KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI

COLLEGE OF HEALTH SCIENCES

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DEPARTMENTOF PHARMACEUTICS

ASSESSING THE STABILITY OF PAEDIATRIC ORAL FORMULATIONS PREPARED AT KOMFO ANOKYE TEACHING HOSPITAL (KATH)

By

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By

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DECLARATION

I hereby declare that, except where it has been cited as reference, this dissertation is the outcome of my own research.

Date..... .....

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DATE..... .....

Professor M.T BAYOR

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Professor M.T BAYOR

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DEDICATION

To My family and all members of APRIDEC MEDICAL OUTREACH GROUP (AMOG) who give free services of quality healthcare to needy and vulnerable people living in deprived communities in Ghana.

ACKNOWLEDGEMENT

I give you thanks God Almighty for my life, Thy will be done.

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LIST OF ABBREVIATIONS

AS CHAM – *Accelerated Stability Chamber*

IPC - *In-process Control*

RT –*Room Temperature*

NA – *Not Applicable*

AUC-*Area Under Curve*

ECL – *Ernest Chemists Ltd*

STD – *Standard*

HIV- *Human immune Virus*

SUSP- *Suspension*

ADR - *Adverse Drug Reaction*

CAP - *Capsule*

CR –*Cold Room*

QC - *Quality Control*

PM- *Production manager*

QA - *Quality Assurance*

KATH - *Komfo Anokye Teaching Hospital*

UV - *Ultraviolet*

SPL - *Sample*

GMP - *Good manufacturing practice*

HPLC – *High Performance Liquid Chromatography*

SCD- *Sickle Cell Anaemia*

CHKD-*Children's Hospital of Kings Daughters*

TAB- *Tablet*

FDA- *Food and Drugs Administration*

HEP. B- *Hepatitis B*

HEP. C- *Hepatitis C*

BMR-*Batch Manufacturing Record*

CMC- *Carboxymethylcellulose*

SYRPALTA- *Oral Syrup vehicle as a base*

ABSTRACT

The study was carried out to determine the microbiological and chemical stability limits of paediatric formulations prepared in Komfo Anokye Teaching Hospital. It was also to determine the microbiological stability of buffer syrup of CMC which could be used as a base or vehicle for paediatric formulations.

Suspensions of eight formulations of paediatric dosages (acetazolamide, spironolactone, propranolol, furosemide, phenobarbitone, hydroxyurea, carbamazepine and lamivudine) were formulated according to the Komfo Anokye Teaching Hospital formulation procedure; the formulations were analysed chemically using HPLC methods and microbiologically by the Agar plate method within 120days.

The outcome showed that formulated suspensions of spironolactone and furosemide were microbiologically and chemically stable up to 30 days. Lamivudine suspension was stable chemically and microbiologically up to 60 days. The acetazolamide suspension was not stable up to 30 days contrary to a study reported by Nahata, 2003, though *syrpalta* was used as the vehicle or base in that research work. Phenobarbitone and propranolol suspensions were highly unstable within30 days therefore refrigeration of these suspensions is necessary to maintain their stability.

The buffer syrup of CMC was microbiologically stable up to one year, therefore can be formulated as ready to use base or vehicle for paediatric preparations.

CHAPTER 1

INTRODUCTION

1.1: BACKGROUND

Compounding formulations of paediatric dosage forms for infants and children continue to remain a significant portion of pharmacist in paediatric settings or units. This is due to the non- availability of approved drug dosage forms appropriate for infants and children (Nahata, 2003). The needs of drug therapy for infants and children are diverse but only about one fourth of the approved marketed drugs have specific indications for use in the paediatric population. The drugs not specifically authorized for use in infants and children are often unavailable in suitable dosage forms for paediatric drug therapy. For instance drugs such as phenobarbitone, aminophylline and spironolactone which are authorized (FDA) for paediatric patients are not available in the appropriate formulations. This is due to the difficulty in formulating them commercially hence drug manufacturers do not consider them economical (Allen I.V Jr, 1996).

Many drugs are unstable in aqueous medium and if a drug‘s approval is acceptable in only the aqueous medium and has a limitation of one year stability, the manufacturer will have difficulty in the distribution system because delays will severely limit the shelf life.

The stability of Paediatric Extemporaneous formulations in the late seventies (70‘s), according to the American Society of Hospital Pharmacists (ASHP, 1979) were identified as:

Class I- Formulations that have published data on stability with reference cited.

Class II- Formulations that the pharmacist by submitting the formula had communicated with the manufacturer about the stability and

Class III - Formulations that are based on only personal experience for preparation.

The class III which is based on the pharmacist experience without reference to data signifies that the stability may be difficult to ascertain because different institutions could be having different criteria for establishing stability hence varying degrees of clinical experience with a given formulation (Nahata, 1990).

In Paediatric oral dosage formulations, it is ideal for the pharmacist to ensure that it is easy to prepare and administer. The formulation should have the appropriate drug concentration and volume for accurate administration. The taste should be palatable for compliance and the formulation should be stable up to the expiry date.

Tablets of many drugs, such as acetazolamide and spironolactone are crushed to prepare suspensions. Rifampicin capsule are used to make its liquid dosage form.

The modifications for paediatric dosage forms should be prepared immediately and used for each dose otherwise proof of stability of reformulated medication should be available. This is necessary because manufacturers provide stability data for only their original product and only few pharmacy units are equipped with the capacity to conduct research on the chemical stability using standard analytical methods (US, NF18.)

Some of the considerations required for formulating oral paediatric dosage formulations from other dosage forms are:

- The ability of the vehicle to suspend the powdered active ingredient(primary importance) which will affect the quality of the suspension,
- The competence of the suspending agent to hold the active particle long enough for an adequate dose to be taken,
- The capability of the formulation being easily re-dispersed after settling and
- The storage condition of the formulation and the compliance of medication when the patient goes home with it (Nahata, 2003).

In the United States of America pharmacists mostly use the combination of Ora-plus and the Ora-sweet (1:1) these are ready to use products as suspending agents. Methylcellulose and Carboxymethylcellulose are also used as suspending agents but the methylcellulose is difficult to work with because of its viscosity. These products have widely been used in stability studies and found suitable and compatible with most drugs. These ready to use suspending agents (oral plus and oral sweet) are not available on our markets. In Komfo Anokye Teaching Hospital from experience, Carboxymethylcellulose or tween 80% is used as suspending agent, aspartame as sweetener and sodium benzoate as a preservative.

Therefore, for drugs that are compatible with Carboxymethylcellulose (examples Furosemide and Spironolactone suspensions) the below formula in table1.1 is used whilst the general formula in table1.2 is used for drugs that are compatible tween 80% (example Ciprofloxacin suspension) (KATH, *drug manufacturing unit records*, 2005).

Table1. 1 **Formulation formulae for CMC Compatible drugs**

Dosage	daily dose x 30			

Raw materials	Quantity	Quantity weighed	Weighed by	Checked by
API tablets	x tablets			
CMC	q.s.			
Sodium benzoate	0.15%			
Aspartame	q.s.			
Flavor	q.s.			
Distilled water to	y mL			

**x and y are based on calculations made from the request*.

Table1. 2 **Formulation formulae for Tween 80% Compatible drugs**

Dosage				
Raw materials	Quantity	Quantity weighed	Weighed by	Checked by
API tablets	x tablets			
Tween 80	q.s.			
Sodium benzoate	0.15%			
Aspartame	q.s.			
Flavor	q.s.			
Normal saline to	y mls			

When a request is received for a formulation to be prepared, the request is validated accordingly, taking into consideration the age of the child, diagnosis, dosage, the availability of adult dosage forms of medication to be used for the formulation, quantity required of adult dosage drug and the cost involved. The necessary calculations are then made to determine the volume and quantity of the suspension required for the duration indicated (KATH, *Extempo.procedure*, 2005).

1.2: RESEARCH PROBLEMS

The Komfo Anokye Teaching Hospital established the Child health department in 1983. And for four decades, paediatric dosage formulations have not been easy to come by for infants and children who require these medications for survival. As a result of few approved drugs available in dosage forms appropriate for infants and children, the Drug Manufacturing unit-KATH, continue to compound formulations generally from the tablets, capsules and sometimes from the intravenous dosage forms to meet the medication needs of these children. However infants and children on long term medication of these formulations have to come weekly, two weekly or monthly for refills due to stability issues with the preparation formulated from adult dosage forms. Although some stability reports are cited and used for the formulations, there is currently no documented report on stability study of the paediatric dosage formulations prepared at KATH. As more infants and children are being diagnosed with diseases that are known in adult life without the appropriate dosage forms of medication there is the need to carry out these studies on the formulation to ascertain the safety of the preparations and their usage.

1.3: JUSTIFICATION

The drug manufacturing unit of the Komfo Anokye Teaching Hospital (KATH) over four decades has been thriving hard to meet some of the medication needs of neonates, infants and children who are brought to the directorate of child health clinics. This is because paediatric dosage forms of some medications are not produced commercially or are not readily available on our markets. The drug manufacturing unit-KATH, therefore prepares some of these paediatric dosage forms from the adult dosage form such as tablets capsules and injections. As a referral Hospital, KATH serves the three regions in the North, the Brong Ahafo, Western region and parts of the Central region of Ghana. Infants and children diagnosed with some congenital heart disease conditions are put on these medications. Hence parents come to the drug manufacturing unit from far and near (weekly, two weekly or monthly) for refills of their wards medications. The study will therefore add to research on paediatric dosage formulations and could lead to a reduced frequency of parents coming to KATH for their wards medication refills and or reduce the time wasting at the manufacturing unit.

In general, the frustration that parents go through to obtain their wards medications which are not readily available commercially on our markets could be reduced.

1.4: RESEARCH OBJECTIVE

The purpose of this study was to determine the chemical and microbiological stability of acetazolamide, spironolactone, furosemide, propranolol, phenobarbitone, carbamazepine, hydroxyurea and lamivudine suspensions as formulated at the **Komfo Anokye Teaching Hospital.**

1.5: SPECIFIC OBJECTIVES

The specific objectives of the study were to formulate:

- Acetazolamide suspension from the tablet dosage form,
- Spironolactone suspension from the tablet dosage form,
- Furosemide suspension from the tablet dosage form,
- Propranolol suspension from the tablet dosage form,
- Lamivudine suspension from the tablet dosage form,
- Phenobarbitone suspension from the tablet dosage form,
- Hydroxyurea suspension from the capsule dosage form,
- Carbamazepine suspension from the tablet dosage form,
- buffer syrup of Carboxymethylcellulose (CMC) Sodium,

Determine the minimum inhibitory concentration (MIC) of sodium benzoate as preservative of the suspensions, syrup of CMC and chemical stability of suspensions within 120days.

CHAPTER 2

2.0: LITERATURE REVIEW

2.1: Paediatric dosage forms

In the United Kingdom between the period of October 1995 to September 2005, of all the active substances that were authorized and issued with a Marketing Authorization (MA) by the European Agency for the Evaluation of Medicinal Products, only 33% were licensed for paediatric use, 23% for use in infants and only 9% were available for the new-born (Ceci*et al*., 2006). Notwithstanding the advancement of technology, the developments of paediatric dosage form medications are still low as compared to the adult dosage form medications. The FDA of USA updated the labeling of 434 drugs for which studies have been completed in children, but only one product intended for premature infants (Davis, Connor and Wood, 2012). Therefore it is an indication that paediatric

dosage form market is comparatively small and segmented by age groups, hence a need for alternate formulations and dosages for each age group (Nahata, 1999a).

