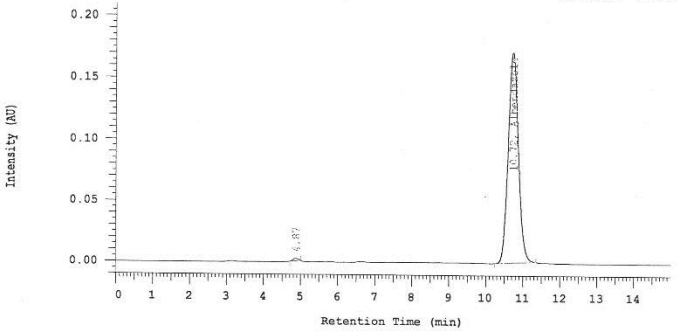
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole Std 2
Injection from this vial: 1 of 1

Analyzed: 07-05-19 05:13 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.87			10493	0.45	0.640	BB
2	10.72	Albendazole	R	1629395	1.00	99.360	BB
				1639888		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7418	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7483	---	7497	---	10736.53	16.00

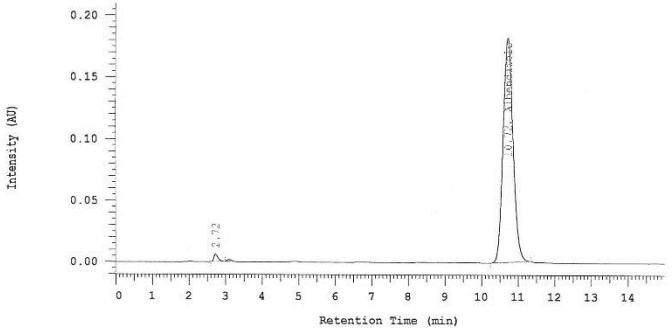
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 1
Injection from this vial: 1 of 1

Analyzed: 07-05-19 05:29 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23747	0.25	1.356	BB
2	10.72	Albendazole	R	1727528	1.00	98.644	BB
				1751275		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.11	7436	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7494	---	7508	---	11394.03	16.00

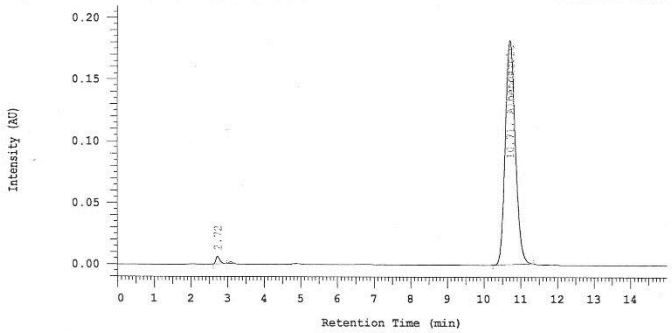
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 2
Injection from this vial: 1 of 1

Analyzed: 07-05-19 05:45 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23739	0.25	1.357	BB
2	10.71	Albendazole	R	1725952	1.00	98.643	BB
				1749691		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.71	Albendazole	3.43	1.13	7419	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.71	Albendazole	7485	---	7498	---	11385.94	16.00

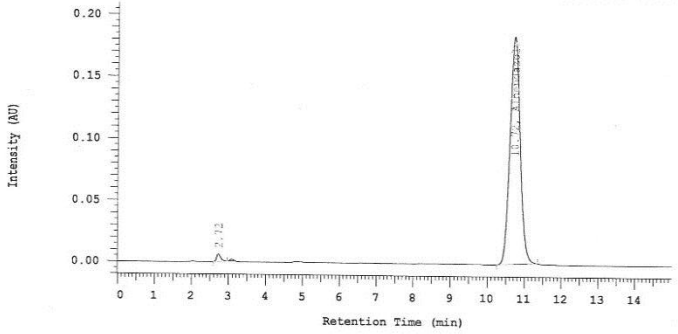
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 3
Injection from this vial: 1 of 1

Analyzed: 07-05-19 06:01 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	24015	0.25	1.356	BB
2	10.72	Albendazole	R	1747240	1.00	98.644	BB
				1771255		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7410	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7473	---	7486	---	11512.44	16.00

Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

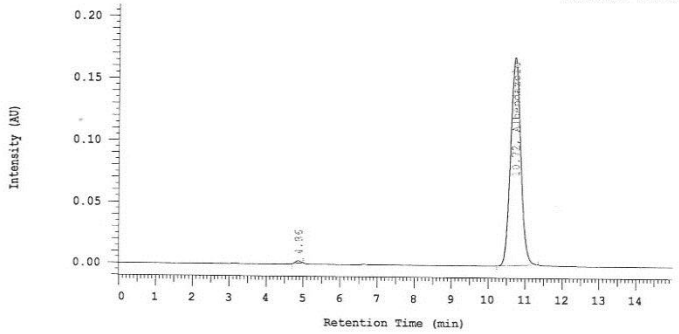
(3)

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole Std 1
Injection from this vial: 1 of 1

Analyzed: 07-05-19 06:17 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.86			10315	0.45	0.638	BB
2	10.72	Albendazole	R	1606186	1.00	99.362	BB
				1616501		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7384	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7441	---	7454	---	10562.16	16.00

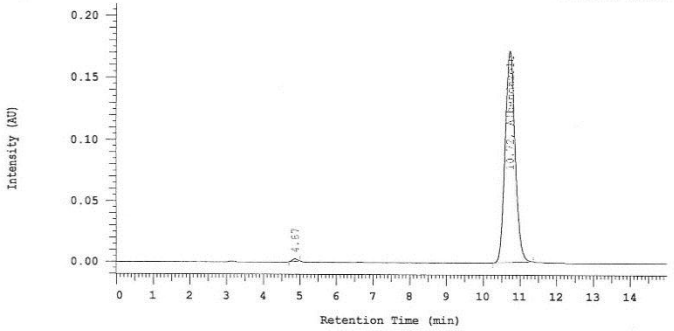
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole Std 2
Injection from this vial: 1 of 1

Analyzed: 07-05-19 06:33 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.87			10633	0.45	0.646	BB
2	10.72	Albendazole	R	1636522	1.00	99.354	BB
				1647155		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7386	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7451	---	7465	---	10763.97	16.00

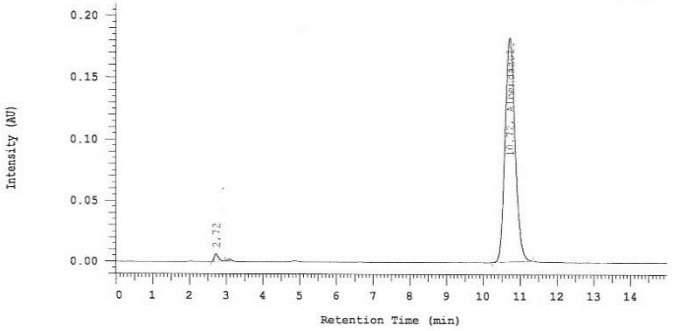
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 1
Injection from this vial: 1 of 1

Analyzed: 07-05-19 06:49 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23868	0.25	1.356	BB
2	10.72	Albendazole	R	1736412	1.00	98.644	BB
				1760280		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7381	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7441	---	7454	---	11416.38	16.00

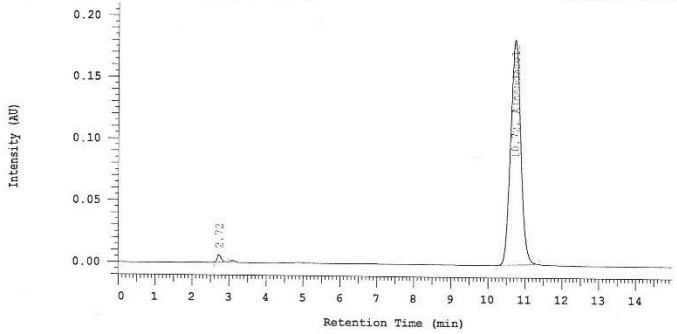
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 2
Injection from this vial: 1 of 1

Analyzed: 07-05-19 07:05 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23968	0.25	1.361	BB
2	10.72	Albendazole	R	1736713	1.00	98.639	BB
				1760681		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.13	7392	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7451	---	7465	---	11414.63	16.00

