

**COMPARATIVE *IN VITRO* QUALITY EVALUATION OF TABLETS OF
THE COMMONLY PRESCRIBED ANTIEPILEPTIC DRUGS,
CARBAMAZEPINE AND PHENOBARBITAL, FROM DRUG RETAIL
OUTLETS IN ADDIS ABABA**

BY

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**A thesis submitted to the School of Graduate studies of Addis Ababa
University in partial fulfillment for the Degree of Master of Science in
Pharmaceutical Analysis and Quality Assurance**

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ADDIS ABABA UNIVERSITY
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DECLARATION

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ACRONYMS

DACA	Drug Administration and Control Authority
FDA	Food and Drug Administration
USPDQI	United States Pharmacopoeia Drug Quality and Information
AUC	Area under the Curve
C _{max}	Maximum Concentration
SAR	Structure-Activity-Relationship
TDM	Therapeutic Drug Monitoring
GC	Gas Chromatography
LC	Liquid Chromatography
TLC	Thin Layer Chromatography
HPTLC	High Performance Thin layer chromatography
LAL	Lumulus Amoebocyte Lysate
ICH	International Conference on Harmonization
USP	United States Pharmacopoeia
RSD	Relative Standard Deviation
USCDC	United States Center for Disease Control
GMP	Good Manufacturing Practices
TGA	Therapeutic Goods Act
API	Active Pharmaceutical Ingredient
BP	British Pharmacopoeia
IP	International Pharmacopoeia
SD	Standard Deviation
PhB	Phenobarbitone
NPCB	National Pharmaceutical Control Bureau
GABA	Gamma Amino Butyric Acid

ABSTRACT

Several literature reports show that up to 30% of epileptic patients may not respond to drug therapy, or inadequate control of their seizures, even if there is increasing prevalence and incidence rates of epilepsy. But why this happens and whether it can be predicted is unknown. Studies show that patients who have many seizures before therapy or who have an inadequate response to initial treatment with antiepileptic drugs are likely to develop refractory epilepsy (resistance epilepsy) of unknown origin. In this investigation, trial has been undertaken for the prediction of the reasons of treatment failures by virtue of controlling the drug quality aspects.

Evaluation studies provide a means of identifying quality differences between same products obtained from various manufacturers. Quality analysis and evaluations are the most important tasks to be performed when various reports of therapy indicate problems and failures of treatment.

Different products of antiepileptic drugs, carbamazepine and Phenobarbital tablets were evaluated for their *in vitro* quality so that a base line data was developed to quote the reasons for the therapeutic failures with antiepileptic drugs from the drug quality point of view. Different brands of carbamazepine tablets and different generic products of Phenobarbital tablets marketed in Addis Ababa were analyzed for their identification, hardness and friability, disintegration, dosage form uniformity (weight variation and/or content uniformity), assay (drug content), and *in vitro* dissolution profiles. In addition, values of $t_{50\%}$ and $t_{90\%}$ for drug release were determined for all the tablets. Weight variation test was performed for all the tablets analyzed while content uniformity test was performed for two of the Phenobarbital tablets: East Africa Pharmaceutical and Cadila products. With respect to identification, hardness and friability, disintegration test and dosage form uniformity, all the tablets evaluated were in good agreement with the official specification. From the carbamazepine tablets analyzed, Tegral was found to be below the drug

content specification, while the other brands of carbamazepine and all the Phenobarbital tablets analyzed were in accordance with their respective specifications. The dissolution tests performed indicated that Tegral tablet and two of the Phenobarbital tablets (East Africa Pharmaceutical and Cadila products) did not release the required drug content within the specified time. Taver from the carbamazepine tablets and the Epharm product Phenobarbital had good dissolution profiles, while the other products showed slower dissolution rates as compared to the above two drugs.

The study indicated that the tablets analyzed were bioinequivalent to each other with respect to the *in vitro* quality studies as some were found to be substandard and some were unable to release the required content at the specified time. Therefore, utilization of the substandard ones or interchange use of the bioinequivalent drug products could have contribution to treatment failures.

The present study will be of paramount importance provided further *in vivo* bioavailability evaluation of the indicated brands and generic tablet formulations are performed and correlated with the *in vitro* findings. Stability studies of the products should be investigated to identify if possible to quantify degraded products, continued quality surveillance of the brand and generic tablet formulations of antiepileptic drugs from different regions of the country should be conducted to ensure quality thereby improve clinical efficacy.

1.1. Epilepsy

1.1.1. Epilepsy and Seizures

Epilepsy is a neurological disorder that affects people in every country throughout the world. Epilepsy is also one of the oldest conditions known to mankind. It is characterized by a tendency to recurrent seizures and it is defined by two or more unprovoked seizures. The belief widely held in many countries is that a person with epilepsy is seized by a supernatural force or power. This ancient belief is reflected in the name of the disorder the word "epilepsy" being derived from the Greek word "epilambanein" which means, "to seize or attack". We now know, however, that seizures are the result of sudden, usually brief, excessive electrical discharges in a group of brain cells (neurons) and those different parts of the brain can be the site of such discharges. The clinical manifestations of seizures will therefore vary and depend on where in the brain the disturbance first starts and how far it spreads. Transient symptoms can occur, such as loss of awareness or consciousness and disturbances of movement, sensation (including vision, hearing and taste), mood or mental function [WHO. 2001].

Seizures may vary from the briefest lapses of attention or muscle jerks to severe and prolonged convulsions. They may also vary in frequency, from less than one a year to several per day. Seizures are classified according to where in the brain they arise, for instance: Partial or focal seizures arise from an electric discharge of one or more localized areas of the brain regardless of whether the seizure is secondary generalized. Depending on their type, they may or may not impair consciousness. Whether seizures are partial or focal, they begin in a localized area of the brain, but then may spread to the whole brain causing a generalized seizure. The electrical discharge, which leads to these seizures, involves the whole brain and may cause loss of consciousness and/or muscle contractions or stiffness. They include what used to be known as "grand mal" convulsion and also the brief "petit mal" absence of consciousness. The other type is status epilepticus, which is characterized by a state in which a person has frequent seizures without recovery of consciousness between each episode. It is a dangerous state and if not treated may lead to brain damage or death [Eeg-Olofsson *et al.*, 1995].

It is unclear why particular seizures occur at a particular age or time and not at other ages or times. Provocative factors, however, are recognized in some patients. For example, certain

flashing lights (television, video games etc.), over-breathing, over-hydration, loss of sleep, and/or emotional and physical stress, may stimulate seizures. Although these are not causes of epilepsy, they may influence the timing and frequency of seizures [WHO, 2001].

Different epileptic syndromes are based on the age of onset, the type of seizure, the presence or absence of detectable brain disease and genetic background. However, medical science is only at an early stage in understanding these different types [Allen *et al.*, 2000].

1.1.2. Etiology of epilepsy

Epilepsy is often, but not always, the result of an underlying brain disease [Artemowicz *et al.*, 2002]. Any type of brain disease can cause epilepsy, but not all people with the same brain disease will have epilepsy. In view of the fact that only a proportion of people who have a brain disease experience seizures as a symptom of that disease, it is suspected that those who do have such symptomatic seizures are more vulnerable due to biochemical/neurotransmitter reasons. There are still many people for whom the cause of their epilepsy cannot, as yet, be identified. In such cases, the theory most commonly accepted is that this epilepsy is the result of an imbalance of certain chemicals in the brain (especially chemical messengers known as neurotransmitters) causing them to have a low convulsive threshold [Henry, 2001, Richard, 2002].

Children and adolescents are more likely to have epilepsy of unknown or genetic origin. The older the patient, the more likely it is that the cause is an underlying brain disease, such as a brain tumour or cerebrovascular disease, or is the result of head injury. Trauma and brain infection can cause epilepsy at any age, and as mentioned previously may account for a higher incidence of epilepsy in developing countries. For example, a common cause in Latin America is neurocysticercosis cysts on the brain caused by tapeworm infection, while in Africa, malaria and meningitis are common causes. In India neurocysticercosis and tuberculosis often lead to epilepsy. Febrile illness of any kind can trigger seizures in young children. About 3% of children who have febrile convulsions go on to develop epilepsy in later life. Studies in Ethiopia indicate that epilepsy is the commonest cause of neurological disability in rural Ethiopia [WHO, 2001, Tekle-Haimanot, *et al.*, 1991].

1. 1. 3. Epidemiology: prevalence, incidence and mortality of epilepsy

Epilepsy knows no geographical, racial or social boundaries. It occurs in men and women and can begin at any age, but is most frequently diagnosed in infancy, childhood, adolescence and old age. Anyone can be affected by seizures. In fact, up to 5% of the world's population may have a single seizure at some time in their lives, but a diagnosis of epilepsy is reserved for those who have recurring seizures, at least two unprovoked ones [Annegers, *et al.*, 1999].

The prevalence of a disorder is the proportion of a population with that disorder at a given point in time. From many studies around the world it has been estimated that the mean prevalence of active epilepsy (i.e. continuing seizures or the need for treatment) is approximately 8.2 per 1,000 of the general population. However, this may be an underestimate as some studies in developing countries (such as Colombia, Ecuador, India, Liberia, Nigeria, Panama, United Republic of Tanzania and Venezuela) suggest a prevalence of more than 10 per 1,000. Thus, it is likely that around 50 million people in the world have epilepsy at any one time. The lifetime prevalence of epilepsy (i.e. the number of people presently in the world who have epilepsy now or have had it in the past or will experience it in the future) is approximately 100 million people [WHO, 2001, Kwan, *et al.*, 2000]. Epidemiological studies in Ethiopia are localized more to the central part of the country and may underestimate the prevalence rate to 5.2 per 1000 [Shibru, *et al.*, 2004].

The incidence of a disorder is the number of new cases at a given time. Studies in developed countries suggest an annual incidence of epilepsy of approximately 50 per 100,000 of the general population [Kotsopoulos, *et al.*, 2002, Hauser, *et al.*, 1993, Hauser, *et al.*, 1996, Olafsson, *et al.*, 1996]. However, studies in developing countries suggest that this figure is nearly double that at 100 per 100,000. One of the main reasons for the higher incidence of epilepsy in developing countries is the higher risk of experiencing a condition, which can lead to permanent brain damage. These conditions include neurocysticercosis, meningitis, malaria, pre and perinatal complications and malnutrition. A study by Tekle-Haymanot, *et al.* (1997) indicated that there are high incident rates in the study areas of Ethiopia comparable to that in the rest of the World [Tekle-Haimanot, *et al.*, 1997].

Epilepsy is associated with an increased risk of mortality. Death may be related to: an underlying brain disease, such as a tumour or infection; seizures in dangerous circumstances, leading to drowning, burns or head injury; status epilepticus; sudden and unexplained causes, or a possible respiratory or cardio-respiratory arrest during a seizure; suicide. Whilst studies on this subject are rare, epilepsy-related deaths in young adults in the UK, for example, are 3 times higher than standard age-related mortality rates [WHO. 2001].

1.1.4. Treatment of Epilepsy

Recent studies in both developed and developing countries have shown that upto 70% of newly diagnosed children and adults with epilepsy can be successfully treated (i.e., their seizures can be completely controlled for several years) with anti-epileptic drugs. Antiepileptic drugs are fully effective in controlling seizures in 50-80% of patients. Patients with epilepsy usually need to take drugs continuously for many years. There is a need for more specific and effective drugs, and several new drugs have been recently introduced for clinical use. The main well established antiepileptic drugs are phenobarbitone, phenytoin, carbamazepine, valproate, and ethosuximide. The newer drugs, whose place in therapy is still being evaluated, include vigabatrin, gabapentin, lamotrigine, felbamate, tiagabine and topiramate [Rang, *et al.*, 2002, Patsalos, 1999]. Studies regarding treatment of epilepsy in rural Ethiopia by Shibru, *et al.*(2004) revealed that Phenobarbitone is the only available antiepileptic drug in the study areas and further reports show that phenobarbitone, phenytoin and carbamazepine are mostly prescribed [Shibru, *et al.*, 2004, Shibru , *et al.*, 2002].

Standard Treatment Guidelines of Ethiopia puts phenobarbitone 60-180 mg/day per oral in divided doses as a first line antiepileptic drug and phenytoin 5mg/kg/day per oral in a single or divided doses OR carbamazepine 600-1,800 mg/day per oral in 2 divided doses as alternative medications [DACA Ethiopia, 2004].

1.1.5. Treatment failures with antiepileptic drugs

After 2-5 years of successful treatment, drugs can be withdrawn in about 70% of children and 60% of adults without relapses. However, up to 30% of people may not respond to drug therapy, or inadequate control of their seizures. Reasons for their anomalous behaviour could not be

predicted. Studies show that patients who have many seizures before therapy or who have an inadequate response to initial treatment with antiepileptic drugs are likely to develop refractory epilepsy (resistance epilepsy), and why this happens is not known. Results from studies in rural central Ethiopia by Tekle-Haimanot, *et al.* (1997) showed some treatment failures leading to high mortality. Other critical problem with antiepileptic drugs is the development of tolerance with the established drugs, thus efficacy and tolerability with the newly discovered antiepileptic drugs is on evaluation and establishment [Tekle-Haimanot, *et al.*, 1997, French, *et al.*, 2004].

Further studies on the new drugs on patients indicated that there is an increase in seizures when a new antiepileptic drug is added to their therapy, which may be the drugs effect [Ernest, *et al.*, 2002]. Thus the possible cause of this inadequate control of seizures and tolerance developments to antiepileptic drugs may be viewed from the quality aspect of the drugs. Reports from pharmaceutical care services show that there are bioavailability problems leading to therapeutic failures. There has been reported a continued breakthrough of seizures associated with subtherapeutic phenytoin concentrations, and non-compliances. Such drug therapy problems may occur because the patient has become refractory to the present drug therapy; the patients is receiving a drug that is effective but not the least costly that he/she could not afford to buy and complete the dosage regimen; the patients serum drug concentration is below the desired therapeutic range. The latter might be attributed to drug release problems, too low drug content of the dosage form, and drug, dose or formulation conversions were inappropriate for the patients [Robert, *et al.*, 1998].