2.1.1: Paediatric Medication

When a drug is not approved for use in infants and children, it is usually not available in an appropriate dosage form, formulation, size or concentration for the paediatric population. Children are often unable to swallow capsules or tablets due to anatomy of their buccal cavity, and consequently some deaths have been reported associated with aspiration of solid dosage forms (Reilly and Walter (1992). Many pharmaceutical preparations contain ingredients that have been reported to cause ADRs for paediatric patients. The range of doses needed may be wide because there is such a wide variation in body mass, biological development and pharmacological features (Nahata, 1991).

It is difficult for parents when their sick or premature infant must be taken away from the family to be cared for because of illness. The worst emotions of parents may arise when a medication is required for treatment and such medication is not available or its required dosage form is commercially not on market. Pharmacists involve in such situations will have to make interventions, which include formulating a required dosage form from other dosage forms available on the market (Nahata *et al.*, 1990).

2.1.2 Paediatric Medication Risks

The knowledge of paediatric drug administration is not well developed or advanced as that of the adult drug administration (Sinha and Cranswick, 2007). Therefore the skill and judgement of physicians and pharmacists are critical in ensuring that paediatric patients receive the appropriate drug, in the best dosage form and the desired dosing regimen. In the absence of specific clinical trial-based data in children, clinicians are forced to rely on experience from adult patients, although children have different pharmacokinetics to adults and their response to many medicines can be unpredictable (Nahata, 1992). In neonates often, the decision to use a drug is based on a number of factors such as the clinical experience of the prescribing physician, an expert opinion, studies in older children, or a pilot study in new-bornsnew-born (Connor and Wood, 2012). The use of Unlicensed and off-label medicine is more likely to occur in new-born infants, who may be predisposed to suffer adverse drug reactions (ADRs) due to their physiological immaturity (Zenk, 1994). The lack of suitably adapted medicines and calculated individualized doses for children may increase the risk of ADRs and ineffective treatment either by under- or over-dosing. Children

are often unable to swallow capsules or tablets due to anatomy of their buccal cavity, and consequently some deaths have been reported associated with aspiration of solid dosage forms (Reilly and Walter, 1992)). Pharmaceutical preparations contain ingredients that have been reported to cause ADRs for paediatric patients. Also the range of doses needed may be wide because there is such a wide variation in body mass, biological development and pharmacological features (Nahata, 1991).

In addition, drug products intended for adults are not often available in a concentration low enough to permit accurate and precise dispensing of small doses (Nahata, 1999a; Nahata, 1999b). Hence measuring errors may occur, and for potent drugs like morphine and digoxin intoxication and deaths in paediatric patients can occur.

2.1.3 Formulated Preparations

Formulated formulations can be considered as one subgroup of unlicensed drugs and it includes modifications to commercially manufactured products such as the preparation of a suspension or powders from tablets, or the preparation of a product from the individual raw materials (Giam and McLachan;2008). The type of reconstitution where medicinal products are made ready for immediate administration (e.g. dissolution of a powder according to the appropriate instructions) is normally not considered as formulating (Pharmaceutical Inspection Convention, 2008). In addition, dividing or grinding solid dosage forms, dissolving tablets in water due to the inability of the patient to swallow the solid dosage form or administering fractions of a liquid, which nurses regularly do on the wards and at the bedside, is not extemporaneous preparation (Giam and McLachan, 2008). The basis for formulating medicine can be traced to the societies of Ancient Egypt, Greece, Rome and especially the Arabian cultures, where advanced levels of medical knowledge were developed (Marriot *et al*., 2010). The majority of prescriptions formulated by pharmacists (according to the order of a physician) for each individual patient are carried out with reference to published studies (Giam and McLachlan (2008). In the neonatal wards, surgical wards and medical wards extemporaneously prepared products or _specials‘ needed for patients still remains a challenge of Pharmacists have to solve.

2. 1.4 Formulating methods

Methods of extemporaneous preparation vary in different European countries (Brion, Nunn and Rieutord, 2003). Liquids are predominantly (> 60% of doses) formulated in Denmark, England, Ireland, Norway and Sweden, capsules in Belgium, Croatia, France and Switzerland and powders

in Finland, Italy and Scotland. One common practice in Germany, Spain and Slovenia involves the preparation of a less well-defined combination of liquids, powders and capsules. In KATH, extemporaneous preparation often means reformulating a solid dosage form into a liquid dosage form for infants, or conversion of tablets into powder with an appropriate dose for children. For instance Spironolactone, captopril, phenobarbital, Furosemide suspensions are formulated on a daily bases. The methods used in these preparations are either the USP or the BP standards or modifications these methods.

2.1.5 Formulations quality risks

In spite of the many improvements in quality, extemporaneous preparation is still confronted by a range of challenging issues especially quality issues when compared to those of registered products or unlicensed use of commercially manufactured products (Giam and McLachlan, 2008). In a British survey of extemporaneous captopril formulations, it was discovered that 22 hospitals were using nine different liquid formulations of captopril while four hospitals crushed the tablets and dispersed the powder in water (Mulla*et al*., 2007). A Canadian study also found a wide variation in the types of captopril formulations used: four of the 14 centres were dispensing solid tablets, two dispensed solid tablets or liquid formulations and eight made different kinds of extemporaneously prepared liquid formulations (Bhatt, Thomas and Mondal, 2011).

As a guide the following quality risks areas of concern must always be considered when formulating paediatric dosage form from other dosage forms.

These are: ADRs of the formulations, alternative routes of administration of the drug, bioavailability (efficacy, safety), accuracy of dosing, incompatibility of the excipient with the API, the manipulation of the adult dose, the microbial challenges of the formulation, losses of activity during preparation process, the skills of the Pharmacist, stability data, strength of drugs and as well the taste of the prepared formulation.

2.1.6 Formulating standards

Currently, there are neither appropriate nor comprehensive published standards about the process of extemporaneous preparation; in fact not all pharmacies formulate according to published formulations (Brion, Nunn and Rieutord, 2003; Giam and McLachlan, 2008). Standardised and verified methods of formulating with suitable instructions should be required (Ernest *et al*., 2012). The variability in the method of preparation should be minimised and there should be adherence

to a method of quality assurance. In order to ensure product quality, it has been recommended that there should be harmonization of extemporaneous formulations and quality control procedures and collected data should be published as standards and uniformly implemented in all countries. The European Pharmacopoeia contains a monograph about Pharmaceutical preparations, which allows the supply of unlicensed products to meet the special needs of individual patients with a suitable level of risk assessment being undertaken when considering this kind of the preparation (European Directorate for the Quality of Medicines & Health Care, 2013).

The USP26/NF21 Chapter 795; Pharmacy Compounding – Non-sterile preparations gives instructions including formulating process, stability, ingredients and quality control. Ten (10) needs for regulation of extemporaneous formulations have also been reviewed by the Australian government (Australian government, 2005 and 2008). In this regard, it is recommended that the GMP Guide be used as a reference for an appropriate quality system for high-risk preparations and the PIC/S GPP Guide be used for low-risk preparations. Suitable sources of stability indicating information include formularies such as Allen‘s compounded formulations, Nahata and Hipple‘s Paediatric drug formulations, Trissel‘s Stability of compounded formulations as well as peer-reviewed journals. The guidance contains usually the formula, conditions of storage and an estimate of shelf life based on chemical stability (Tuleu, 2007). However, there is often a lack of information about physical and microbiological stability and exact details of formulating.

2.1.7 Uniformity and Stability of Extemporaneous preparations

It has to be ensured that a formulation packaged in a specific container will remain within its physical, chemical and microbiological specifications during storage. The main causes of limited stability are:

1) Loss of drug (e.g. degradation),

2) Loss of vehicle (e.g. evaporation),

3) Loss of uniformity (e.g. caking of a suspension),

4) Change of organoleptic characteristics (e.g. appearance),

5) Change of bioavailability,

6) Appearance of an irritant or toxic degradation product (Tuleu,*et al*., 2007).

Interactions between drug substance and excipient may also induce instability. Extemporaneous preparations are often given arbitrary shelf lives or shelf lives based on published information (Tuleu, *et al*., 2007).

Formulators have to rely on drug-specific and general stability documentation and literature, but often have to estimate this information by considering the drug and its degradation mechanism, packaging container, the expected storage conditions, and the intended duration of therapy.

At KATH the preparations are usually packed in the amber plastic bottles, corked and checked for leakages. When dispensed patients are instructed to store in the fridge (preferably), however a larger number of these patients do not have access to fridges. Others have fridges but are faced with erratic power supply issues. In this view, a good number of the clients simply store these preparations at any ordinary room temperature which is often in the range of 27±3^{o}C (***Meteorological Department, Ghana reports*)**. These temperatures are weather dependent.

2.1.8 Chemical stability of formulation

Each active ingredient has to retain its chemical integrity and labeled potency within the specified limits. A reduction of content down to 90% of theoretical value (with 95% confidence limits) is generally regarded as the maximum reduction acceptable (Mehta, 1993; Barnes, 2007).

2.1.9 Physical Stability of formulation

Physical stability means that the original physical properties, including appearance, palatability, uniformity, dissolution and suspendability, have been retained in the formulation. For powders, the physical changes indicating instability include caking instead of free flowing, discoloration and release of pressure upon opening (Allen, 2002). Physical instability in suspensions is expressed as caking of sediment or particle growth, which leads to inaccuracy of dose, poor appearance and grittiness (Barnes (2007). The adhesion of suspension particles to container walls has also been noted as a problem, particularly with low-dose drugs

2.2: Microbiological Stability of formulation

Pharmacopoeia requirements for microbiological quality of non-sterile oral preparations include:

- Not more than 102 (maximum acceptable count is 200) aerobic microbes
- Not more than 101 (maximum acceptable count is 20) yeasts or moulds per gram or per milliliter in aqueous formulations,

- Not more than 103 (maximum acceptable count is 2000) aerobic microbes and
- Not more than 102 (maximum acceptable count is 200) yeasts or moulds per gram or per milliliter in non-aqueous formulations and the absence of Escherichia coli in both types of dosage forms (European Directorate for the Quality of Medicines & Health Care, 2013). The prevention of the presence or growth of microorganisms in the product is achieved by hygienic production, sterilization and suitable preservatives incorporation to products.

2.3 KOMFO ANOKYE HOSPITAL (KATH) CHILD HEALTH

As a department of Child Health, established in 1983, It is developed into a Directorate. _Child Health' as it is known at KATH, has become the referral Centre for neonates, infants and children from the three regions in the north, the Brong Ahafo region and parts of the central and western regions aside catering for the Ashanti region and training clinical students (medical, dental, nursing etc.). On a yearly basis the department provides various forms of services to nearly 11,276 in-patient and 20,239 out-patients (Report: Directorate of Child Health-KATHKNUST/SMS, KUMASI, 2015).