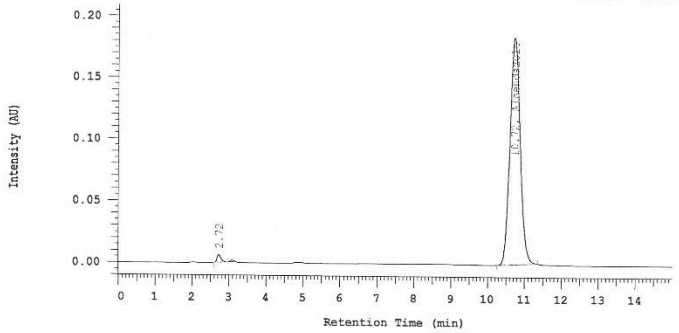
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 3
Injection from this vial: 1 of 1

Analyzed: 07-05-19 07:22 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	24054	0.25	1.355	BB
2	10.72	Albendazole	R	1751438	1.00	98.645	BB
				1775492		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.13	7382	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7441	---	7454	---	11521.63	16.00

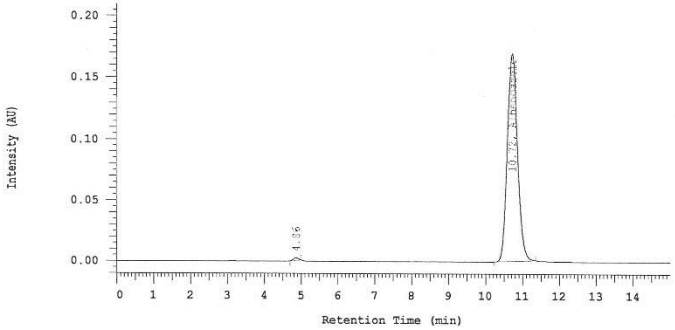
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole Std 1
Injection from this vial: 1 of 1

Analyzed: 07-05-19 07:38 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.86			10357	0.45	0.636	BB
2	10.72	Albendazole	R	1618369	1.00	99.364	BB
				1628726		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7347	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7399	---	7412	---	10613.34	16.00

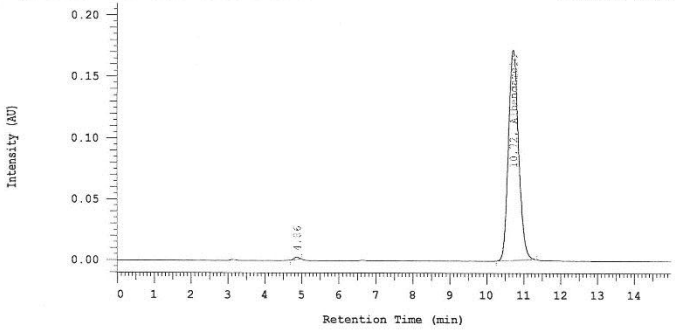
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Albendazole Std 2
Injection from this vial: 1 of 1

Analyzed: 07-05-19 07:54 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	4.86			10671	0.45	0.647	BB
2	10.72	Albendazole	R	1638932	1.00	99.353	BB
				1649603		100.000	

Peak rejection level: 10000

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.13	7358	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7420	---	7433	---	10763.16	16.00

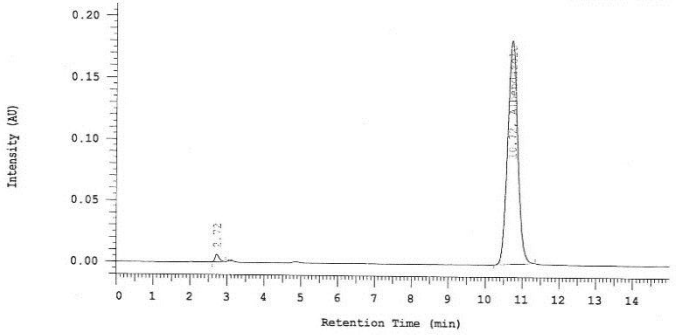
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 1
Injection from this vial: 1 of 1

Analyzed: 07-05-19 08:10 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23734	0.25	1.354	BB
2	10.72	Albendazole	R	1729054	1.00	98.646	BB
				1752788		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.11	7400	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7462	---	7475	---	11381.56	16.00