1.2. Pharmaceuticals Quality, Effectiveness and Trading

1.2.1. Therapeutic Effectiveness and Quality of Pharmaceuticals

For a drug to be effective, enough of it needs to reach its site(s) of action and stay there long enough to be able to exert its pharmacological effects. Many factors have been found to influence the rate and extent of absorption, and hence the time course of a drug in the plasma and, therefore, at its site(s) of action. These include the foods eaten by the patient, the effect of the disease state on drug absorption, the age of the patient, the sites of absorption of the administered drug, the co administration of other drugs. In addition, the physical and chemical properties of the drug, the type of dosage form, the composition and method of manufacture of

the dosage form, the size of the dose and frequency of administration are among the factors, which are almost addressed in quality assessment studies [Ashford, 2002, FDA, 1997].

The presence of substandard pharmaceutical products in the drug distribution chain may produce a danger to public health. Drug quality reports by the United States Pharmacopoeia Drug Quality and Information Program in different countries e.g. Benin, Ghana, Nigeria, Bangladesh, Cambodia, China revealed that a large number of drugs failed quality testing. Some of these drugs were found to contain active ingredients outside the appropriate limits and most of them below the limits. Such drug products have therapeutic as well as social and economic implications [WHO, 1999, USPDQI, 2003].

The quality of drugs in less developed settings is inadequate. Reasons for the availability of poor quality drugs could be the widespread counterfeiting of medicines, decomposition of the active ingredient in drug dosage form due to high temperature and humidity of the storage condition, and inadequate quality assurance systems during the manufacture of pharmaceutical products [Robert, 2001, Risha, *et al.*, 2003]. For instance, in the study of effects of humidity and temperature on the *in vitro* quality of carbamazepine tablets revealed that high humidity and temperature have a profound effect on tablet disintegration and dissolution which are affecting the release pattern and which in turn affect bioavailability and effectiveness of the drugs. Stability of the drugs also depends on packaging and storage [Wang, *et al.*, 1993, al-Zein, *et al.*, 1999]. Counterfeiting can also influence drug quality because such drugs have bypassed regulatory control for quality assurance, thus resulting in products with correct active ingredient but too little or with wrong ingredient or with fake packaging [WHO, 1992, Albert, *et al.*, 2004].

1.2.2. Pharmaceuticals Quality

Pharmaceuticals play an important role in improving human health and promoting well-being. However, to produce the desired effect, they have to be safe, efficacious and of acceptable quality, and have to be used rationally. The use of ineffective and poor quality drugs will endanger therapeutic treatment and may lead to treatment failures. Thus, the production, storage, and distribution of drugs in each country need to be regulated by the government drug regulatory authority. Challenges to these drug regulatory authorities are the flourishing of many

pharmaceutical industries and distribution channels during the past few years in the world, leading to an increased number of products circulating in national and international drug markets. In the same manner, the presence of counterfeit and substandard drugs in those markets has increased substantially as a result of ineffective regulation of the manufacturers and trading of pharmaceutical products by both exporting and importing countries [WHO, 1999].

Marketing of poor quality drugs is high in developing countries, especially of Africa and Asia because of weak drug regulatory systems [Reidberg, *et al.*, 2001]. Thus, in countries like Ethiopia, where drug regulatory control is weak, the quality of marketed drug products cannot be guaranteed. Quality assessment studies on some of the marketed drug products could give an insight into the quality of the pharmaceutical products marketed within the distribution chain and consumed. Such studies could provide basis for corrective measures taken by drug regulatory authorities.

1.3. Antiepileptic drugs: Carbamazepine and Phenobarbitone

The term antiepileptic is used synonymously with anticonvulsant to describe drugs that are used to treat epilepsy. Studies have shown that particular types of epileptic seizures in humans respond best to a particular antiepileptic drug. The mechanism of action of each antiepileptic drug varies from drug to drug and thus their choice for therapeutic significance. The main drugs in current use like carbamazepine and phenytoin act by blocking Calcium ion (Ca^{++}) channels thus reducing electrical excitability of cell membranes; and phenobarbitone act by enhancing Gamma Amino Butyric Acid (GABA) action thus reducing electrical activity of neurons within a chemically induced epileptic focus [Rang, *et al.*, 2002, Mervyn, *et al.*, 1989]. It could be better, therefore, to consider carbamazepine and phenobarbitone from these drugs.

1.3.1. Carbamazepine

Carbamazepine is a white or yellowish white crystalline powder with molecular weight of 236.3 daltons. It may exhibit polymorphism, which has considerable effect on the physical properties and release pattern of the drug. Comparative study for photostability of carbamazepine polymorphs indicated that the surface of pellets of all crystalline forms turned gradually from white to yellow orange upon exposure to light, revealing unstability of the drugs polymorphs to

light. The drug has a melting point range between 189 and 193°C [Clarke, 1986, Matsuda, *et al.*, 1994].

1.3.1.1. Chemistry of Carbamazepine

Carbamazepine is related chemically to the tricyclic antidepressants. It is a derivative of iminostilbene (Fig.1. 1) with a carbamyl group at the 5-position (Fig1.2). This moiety is essential for the potent antiseizure activity of carbamazepine [Joel, *et al.*, 2001].

Carbamazepine is soluble in the more polar organic solvents such as propylene glycol, ethanol, chloroform and acetone, but very poorly soluble or practically insoluble in water. For most practical purposes the molecule is neutral and does not ionize in an aqueous environment [Mervyn, *et al.*, 1989].

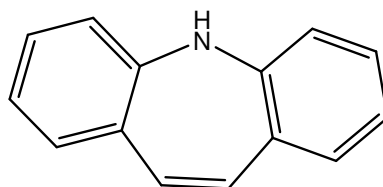


Figure 1.1: The Structural Formula of Iminostilbene

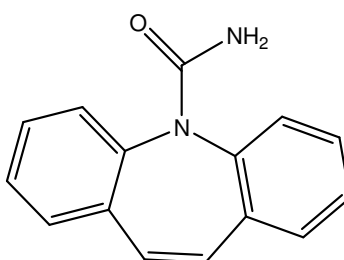


Figure 1.2: The Structural Formula of Carbamazepine

1.3.1.2. Pharmacokinetic profile of Carbamazepine

Carbamazepine is given by mouth, nearly always as tablets. No parenteral preparations are available. The absorption half time of the drug from tablets averaged 1.72 hr with mean value

range of 1.42 ± 0.34 hrs. The mean T_{max} increases as the dose of carbamazepine increases, indicating dose dependent absorption half time. Carbamazepine particle size in tablet preparations can influence the rate of absorption of the drug. Dam, *et al* (1981) noted that there are differences in steady state plasma drug levels produced by different brands of carbamazepine tablets [Mervyn, *et al.*, 1989, Dam, *et al.*, 1981]. Research results indicate that carbamazepine has an oral bioavailability of 58-85%. Bioavailability studies with different brands and generic formulations of carbamazepine tablets revealed that there are significant differences in the rate and extent of absorption and bioinequivalencies with each other. These differences were critical for carbamazepine tablets with a history of clinical failures when their bioavailability profiles were compared with the innovator product [Morselli, *et al.*, 1975, Oluke, *et al.*, 1996, Meyer, *et al.*, 1992, Saavedra, *et al.*, 1990, Revankar, *et al.*, 1999, Meyer, *et al.*, 1998].

1.3.2. Phenobarbitone

Phenobarbitone is a white or colorless crystalline powder with molecular weight of 232.23 daltons. It has somewhat bitter taste and may exhibit polymorphism. Studies on the effect of polymorphism of drugs on the physical properties and drug release of Phenobarbital tablets indicated that the physical properties of the tablets were different according to the modification of the tablets. Phenobarbitone has a melting point range of 174-178°C [Mervyn, *et al.*, 1989, Clarke, 1986, Szabo-Revesz, *et al.*, 1987].

1.3.2.1. Chemistry of phenobarbitone

Phenobarbitone is a barbituric acid derivative, 5-phenyl-5-ethylbarbituric acid (Fig1. 3). The Structural Activity Relationship (SAR) of barbiturates indicates that maximal antiseizure activity is attained by phenyl group at position 5 [Joel, *et al.*, 2001].

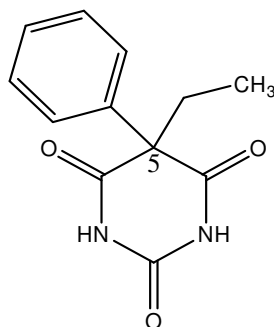


Figure 1.3: The structural formula for phenobarbitone

Phenobarbitone is poorly soluble in water but dissolves in organic solvents such as ethanol, diethylether and chloroform. It has a pka value of 7.2. It is sometimes administered as its sodium salt, which is more water soluble than the parent substance [Mervyn, *et al.*, 1989].

1.3.2.2. Pharmacokinetic profile of phenobarbitone

Orally administered phenobarbitone is fully bioavailable. The maximum plasma phenobarbitone level is attained 6-12 hrs after an oral dose of the drug. Pharmacokinetic studies in healthy volunteers showed that phenobarbitone administered as a tablet has almost complete bioavailability. The absorption rate of the drug usually depends on formulation. Absorption from suspension dosage form is faster than that from the tablet which is confirmed from higher concentration maximum (C_{max}) and Area under the Curve (AUC) for the former [Mervyn, *et al.*, 1989, Shah, *et al.*, 1994]. Drug product equivalence and clinical significance of plasma levels of phenobarbitone revealed that there were increased plasma levels following the change between the tablets which were matched by a decrease in the number of seizures. Minor changes between any two different tablet formulations could influence drug absorption and thus drug bioavailability [Stewart, *et al.*, 1975, Lazowski, *et al.*, 1978, Meyer, *et al.*, 1984].

1.3.3. Therapeutic Drug Monitoring (TDM) of carbamazepine and phenobarbitone

The application of pharmacokinetic principles in conjunction with monitoring of plasma drug concentrations has led to major advances in the treatment of epilepsy with antiepileptic drugs like carbamazepine and phenobarbitone. Since antiepileptic drugs have a narrow therapeutic index and complex pharmacokinetic properties, wide fluctuations in their plasma concentration can lead to either toxic effects or loss of therapeutic efficacy [Gogtay, *et al.*, 1999].

Carbamazepine and phenobarbitone are currently considered to be drugs of choices for different types of epileptic seizures. Large interindividual differences in apparent plasma half-life linked to a narrow therapeutic range make these drugs suitable candidates for Therapeutic Drug Monitoring (TDM). Therapeutic drug monitoring helps the physicians in optimizing the dose for a better seizure control in epileptic patients. TDM involves quantification of serum drug concentration. Anticonvulsants are currently quantified in serum by different techniques of physicochemical methods including gas chromatography (GC), liquid chromatography (LC), thin

layer chromatography (TLC), high performance thin layer chromatography (HPTLC), etc [Garg, *et al.*, 2000, USCDC, 1985].

1.4. Quality Control Parameters and Properties of Tablet dosage forms

The need for precisely defined and acceptable specifications for production control during manufacturing processes and for the final products, in order to assure reproducibility in the wide context of drug safety, is recognized. This is required not only by the pharmaceutical industry but also by national drug regulatory bodies and international organizations actively concerned with the quality control of medicines moving in the international commerce. The assurance of quality of medicines is the primary responsibility of the manufacturers (internal quality control). However, it is recognized in most countries that the national health authorities must exercise comprehensive surveillance by legislative methods over pharmaceutical manufacturers within their jurisdiction, in order to ensure observance of good manufacturing practices and quality control of products. During research, development and formulation, physicochemical analysis and analytical profiles of drug substances provide good quality control data on which good decisions can be established [Deasy, *et al.*, 1976, Ayers, *et al.*, 1977].

Assessment of quality, safety and efficacy constitutes an important component of pharmaceutical product evaluation, which is based on quality control tests. The minimum quality control tests for pharmaceutical preparations are illustrated in Table 1.1 [Ayers, *et al.*, 1981, Berita Ubat-ubatan, 1999].

Tablets have some apparent features, like certain amount of hardness and resistance to friability to withstand mechanical shocks encountered during their production, packaging and handling prior to use. In addition to these apparent features, they must meet other physical specifications and quantity standards. These include criteria for tablet dosage form uniformity (weight variation, content uniformity), disintegration, and drug dissolution [Howard, *et al.*, 1999, Lachman, *et al.*, 1990]. Each property will influence the other as hardness has influence on both friability and drug dissolution. All are tablet properties that can be utilized as parameters for drug quality control [Souto, *et al.*, 1989].

Table 1.1 Minimum Quality Control Tests for Pharmaceutical Preparations

Types of pharmaceutical preparations	Types of tests
1. Tablet, Capsule, Lozenges	Identification of active ingredient (s), Uniformity of weight, Friability, Hardness, Disintegration, Dissolution, Assay of active ingredient (s).
2. Injectable (Liquid)	Identification of active ingredient(s), pH, Extractable volume, Particle Count, Pyrogen / LAL, Sterility, Effectiveness of microbial preservatives, Assay of active ingredient (s)
3. Cream, Ointment	Identification of active ingredient (s), Viscosity, Homogeneity, pH, Release rate, Sterility, Microbial limit test, Effectiveness of preservatives (if present)
4. Aerosol, Inhalation, Spray	Identification of active ingredient (s), Net contents, Particle size and tests for foreign particles, Delivery rate, Leak testing, Pressure testing, Limit test for degradation products/ impurities (where applicable), Moisture determination, Assay of active ingredient (s)

1.4.1. Hardness and Friability

Tablets require certain degree of strength and resistance to friability to withstand mechanical shocks of handling during manufacturing, packaging, shipping and utilization by the patient. Adequate tablet hardness and friability are necessary requisites for customer acceptance [Getie, *et al.*, 1998] and have adequate impact on drug product quality. There are factors, which may alter tablet hardness and friability. These are changes in particle size, distribution of the granulation mix and lubricants, etc. Large particles of low density will produce softer tablets whereas smaller particles of high density granules will produce relatively stronger tablets

[Lachman, *et al.*, 1990, James, 1996]. Studies with Phenobarbital tablets quality control showed that duration of mixing with lubricant, maximal compression force and compression rate have influence on various properties of the tablets. These variables of tablet manufacturing process are found to have a marked influence on hardness, friability, disintegration, and dissolution properties [Souto, *et al.*, 1989, Shah, *et al.*, 1977].