The Directorate is made up of the units and subunits which include the:

I. Nephrology Unit
II. Gastroenterology Unit
III. Infectious Disease
IV. Neurology
V. Oncology
VI. Haematology
VII. Cardiology
VIII. Pulmonology
IX. Endocrinology
X. Paediatric and emergency unit (PEU)
XI. Paediatric intensive care unit (PICU)
XII. **Mother and Baby Unit** (MBU) which is made up of:
    - *High Dependency Unit,*
    - *Low Dependency Unit and*
    - *Septic Unit*

2.4 DIRECTORATE CLINIC DAYS

Table2. 1 **KOMFO ANOKYE PAEDIATRIC CLINIC DAYS**

DAY	NAME OF CLINIC
MONDAY	Cardiac Clinic Respiratory Clinic Endocrinology Clinic General C5 Consultation Sickle Cell Clinic Adolescent Retroviral Clinic Oncology Clinic
TUESDAY	Neurology Clinic General B5 Consultation All New-born from 0-3months Childhood TB Renal /Nephrology Clinic
WEDNESDAY	Nutrition Clinic General B4 Consultation Neonatology Clinic HIV, Hep.B, Hep.C, Syphilis exposed clinic Cleft Lip and Palate Clinic Asthma Clinic

THURSDAY	Paediatric Oncology Clinic
FRIDAY	Neurology Clinic, Hematology Clinic, General B5 Consultation, All new-born up to 3 months/ Paediatric Retroviral Clinic

The Monday clinics are quite busy days as most referrals on the weekends can only, (mostly) report to KATH on the Mondays to be seen at the specialist clinics.

—Chronic health conditions (both chronic illnesses and chronic physical disabilities) are generally defined as those conditions that last > 12 months and are severe enough to create some limitations in usual activity. It has been estimated that chronic health conditions affect 10 to 30% of children, depending on the criteria. Examples of chronic illnesses include asthma, cystic fibrosis, congenital heart disease, diabetes mellitus, attention-deficit/hyperactivity disorder, and depression. Examples of chronic physical disabilities include meningomyelocele, hearing or visual impairments, cerebral palsy, and loss of limb function (The Merck Manual of Diagnosis & Therapy; 19th edition, Chapter 270). These Children with chronic health conditions may experience limitations in some activities; frequent pain or discomfort; abnormal growth and development; and more hospitalizations, outpatient visits, and medical treatments.

At KATH, paediatric outpatients with chronic health conditions visit the clinics quarterly in the year (every 3months). However those on drug treatment of which appropriate dosage forms of medication are not available will have to visit the Pharmacy every month for their **drug formulation** refills (Example, congenital heart disease, diabetes mellitus, etc.). Others may even revisit the Pharmacy two weekly or weekly depending on the formulations stability information available (Jessica Cha *et al* 2001).

2.5 Paediatric formulation prepared at KATH

The table below shows Paediatric oral formulations prepared at KATH from January to June 2016.

Table2. 2**PAEDIATRIC FORMULATIONS PREPARED AT KATH FROM 1ST JANUARY TO 30TH JUNE, 2016**

SUSPENSION FORMULATED	Jan.	Feb.	March	April	May	June	**TOTAL**	**% of 1534**
Acetazolamide	**3**	**7**	**2**	**12**	**14**	**10**	**48**	**3.1248**
Acyclovir		3	3	2	1		9	0.5859
Spironolactone	**59**	**43**	**51**	**68**	**76**	**65**	**362**	**23.5662**
Allopurinol			1	2	1		4	0.2604
Amlodipine					2	1	3	0.1953
Artane				2			2	0.1302
Atenolol				1	1		2	0.1302
Azathiopine	1						1	0.0651
Azithromycin		1					1	0.0651
Buscopan				3			3	0.1953
Carbamazepine	**5**	**5**	**8**	**7**	**7**	**8**	**40**	**2.604**
Ciprofloxacin	5	2	6	4	3	1	21	1.3671
Clindamycin	2	4		1			7	0.4557
Clonazepam	1		2		4		7	0.4557
Dexamethasone						1	1	0.0651
Deoxycholic acid				1	2	1	4	0.2604
Digoxin	1	1	1	1	1		5	0.3255
Enalapril	5	7	5	5	5	3	30	1.953
Fluconazole		1	1	1			3	0.1953
Frusemide	**67**	**52**	**60**	**70**	**54**	**71**	**374**	**24.3474**
Haloperidol				3			3	0.1953
Hydroxyurea oral Susp.*	**63**	**65**	**70**	**61**	**60**	**73**		**27.0816**
Hydroxyurea oral Sup	**4**	**2**	**1**	**3**	**8**	**6**	**416**	
Isoniazid					1		1	0.0651

Itraconazole oral susp.					1		1	0.0651
Lamivudine	**6**	**4**	**8**	**2**	**5**	**4**	**29**	**1.8879**
Levetracetam		1			1		2	0.1302
Lisinopril			1	1	1	1	4	0.2604
Loperamide			1				1	0.0651
Losartan					1		1	0.0651
Methyldopa						1	1	0.0651
Nitrofurantoin	2	1	1	2		5	11	0.7161
Ofloxacin	6						6	0.3906
Omeprazole		4	1	7	8	2	22	1.4322
Pen V				1		1	2	0.1302
Phenobarbitone Oral Susp	**18**	**1**	**10**	**9**	**12**	**11**	**61**	**3.9711**
Phenytoin						1	1	0.0651
Prednisolone	2	1			2	3	8	0.5208
Propranolol	**1**	**4**	**5**	**5**	**1**	**7**	**23**	**1.4973**
Pyridoxine					1		1	0.0651
Sildenafil	1	5	3	2		1	12	0.7812
Sodium Valporate						1	1	0.0651
TOTAL	**1534 (*from drug manufacturing unit second quarter,2016 report)***							

**bulk formulations prepared for paediatric Oncology unit/ Child Health*

The above data indicated that, the acetazolamide, furosemide, spironolactone, phenobarbitone, carbamazepine, propranolol, lamivudine, and the hydroxyurea suspensions formed 88.1% the paediatric oral suspension formulations prepared in the first half of 2016. The acetazolamide, furosemide and spironolactone are diuretics used in the treatment/management of congenital cardiovascular anomalies (in neonates, infants, and children). Lamivudine and hydroxyurea are used in the management of HIV (anti-retroviral) and sickle cell anaemia (oncology) respectively. Carbamazepine and phenobarbitone are used as anticonvulsants whilst the propranolol a betablocker is used to treat tremors, angina (chest pain), and hypertension (high blood pressure), and heart rhythm disorders (Lexicomp, 22nd edition).

The dosages of the above medications required in paediatric drug management are small such that their dosage formulations are not available on the market hence the need to formulate at the drug manufacturing unit of Pharmacy, KATH. In situations where inadequate information is given to

patients (parent of the sick child) at the consulting clinics, they roam for several days in search for these medications not available on market before being redirected back to KATH drug manufacturing unit. Therefore the top eight of the formulations were selected for study.

2.6: DOSAGES OF INFANT AND PAEDIATRIC ORAL FORMULATIONS

Table2. 3**PAEDIATRIC DOSAGES OF FORMULATIONS**

NUMBER	MEDICATION	USE/ACTION	DOSAGE
1	Acetazolamide	Diuretic/reduction of fluid in the body	5mg/kg/dose in 6-12hourly
2	Furosemide		1mg/kg/dose 12hourly
3	Spironolactone	K^+ sparing diuretic	1-3mg/kg/day 12hourly
4	Propranolol	Beta blocker	0.5-1mg/kg/day(not >8mg/kg/day
5	Carbamazepine	Anticonvulsant	10-20mg/kg/day
6	Phenobarbitaone		10mg/kg/day 12hourly
7	Hydroxyurea	Sickle cell disease	10 to 20 mg/kg/day
8	Lamivudine	HIV	4mg/kg 12lourly(8mg/kg /day

2.7: CHEMICAL FORMULAS AND STRUCTURE OF APIs

Structure of Hydroxyurea **Structure of Lamivudine**

Figure G

Formula: $CH_4N_2O_2$

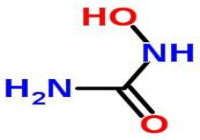
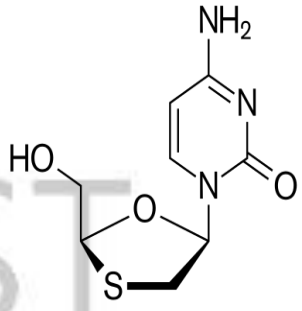
Molar Mass:  76.10 g/mol

Figure H 

Formula: $C_8H_{11}N_3O_3S$

Molar mass: 229.26 g/mol

Structure of Furosemide

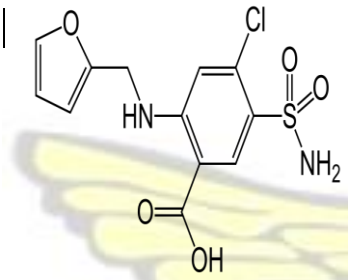


Figure C

Formula: $C_{12}H_{11}ClN_2O_5S$

Structure of Propranolol

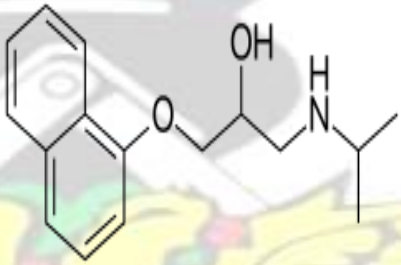
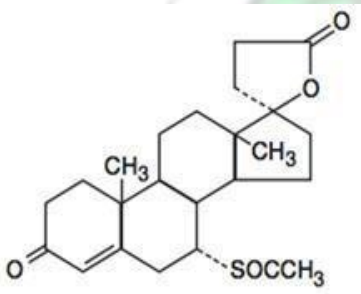


Figure D

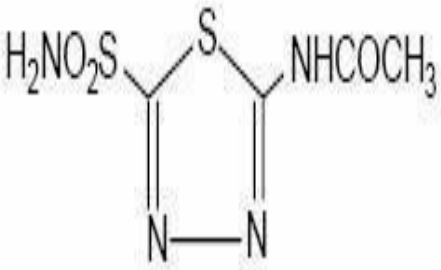
Formula: $C_{16}H_{21}NO_2$

Molar Mass: 259.34 g/mol



Structure of Spironolactone

Figure E



Structure of Acetazolamide

Figure F

Molecular Weight : 222.25g/ mol

Molecular Weight: 416.57g/mol Molecular Formula: C4H6N4O3S2 Molecular Formula: C24H32O4S

Structure of Phenobarbitone

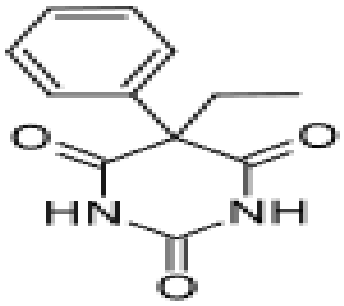


Figure G

Formula: C12H12N2O3
Molar Mass:232.24g/mol

Structure of Carbamazepine

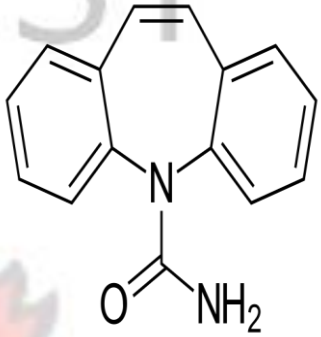


Figure H
Formula: $C_{15}H_{12}N_2O$

Molar Mass: 236.27 g/mol

2.8 Formulating paediatric oral suspensions

Liquid preparations for oral use include oral solutions and oral suspensions (European Directorate for the Quality of Medicines & Health Care, 2013). For infants and children under 5 years old, pharmaceutical liquids are traditionally preferred for oral administration (Allen, 2008). Liquid preparations have advantages of ease of administration compared to solid dosage forms, especially for children but usually dissolved drugs are more susceptible to degradation than when they are in the solid state (Tuleu, 2007). Oral liquids are comparatively quick to formulate. A small-scale preparation of an extemporaneous liquid dosage form from a tablet or capsule involves crashing of the tablets or emptying the contents of the capsules into a mortar and manipulating with other excipient to obtain a suspension (Nahata and Allen, 2008).