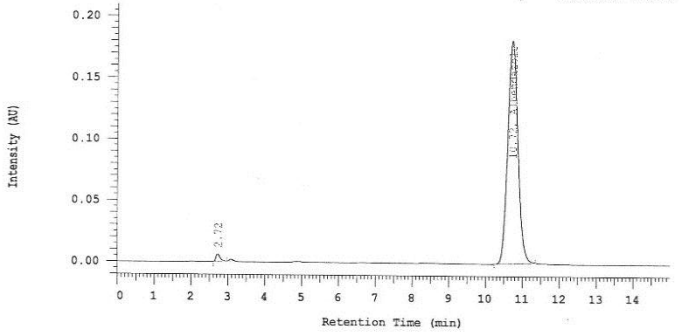
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 2
Injection from this vial: 1 of 1

Analyzed: 07-05-19 08:26 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23782	0.25	1.357	BB
2	10.72	Albendazole	R	1728589	1.00	98.643	BB
				1752371		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.12	7400	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7451	---	7465	---	11380.38	16.00

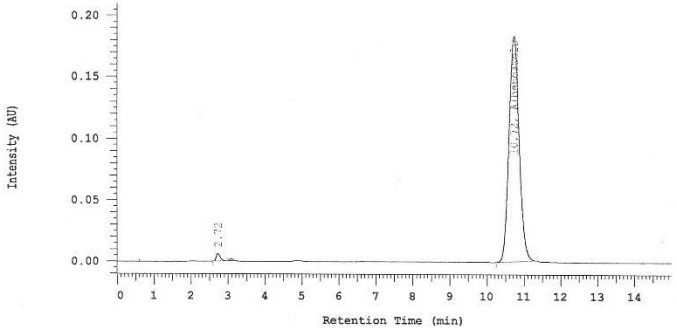
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Zentel Susp Test 3
Injection from this vial: 1 of 1

Analyzed: 07-05-19 08:42 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name		Area	RRT	Area %	BC
1	2.72	.	U	23948	0.25	1.354	BB
2	10.72	Albendazole	R	1745313	1.00	98.646	BB
				1769261		100.000	

Peak rejection level: 10000
Note: (R) Reference Compound
Note: (K) Identified by RT window. Quantified with RF of the Reference.
Note: (U) Unidentified. Quantified with RF of the Reference.

RT (min)	Name	k'	Asym	N (USP)	Res (USP)	Alpha
10.72	Albendazole	3.43	1.13	7387	---	---

RT (min)	Name	N (EUP)	Res (EUP)	N (JP)	Res (JP)	S/N	Noise (uV)
10.72	Albendazole	7441	---	7454	---	11483.94	16.00

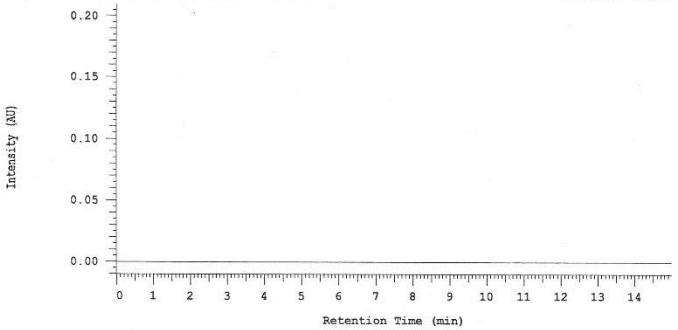
Asymmetry warning outside the range of: 0.800 to 2.000
No. of theoretical plates warning at less than: 2000
Resolution warning at less than: 0.800
Signal to noise ratio warning at less than: 3

Application: Albendazole Series: 0045 Report: modified System: HPLC E

Zentel Suspension Control Sample Assay

Reported: 08-05-19 09:09 AM
Processed: 08-05-19 09:07 AM
Sample Name: Mobile Phase Blank
Injection from this vial: 1 of 1

Analyzed: 07-05-19 08:58 PM
Series:0045
Volume: 20.0 ul



No.	RT	Name	Area	RRT	Area %	BC
			0		0.000	

Peak rejection level: 10000
Note: Reference compound not found.