1.4.2. Uniformity of dosage units: content uniformity and weight variation

This term includes both the mass of the dosage form and the content of the active substance in the dosage form. These are expressed in terms of content uniformity or weight variation; which are among the parameters of tablet quality control [ICH, 1999, Irem, *et al.*, 2000]. Weight variation requirements may be applied where the product to be tested contains 50 mg or more of an active ingredient comprising 50% or more, by weight, of the dosage form unit, and otherwise content uniformity. The United States Pharmacopoeia (USP) contains a test for the determination of dosage form uniformity for uncoated tablets. Ten tablets are weighed individually and the average weight calculated. The tablets are assayed and the content of active ingredient in each of the ten tablets is calculated assuming homogeneous drug distribution. USP also provides that for content uniformity ten dosage units are individually assayed for their content according to the assay method described in the individual monograph. Unless otherwise specified in the monograph, the requirements for content uniformity are met if the amount of active ingredient in each dosage unit lies within the range of 85% to 115% of the label claim and relative standard deviation (RSD) is less than 6.0 % [USP, 2003, Kovacs, *et al.*, 1980].

1.4.3. Disintegration

For the active medicinal agent in a tablet to become fully available for absorption, the tablet must first disintegrate and discharge the drug to the body fluids for dissolution. Tablet disintegration also provides drug particles with an increased surface area for localized activity within the gastrointestinal tract [Howard, *et al.*, 1999]. Before a tablet goes into solution it must breakdown into smaller particles or granules by the process of disintegration. Complete tablet disintegration is the state in which any residue of the tablet is a soft mass having no palpably firm core. Disintegration testing is more appropriate when a relationship to dissolution has been established and it is a limiting factor of drug dissolution, particularly with low aqueous solubility drugs like

carbamazepine. For tablets to be disintegrated, it is necessary to overcome the cohesive forces introduced into the mass by compression and by any binder present, which is usually practiced by incorporating disintegrants. Disintegration could be affected by formulation factors and properties and concentration of excipients, in particular. Studies done on paracetamol and oxytetracycline tablets revealed that variations with various formulation and processing variables and excipients led to variations in physical properties like disintegration [Lachman, *et al.*, 1990, Esezobo, *et al.*, 1977, Esezobo, 1985]. Porosity, hydrophilicity, swelling ability of particles and interparticle forces are important factors for tablet disintegration. Tablet porosity is related to water absorption, which is an important step of disintegration process. There are factors related to the inner structure of the tablets and hydrophilicity of excipients affecting wettability of the formulation and playing a vital role in the process of disintegration [Yunxi, *et al.*, 1996, Fell, *et al.*, 1978, Lopez-Solis, *et al.*, 2001, Bi, *et al.*, 1999].

1.4.4. Assay for the active ingredients

Assay is a critical step in analytical sciences. It is the determination of the strength or content of the active ingredient within the dosage form. The quantitative determination of a drug dosage form is preferentially performed by physicochemical methods. Such analytical techniques are very diverse and successfully applied to the assay of the active medicinal agent in the pharmaceutical tablets. The technique should provide specificity, accuracy, precision and sensitivity to the particular ingredient of interest within the dosage form [USCDC, 1985, Sang, *et al.*, 2003, John, 2002].

1.4.5. Dissolution and Dissolution tests

1.4.5.1. Dissolution process and rate

Dissolution is defined as the process by which a solid substance enters into the solvent to yield solution, i.e., the process by which a solid substance dissolves [Banakar, 1992]. The process involved in the dissolution of solid dosage form could be described as in Figure 1.4. The scheme illustrates that the tablets must break down into smaller particles through disintegration and render greater surface area to the dissolving media to bring the tablets into solution. Thus, this solution will be absorbed into the blood stream that dissolution must be related to the availability of the drug to the body [Alton, 2002]. The amount of drug substance that goes into solution per

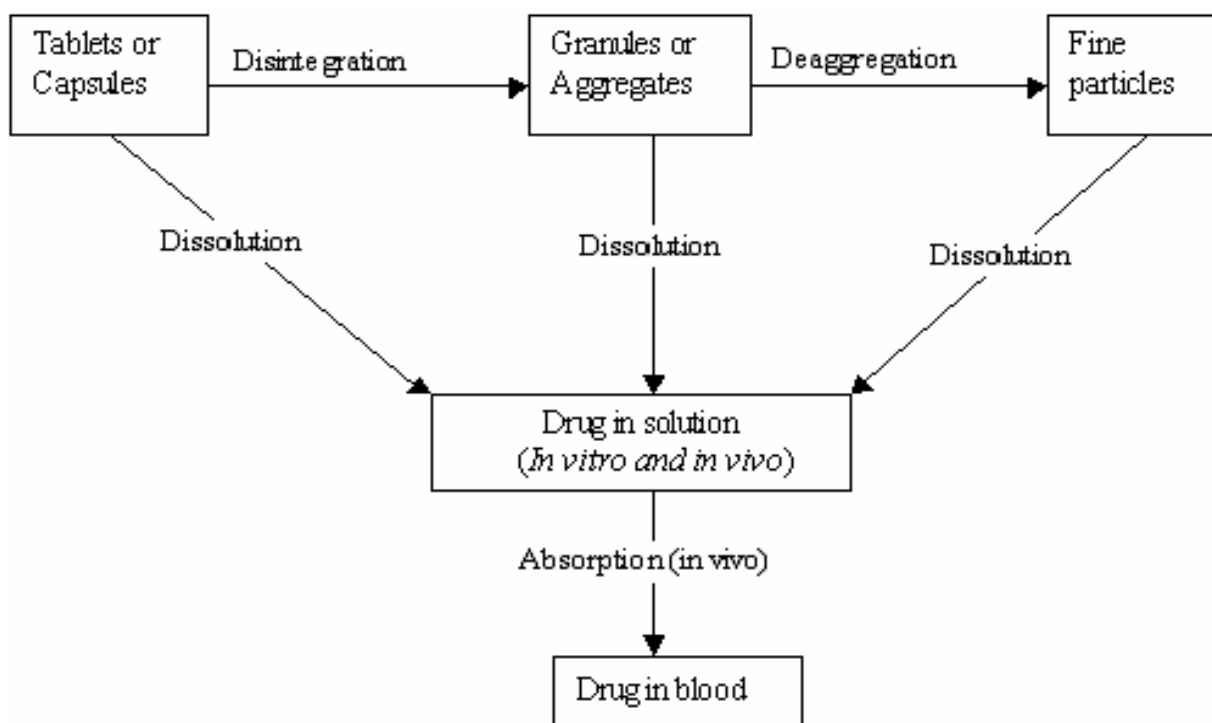


Figure 1.4: Schematic Illustration of Dissolution of solid dosage forms

unit time under standardized conditions is called dissolution rate. This depends on several factors like ageing, excipient type (surfactants, disintegrants, etc.), tablet integrity, other drugs, etc. Some excipients like sorbitol and sodium lauryl sulfate increase dissolution rate. A study with paracetamol tablet showed that mode of excipient incorporation also influences the rate. External addition of aerosil led to increased dissolution rate while internal addition resulted in decreased dissolution rate of the tablet. Naggar, *et al.* (1980) studied the effect of some drugs on the dissolution rate of nitrofurantoin. The study showed that drugs like nicotinamide and sodium salicylate have increased the dissolution rate of the drug. Aminophenazone, paracetamol, and aminophylline possessed a retardation effect on the rate of dissolution of the drug [Esezobo, 1985, Chowhan, 1980, Gordon, *et al.*, 1993, Mandal, 1996, Naggar, *et al.*, 1980].

1.4.5.2. Factors affecting tablet dissolution

There are various intrinsic characteristics of the drug affecting dissolution. These could be primarily solubility and permeability of the drug substance in the dissolution medium. High solubility and high permeability of drugs are necessary for the appropriate and rapid *in vitro*

release requirements [FDA, 1997, Lawrence, *et al.*, 2001, Oluwatoyin, *et al.*, 2003]. The process of dissolution of an active ingredient from a solid pharmaceutical dosage forms particularly tablets involves several intermediate physicochemical steps of the drug such as wetting, swelling, solubility and diffusion. These steps influence the way the tablets disintegrate to expose the drug contents to the medium so that *in vitro* release is possible. Surface area of solids influences fluid flow properties in the dissolution process. Fluid flow properties in turn are influenced by particle size, shape and density. Usually, the dissolution rate increases with decrease in particle size. However, hydrophobic properties of liquid-solid interface, and mutual interference in the particulate motion have a retarding effect on dissolution that smaller particles, in such cases, may exhibit slower dissolution rates [Lachman, *et al.*, 1990, Banakar, 1992, Johnson, *et al.*, 1991, Gordon, *et al.*, 1987].

In an effort to produce tablet formulations of drugs, there is involvement of several interacting formulation and processing variables, which have critical effect on dissolution and release profiles of the products. These variables may vary in conditions and situations from manufacturers to manufacturers that release and dissolution profiles of different manufacturers may differ [Gordon, *et al.*, 1987, Yamamoto, *et al.*, 2000]. Processing and formulation factors which influence dissolution include: nature of diluents, process of mixing, granule size and distribution, nature of disintegrants, nature and concentration of lubricants, presence or absence of surface active agents, physical properties of the drug, flow of granulation through hopper and dies and compressional force in production [Lachman, *et al.*, 1990, Esezobo, 1985, Chowhan, 1980, Mandal, 1996, Naggar, *et al.*, 1980, FDA, 1997]. There are various scientific reports on the effects of processing and formulation variables on dissolution. Reports show that hydrophobic lubricants like magnesium stearate retarded dissolution while water soluble lubricants like sodium lauryl sulfate enhanced dissolution rate [Esezobo, 1985, Iranloye, *et al.*, 1978]. It was reported that the type and concentration of binders used in the formulation affect dissolution rates. An increase in binder concentration resulted in a decrease in dissolution rates of the tablets [Chowhan, 1980, Endale, *et al.*, 1996]. The type and mode of incorporation of disintegrants used has been reported to have a pronounced effect on dissolution rates [Gordon, *et al.*, 1993, Khan, *et al.*, 1976]. Studies on the effects of manufacturing process variables on dissolution on the *in vitro* dissolution characteristics of drugs indicated the influence of various process variables on drug

release from the tablets. The results indicated that a change in the manufacturing process could yield significantly dissimilar dissolution profiles for the same formulation [Huang, *et al.*, 2003].

1.4.5.3. Dissolution Test as Quality Control Parameters

Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Thus *in vitro* dissolution may be relevant to the prediction of *in vivo* performance. *In vitro* dissolution tests for immediate release dosage forms, such as tablets, are used to assess lot to lot quality of drug product and ensure continuing drug product quality and performance after certain changes such as changes in formulation, the manufacturing process and the site of manufacture [FDA, 1997]. Dissolution tests are valuable tools in ensuring the quality of a drug product. Generally, product to product variations are due to formulation factors such as particle size differences, excessive amounts of lubricants and coatings. These factors are reactive to dissolution testing [Huang, *et al.*, 2003].

Dissolution testing is performed as a quality control test to check whether they comply with pharmacopieal specifications or not. Pharmacopieal *in vitro* dissolution specifications are established to ensure product to product and batch to batch consistency of pharmaceutical products and to signal potential problems with *in vivo* bioavailability [USP, 2004].

In general, *in vitro* dissolution rate measurements have been used in a variety of ways including:

- To study the effects of physicochemical variables of the pure drug on dissolution rates.
- To study variables such as manufacturing processes, tablet coating, excipients, on dissolution characteristics of the dosage form.
- To screen potential dosage form candidates for *in vivo* bioavailability studies.
- As a retrospective study to explain clinical failures of a particular dosage form.
- As a sensitive quality control procedure to detect changes in the release characteristics due to lot-to-lot variations, formulation changes, or storage conditions, which may or may not be detected in less sensitive *in vivo* absorption.

- To provide an indication of differences in *in-vivo* absorption characteristics of the drug and serve as a secondary standard to detect dosage forms with a potential for poor bioavailability [Jollow, *et al.*, 1972].

1.4.5.3.1. Dissolution characteristics of Carbamazepine

Dissolution tests are measures of the amount of drug released into the dissolution medium with time. For routine quality control tests single point (release of the drug at the pharmacopieally specified time) could be used, especially for highly soluble and rapidly dissolving drugs. Two point specifications could be used for characterization of the quality of drug products and as a routine quality control test for certain types of drugs such as carbamazepine. For carbamazepine, USP specifies the drug release at two points: 15 minutes (drug release between 45%- 75%) and 60 minutes (drug release greater than 75%). For accepting product similarity and to waive bioequivalence requirements especially for lower strengths of a dosage form, usually dissolution profile comparisons are performed. Dissolution profiles involve measurements of the amount of drug release at different points in increasing time order [USP, 2004, FDA, 1997, Michael, *et al.*, 2003].