The selected paediatric suspensions formulations for this study were furosemide, acetazolamide, spironolactone, propranolol, carbamazepine, phenobarbitone, hydroxyurea and lamivudine. These medications are used in the management of paediatric Hypertension, cardiac arrhythmia, sickle cell disease (oncology) and paediatric HIV /Hep B infections. The formula below was adopted to prepare each of the formulations of the require dosage in a volume of one litre.

Tb/cap of drug in (mg)……quantity of tab /cap (weight in mg/g)

CMC (low Viscosity) …….2.40 g

Sodium Benzoate ……2.0 g

Aspartame ……1.32 g

Purified water to …….1000mL

The dosage strengths of the formulations were:

Acetazolamide 5mg per mL, Spironolactone 1mg per mL, Furosemide 1mg per mL, Propranolol 1mg per mL, Phenobarbitone 3mg per mL , Carbamazepine 10mg per mL, Hydroxyurea 10mg per mL and Lamivudine 10mg per mL

In the preparation of the above formulations, all the GMP standards were adhered to. Plastic amber bottles were used for packaging and the formulations stored at room temperature (27±3ºC) for the periods required for the study.

CHAPTER 3

EXPERIMENTATION

3.0: WORKING ENVIRONMENT

An Aseptic preparation room was used.

3.1: MATERIALS

3.1.1: Active Pharmaceutical Ingredients (API's) as standards

The API's used as standards were obtained from the manufacturing unit of Ernest Chemists Ltd (Furosemide, Propranolol, Acetazolamide, Phenobarbitone and Spironolactone) and Global Pharma Health Fund (GPHF) provided the Lamivudine tablet standard.

The API's from Ernest Chemists Ltd were of the following percentage purity by their analysis:

API	Percentage purity (%)
Acetazolamide	99.25
Furosemide	99.20
Propranolol	99.04
Spironolactone	99.57

Phenobarbitone	99.87

3.1.2: Pharmaceutical Ingredients for Formulation of Suspensions

The most common tablets of Propranolol, Spironolactone and Acetazolamide on the market within reach of patients who attend KATH were used for the formulations of the suspensions.

Tablet	**Strength**
Acetazolamide	250mg
Furosemide	40mg
Propranolol	40mg
Spironolactone	50mg
Phenobarbitone	60mg
Carbamazepine	200mg
Hydroxyurea	500mg
Lamivudine	150mg

3.1.3: Additives used in the Formulation of the suspensions

In the reformulation of the suspensions; carboxyl methylcellulose was employed as the suspending agent, Aspartame as a sweetener, Sodium benzoate as the preservative, sunset yellow as colour, 200mL plastic bottles with closures and purified water as the vehicle for formulation.

3.1.4: SOLVENTS/CHEMICALS FOR HPLC ANALYSIS

Chemicals

Potassium hydrogen phosphate, Sodium dodecyl Sulphate, Glacial Acetic acid, Sulphuric acid , Sodium hydroxide , Ammonium Acetate, Phosphoric Acid and Sodium Acetate were all obtained from BDH Laboratory Chemicals Limited, Poole, England.

Solvents

Methanol, HPLC grade; Acetonitrile, HPLC grade and water, HPLC grade were all obtained from KNUST Central Laboratory.

3.1.5: Microbiological analysis

Nutrient Agar and sterile water were obtained from the Microbiology unit, department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical sciences, KNUST.

3.1.6: EQUIPMENT AND APPARATUS

Equipment/ Apparatus

- Microprocessor pH meter —SCHOTT instruments Lab 860by Jos. Hansens and Soehne GmbH
- Filters
- Digital analytical balance — Adam Equipment Co. Ltd. Milton Keynes, UK.
- HPLC — Perkin Elmer s, with PDA Detector; by Jos.Hansen
- Electronic balance-Sartorius Te214s
- Column C18, 3.9 x 30 cm, made in Ireland
- Column C8, 4.6cm x 15cm, 5um (Phenomener)
- Column oven-eppendorf-CH500, USA
- Automatic voltage regulator (stabilizer) Serial no. C00503 HT
- Syringe- Syringe Care
- Temperature controlled Water bath
- Volumetric flask(10mL,25mL,50mLand 100mL
- Beaker 1000mL
- Funnel
- 50ml beakers
- Micro-pipettes (200umL and 1000umL)
- Siemens Ultraclear RO water treatment apparatus by Jos Hansen and Soehne GmbH2mL syringes
- Agar Plates
- Laminar flow cabinet
- Electrical oven-Thermo scientific by Jos.Hansen
- Autoclave, by Certoclav Sterilizer GmbH
- Volumetric Flasks (10mL,25mL 50mL and 100mL

- Incubator-Thermo Scientific, Jos Hansen
- 1000mL beaker
- Aluminium foil
- Sonicator-Elma S15, Elmasonic by Jos.Hansen
- Porcelain mortar and pestle
- Wash bottle
- Magnetic stirrer

3.1.7: METHODS

3.1.7.1: Preparation of formulations

The formulation of the suspensions was made using the below modified USP-NF methods for formulating paediatric oral dosage forms with a mortar and pestle from other dosage formulations:

MODIFIED FORMULAE:

Active ingredient (Tablet form)… weight in gram

CMC (low Viscosity) …….2.40 g

Sodium Benzoate ……..……2.00 g

Aspartame ……..…1.32 g

Purified water to ……. 1000mL

3.1.7.2: Acetazolamide suspension (5mg/mL) from commercially available tablets

Tab. Acetazolamide 250 mg ……20 tablets (5000mg/5.0g)

CMC (low Viscosity) ……2.40 g

Sodium Benzoate ……2.00 g

Aspartame ……..……1.32 g

Purified water to …….1000mL

Procedure:

The twenty (20) tablets of 250 mg of Acetazolamide) were removed from the blister packs into a porcelain mortar. The tablets were crushed and triturated into a fine powder using a porcelain pestle. 2.0 g and 1.32 g of sodium benzoate and aspartame were respectively weighed accurately and added to the finely triturated Acetazolamide. Further triturating was done to mix both the sodium benzoate and aspartame well with the Acetazolamide. 2.4 g of Carboxymethylcellulose (CMC) (low viscosity grade) was accurately weighed into a 1000mL measuring cup. 500mL of purified water was added and mixed well with a magnetic stirrer to obtain a uniform syrup-like solution. The content of mortar was carefully transferred into the syrup-like solution of CMC and stirred well; about 100 mL of purified water was used to rinse the mortar and added to the content of the measuring cup. More purified water was added to the 900 mL mark and further mixed well under the magnetic stirrer.

The cup was then made up to the 1000mL mark with purified water and manually stirred with a rod and the pH of the suspension was measured as 6.20.

The suspension was then filled into clear, amber plastic 200ml bottles, sealed with their closures and labelled.

3.1.7.3: Spironolactone suspension (1mg/mL) from commercially available tablets

Tb. Spironolactone 50mg……..…20 tablets (1000 mg/1.0g)

CMC (low Viscosity) ……2.40 g

Sodium Benzoate ……..…2.00 g

Aspartame ……..….1.32 g

Purified water to ……..1000mL

Procedure:

The twenty (20) tablets of 50mg of Spironolactone were removed from the blister packs into a porcelain mortar. The tablets were crushed and triturated into a fine powder using a porcelain pestle. 2.0 g and 1.32 g of sodium benzoate and aspartame were respectively weighed accurately and added to the finely triturated spironolactone. Further triturating was done to mix both the

sodium benzoate and aspartame well with the spironolactone. 2.4 g of Carboxymethylcellulose (CMC) (low viscosity grade) was accurately weighed into 1000 mL measuring cup. 500 mL of purified water was added and mixed well with a magnetic stirrer to obtain a uniform syrup-like solution. The content of mortar was carefully transferred into the syrup-like solution of CMC stirred well; and about 100 mL of purified water was used to rinse the mortar and added to the content of the measuring cup. More purified water was added to the 900mL mark and further mixed well under the magnetic stirrer.

The cup was then made up to the 1000mL mark and manually stirred with a rod and the pH of the suspension was measured as 6.88.

The suspension was then filled into clear, amber plastic 200mL bottles, sealed with their closures and labelled.

3.17.4: Furosemide suspension (1mg/mL) from commercially available tablets

Tb. Furosemide 40 mg……...…25 tablets (1000 mg/1.0g)

CMC (low Viscosity) …….2.40 g

Sodium Benzoate …….2.0 g

Aspartame ……..1.32 g

Purified water to ……..1000mL

Procedure:

The twenty-five 25tablets of 40mg Furosemide were removed from the blister packs into a porcelain mortar. The tablets were crushed and triturated into a fine powder using a porcelain pestle. 2.0 g and 1.32 g of sodium benzoate and aspartame were respectively weighed accurately and added to the finely triturated Furosemide. Further triturating was done to mix both the sodium benzoate and aspartame well with the furosemide. 2.4 g of Carboxyl methylcellulose (CMC) (low viscosity grade) was accurately weighed into 1000 mL measuring cup. 500 mL of purified water was added and mixed well with a magnetic stirrer to obtain a uniform syrup-like solution. The content of mortar was carefully transferred into the syrup-like solution of CMC stirred well; and

about 100 mL of purified water was used to rinse the mortar and added to the content of the measuring cup. More purified water was added to the 900 mL mark and further mixed well under the magnetic stirrer.