Reports indicated that carbamazepine tablets with lower dissolution profiles when given to epileptic patients were associated with the breakthrough of seizures and higher incidence of neurological side effects. This could reveal that those drugs with better dissolution profiles have better bioavailability than those with lower dissolution profiles. Poor control of epileptic seizures with those carbamazepine tablets with smaller dissolution profile was also reported [Oluka, *et al.*, 1996, Meyer, *et al.*, 1992, Hartely, *et al.*, 1991]. A lot of products after postmarketing drug surveillance could fail pharmacopieal dissolution test specification and may get very different dissolution profiles with each other indicating bioavailability differences when bioavailability tests are performed on such products [Oluka, *et al.*, 1996, Meyer, *et al.*, 1992, Stewart, *et al.*, 1975, Soryal, *et al.*, 1992].

1.4.5.4. Dissolution, Bioavailability and Bioequivalence

Drug release properties, which can be characterized by dissolution, have influence on the systemic exposure of drug products, i.e. absorption is governed by the amount of drug substances

in solution (solubility). Thus, the rate-limiting step in absorption could be the rate at which the solid drug enters the bulk solution [Lawrence, *et al.*, 2001, Jollow, *et al.*, 1972]. Dissolution influences the amount of the drug substance that goes to solution before it is absorbed and thus governs the blood levels of the drug. Therefore, it can function as the *in vitro* controlling factor in determining the magnitude of pharmacological response elicited, i.e., the clinical effectiveness which can be addressed by bioavailability. Bioavailability is a pharmacokinetic term that describes the rate and extent to which the active drug ingredient is absorbed from a drug product and becomes available at the site of drug action. Since pharmacological response is generally related to the concentration of a drug from a dosage form at the receptor site, the bioavailability of the drug is a critical element of a drug product's clinical efficacy. Before rendering this therapeutic effect, the drug must be absorbed, and before absorption, the drug must be dissolved to solution (dissolution) [Banakar, 1999].

Literatures showed that there might be variations in clinical response among orally administered drug products that contain chemically equivalent amounts of a drug due to differences in their dissolution rates. This is a useful indication of the influence of dissolution rate on absorption and bioavailability of drugs for clinical use [Banakar, 1992].

The physical and chemical characteristics of a drug together with formulation factors are affecting bioavailability because they can affect dissolution and the absorption characteristics of the drug. There may be effects of dosage form type, particle size, diluents and process on the bioavailability of the drug products [Mendes, *et al.*, 1978]. Neuvonen, *et al.* (1977) indicated that particle size and several other formulation factors are important for dissolution rate and absorption characteristics of phenytoin products and confirmed that the properly performed measurement of the *in vitro* dissolution rate can be used as a preliminary screening test in predicting the bioavailability of pharmaceutical drug products [Chakrabarti, *et al.*, 1980, Neuvonen, *et al.*, 1977]. Usually pharmaceutical products of the same dosage form and strength but with different bioavailability data could be marketed for therapeutic purposes. For instance, a study by Stewart and co-workers (1975) indicated that there was significant increase in plasma phenytoin levels following change of tablets from generic to a brand product [Stewart, *et al.*, 1975]. In order for a drug product to be interchangeable with the pioneer (innovator or brand

name) product, it must be both pharmaceutically equivalent and bioequivalent to it. According to FDA, "Pharmaceutical equivalents" are drug products that contain identical active ingredients and are identical in strength or concentration, dosage form and route of drug administration. However, pharmaceutical equivalents do not necessarily contain the same inactive ingredients; various manufacturers' dosage forms may differ in colour, flavour, shape, and excipients. The terms "Pharmaceutical equivalents" and "Chemical equivalents" are often used interchangeably [Banakar, 1999].

"Bioequivalence" is a comparison of the bioavailability of two or more drug products. Thus, two products or formulations containing the same active ingredient are bioequivalent if their rates and extents of absorption are the same. For a drug product to be considered bioequivalent to a pioneer product there must be no statistical differences (as specified in the accepted criteria) between their plasma concentration-time profiles, with some degree of acceptable tolerance limits. Since *in vitro* dissolution test is designed to accurately reflect the dissolution process in the gastrointestinal tract, dissolution profiles could be utilized to predict the plasma concentration-time profile and thus reflect a drug's bioavailability [Welling, 1991, FDA, 1994].

In general, the FDA considers two products to be "Therapeutic equivalents" if they each meet the following criteria:

- They are pharmaceutical equivalents,
- They are bioequivalent (demonstrated by a bioavailability measurement or an *in vitro* dissolution),
- They are in compliance with compendial standards for strength, quality, and identity,
- They are adequately labeled, and
- They have been manufactured in compliance with Good Manufacturing Practices (GMP) [Banakar, 1999].

Dissolution testing, therefore, has not only been recognized as a valuable quality control test but also proved itself as a useful indicator of differences in bioavailability. This is because of the fact that drug absorption after oral administration depends on the release of the drug substance from

the drug product, the dissolution or solubilization of the drug under physiological conditions and the permeability across the gastrointestinal tract. The dissolution rate of per-oral solid dosage forms is the rate-limiting step in the absorption process [Endale, *et al.*, 1996]. Whenever a significant difference in bioavailability has been found among expectedly identical products, the dissolution test most of the time has been able to discriminate among these products. In fact, dissolution is so sensitive to formulation factors that bioequivalent formulations show differences in their dissolution profiles [Amidon, *et al.*, 1995, FDA, 2000].

1.5. Bioavailability assessment and *in vitro/in vivo* correlations

Pharmaceutical scientists have for many years been attempting to establish a correlation between some physicochemical properties of a dosage form and the biological availability of the drug from that dosage form. The term commonly used to describe such relationship is *in vitro/in vivo* correlation (see section 1.5.2). If such correlation could be established, it would be possible to use *in vitro* data to predict a drug's *in vivo* bioavailability. This would drastically reduce, or in some cases, completely eliminate the need for bioavailability tests. The desirability for this becomes clear when one considers the cost and time involved in administering drugs to healthy subjects or patients. It would certainly be preferable to be able to substitute quick, inexpensive *in vitro* tests for *in vivo* bioavailability studies [Banakar, 1999].

1.5.1. *In vitro* methods of assessing bioavailability

Evidences provided us with formulation variables that can greatly affect the bioavailability and thus the clinical efficacy of many important therapeutic agents [Chakrabarti, *et al.*, 1980, Neuvonen, *et al.*, 1977]. Various *in vivo* and *in vitro* physical methods have been developed to assess these effects. The release of a drug from the dosage form into solution in the gastrointestinal fluids is often the rate-limiting step in determining the rate and extent of absorption and thus *in vitro* measurements on disintegration and dissolution provide a rapid, sensitive and reproducible means to study and assess the bioavailability without involvement of human experimentation [Jollow, *et al.*, 1972].

1.5.1.1. Disintegration test method of assessing bioavailability

The early attempts to establish an indicator of drug bioavailability focused on disintegration as the most pertinent *in vitro* parameter. It is true that a solid dosage form must disintegrate before significant dissolution and absorption can occur, meeting the disintegration test requirements. However, this only ensures that the tablet dosage form will break up into sufficiently small particles in a specified length of time. It does not ensure that the rate of dissolution of the drug is adequate to produce suitable blood levels of the active ingredient. Therefore, while the test for tablet disintegration is very useful for quality control purposes in manufacturing, it is a poor index of bioavailability [Rasma, 1999]. Such testing is intended to demonstrate the effective breakup of the solid formulation (tablet) [performance of disintegration after administration]. The test procedure should be described in the pharmacopoeia and requirements and disintegration test specifications for maximum disintegration time should also be included in the general monographs of the pharmacopoeia [EMA, 1999, TGA, 1989].

1.5.1.2. Dissolution test for assessing bioavailability

Dissolution test is currently considered to be the sensitive and reliable *in vitro* parameter most likely to correlate with bioavailability. Since dissolution of a dosage form *in vivo* is often a rate-limiting factor determining the physiologic availability of a drug, measurement of the *in vitro* dissolution rate is more likely to offer a meaningful indication of physiologic availability. Dissolution testing is implemented in the assessment and evaluation of the release rates and bioavailability of a variety of conventional tablets. It is believed that if a tablet does not dissolve properly in *in-vitro* test, it certainly will not do so in *in-vivo* tests for dissolution [Banakar, 1992]. This could be evidenced by a study by Brandau and Wehnert in their studies on dissolution and availability of phenytoin preparations. The study indicated that there was a correlation between the *in vitro* dissolution and the bioavailability of the preparations. The research showed that the results of the *in vitro* test gave good information about bioavailability [Brandau, *et al.*, 1979].

1.5.1.2.1. Official dissolution methods

Dissolution tests provide an indication for differences in *in-vivo* absorption characteristics of the drug and serve as a secondary standard to detect dosage forms with a potential for poor bioavailability. It involves official methods. There are two official USP dissolution methods:

Apparatus-1 (Basket method) and Apparatus-2 (Paddle method) (Figure 1.5 and 1.6). Carbamazepine and Phenobarbital tablets dissolution study is carried out using Dissolution apparatus II. Another official method is USP apparatus-3, which has been evaluated to produce similar dissolution profiles to USP apparatus-2 [USP, 2004, Lawrence, *et al.*, 2002].

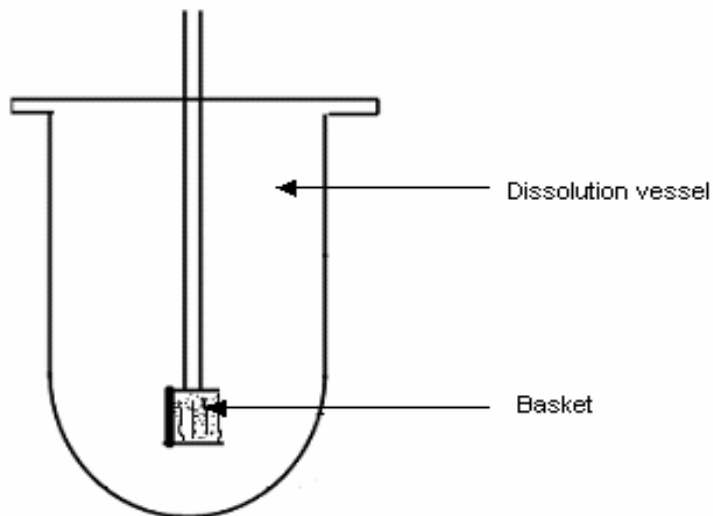


Figure 1.5: Schematic representation of dissolution Apparatus I (Basket method)

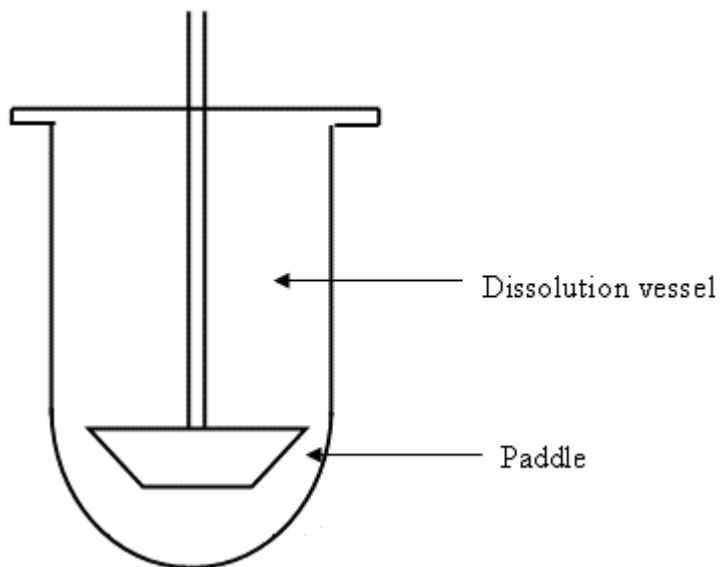


Figure 1.6: Schematic representation of dissolution Apparatus II (Paddle method)

1.5.1.2.2. Limitations of dissolution testing

Limitation with dissolution testing could be problems, which make *in vitro* dissolution correlation with *in vivo* bioavailability difficult. These could be related firstly to instrument variance and the absence of a standard method. There are tests described in the USP, but a few of the large number of dissolution methods are proposed to predict bioavailability. Since dissolution rate of a dosage form is dependent on the methodology used in the dissolution test, changes in the apparatus, dissolution medium, etc can dramatically modify the results. Another significant problem is related to the difference between the *in vitro* and *in vivo* environment in which dissolution occurs. *In vitro* studies are generally carried out under controlled conditions in one, or perhaps two, standardized solvents. The *in vivo* environment, the gastrointestinal tract, on the other hand, is a continuously changing complex environment. There are many variables, which can affect the dissolution rate of a drug in the gastrointestinal tract, including pH, enzyme secretions, surface tension, motility, presence of other substances and absorption surfaces. Thus, drugs frequently dissolve in the body at rates quite different from those observed in an *in vitro* test situation. Most of the official dissolution tests tend to be acceleration dissolution tests, which bear limited or no relationship with *in vitro* dissolution [Banakar, 1999].

1.5.2. *In vitro-in vivo* correlations

When dissolution tests are utilized to assess possible differences in bioavailability of pharmaceutical products, the main requirement is a high degree of correlation between *in vitro* and *in vivo* methods and the selectivity of the method to reject poor products but not acceptable ones. It will be much more difficult to find general methods that will be applicable to all products as a compendial standard to assure comparable performance of many different brands of drugs than a method that can be used by a particular manufacturer for his product alone [Banakar, 1992].

There are, thus two important characteristics of a given drug dissolution method: the sensitivity of the method to detect differences and the correlation of observed differences to those in *in-vivo* bioavailability. The sensitivity and extent of correlation between *in vitro* dissolution methods and *in vivo* absorption parameters will depend on the complex inter-relationship between the

numerous variables of the drug and the dosage form, the apparatus and experimental conditions of the *in vitro* method and the mechanism of absorption in the species tested [Banakar, 1999].. Various literature reviews indicated that there are good *in vitro/in vivo* correlations with drugs like nitrofurantoin, chlorothiazide, methenamine, and carbamazepine tablets with the pharmacokinetic data taken for all the products in healthy volunteers [Karasulu, *et al.*, 1996, Yau, *et al.*, 1981, Gladigau, *et al.*, 1978, Lake, *et al.*, 1999].

This work is initiated to investigate the Comparative Quality Assessment Studies which depends on Identification and Evaluation of Physical Properties and *In vitro* Dissolution Profiles of Different Products of the Commonly Prescribed Antiepileptic Drugs: Carbamazepine and Phenobarbital tablets in Various Drug Retail Outlets in Addis Ababa.

1.5. Objectives

1.5.1. General Objectives

To assess the quality of imported and locally manufactured antiepileptic drugs by confirming whether they comply with the Pharmacopoeial specifications or not and determine the level of substandard products within the distribution chain.

1.5.2. Specific Objectives

The specific objectives of the study are:

- To identify the active pharmaceutical ingredient of different formulations of the commonly prescribed antiepileptic drugs: Carbamazepine and Phenobarbital tablets.
- To evaluate the physical properties [hardness, friability, uniformity of dosage form (weight variation or content uniformity)] of the different tablets.
- To evaluate and compare disintegration time of the tablet formulations.

- To assay and quantity of Active Pharmaceutical Ingredient (API) in different products the tablets.
- To study the dissolution profiles of the tablets of different manufacturers.

2.1. Materials and Methods

2.1.1. Materials

2.1.1.1. Tablets and reference standards

The following tablets of Carbamazepine and Phenobarbitone were purchased from drug retail outlets in Addis Ababa [Table 2.1 and Table 2.2].

Table 2.1 Carbamazepine 200mg tablets

<i>Brand</i>	<i>Manufacturer</i>	<i>Batch No</i>	<i>Mfg. date</i>	<i>Exp. date</i>	<i>Country</i>	<i>Local agent</i>
Tegretol®200mg	Novartis pharma AG	105	10/2002	10/2007	Switzerland	Ageca
Tegral®200mg	Chemical Industries Development	T2094	07/2003	07/2007	Egypt	Ramada Trading
Taver®200mg	Medochemie Ltd, Limassol	A8C014	03/2004	03/2009	Cyprus	Pharmid

Table 2.2 Phenobarbitone 100mg tablets

<i>Generic name</i>	<i>Manufacturer</i>	<i>Batch No</i>	<i>Mfg. date</i>	<i>Exp. date</i>	<i>Country</i>	<i>Local agent</i>
Phenobarbitone BP 100 mg	Epharm	406285-2	06/2004	06/2007	Ethiopia	Pharmid
Phenobarbitone BP 100 mg	Cadila Pharmaceuticals	E2005	11/2002	10/2006	India	Pharmid
Phenobarbitone BP 100 mg	East Africa Pharmaceuticals PVT. Ltd. Co.	20/091	01/2002	01/2005	Ethiopia	Pharmid

The USP reference standard of phenobarbital and working standard of carbamazepine were kindly donated by Ethiopian Pharmaceutical Manufacturing Company (Epharm) and Ethiopian Drug Administration and Control Authority (DACA), Department of Drug Quality Control and Toxicology, respectively.

2.1.1.2 Solvents / chemicals/ reagents

Acetone (TECHNO PHARMACHEM[®], 231295, India) Ammonia (Reidel-de Haen AG, 05002, Germany), Ethanol (Alpha CHEMIKA[®], Product No 04856, India), Cobalt (II) chloride (PROLABO[®] Products, No 22.896, France), Nitric acid (ANALYTICALS CARLO ERBA, Code 408071, Italy), Sulfuric acid (ANALYTICALS CARLO ERBA, Code 410306, Italy), Sodium Nitrate (MERCK 7464527, Germany), Methanol (SIGMA-ALDRICH LABO CHEMIKALIEN GmbH, Germany), Sodium lauryl sulphate (Riedel-deHaen[®], 62862 SIGMA-ALDRICH LABO CHEMIKALIEN GmbH, Germany), Boric acid [LOBA CHEMIE PVT Co., Batch No 59264, India], Sodium carbonate(anhydrous) (TECHNO PHARMACHEM, 231265, India), Silver nitrate (BDH, General Purpose reagent, Product 30087, England), Potassium chloride [BDH AnalaR[®], England], Sodium hydroxide (LOBA CHEMIE PVT Co., Batch No 59265, India) , Sodium Chloride (AVONDALE LABORATORIES, 930102, England)

2.2.1.3. Instruments

Melting Point Apparatus (STAURT SCIENTIFIC Melting Point Apparatus SMP3, United Kingdom), Hardness Tester (CALEVA[®] THT-2, British), Friability Tester (ERWEKA[®] TAR 20, Germany), Disintegration Tester (CALEVA[®] DISTIL 1.14, British), Analytical Balance (AB204-S[®] and WAGTECH[®] AAA250L, England; Sartorius CP224S, Germany), UV Spectrophotometer (CECIL[®] CE 1021 Spectrophotometer, England and SPECTRONIC[®] GENESYS[™] 5 Spectrophotometer, New York), Automatic Potentiometric Titrator (METRHOM[®] 799 GPT Titrino, Switzerland), Dissolution Apparatus (CALEVA[®] 10ST Dissolution Tester, British and PHARMA TEST Dissolution Tester PTWII, Germany), pH meter (SCHOTT[®] CG843P Laboratory pH meter, Germany), Vacuum Oven (JOUAN[®] Vacuum Oven E-50, Germany).

2.1.2. Methodology

The following quality parameters were evaluated using official and non-official methods [BP 2001, IP 2003, USPXXVII, Clarke, 1986].

2.1.2.1. Identification tests

a) Carbamazepine

The identification test for carbamazepine tablets was performed according to British Pharmacopoeia (BP) 2001 that is summarized as follows. A quantity of powdered tablet containing 0.2 g of carbamazepine was boiled with 15 ml of acetone. The hot solution was filtered and the filter was washed with two 5 ml quantities of hot acetone. The combined filtrates was evaporated to 5 ml and cooled in ice. The crystals were dried in an oven by keeping the temperature at 70°C and a pressure of 2 kpa for 30 minutes. Then the melting point of the crystals was taken in a melting point apparatus. About 0.1 g of these crystals was heated with 2 ml of nitric acid in a water bath for 3 minutes and the colour formed was observed and recorded. On the other hand, the fluorescence of the powdered tablets was observed under ultraviolet light at 366 nm, according to the BP specification. The UV spectrum of the solution of powdered tablets prepared in the same procedure was also taken as a means of identification [BP, 2001].

b) Phenobarbital

Phenobarbital identification test was performed according to the specification in The International Pharmacopoeia (IP) 2003. 10 ml of dehydrated ethanol was added to a quantity of powdered tablets equivalent to about 0.4 g of Phenobarbital, and the mixture was shaken and filtered. The filtrate was evaporated to dryness and the residue was dried at 105°C for 1 hour in an oven to be utilized for identification tests. The melting point of the residue was taken and 20mg of the residue was dissolved in 5 ml methanol. One drop of Cobalt (II) Chloride and 3-4 drops of ammonia were added to the solution and the colour produced was observed and recorded. On the other hand, 2 ml of sulfuric acid and 20 mg of sodium nitrate were added to 0.2 g of the residue and allowed to stand for 30 minutes. The colour produced was taken as one of the identification test requirements [IP, 2003].

Ultraviolet spectrophotometric spectrum scan of the Phenobarbital tablets was taken according to the procedure indicated in Clarke's Isolation and Identification of drugs, which provides that Phenobarbital in 1M NaOH (pH=13) has $A(1\%, 1\text{cm})=342$ at a wavelength maximum λ_{max} of 254 nm. Based on this fact, and considering the UV spectrophotometric linearity range (0.4-0.5),

1M Sodium hydroxide (NaOH), pH=13, was prepared and the tablets powder equivalent to 7.5mg Phenobarbital was dissolved in 25 ml of the prepared sodium hydroxide solution. The mixture was filtered quantitatively and made upto volume in a 50 ml volumetric flask. 5ml of this solution was taken and diluted to 50ml. The final diluted solution UV spectrophotometric spectrum was then taken [Clarke, 1986].

2.1.2.2. Tablet hardness and friability

Six tablets were individually placed carefully in a hardness tester and the degree of force required to break the tablets was recorded from the resulting visual reading display screen of the instrument [Howard, *et al.*, 1999].

Twenty tablets from each product (brand and/or generic) were dedusted and weighed initially before undergoing friability test on analytical balance. The tablets were then placed in the drum of the friability tester and rotated at 25 revolutions per minute (100 times). The tablets from each product batch were dedusted and reweighed. The percent loss of total weight was calculated [James, 1996].

2.1.2.3. Dosage form uniformity test (weight variation and content uniformity)

The uniformity of dosage units was demonstrated according to USP by weight variation and/or content uniformity.

For the determination of dosage form uniformity by weight variation, 10 tablets from each product were individually weighed, and the average weight was calculated. The content of the active ingredient in each of the ten tablets was calculated from the results of the assay obtained as directed in the individual monographs assuming homogeneous distribution of the active ingredient. The percent weight variation and standard deviations [standard deviation (SD) and relative standard deviation (RSD)] were calculated.

The determination of dosage form uniformity by content uniformity tests were performed on ten individual tablets from each product that contain an active ingredient less than 50% by weight of the tablets [Phenobarbital (Cadila) and Phenobarbital (East Africa Pharmaceuticals PVT. Ltd.

Co.)). Each of the ten tablets of the two products were powdered and dissolved in 20ml methanol and 7.5 ml of freshly prepared solution of 30 mg of anhydrous sodium carbonate per ml. The Phenobarbital content was determined potentiometrically by titrating with 0.1M silver nitrate solution (International Pharmacopoeia (IP) 2003). The percent of drug contained in each tablet relative to the labeled potency (100mg) was calculated and checked if it was within the range specified in USP XXVII (85-115%).

2.1.2.4. Disintegration test

The mean disintegration times of six tablets from each product were determined using CALEVA[®] disintegration apparatus (CALEVA[®] Distil 1.14). The disintegration media for all the products comprised of distilled water maintained at 37±1°C. Tablets were considered completely disintegrated when all particles passed through the wire mesh.

2.1.2.5. Assay of the tablets

a) Carbamazepine

For the assay of the carbamazepine tablets BP 2001 was used. Twenty tablets from each brand product were weighed and powdered. A quantity of the powder containing 60 mg of carbamazepine was boiled with 25 ml of 96% ethanol for a few minutes. The hot mixture was stirred in a closed flask for 10 minutes and filtered through sintered glass. The flask and the filter were washed with 96% ethanol and sufficient 96% ethanol was added to the cooled filtrate to produce 100 ml. 5 ml of this solution was diluted to 250 ml with 96% ethanol and the absorbance of the resulting solution was measured using an ultraviolet spectrophotometer (SPECTRONIC[®] GENESYS[™] 5 Spectrophotometer, New York) at the wave length maximum, λ_{\max} , of 285 nm. Then the content of carbamazepine C₁₅H₁₂N₂O was calculated taking 490 as the value of A (1%, 1cm) at the λ_{\max} of 285 nm [BP 2001].

b) Phenobarbital

The assay of Phenobarbital tablets was performed in accordance with IP 2003. Twenty tablets from each generic products of Phenobarbital were weighed and powdered. 40 ml methanol and 15 ml of a freshly prepared solution of 30 mg of anhydrous sodium carbonate per ml was added to a quantity of powdered tablets equivalent to about 0.2 gram of Phenobarbital. The solution

was then titrated with 0.1M volumetric solution of silver nitrate, which was initially standardized with sodium chloride according to the BP 2001, determining the end point potentiometrically.

Standardization of 0.1M silver nitrate solutions

About 17.0 gram of silver nitrate was dissolved in distilled water in a 1L volumetric flask and sufficient distilled water was added to make up to volume so that the expected 0.1M silver nitrate solution was to be obtained. To ascertain the exact concentration of silver nitrate solution, 0.1 gram of sodium chloride (AVONDALE LABORATORIES, 930102, England) was dissolved in 30 ml of distilled water and titrated with the silver nitrate solution determining the end point potentiometrically.

2.1.2.6. Dissolution studies

The dissolution rates of the tablets (carbamazepine and Phenobarbital) were determined according to USP XXVII specifications. The dissolution medium consisted of 900 ml of 1% sodium lauryl sulfate in a thermostatically controlled water bath at $37\pm 0.5^{\circ}\text{C}$ that was being stirred at 75 rpm for the different brands of carbamazepine tablets using dissolution apparatus II (PHARMA TEST Dissolution Tester PTWII, Germany). For the different generic products of Phenobarbital tablets the dissolution apparatus II (CALEVA[®] 10ST Dissolution Tester, British) was used. The medium consisted of 900 ml distilled water in a thermostatically controlled water bath at $37\pm 0.5^{\circ}\text{C}$ that was being stirred at 50 revolution per minute.

The amount of carbamazepine and Phenobarbital released from the respective tablet products put in dissolution media were determined by sample withdrawal at different times. Samples (5 ml) were withdrawn after 5, 10, 15, 20, 30, 45, and 60 minutes for Phenobarbital tablets while an additional sample was taken further at 75 minutes for carbamazepine tablets, and an equivalent amount of water and 1% sodium lauryl sulfate solution were immediately introduced, respectively, as replacement. The samples were filtered and suitably diluted with 1% sodium lauryl sulfate for carbamazepine samples and freshly prepared alkaline borate buffer (pH=9.6) for Phenobarbital tablet samples for the assay of drug contents.