The cup was then made up to the 1000 mL mark and manually stirred with a rod and the pH of the suspension was measured as 5.30

The suspension was then filled into clear, amber plastic 200mL bottles, sealed with their closures and labelled

3.1.7.5: Lamivudine suspension (10mg/mL) from commercially available tablets

Tb. lamivudine 150 mg…….100 tablets (15000 mg/15.0g)

CMC (low Viscosity) …….3.60 g

Sodium Benzoate …….3.0 g

Aspartame ……..1.98 g

Purified water to ……..1500mL

Procedure:

The one hundred (100) tablets of 150mg Lamivudine were removed from the blister packs into a porcelain mortar. The tablets were crushed and triturated into a fine powder using a porcelain pestle. 3.0 g and 1.98 g of sodium benzoate and aspartame were respectively weighed accurately and added to the finely triturated Lamivudine. Further triturating was done to mix both the sodium benzoate and aspartame well with the Lamivudine. 3.6 g of Carboxymethylcellulose (CMC) (low viscosity grade) was accurately weighed into 2000 mL measuring cup. 750 mL of purified water was added and mixed well with a magnetic stirrer to obtain a uniform syrup-like solution. The content of mortar was carefully transferred into the syrup-like solution of CMC stirred well; and about 200 mL of purified water was used to rinse the mortar and added to the content of the measuring cup. More purified water was added to the 900 mL mark and further mixed well under the magnetic stirrer.

The cup was then made up to the 1500 mL mark and manually stirred with a rod and the pH of the suspension was measured as 6.10.

The suspension was then filled into clear, amber plastic 200mL bottles, sealed with their closures and labelled

3.1.7.6: Propranolol suspension (1mg/mL) from commercially available tablets

Tb. Propranolol 40 mg……..…25 tablets (1000 mg/1.0g)

CMC (low Viscosity) ……...2.40 g

Sodium Benzoate ……2.0 g

Aspartame ……1.32 g

Purified water to ……1000mL

Procedure:

The twenty-five 25tablets of 40mg Propranolol were removed from the blister packs into a porcelain mortar. The tablets were crushed and triturated into a fine powder using a porcelain pestle. 2.0 g and 1.32 g of sodium benzoate and aspartame were respectively weighed accurately and added to the finely triturated propranolol. Further triturating was done to mix both the sodium benzoate and aspartame well with the Propranolol. 2.4 g of Carboxymethylcellulose (CMC) (low viscosity grade) was accurately weighed into 1000 mL measuring cup. 500 mL of purified water was added and mixed well with a magnetic stirrer to obtain a uniform syrup-like solution. The content of mortar was carefully transferred into the syrup-like solution of CMC stirred well; and about 100 mL of purified water was used to rinse the mortar and added to the content of the measuring cup. More purified water was added to the 900 mL mark and further mixed well under the magnetic stirrer.

The cup was then made up to the 1000mL mark and manually stirred with a rod and the pH of the suspension was measured as 5.80

The suspension was then filled into clear, amber plastic 200mL bottles, sealed with their closures and labelled

3.1.7.8: Phenobarbitone suspension (3mg/mL) from commercially available tablets

Tb. phenobarbitone 60 mg…….50 tablets (3000 mg/3.0g)

CMC (low Viscosity) ……..2.40 g

Sodium Benzoate ……..2.0 g

Aspartame ……1.32 g

Purified water to ……1000mL

Procedure:

The fifty 50tablets phenobarbitone 60mg loose tablets were taken into a porcelain mortar. The tablets were crushed and triturated into a fine powder using a porcelain pestle. 2.0 g and 1.32 g of sodium benzoate and aspartame were respectively weighed accurately and added to the finely triturated phenobarbitone. Further triturating was done to mix both the sodium benzoate and aspartame well with the phenobarbitone. 2.4 g of Carboxymethylcellulose (CMC) (low viscosity grade) was accurately weighed into 1000 mL measuring cup. 500 mL of purified water was added and mixed well with a magnetic stirrer to obtain a uniform syrup-like solution. The content of mortar was carefully transferred into the syrup-like solution of CMC stirred well; and about 100 mL of purified water was used to rinse the mortar and added to the content of the measuring cup. More purified water was added to the 900 mL mark and further mixed well under the magnetic stirrer.

The cup was then made up to the 1000 mL mark and manually stirred with a rod and the pH of the suspension was measured as 5.90

The suspension was then filled into amber clear plastic 200mL bottles, sealed with their closures and labelled.

3.1.7.9: Hydroxyurea suspension (10mg/mL) from commercially available capsules

Tb. Hydroxyurea 500 mg……20 capsules (10000 mg/10.0g)

CMC (low Viscosity) ……2.40 g

Sodium Benzoate ……2.0 g

Aspartame ……1.32 g

Purified water to ……1000mL

Procedure:

The twenty (20) capsules of Hydroxyurea 500mg were removed from their blister packs and the capsule content removed from the shells into a porcelain mortar. The powder was then triturated into a finer powder using a porcelain pestle. 2.0 g and 1.32 g of sodium benzoate and aspartame were respectively weighed accurately and added to the finely triturated Hydroxyurea. Further triturating was done to mix both the sodium benzoate and aspartame well with the Hydroxyurea. 2.4 g of Carboxymethylcellulose (CMC) (low viscosity grade) was accurately weighed into 1000 mL measuring cup. 500 mL of purified water was added and mixed well with a magnetic stirrer to obtain a uniform syrup-like solution. The content of mortar was carefully transferred into the syrup-like solution of CMC stirred well; and about 100 mL of purified water was used to rinse the mortar and added to the content of the measuring cup. More purified water was added to the 900 mL mark and further mixed well under the magnetic stirrer.

The cup was then made up to the 1000 mL mark and manually stirred with a rod and the pH of the suspension was measured as 6.20

The suspension was then filled into clear, amber plastic 200mL bottles, sealed with their closures and labelled.

3.1.8.0: Carbamazepine suspension (10mg/mL) from commercially available tablets

Tb. Carbamazepine 200 mg ……50 tablets (10000mg/10.0g)

CMC (low Viscosity) …….2.40 g

Sodium Benzoate …….2.0 g

Aspartame ……..1.32 g

Purified water to ……..1000mL

Procedure:

The Fifty (50) tablets of 200 mg of Carbamazepine were removed from the blister packs into a porcelain mortar. The tablets were crushed and triturated into a fine powder using a porcelain pestle. 2.0 g and 1.32 g of sodium benzoate and aspartame were respectively weighed accurately and added to the finely triturated Carbamazepine. Further triturating was done to mix both the sodium benzoate and aspartame well with the Carbamazepine. 2.4 g of Carboxymethylcellulose (CMC) (low viscosity grade) was accurately weighed into a 1000mL measuring cup. 500 mL of purified water was added and mixed well with a magnetic stirrer to obtain a uniform syrup-like solution. The content of mortar was carefully transferred into the syrup-like solution of CMC and stirred well; about 100 mL of purified water was used to rinse the mortar and added to the content of the measuring cup. More purified water was added to the 900 mL mark and further mixed well under the magnetic stirrer.

The cup was then made up to the 1000 mL mark with purified water and manually stirred with a rod and the pH of the suspension was measured as 6.20.

The suspension was then filled into clear, amber plastic 200ml bottles, sealed with their closures and labelled.

3.1.8.1: Formulation of buffer syrup (without API)

CMC (low Viscosity) ……..13.0g

Sodium Benzoate ……...2.0g

Citric Acid ……..…2.0g

Sodium Citrate ……..0.8g

Aspartame ……1.32g

Sunset yellow …….. *QS

Purified water to ……1000mL

Sufficient quantity

Procedure:

2.0 g of sodium benzoate, 2.0g of Citric acid and 0.8g of Sodium citrate were accurately weighed into a mortar and triturated with about 300mL of water.1.32g of aspartame and 13.0g of were respectively weighed into a 1000mL cup. The content of the mortar was carefully transferred into the cup and mortar rinsed and added. More purified was used to make up the volume to the 750 mL mark. The content of the cup was then mixed well with a magnetic stirrer to obtain a uniform syrupy solution. Sufficient amount of sun-set yellow colour was then added to make the syrup elegant. More purified water was added to make up to the 1000 mL mark and manually stirred with a rod. The pH of the syrup was measured 4.27. The pH of syrup resisted changes up on small addition of acid or base. The syrup was then filled into amber clear plastic 200ml bottles, sealed with their closures and labelled.

3.1.8.2: Storage and analysis of suspensions Formulated

The formulations prepared were stored at the same room temperature (27±3^{o}C) condition but different durations and analysed for percentage content and microbiological quality as shown below.

Table3. 1**RESULTS ENTRY SHEET**

	STORAGE DURATION IN DAYS				
SUSPENSION	T0	T30	T60	T90	T120

ACETAZOLZMIDE-(ACE)					
SPIRONOLACTONE-(SPIRO)					
FUROSEMIDE-(FURO)					
LAMIVUDINE-(LAM)					
PROPRANOLOL-(PROP)					
PHENOBARBITONE-(PHENO)					
HYDROXYUREA-(UREA)					
CARBAMAZEPINE-(CARBA)					
SYRUP OF CMC					

3.1.8.3: Microbiological quality

The work environment was equipped with a Laminar flow cabinet which was cleaned and disinfected. The surrounding work bench surfaces were also disinfected and protective clothing put on (including hair cap and hand cloves) to prevent contamination from the surroundings.

The formulations were preserved with sodium benzoate 0.2% hence Nutrient agar (NA) which is a general purpose medium was employed for this test. The NA was melted and stabilized at approximately 45-50^{o}c. After stabilization enough melted agar was poured into each sterile Petri dish to cover the bottom - about 1/8" to 1/4" deep and the lid replaced immediately.

The agar plates were then placed on the counter top to cool but not to set completely (USP 31/NF 26, (United States Pharmacopeia 2008).

1ml each of the formulations (without dilution) was then aseptically transferred into the labelled Petri dishes with the agar using sterile syringes. The plates were swirled to aid the mixture of the samples and the agar. The lids of the Petri dishes were removed just long enough to allow contamination from air-borne particles outside the laminar flow cabinet. The Petri dishes were then covered and left undisturbed to enable the content set completely before incubation.

Each test was done in duplicate and the entire processes was repeated for the formulations after a tenfold dilution was carried out to inactivate the preservative 0.2% Sodium benzoate (that is 1mL of each sample added to 9mL of sterile water) and used for the test (USP <62>). Control test for the water used in diluting the suspensions and the agar use as culture media were also concurrently tested for microbial contamination. The test dishes were incubated by turning the plate upside down in the incubator (37ºC) for 36 hours.

The microbiological stability of the suspensions was performed according to the USP 31/NF 26(United States Pharmacopeia 2008), Using the agar plate method.

3.1.8.4 Spectrophotometric assay of formulations

High performance liquid Chromatography (HPLC) was used to analyse the suspensions to determine the percentage content of the active pharmaceutical ingredient present immediately after the formulation and at the end of the storage duration and condition set out (USP 38).

Challenges

The limited resource and challenges such as non-availability of some reagents and chemicals made it impossible to run Spectrophotometric assays on two of the formulations, namely Hydroxyurea and Carbamazepine. However microbial quality was carried out on the suspensions and both were stable up to 90 days when stored at room temperature.