The assay for carbamazepine released from the tablets was performed by measuring the absorbance at 288 nm using SPECTRONIC® GENESYS™ 5 Spectrophotometer, New York Spectrophotometer. 1% sodium lauryl sulfate was used as a blank and the necessary correction for dilution was made when calculating the amounts of drug released. The drug content was calculated using comparison method with the reference standard of carbamazepine.

Amount of Phenobarbital released from the tablets was assayed by measuring absorbance at 240 nm using CECIL® CE 1021 Spectrophotometer (England). Alkaline borate buffer (pH=9.6) was used as blank and drug content calculation was made by comparison with USP Phenobarbital reference standard and making all the necessary correction for dilution.

Preparation of alkaline borate (pH=9.6) buffer

Boric acid and potassium chloride solution, 0.2M, was initially prepared by dissolving 12.37 gram of extra pure boric acid (H_3BO_3) [LOBA CHEMIE PVT Co., Batch No 59264, India] and 14.91 gram of potassium chloride (KCl) [BDH AnalaR®, England] in distilled water, and diluted with water to 1000 ml. Secondly, 0.2M sodium hydroxide solution was prepared by dissolving 8 gram of extra pure sodium hydroxide (NaOH) [LOBA CHEMIE PVT Co., Batch No 59265, India] pellets in 1000 ml of distilled water. Then, 50 ml of the boric acid and potassium chloride solution (0.2M) was placed in a 200 ml volumetric flask. 36.9 ml of sodium hydroxide solution (0.2M) was added and made upto volume with distilled water to produce an alkaline borate buffer (pH=9.6). The pH was checked using pH meter.

2.2. Data Analysis

The data were analyzed using Microsoft Excel-2000, GraphPad InStat Demo- [DATASET-1SD] and Origin® Graphing and Scientific Software and comparison was made between content uniformity, assays, and dissolution profiles of the tablets using GraphPad InStat Demo- [DATASET-1SD].

3.1. Identification test

The ultraviolet Spectrophotometric spectrum of the investigated tablets (Carbamazepine and Phenobarbital) showed absorption maxima as indicated in Table 3.1.

Table 3.1 Absorption maxima [λ_{\max}] of Carbamazepine and Phenobarbital tablets

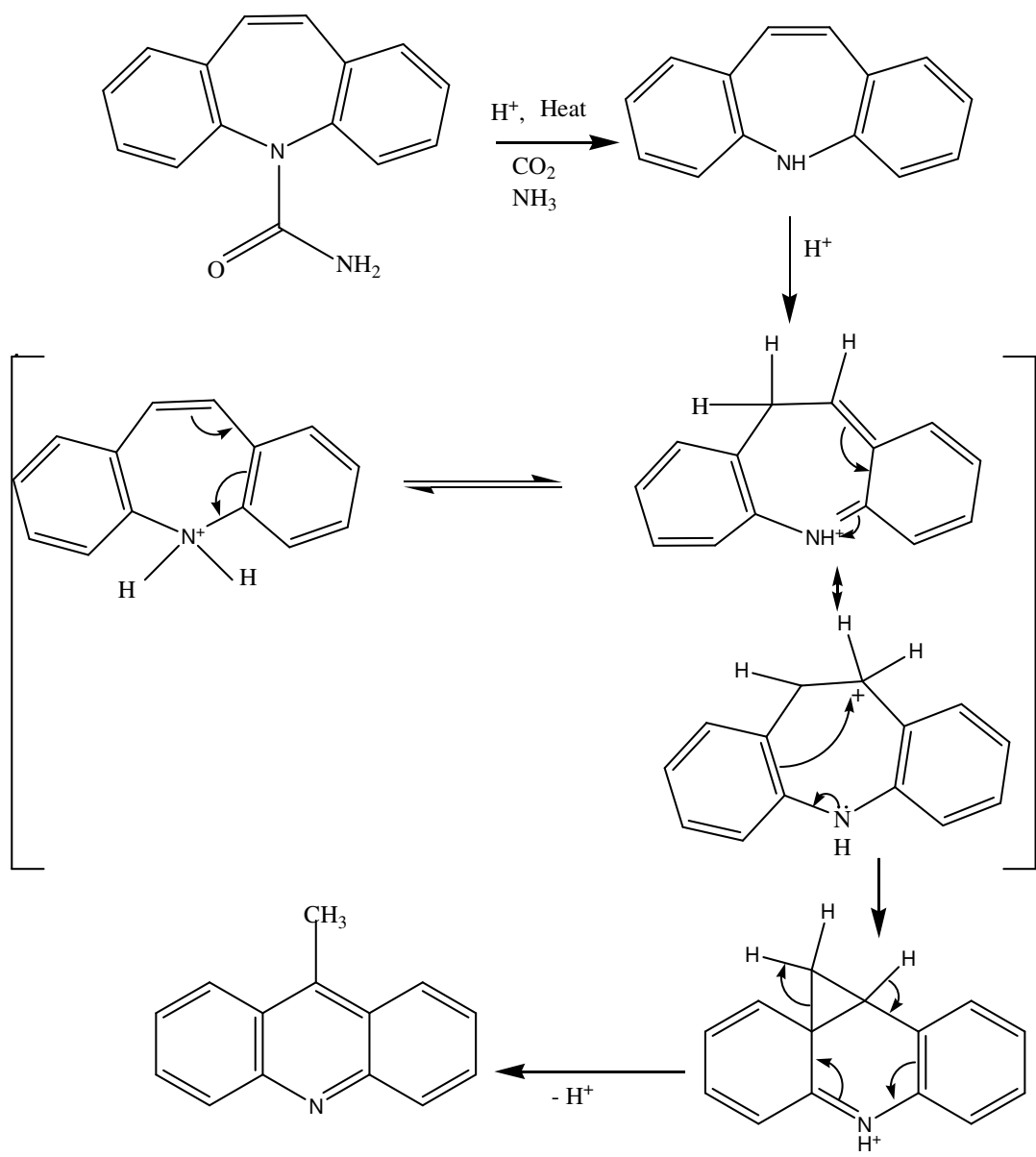
Drugs	Carbamazepine tablets [Conc. 0.01 mg/ml]				Phenobarbital tablets [Conc. 0.015 mg/ml]			
	Tegretol	Tegral	Taver	Ref.	PhB, Epharm	PhB, Cadila	PhB, East Africa	Ref.
Absorption λ_{\max} , nm	285	285	286	285	256	256	256	256

Two of the carbamazepine tablets, Tegretol and Tegral, have shown absorption λ_{\max} at 285 nm and Taver at 286 nm. All the Phenobarbital tablets showed absorption maxima at λ_{\max} of 256. All the tablets pass the UV Spectrum identification test as all are in the range $\lambda_{\max} \pm 2\text{nm}$ (BP 2001). The UV spectrum of the tablets is annexed.

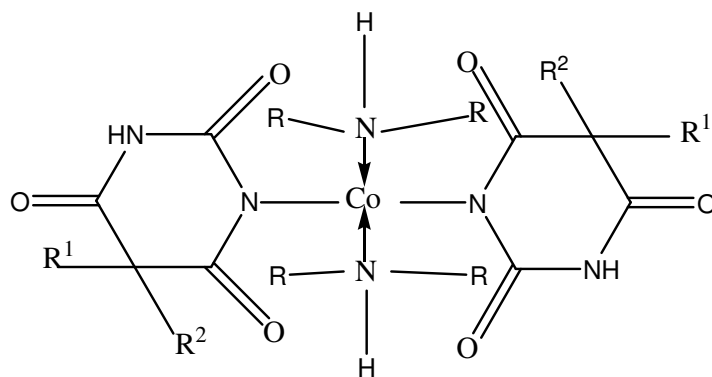
The melting points of the tablets are indicated in Table 3.2. All of the investigated tablet formulations pass their respective melting point specifications, 191°C for carbamazepine and 174°C for Phenobarbital tablets according to BP 2001 and IP 2003, respectively.

The powdered tablets of carbamazepine tablets when seen under ultraviolet light at the wavelength of 365 nm, all the three investigated brands show intense blue fluorescence complying with the BP 2001 specification requirement. Upon reaction with nitric acid all the three carbamazepine (Tegretol, Tegral and Taver) tablet formulations analyzed gave orange red colour, which is also in accordance with the BP specification.

Structural specific identity reaction depends on heating an acidic solution (HCl or HNO₃) resulting in the formation of 9-Methylacridine as the final product. The reaction involves rearrangement of the molecule after hydrolysis of the coloured carbamate moiety as illustrated in the following scheme:



One of the known identification methods of barbituric acid derived drugs like Phenobarbital is what is known as Zwikker reaction. The reaction depends on treatment of the respective barbituric acid derivatives with alkaline solution of Co (II) salts. A coloured complex will be formed, having the general formula $(Barb)_2Co$ and the chelate is stabilized as solvate or diamine complex. BP and IP have adopted this method on the basis of its high sensitivity and relative simplicity. The coloured complex is illustrated in the following scheme [Roth, *et al.*, 1981]:



For all the three tablets of Phenobarbital violet colour was produced upon addition of methanol, cobalt chloride and ammonia solutions. Yellow colour was produced with the reaction with sulfuric acid and sodium nitrate, which is satisfying the IP 2003 specification.

Table 3.2 Melting point of Carbamazepine and Phenobarbital tablets

Drugs		Melting Point Reading (°C) [n = 5]
Carbamazepine tablets	Tegretol	190.8 ± 0.1
	Tegral	190.7 ± 0.1
	Taver	190.7 ± 0.2
Phenobarbital tablets	PhB, Epharm	173.5 ± 0.1
	PhB, Cadila	173.6 ± 0.2
	PhB, E. Africa	173.5 ± 0.2
References	Carbamazepine working standard	190.9 ± 0.1
	PhB USP reference standard	173.8 ± 0.2

In general, all the tablets analyzed, carbamazepine (Tegretol, Tegral and Taver) and Phenobarbital (Epharm, Cadila and East Africa), passed the BP 2001 and the IP 2003 identification tests, respectively.

3.2. Hardness and Friability Test

The mean hardness values of the tablets of carbamazepine and Phenobarbital in this study are shown in Table 3.3. It is indicated that for the carbamazepine tablets studied the lowest hardness is that of Tegretol which is 60.7 (\pm 2.0) N, and the highest is that of Tegral (103.5 \pm 9.4 N). Taver showed a hardness of 89.2 \pm 6.6 N. The hardness test for the Phenobarbital tablets indicates maximum value of 84.7 \pm 11.6 N for the East African pharmaceutical Pvt. Ltd. Company product and a value of 65 \pm 7.2N for the Epharm product. The Cadila product Phenobarbital tablet gave an average hardness value of 50.5 \pm 7.8N.

Similarly, the weight loss of the tablets, after friability test expressed as percent friability, is also indicated in Table 3.3. The lowest percent friability for the carbamazepine tablets has been obtained for Taver (0.07) while Tegretol and Tegral percent friability are 0.47 and 0.51, respectively.

Table 3.3 Summary of hardness and friability tests of the tablets

Tablet name		Hardness (N*) [\pm RSD]	Friability F%
Carbamazepine tablets	Tegral	103.5 (9.4)	0.5126
	Tegretol	60.7 (2.0)	0.4688
	Taver	89.2 (6.6)	0.0740
Phenobarbital tablets	Phenobarbitone (East Afr.)	84.7 (11.6)	0.2306
	Phenobarbitone (Epharm)	65.0 (7.2)	0.1722
	Phenob. (Cadila)	50.5 (7.8)	0.2942

*N: Newton

The percent friability of the Phenobarbital tablets analyzed is 0.17 for the Epharm product, and 0.23 and 0.29 for the East African Pharmaceuticals product and the Cadila product, respectively.

Usually, tablets require a certain degree of strength and resistance to friability to withstand mechanical shocks of handling during manufacturing, packaging, shipping, storage and finally consumption by the patient. Adequate tablet hardness as well as reasonable friability is the necessary requisites for a consumer acceptance. For conventional compressed tablets, a hardness of 50N is the minimum requirement for a satisfactory tablet product and must not lose more than 1% of its weight after friability test. Limits of < 1% friability are often set, but < 0.1% is a realistic goal (James, 1996).

Phenobarbital tablet of Cadila product hardness is very close to the lower limit 50N. The % friability of Taver is 0.07, which is <0.1%, an ideal specification limit. Generally, all the studied tablets passed the hardness and friability test specification of tablet dosage forms [BP 2001].

3.3. Disintegration test

Carbamazepine and Phenobarbital tablets, like any other solid dosage forms, need to disintegrate in the gut and go into solution before they are completely absorbed. Disintegration, therefore, is an important process in making the drug available for absorption. The official requirement in the British Pharmacopoeia disintegration test is that uncoated tablets should disintegrate in less than 15 minutes (BP 2001). As shown in Table 3.4, the disintegration times for the studied Carbamazepine tablets are: Tegretol (1.0 ± 0.0 minute), Tegral (4.5 ±1.5 minutes) and Taver (4.3 ± 0.5 minutes) and tablets are: Phenobarbital EPHARM (2.8±0.4minutes), Phenobarbital East

Table 3.4 Disintegration time (minute) of Carbamazepine and Phenobarbital tablets

Drugs	Disintegration time (minute)					
	Carbamazepine tablets (±SD)			Phenobarbitone tablets (±SD)		
	Tegral	Tegretol	Taver	East Africa	Epharm	Cadila
Average	4.5 (1.5)	1.0 (0.0)	4.3 (0.5)	4.8 (0.8)	2.8 (0.4)	2.3 (0.5)

that of Phenobarbital East African Pharmaceuticals (4.8 ± 0.8 minutes) and Phenobarbital Cadila (2.3 ± 0.5 minutes).

As evident from the results, all the tablets pass the BP specification. The results indicate that disintegration time for carbamazepine tablets increases in the order Tegretol (1.0 minute), Taver (4.3 minutes) and Tegral (4.5 minutes) while that of Phenobarbital tablets increases in the order of Cadila (2.3 minutes), Epharm (2.8 minutes) and East African Pharmaceuticals (4.3 minutes). The values from the hardness test of the tablets also increase in the same order: Tegretol (60.7N), Taver (89.2N) and Tegral (103.5N) for carbamazepine tablets; and Cadila (50.5N), Epharm (65.0N) and East African Pharmaceuticals (84.7N) for Phenobarbital tablets. Thus, the results of the study indicate that there is direct relationship between disintegration time and hardness.

3.4. Weight variation and Dosage unit uniformity

3.4.1. Weight variation

As illustrated in Table 3.5, the average weight of the tablets varies from product to product. For Carbamazepine tablets, the average weight is 256.8 mg (Taver), 276.2 mg (Tegral) and 279.2 mg (Tegretol) where the official standard strength for all the three products is 200 mg. Similarly, for the Phenobarbital tablets the average weight is 152.5 mg (Epharm), 203.4 mg (East Africa Pharmaceutical) and 214.2 mg (Cadila). The variations in average weights of the tablets could be accounted due to the use of different kinds and/or amounts (varying proportions) of excipients in the tablets investigated. In case of the analyzed brands of carbamazepine tablets analyzed, the weight variation may be because of the use of different kinds/varying proportion of excipients by manufacturers. For Phenobarbital tablets, all of the products analyzed are generic, which means the different manufacturers used the same excipient and formula to manufacture the products. However, they may use varying proportions of the same kind of excipient or there may be variations in Good Manufacturing Practices (GMP), which possibly contribute to the weight variations. The GMP influences especially processing and manufacturing variables that have direct or indirect effect on the tablets weight and responsible for the weight variations.

The percent mean deviations (RSD) for the products tested is less than 1% for the three carbamazepine tablets and less than 4% for the Phenobarbital tablets analyzed. The compendial

Table 3.5 Weight variation and dosage uniformity of carbamazepine and Phenobarbital tablets

Drugs		a	b	c
Carbamazepine tablets	Taver	256.8 (1.0)	99.7 (1.0)	-
	Tegral	276.2 (0.8)	90.7 (0.8)	-
	Tegretol	279.2 (0.5)	95.9 (0.5)	-
Phenobarbital tablets	Epharm	152.5 (3.8)	94.1 (3.8)	-
	E. Africa	203.4 (2.1)	97.2 (2.1)	95.1 (2.5)
	Cadila	214.2 (1.3)	91.1 (1.3)	90.9 (4.1)

Key: a) Tablet average weight, mg (\pm RSD)

b) %content (\pm RSD) from weight variation method

c) %content (\pm RSD) from content uniformity method.

specification for uniformity of weight is that the weight of each of the tablets analyzed should not deviate from the average value by more than 5.0% (USP XXVII). This implies that all the tablets comply with USP XXVII specifications for weight variation. The relatively small weight variations indicate reasonable control system in the GMP of the manufacturers.

Statistical comparison of the weights of the three brands of the carbamazepine tablets and the three generic products of Phenobarbital tablets indicate that at 95% confidence interval there is significant difference in the weight of Tegretol and Tegral, East Africa Pharmaceutical Phenobarbital and Epharm Phenobarbital, and Epharm and Cadila Phenobarbital ($P < 0.05$). Extremely significant differences have been observed for the weights of Tegretol and Taver, Tegral and Taver, and East Africa Pharmaceutical Phenobarbital and Cadila Phenobarbital

($P < 0.001$). The statistical results indicate that there are variations between individual tablets of the products investigated.

3.4.2. Dosage unit uniformity

The uniformity of dosage units can be demonstrated by either of the two methods, weight variation tests or content uniformity test. For tablets of high dose drugs (pharmaceutical products like tablets, capsules, etc., which contain 50 mg or more of an active ingredient comprising 50% or more, by weight, of the dosage form unit), weight variation test is recommended by the USP XXVII, as an effective means of assuring uniform potency. For tablets of low dose drugs (tablet formulations containing less than 50 mg of an active ingredient comprising less than 50% by weight of the dosage form), excipients make up the bulk of the tablets weight. In this case, therefore, content uniformity test has to be performed. According to USP XXVII specification, the requirements for dosage form uniformity are met if the amount of the active ingredient in each of the ten dosage units as determined from the weight variation or content uniformity method lies within the range of 85.0% to 115.0% of the labeled claim and the relative standard deviation (RSD) less than or equal to 6.0%.

The percentage contents of different brands of carbamazepine tablets and different generic products of Phenobarbital tablets calculated using weight variation and/or content uniformity method is indicated in Table 3.5. The percentage content according to the weight variation method was calculated for all of the tablets as they contain 200 mg (carbamazepine tablets) and 100 mg (Phenobarbital tablets), both of which contain more than 50 mg weight. The percentage contents for the carbamazepine tablets are Tegral (89.9-91.5%), Tegretol (95.4-96.4%), and Taver ((98.7-100.7%); and that of Phenobarbital tablets are East Africa Pharmaceuticals Phenobarbital (95.1-99.3%), Epharm Phenobarbital (90.4-97.9%), and Cadila Phenobarbital (89.8-92.4%). These results indicate that all the tablet products analyzed pass the USP XXVII specification requirements for dosage form uniformity by weight variation.

In order to achieve the relatively wider content uniformity range (i.e. 85.0-115.0% of the labeled potency) for potent low dose drugs, it needs special effort to uniformly distribute them throughout the powder mixture, to avoid segregation or drug excipient separation during the

manufacturing process or to control all the variables that bring about tablet weight variation. These variables could be utilized as a quality control parameter so that the marketed pharmaceutical products should fulfill the requirements.

To check whether or not weight variation method is sufficient to assure dosage form uniformity, the claimed potency, 200 mg for the carbamazepine tablets and 100 mg for the Phenobarbital tablets, was divided for the weight of each tablet. The results showed that for tablets of East Africa pharmaceuticals Phenobarbital (49.2 %) and Cadila Phenobarbital (46.7 %), the proportion of active ingredient to the tablet weight was found to be less than 50 % (Table 3.6). Thus, content uniformity was utilized for further dosage unit uniformity test, as indicated in Table 3.5. The percentage contents calculated using content uniformity method for these tablets were Cadila Phenobarbital (86.8-95.0 %) and East Africa pharmaceuticals Phenobarbital (96.2-95.0 %). These results reveal that content uniformity has higher strength than weight variation in determining dosage form uniformity as lower percentage content ranges for content uniformity (86.8-95.0 %) when compared to the weight variation result (89.8-92.4 %) for Cadila Phenobarbital were obtained. Even at these lower ranges both the tablets also pass the requirements for dosage form uniformity using content uniformity method.

Table 3.6 Percent claim potency of the active ingredient per tablet weight of Carbamazepine (200mg) and Phenobarbital (100mg) tablets

Tablet type	Carbamazepine tablets			Phenobarbital tablets		
	Tegral	Tegretol	Taver	East Africa	Epharm	Cadila
Average weight, mg	276.2	279.2	256.8	203.4	152.5	214.2
%Claim potency per tablet weight	72.4	71.6	77.9	49.2	65.6	46.7

Statistical analysis of the percentage contents obtained from the tablets analyzed by both weight variation method and content uniformity method indicate that there has been extremely significant differences between the percentage contents calculated using weight variation method from the three brands of carbamazepine tablets as well as between that of the three generic

products of Phenobarbital tablets. The percentage content using content uniformity test for the two generic products: Cadila and East Africa Pharmaceuticals was compared using unpaired t-test and found to have very significant difference ($P=0.0082$) at 95% confidence interval. The results of the percentage contents of both methods (weight variation and content uniformity) for these two products were also compared and found that there is no significant difference ($P=0.9014$) for the Cadila product while significant difference was obtained for the percentage contents of East Africa Pharmaceuticals Phenobarbital ($P=0.0453$). The statistical results indicate that the products analyzed are not equivalent with respect to dosage form uniformity.

3.5. Assay of the tablets

Assay of active ingredients in pharmaceutical products is possible using different instrumental methods. For the assay of the investigated products, carbamazepine and Phenobarbital tablets, different official Pharmacopias set techniques like gravimetric analysis, uv spectrophotometric analysis, potentiometric titration and the like. In this study, depending on cost and availability of the instruments, the methods described in BP 2001 and IP 2003 have been chosen. The British Pharmacopoeia states that carbamazepine tablets must contain not less than 95.0% and not more than 105.0% of carbamazepine stated on the label while the International Pharmacopoeia states that Phenobarbital tablets must contain not less than 90.0% and not more than 110.0% of the amount of Phenobarbital of the label claim [BP, 2001, IP, 2003]. The results of analysis of the three brands of carbamazepine tablets and the three generic products of Phenobarbital tablets analyzed according to the BP 2001 and IP 2003 methods, respectively, are represented in Table 3.7. From the results of assay of carbamazepine tablets, Tegretol ($95.9 \pm 0.4\%$) and Taver ($99.5 \pm 1.0\%$) passed the BP specification i.e. they contain the required amount of carbamazepine while Tegral ($91.9 \pm 0.7\%$) fail to pass the specification on percentage content as it has lower percentage than the BP specification limit. The highest content has been achieved for Taver tablets.

The assay results for Phenobarbital tablets indicate that all the tablets analyzed pass the IP specification. The highest percentage content was obtained from the East Africa Pharmaceuticals Phenobarbital product ($98.0 \pm 0.1\%$), while intermediate value and lower value have been

Table 3.7 Assay results of carbamazepine and Phenobarbital tablets [n = 3]

Drugs	Product type	Assay Result, %w/w (\pm RSD)
Carbamazepine tablets, 200mg	Tegretol	95.9 \pm 0.4
	Tegral	91.9 \pm 0.7
	Taver	99.5 \pm 1.0
Phenobarbital tablets, 100mg tabs	Epharm	93.7 \pm 0.1
	East African Pharmaceutical	98.0 \pm 0.1
	Cadila	91.4 \pm 1.0

obtained for Epharm Phenobarbital (93.7 \pm 0.1%) and Cadila Phenobarbital (91.4 \pm 1.0%), respectively.

Table 3.8 Statistical analysis results of assay values of different carbamazepine and Phenobarbital tablets [n = 3]

Drugs		Minimum	Maximun	Mean	Standard deviation	Median
Carbamazepine tablets	Tegretol	95.4	96.5	95.9	0.4	95.8
	Tegral	91.4	92.0	92.0	0.7	91.6
	Taver	98.3	100.7	99.3	0.9	99.5
Phenobarbital tablets	Epharm	93.6	93.8	93.7	0.1	93.8
	East Africa	97.9	98.1	98.0	0.1	98.0
	Cadila	90.4	91.9	91.4	0.9	91.9

P<0.001 for comparison of: Tegretol vs Tegral, Tegretol vs Taver, Tegral vs Taver, Epharm PhB vs East Africa PhB and East Africa PhB vs Cadila PhB and P<0.01 for Epharm PhB vs Cadila PhB at 95% confidence interval.

Statistical analysis of the assay results using GraphPad InStat Demo- [DATASET-1SD] and comparison of the different brands of carbamazepine tablets and the different generic products of Phenobarbital tablets at 95% confidence interval is indicated in Table 3.8. With 95% confidence interval the assay results showed that there is extremely significant difference in percentage content of the three brands of carbamazepine tablets analyzed: Tegretol vs Tegral, Tegretol vs Taver, Tegral vs Taver; and from the generic products of Phenobarbital tablets: East Africa Pharmaceuticals Phenobarbital vs Epharm Phenobarbital, and Africa Pharmaceuticals Phenobarbital vs Cadila phenobarbital ($P < 0.001$). At the same confidence interval (95%), there is very significant difference with $P < 0.01$ between Epharm Phenobarbital and Cadila Phenobarbital.

The statistical analysis as shown above reveals that the different brands of carbamazepine tablets analyzed: Tegretol, Tegral and Taver; and the three different generic products of phenobarbital tablets tested: Epharm, East Africa and Cadila have showed no equivalency with respect their assay values.

3.6. Dissolution studies

3.6.1. Dissolution profile of carbamazepine tablets

The dissolution profile of carbamazepine tablets is depicted in Figures 3.1. The USP specification for the release of carbamazepine from the brands of carbamazepine tablets is that between 45% and 75% of the label claim of carbamazepine should be dissolved in 15 minutes; and not less than 75% of the labeled amount of carbamazepine is dissolved in 60 minutes. From the three brands of carbamazepine tablets analyzed both Tegretol and Taver passed the above USP requirement but Tegral has failed to release its contents in the tolerance limits specified as a dissolution requirement. Tegretol tablet released 58.3% of its content at the 15th minute and 80.6% at the 60th and Taver tablets released 77.6% at the 15th minute and 84.1% at the 60th minute. Tegral tablet has released 43.0% of its content at the 15th minute and 71.6% at the 60th minute. These results are below the tolerance limits at both time references.

The dissolution data of carbamazepine tablets when treated statistically revealed that at 95% confidence interval there is extremely significant difference between the three tablets ($P < 0.05$) at the pharmacopoeially specified times, 45 and 60 minutes.