3.1.8.5: Spectrophotometric assay of Acetazolamide suspension using HPLC (Perkin Elmer)

Composition suspension: 5mg/mL Analytical method: **USP 38**, page 2042

Chromatographic Conditions:

Column: Zorbax 5B-C 18 (3.9x 300 mm) 5µ

Temperature: 28ºC.
Wavelength: 254 nm

Injection volume: 20µL

Flow rate: 2mL/minute

Mobile phase: A mixture of 4.10g of Sodium Nitrate anhydrous in 950 mL water, 20 mL of methanol and 30 mL of Acetonitrile was prepared and the pH adjusted to 4.0

10mL of 1.0M of NaOH solution was used to dissolve the STD and SPL and sonicated for 5 minutes. The mobile phase was used to make up the volume to 100mL. The resulting solution of the SPL was filtered and injected for analysis (USP 38).

3.1.8.6: Spectrophotometric assay of Spironolactone suspension using HPLC (Perkin Elmer)

Composition suspension: 1mg/mL Analytical method: **USP 38**, page 5348

Chromatographic Conditions:

Column-Agilent Pep-C 18 (3.9x 300 nm) 5µ

Temperature-28^{o}C

Wavelength-230 nm

Injection volume-20µL

Flow rate-2mL/minute

Mobile phase- Methanol: Water, 70:30

Solvent- Acetonitrile: water, 50:50

Weight of standard (STD) taken: 0.0504g.

50 mL of Spironolactone suspension (SPL) ≡50mg suspension was pipetted for analysis.
About 30 mL of solvent solution was used to dissolve the STD and SPL and sonicated for 15 minutes. After which the resulting solutions both STD and SPL were left to cool and more of the solvent was used to make up the volume to 100mL. The resulting solutions were filtered and injected for analysis. The resulting solution of the SPL was filtered and injected for analysis (USP 38).

3.1.8.8: Spectrophotometric assay of Furosemide suspension using HPLC (Perkin Elmer)

Composition suspension: 1mg/mL

Analytical method: **USP 38**, pages 3628-3629 **Chromatographic**

Conditions:

Column: Zorbax 5B-C 18 (3.9x 300 mm) 5µ Temperature:

28ºC.

Wavelength (λ): 230 nm.

Injection volume: 20µL.

Flow rate: 2mL/minute

Mobile phase: Mixture of water 81%; Acetonitrile 18% and acetic acid 1%.

Solvent: mixture of 22 mL of water, 22 mL Acetonitrile and 1 mL acetic acid.

Weight of standard (STD) taken: 0.025g.

25 mL of Furosemide suspension (SPL) ≡25 mg suspension was pipetted for analysis.

About 30 mL of solvent solution was used to dissolve the STD and SPL and sonicated for 10 minutes. After which the resulting solutions both STD and SPL were left to cool and more of the solvent was used to make up the volume to 100mL. The resulting solutions were filtered and injected for analysis. The resulting solution of the SPL was filtered and injected for analysis (USP 38).

3.1.8.9: Spectrophotometric assay of Lamivudine suspension using HPLC (Perkin Elmer)

Composition suspension: 5mg/mL

Analytical method: **IP, 2015**

Chromatographic Conditions:

Column: Zorbax 5B-C 18 (3.9x 300 mm) 5µ

Temperature: 35ºC.

Wavelength: 277 nm

Injection volume: 20µL

Flow rate: 1mL/minute

Mobile phase: 1.9g of Ammonium Acetate was dissolved in 1000mL of water and the pH adjusted to 3.8 with glacial acetic acid. 50mL of the resulting buffer was taken and added to 950mL of Methanol. The solution was then used to prepare both STD and SPL and sonicated for 5 minutes filtered and injected for the analysis (USP 38).

3.1.9.0: Spectrophotometric assay of Propranolol suspension using HPLC (Perkin Elmer)

Composition suspension: 3mg/mL

Analytical method: **USP 38**, pages 5058-5060.

Chromatographic Conditions:

Column: Phenomener-C 8 4.6x 150 mm) 5µ Temperature: 28^{o}C.

Wavelength: 290 nm

Injection volume: 20µL

Flow rate: 1.5mL/minute

Mobile phase: 2g of Sodium dodecyl Sulphate was dissolve in 72mL of 0.15M of Phosphoric acid in a 1000mL beaker. 360 mL each Acetonitrile and Methanol was measured separately and added. Distilled water was used to make to 1000mL. The solution was filtered. The mobile phase was used to prepare the SPL and STD, filtered and injected for analysis (USP 38).

3.1.9.1: Spectrophotometric assay of Phenobarbitone suspension using HPLC (Perkin Elmer)

Composition suspension: 5mg/mL

Analytical method: **USP 38**, page 4836-4837 **Chromatographic**

Conditions:

Column: Zorbax 5B-C 18 (3.9x 300 mm) 5µ

Temperature: 60ºC.

Wavelength: 235 nm

Injection volume: 5µL

Flow rate: 1mL/minute

Mobile phase: 300mL of Acetonitrile and 700 mL of water were accurately measured into a 10000mL beaker. The solution was adjusted to a pH of 3 with dilute Sulphuric. Part of this solution (mobile phase) was then use to prepare the STD and SPL filtered and injected for analysis (USP 38).

3.2: RESULTS AND CALCULATIONS

3.2.1: Microbial Stability using agar plate method

Table3.2**Results of acetazolamide Suspension 5mg/mL Microbial Quality**

STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	**GROWTH**
0	**0**	**-**	**10**	**+**
30	0	-	10	+
60	0	-	10	+
90	0	-	10	+
120	0	+	10	+

WATER (water for dilution) ONLY		-		
AGAR (culture media) ONLY		-		

Growth: +; No Growth:-

Table3.3**Results of Spironolactone Suspension 1mg/mL Microbial Quality**

STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	**GROWTH**
0	**0**	**-**	**10**	**+**
30	0	-	10	+
60	0	-	10	+
90	0	+	10	+
120	0	+	10	+
WATER ONLY		-		
AGAR ONLY		-		

Growth: +; No Growth:-

				GROWTH
				+

Table3.4**Results of Hydroxyurea Suspension 10mg/mL Microbial Quality**

STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	
0	**0**	**-**	**10**	
30	0	-	10	+
60	0	-	10	+
90	0	-	10	+
120	0	+	10	+
WATER ONLY		-		
AGAR ONLY		-		

Growth: +; No Growth:-

Table3.5**Results of Propranolol Suspension 1mg/mL Microbial Quality**

STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	**GROWTH**
0	**0**	**-**	**10**	**+**
30	0	-	10	+
60	0	-	10	+
90	0	-	10	+

Growth: +; No Growth:-

		GROWTH		
				+
120	0	+	10	+
WATER ONLY		-		
AGAR ONLY		-		

Table3.6**Results of Lamivudine suspension 50mg/mL Microbial Quality**

STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	
0	**0**	**-**	**10**	
30	0	-	10	+
60	0	-	10	+
90	0	-	10	+
120	0	+	10	+
WATER ONLY		-		
AGAR ONLY		-		

Growth: +; No Growth:-

Table3.7**Results of Phenobarbitone suspension 3mg/mL Microbial Quality**

Growth: +; No Growth:-

				GROWTH
STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	**+ GROWTH**
0	**0**	**-**	**10**	**+**
30	0	-	10	+
60	0	-	10	+
90	0	-	10	+
120	0	+	10	+
WATER ONLY		-		
AGAR ONLY		-		

Table3.8**Results of Furosemide suspension 1mg/mL Microbial Quality**

STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	
0	**0**	**-**	**10**	
30	0	-	10	+
60	0	-	10	+
90	0	-	10	+
120	0	+	10	+

Growth: +; No Growth:-

				GROWTH
				+
WATER ONLY		-		
AGAR ONLY		-		

Growth: +; No Growth:-

Table3.9**Results of Carbamazepine suspension 1mg/mL Microbial Quality**

STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	**GROWTH**
0	**0**	**-**	**10**	**+**
30	0	-	10	+
60	0	-	10	+
90	0	-	10	+
120	0	+	10	+
WATER ONLY		-		
AGAR ONLY		-		

Growth: +; No Growth:-

Table3.10 **RESULTS OF MICROBIAL QUALITY OF BUFFER SYRUP OF CMC**

STORAGE DURATION/DAY	**DILUTION FACTOR(DF)**	**GROWTH**	**DF**	**GROWTH**
0	**0**	**-**	**10**	**+**
30	0	-	10	+
60	0	-	10	+
90	0	-	10	+
120	0	+	10	+
WATER ONLY		-		
AGAR ONLY		-		

Growth: +; No Growth

3.3: GRAPHICAL REPRESENTATION OF HPLC ANALYSIS OF SUSPENSIONS

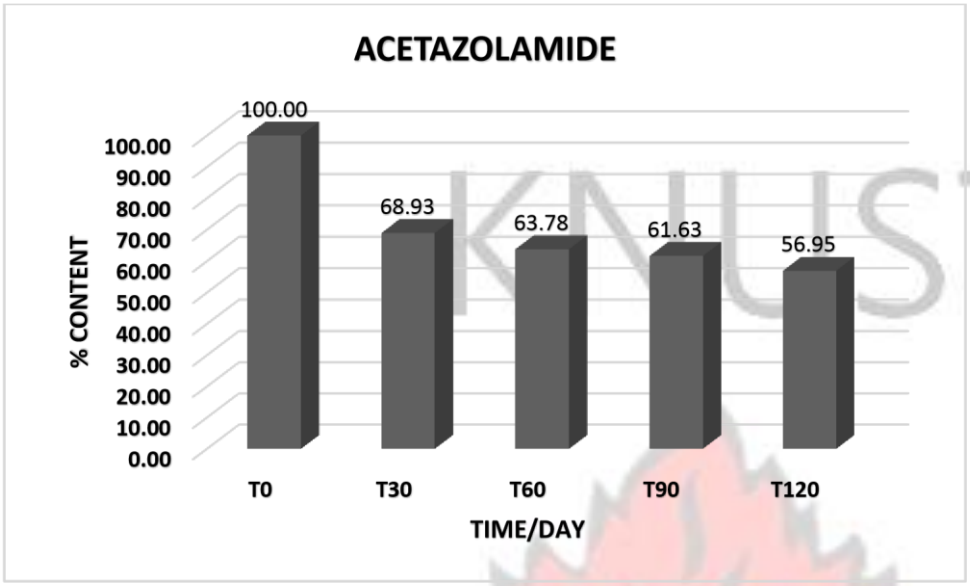


Figure 1 ***Graph showing content stability of Acetazolamide suspension after storage period***

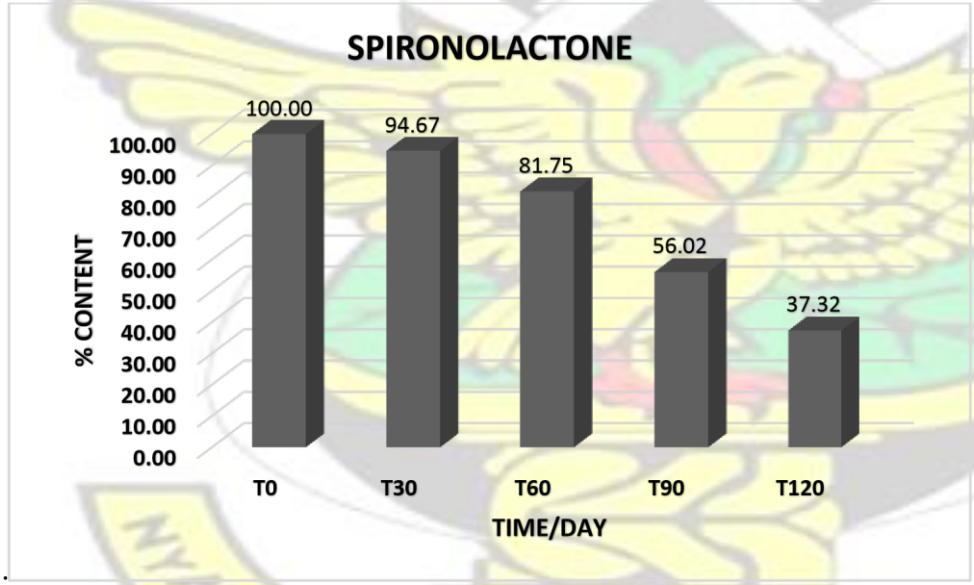


Figure2 ***Graph showing content stability of Spironolactone suspension after storage period***

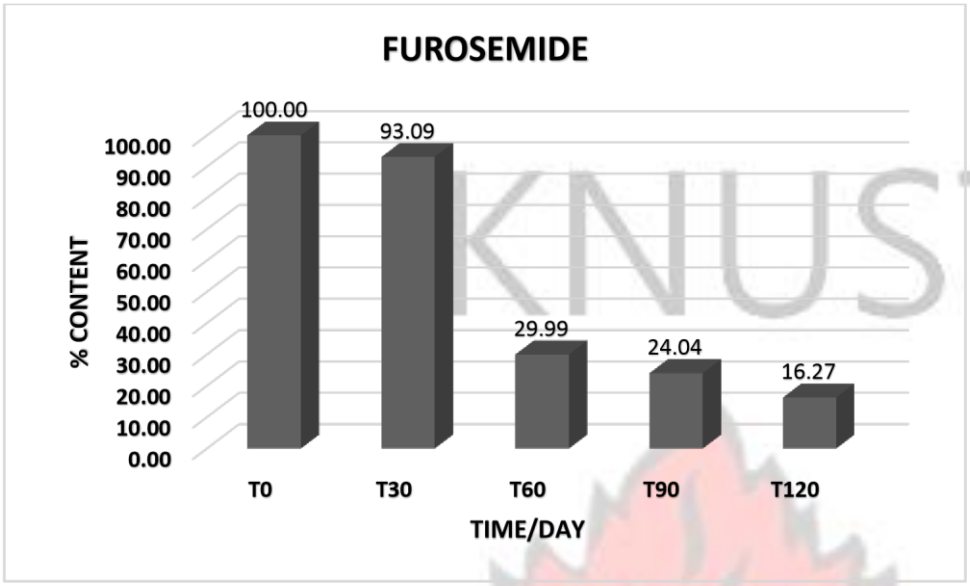


Figure 3 ***Graph showing content stability of Furosemide suspension after storage period.***

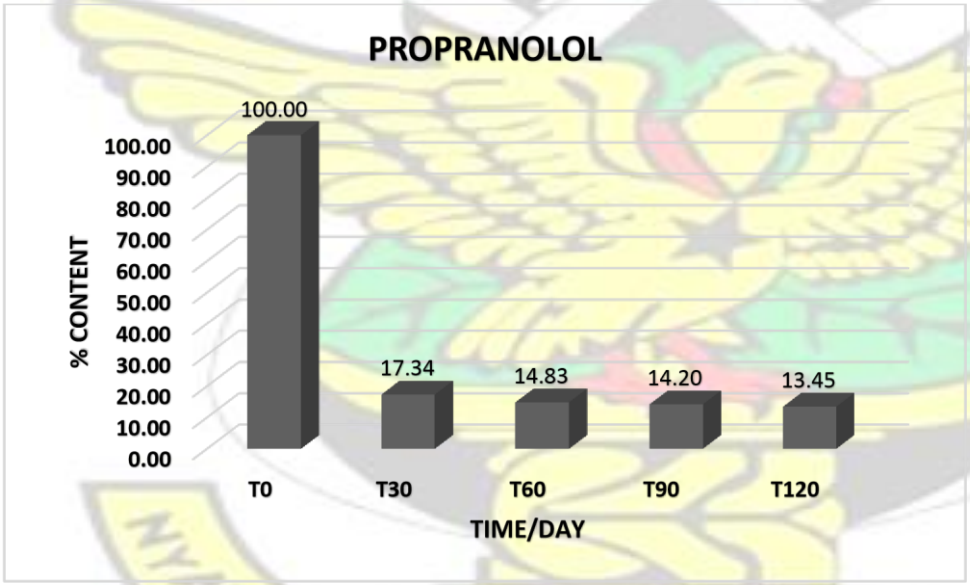


Figure 4 ***Graph showing content stability of Propranolol suspension after storage period***

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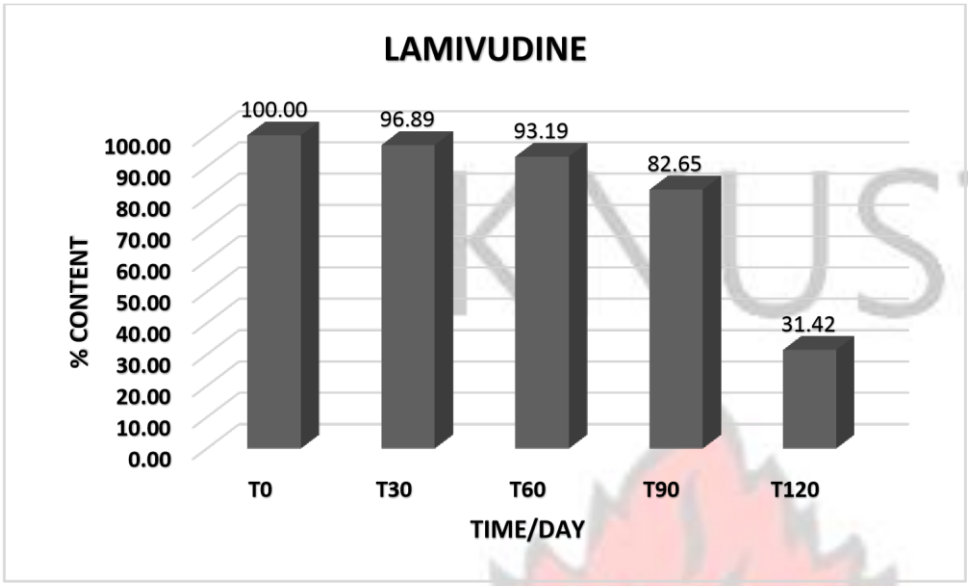

Figure 5 ***Graph showing content stability of Lamivudine suspension after storage period***

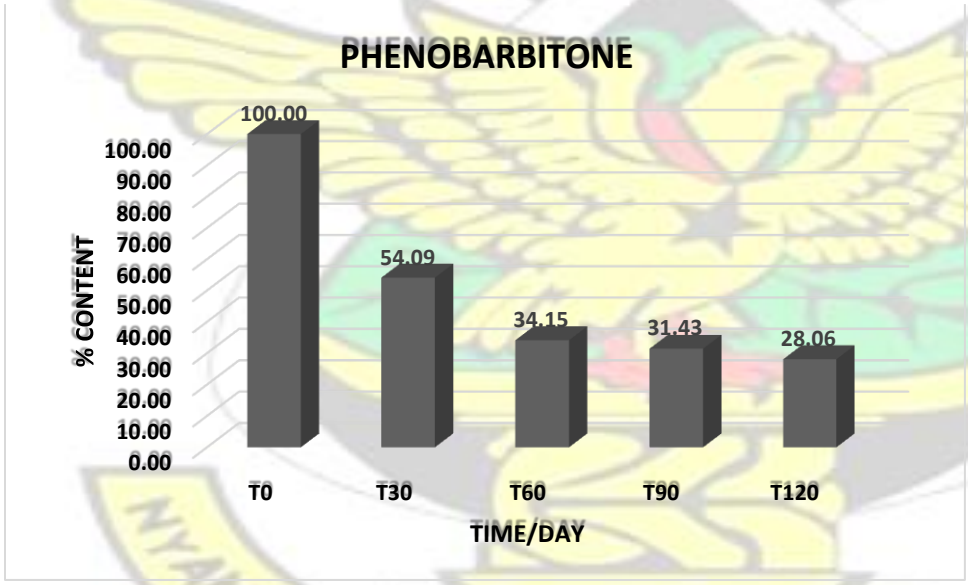

Figure 6 ***Graph showing content stability of Phenobarbitone suspension after storage period***

3.4: DISCUSSION

3.4.1: Microbiological stability of suspensions preserved with 0.2% Sodium Benzoate.

Sodium benzoate is a micro static preservative. Its action is by the absorption of benzoic acid into the cells of microorganism (Krebs*et al.*, 1983). If the pH of the intracellular is 5 or lower, the anaerobic fermentation of glucose through phosphofructokinase decreases drastically hence the inhibition of growth and survival of microorganisms that cause instability in pharmaceutical products.

3.4.2: Microbiological stability of Acetazolamide suspensions

The microbial stability analysis of the acetazolamide suspensions (table 3.2) showed that 0.2% of sodium benzoate used as a preservative is capable of inhibiting the growth of microorganisms up to 90 days (T90) of preparation and storage. This is because at T0, T30, T60, T90 and the controls, all had No Growth (-) when exposed to contamination and incubated on nutrient agar. However T120 and all the all the Acetazolamide suspensions after a ten (10) fold dilution, exposed to contamination and incubated on agar show positive growth (+). The dilution served as an inactivation of the benzoate as preservative.

3.4.3: Microbiological stability of Spironolactone suspensions

The microbial stability analysis of the spironolactone suspensions (table 3.3) revealed that 0.2% of sodium benzoate is capable of protecting the suspensions from spoilage up to day 60 (T60) . At T0, T30, T60 and the controls had No Growth (-) whilst T90, T120 and all the ten (10) fold diluted suspensions showed growth (+) after incubation. Therefore suspensions of spironolactone prepared from the tablet dosage forms and store at ordinary room temperature could only be microbiologically stable for up to 60 days.

3.4. 4: Microbiological stability of Phenobarbitone suspensions

The phenobarbitone suspensions evaluated for microbial stability (table 3.4) showed that at T0, T30, T60, T90 and the controls had No growth (-) after the incubation period whereas the T120 and all the phenobarbitone suspension diluted (10 fold) exhibited microbial growth(+). This is an indication that phenobarbitone suspension prepared from tablets can be stable microbiologically up to 90days using 0.2% sodium benzoate as preservative. Hence if, the suspensions are also chemically stable for that long, then formulations could be prepared and given to patients over 60 days to reduce their frequent revisit to obtain refills.

3.4.5: Microbiological stability of Propranolol suspensions

The propranolol suspensions (table 3.5) at T0, T30, T60, T90 and the controls after the incubation period showed that the 0.2% Sodium benzoate as a preservative protected the suspensions from microbial spoilage. However, the T 120 and the diluted (DF 10) suspensions were susceptible to microbial spoilage. This was an indication that the dilution inactivated the benzoate hence the microbial attack on the suspensions. Propranolol suspensions made from tablet dosage forms could be stable for three month (90 days) when stored at room temperature.