Table 3.9 Percent drug release of different brands of carbamazepine tablets at different times

Time (minute) of sampling	% Carbamazepine Released		
	Tegretol	Tegral	Taver
0	0	0	0
5	44.8	20.9	34.2
10	51.7	33.4	69.6
15	58.3	43.0	77.6
20	63.2	48.1	80.6
30	70.7	56.7	84.0
45	75.7	64.8	84.0
60	80.6	71.6	84.1
75	82.5	75.4	84.2

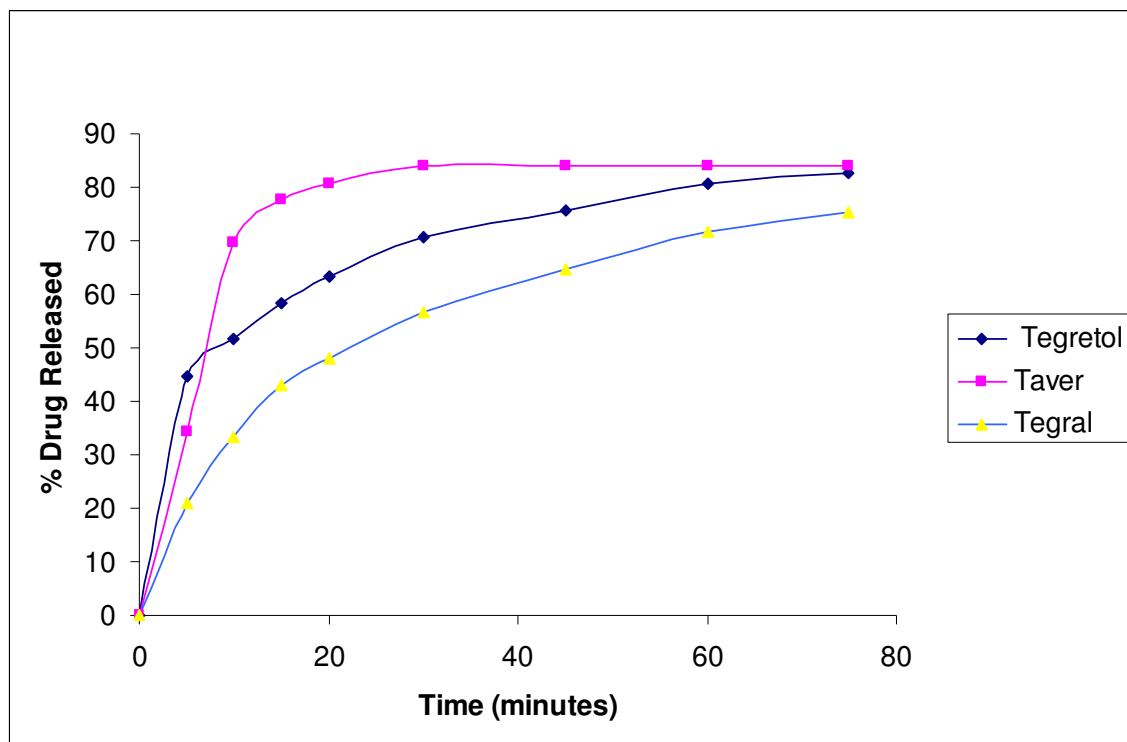


Figure 3.1: Dissolution profiles of different brands of carbamazepine tablets

3.6.2. Dissolution profile of Phenobarbital tablets

The dissolution profile of the different generic products of Phenobarbital tablets are shown in Table 3.10 and Figures 3.2. The USP specification for the release of Phenobarbital from the tablet pharmaceutical products of phenobarbital is that less than 75% of the label claim of phenobarbital should be dissolved in 45 minutes.

From the three generic products of Phenobarbital tablets analyzed it is only the Epharm product which passed the above USP requirement but the East Africa Pharmaceuticals Phenobarbital and the Cadila Phenobarbital have failed to release their contents in the time specified the tolerance limits required as a dissolution requirement. The Epharm product Phenobarbital tablet released 87.0% of its content at the 45th minute, which is in the requirement range. The East Africa Pharmaceuticals Phenobarbital tablet and the Cadila Phenobarbital tablet have released 61.7% and 19.3%, respectively, at the 45th minute, which are results below the tolerance limits at the pharmacopoeially specified time references.

Table 3.10 Percent drug release of different generic products of Phenobarbital tablets at different times.

Time (minute) of sampling	% Phenobarbital Released		
	Epharm	East Africa pharm.	Cadila
0	0	0	0
5	27.2	17.0	6.1
10	58.5	34.8	7.8
15	74.3	44.9	10.4
20	78.7	51.2	14.5
30	82.1	56.3	16.9
45	87.0	61.7	19.3
60	87.2	63.4	20.3

The statistical treatment of the release profile at the pharmacopoeially specified time, 45 minutes, of the different generic products of phenobarbital tablets indicated that at 95% confidence interval there is extremely significant difference ($P < 0.001$) between all the three.

These statistical results indicate that all of the three generic products of phenobarbital are not equivalent with respect to their *in vitro* release profile.

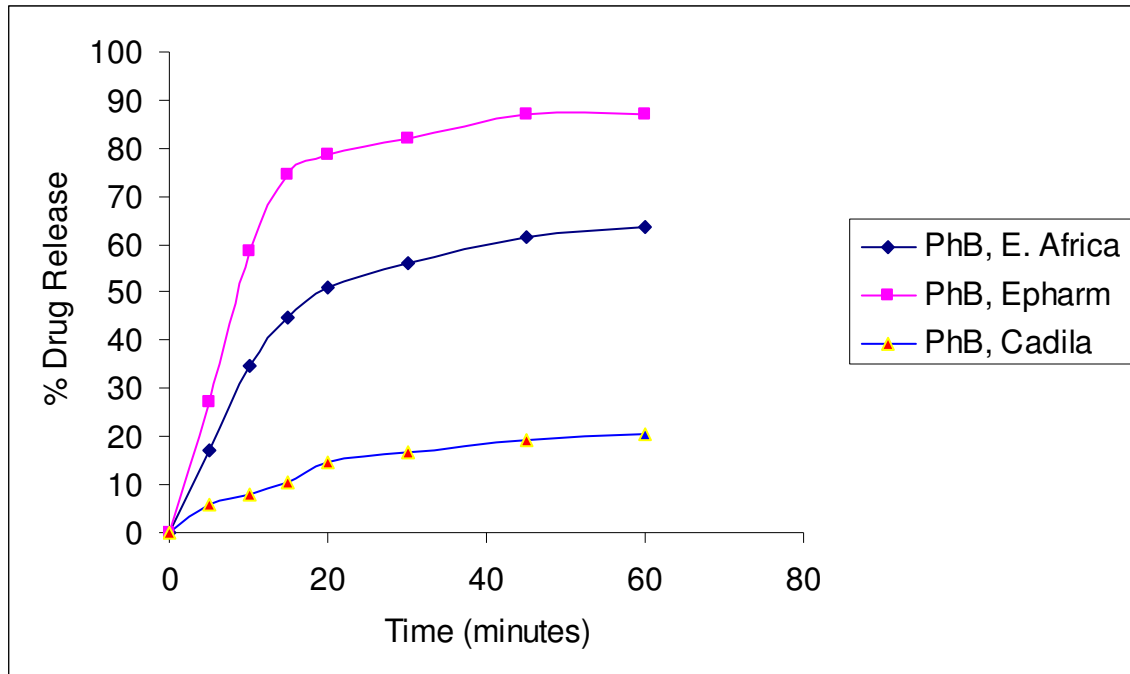


Figure 3.2: Dissolution profiles of different generic products of Phenobarbital tablets

3.6.3. Dissolution parameters

The $t_{50\%}$ and $t_{90\%}$ are the dissolution parameters that can be utilized to analyze dissolution profile of different pharmaceutical products. The $t_{50\%}$ is the time required for release of 50% of the contents of the tablets analyzed and $t_{90\%}$ is the time required for the release of 90% of the contents. Very long $t_{50\%}$ and $t_{90\%}$ values of pharmaceutical products dissolution profile indicate that the product may manifest lower rate and extent of bioavailability in the body.

The $t_{50\%}$ and $t_{90\%}$ of the different brands of carbamazepine tablets and the different generic products of Phenobarbital tablets analyzed were calculated using Origin® Graphing and Scientific Software and are shown in Table 3.11. The results of carbamazepine tablets showed that, since 50% release has been achieved within relatively short period of time for Taver ($t_{50\%}$ =7.1) and Tegretol ($t_{50\%}$ =9.6), they are expected to be absorbed and became bioavailable *within* a short period of time. Relatively longer $t_{50\%}$ value (22.4min) has been obtained for Tegral implying slower rate and extent of bioavailability relative to Taver and Tegretol. Similar trends could happen with respect to $t_{90\%}$ values.

Table 3.11 The $t_{50\%}$ and $t_{90\%}$ of carbamazepine and Phenobarbital tablets

Product		Dissolution parameter	
		$t_{50\%}$ (min)	$t_{90\%}$ (min)
Carbamazepine tablets	Taver	7.1	117.8
	Tegretol	9.6	146.9
	Tegral	22.4	127.3
Phenobarbital tablets	Epharm	9.5	96.1
	East Africa Pharmaceuticals	19.2	141.8
	Cadila	83.3	352.6

Epharm Phenobarbital product has shorter $t_{50\%}$ (9.5 min) and $t_{90\%}$ (96.1 min) relative to the other two Phenobarbital products. East Africa Pharmaceuticals Phenobarbital has intermediate $t_{50\%}$ (19.2 min) and $t_{90\%}$ (141.8 min) values with respect to the larger $t_{50\%}$ (83.3 min) and $t_{90\%}$ (352.6 min) of the Cadila product. From these values we can expect that the rate and extent of bioavailability of the Epharm product Phenobarbital is relatively higher than that of the two products: East Africa Pharmaceuticals and Cadila Phenobarbital.

In summary, proof of bioequivalence can involve *in vitro* dissolution studies, *in vivo* bioavailability studies, or combination of these. Carefully designed *in vitro* dissolution tests can be used as an index of the physiological availability of active constituents from orally administered formulations. In addition, in at least some situations, *in vitro* tests have been used to meet FDA quality requirements for proof of bioequivalence. However, an *in vivo* dissolution and absorption tests would likely give a more complete evaluation of solid formulations than could an *in vitro* test. Therefore, further *in vivo* studies should be conducted for obtaining a complete picture of bioequivalence of the carbamazepine and Phenobarbital tablets analyzed [Getie, *et al.*, 1998].

4. Conclusion

In general, the overall aspect of the study has attempted to make a comparative assessment of the quality in terms of identification, physical properties and *in vitro* bioequivalence of some antiepileptic drug products, carbamazepine and Phenobarbital tablets, available in drug retail outlets in Addis Ababa. All the tablets met the quality specification with respect to identification, hardness and friability, disintegration, and dosage form uniformity (weight variation and/or content uniformity). With respect to drug content (assay), from the carbamazepine tablets analyzed, Tegral was found to be out of the specified tolerance limit, while the other carbamazepine tablets and all the Phenobarbital tablets evaluated were within the tolerance limits of content. Thus, Tegral was found to be substandard. The brand was also found to be not within the tolerance limits of dissolution test requirement since it did not release the specified contents within the specified time. The East Africa pharmaceuticals Phenobarbital and the Cadila Phenobarbital have also result outside the dissolution test specifications. Thus, the different brands of carbamazepine tablets and the different generic products of Phenobarbital tablets analyzed were found to be bioinequivalent with respect to the *in vitro* dissolution test.

Therefore, one possible cause of the inadequate control of seizures and tolerance developments to antiepileptic drugs may be attributed to the quality aspect of the drugs. The different brands and generic tablet formulations utilized by the epileptic patients if not bioequivalent, but interchangeably utilized, or use of substandard drugs could lead to treatment failures. The present study will be of paramount importance provided further *in vivo* bioavailability evaluation of the indicated brands and generic tablet formulations are performed and correlated with the *in vitro* findings.

5. Suggestions for further work

- The *in vivo* bioavailability of the investigated tablet products should be studied in order to obtain their complete picture on their bioequivalence and clinical efficacy.
- Stability studies of the products should be investigated to identify if possible to quantify degraded products.
- Continued quality surveillance of the brand and generic tablet formulations of antiepileptic drugs from different regions of the country should be conducted to ensure quality thereby improve clinical efficacy.
- Any drugs particularly from different boarder regions which are expected to be susceptible to drug smuggling should be assessed to get an overall control of counterfeit drug product chains.

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ANNEX

The UV Spectrophotometric Spectra of Carbamazepine working standard; Phenobarbital reference standard; Carbamazepine in the tablets of Tegretol, Tegral, Taver; and Phenobarbital in the generic tablets of Epharm PhB, East Africa Pharmaceuticals PhB and the Cadila PhB are depicted below. In addition here attached is the potentiometric titration curves of the investigated generic tablet formulations of Phenobarbitone.

1. UV Spectrum of Carbamazepine working standard
2. UV Spectrum of Phenobarbital reference standard
3. UV Spectrum of Carbamazepine in Tegretol tablets
4. UV Spectrum of Carbamazepine in Tegral tablets
5. UV Spectrum of Carbamazepine in Taver tablets
6. UV Spectrum of Phenobarbital in Epharm PhB generic tablets
7. UV Spectrum of Phenobarbital in East Africa Pharmaceuticals PhB generic tablets
8. UV Spectrum of Phenobarbital Cadila PhB generic tablets
9. The potentiometric titration curve of Epharm PhB
10. The potentiometric titration curve of East Africa Pharmaceuticals PhB
11. The potentiometric titration curve of Cadila PhB

Annex 1. UV Spectrum of Carbamazepine working standard

Annex 2. UV Spectrum of Phenobarbital reference standard

Annex 3. UV Spectrum of Carbamazepine in Tegretol tablets

Annex 4. UV Spectrum of Carbamazepine in Tegral tablets

Annex 5. UV Spectrum of Carbamazepine in Taver tablets

Annex 6. UV Spectrum of Phenobarbital in Epharm PhB generic tablets

Annex 7. UV Spectrum of Phenobarbital in East Africa Pharmaceuticals PhB generic tablets

Annex 8. UV Spectrum of Phenobarbital Cadila PhB generic tablets

Annex 9. The potentiometric titration curve of Epharm PhB

Annex 10. The potentiometric titration curve of East Africa Pharmaceuticals PhB

Annex 11. The potentiometric titration curve of Cadila PhB