3.4.6: Microbiological stability of Lamivudine suspensions

The lamivudine suspensions (table 3.6) were microbiologically stable from day zero (T0) preparation up to day 90 (T90) after storage. At T0, T30, T60, T90 and all the diluted (DF 10) suspensions showed microbial growth after being exposed to contamination and incubated for 36 hours. Therefore diluted (DF 10) suspensions of T0, T30, T60, T90 and T120 all showed growth (+) after microbial quality test.

3.4.7: Microbiological stability of Hydroxyurea suspensions

The hydroxyurea suspensions (table 3.4) were found to be microbiologically stable at T0, T30, T60, T90 and the controls as these showed No growth (-) after being exposed to contamination and incubated on nutrient agar. This is an indication that hydroxyurea suspension could be prepared and preserved with 0.2% Sodium benzoate and the microbial quality maintained up to 90 days (T90). The suspension T120 and all the diluted (DF10) hydroxyurea suspensions showed growth (+) after exposure to contamination and incubated on Nutrient Agar for period selected for the study.

3.4.8: Microbiological stability of Carbamazepine suspensions

The carbamazepine suspensions (table 3.9) showed microbial stability with 0.2% of Sodium benzoate as a preservative. The suspensions at T0, T30, T60, T90 and the controls had No growth (-) after the incubation period. The T120 and all the diluted (DF10) suspension showed growth (+) or instability.

3.4.9: Microbiological stability of Furosemide suspensions

The furosemide suspensions (Table 3.2.8) preserved with 0.2% of sodium benzoate was microbiologically stable after exposure to contamination and incubated for the period of study

(36hour). The furosemide suspensions of day zero (T0) up to day 90(T90) and the controls showed No growth (-) whilst that of T120 and the diluted (DF10) had growth (+) indicating contamination after exposure to contamination and culture on nutrient agar.

3.5.0: Microbiological stability of buffer Syrup of CMC suspensions

The syrup prepared from CMC (Table 3.2.9) and preserved with 0.2% sodium benzoate was microbial stable up to one year. That is at T0, T30, T60, T90, T120 and up to one (1) year and controls showed No growth (-) after being exposed to contamination and cultured on nutrient agar. The diluted (DF 10) syrups showed growth (+) when it was cultured for microbial quality after exposure to contamination. The pH of the buffer syrup was stable as it was 4.20± 0.05.

3.5.1: General Comments on the microbial stability of formulations

The suspensions were formulated using: 0.24% CMC (suspending agent), 0.13% Aspartame (sweetener) and 0.2% Sodium benzoate as the preservative which proved to be effective in all the formulations up to 90days. This is an indication that the formulations are capable of retaining their microbiological stability for over 60 days (T60) therefore a two months refill could be formulated for patients without compromising its microbial stability. The buffered syrup was microbiologically stable up to one year (12 month) hence a stock formulation the buffer syrup could prepared and kept for use when required without microbial quality challenges.

3.5.2: Chemical stability of the formulated suspensions

The instability of pharmaceutical products may be as a result of drug or excipient degradation. Hence no pharmaceutical product is stable indefinitely as majority of products are stable for only a time limit. The degradation of pharmaceutical products is exacerbated by poor formulation process, packaging and inappropriate storage conditions. Instability of pharmaceutical products results in loss of active drug, loss of vehicle (if volatile), loss of content uniformity, reduced bioavailability, loss of elegance of product and possible production of toxins.

The nature of solvent, in which the drug is formulated, can affect the chemical degradation of the pharmaceutical product. Also the irreversible loss of electrons (due to presence of radicals) and the effects of atmospheric oxygen can cause the oxidation of the pharmaceutical product hence degradation of it (Aulton *et al.*, 1995).

Light, temperature and humidity also influence chemical degradation of pharmaceutical products therefore the appropriate storage conditions must carefully be selected to reduce degradation so as to improve on the stability of the product.

3.5.3: Chemical stability of Acetazolamide suspension by HPLC Analysis

The percentage content calculation (figure 1) of Acetazolamide was done with T0 percentage content (100%) as the reference. The acetazolamide suspension formulated on day zero (T0) /or the fresh preparation was analyzed and the 100% content assigned to it. After then define periods of storage the suspensions were re-analyzed and the percentage content compared to that of the content at T0. The acetazolamide after, T30, T60, T90 and T120 days degraded by 31.07%, 36.22%, 38.37% and 43.05% respectively. This was an indication there was an initial drastic degradation by the 30th day. However after the 30th day the degradation was less drastic (slower). In general by the 120th day (T120) the degradation was more than 40% of that of T0 percentage content (USP, 38). The Acetazolamide suspensions, from the results (table 3.3.1) was fairly stable after the 60th day (T60) up to the 90th day (T90) indicating the rate of degradation was higher from T0 to T30 but lower from the T60 to T90. The acceptance criteria is 90%- 110% (USP; 38) for Acetazolamide oral suspension stored at a controlled room temperature or in a cold place. It is reported that acetazolamide oral suspension formulated using the 250mg tablets, syrup (30%), methylcellulose (0.3%),Veegum (1%) and parabens concentrate was stable for 79 days (Nahata, 2003). Therefore, modifying the method of preparation and storage condition, the stability of the formulation could be enhanced.

3.5.3: Chemical stability of Spironolactone suspension by HPLC Analysis

The stability of spironolactone suspension formulated and analyzed (figure 2) showed that, from day zero (T0) to the 30th day (T30) the degradation was 5.33% and by the 60th day (T60), the degradation was 18.25%. The rate of degradation of the spironolactone suspension was fairly low from day zero (T0) to the 60th day (T60) than from T60 to T120 (refer to table 3.3.2). The degradation of the spironolactone suspension by the 120th day (T120) was 62.68%, leaving an active drug content of 37.32% of Spironolactone.

The acceptance criteria of Spironolactone suspension is 90%-110% at a storage condition of 2^{o}c to 8^{o}c (USP; 38). Spironolactone oral suspension formulated from 25mg tablets, methylcellulose (03%), and simple syrup (40%) was stable for 91days at room temperature (Nahata, 2003).

3.5.4: Chemical stability of Furosemide suspension by HPLC Analysis

The furosemide suspension formulated analyzed (figure 3) were found to have degraded by 6.91% from day zero (T0) to the 30th day (T30). The rate of degradation from T30 to T120 was very high. A degradation of 83.73% was recorded by the 120th day (T120) leaving an active content of 16.27%. The acceptance criteria for Furosemide suspension is 90% to 110% (USP; 38). Oral furosemide liquid prepared from injection with *syrpalta* (oral syrup vehicle) as a base and stored at room temperature was stable for 30days

3.5.5: Chemical stability Propranolol suspension by HPLC Analysis

The degradation of the propranolol suspension (figure 4) was very high, by the 30th day (T30) 82.66% had degraded leaving an active content of 17.34%. This indicates that less than 20% of the active Propranolol was available by the 120th day (T120). Propranolol suspension formulated from tablets (Nahata, 2003), stabilized with 1% citric acid and refrigerated is stable for 84 days. The acceptance criteria for propranolol suspension is 90% to 110% (USP; 38). The instability of the suspension could and combined effect of temperature (storage condition) and no stabilization with citric acid.

3.5.6: Chemical stability of Lamivudine suspension by HPLC Analysis

The result of lamivudine suspension (figure 5) showed that Lamivudine suspension is highly stable, from day zero (T0) to the 90th day (T90). The percentage active of Lamivudine by day 90(T90) was still more the 80% of the day zero (T0) content. However at T120, the Lamivudine that degradation from the suspension was 68.58%, leaving an active content of 31.42% indicating that after day 90 (T90) the degradation rate was very high. The acceptance criteria for the Lamivudine 90% to 110% (IP; 2015)

3.5.7: Chemical stability of Phenobarbitone suspension by HPLC Analysis

The stability phenobarbitone (figure 6) was very low from day zero (T0) to the 30th day (T30). The percentage of phenobarbitone in the suspension after 30 days was 54.09% indicating 45.91% of the active had degraded and by the 60th day (T60) over 65% of the active had degraded. Phenobarbitone oral suspension formulated from powder (API) with 20% glycerine and 30% sorbitol and refrigerated is stable for 30days (Nahata, 2003). Therefore the instability as observed could be attributed to the storage condition at room temperature (27±3^{o}C).

SUMMARY

The study was carried to determine the limits to which paediatric formulations prepared in Komfo Anokye Teaching Hospital are stable microbiologically and chemically. It was also to decide whether or not, syrup of CMC can be prepared and stock as ready to use vehicle or base which could be used for formulations when require to reduce waiting time of clients or patients.

Suspensions of eight oral paediatric dosage formulations (acetazolamide, spironolactone, propranolol, furosemide, phenobarbitone, hydroxyurea, carbamazepine and lamivudine) were formulated according to KATH formulation procedure; the formulations were analysed microbiologically by the Agar plate method and chemically (except hydroxyurea and carbamazepine) using HPLC methods.

The outcome showed that formulated suspensions of spironolactone and furosemide were microbiologically and chemically stable up to 30 days. Lamivudine suspension was stable chemically and microbiologically up to 60 days. The acetazolamide suspension was not stable up to 30 days contrary to a study reported by Nahata, 2003, though *syrpalta* was used as the vehicle or base in that research work. Phenobarbitone and propranolol suspensions were highly unstable within 30 days therefore refrigeration of these suspensions and possible stabilization is necessary to maintain their stabilities (Nahata, 2003).

The buffer syrup of CMC was microbiologically stable up to one year, therefore can be formulated as ready to use base or vehicle for preparations.

3.6: CONCLUSION

The 0.2% of Sodium benzoate used as a preservative for the formulations (Acetazolamide, Spironolactone, Furosemide, Hydroxyurea, Phenobarbitone, Propranolol, Lamivudine and Carbamazepine) was in general adequate to protect the formulations from microbiological instability up to the 90th day (T90) of the preparation. Hence patients from distance areas could be served with at least a 60days refill of their medication and reduce the frequent visit to KATH for refills. The buffer syrup formulated was physically and microbiological stable up to one year hence a stock of the buffer could be prepared and used as required. This will go a long way to reduce the waiting time of patients who come for medication refill.

The Lamivudine suspension was chemically most stable up to 90th day (T90) followed by that of Spironolactone and furosemide suspensions which were stable up to 60days (T60). Acetazolamide suspension was stable up the 30th day (T30). Phenobarbitone and propranolol suspensions were highly unstable within the 30days of formulation and storage at room temperature.

3.7: RECOMMENDATIONS

- The procedure and formulation methods for paediatric dosage forms at KATH may need to be reviewed. It is necessary for continuous education of mothers or parents of infants and children who are given these preparations on the proper storage of these preparations.
- The mothers should be taught how to detect instability of these preparations and report any changes observed during usage of the preparation.
- Buffer syrup of CMC could be prepared and stocked for use especially in Emergencies.
- There should be continuous study and evaluation of the stability of these preparations, which should be documented.
- Parents receiving formulated medications for their wards should be advised, as a precaution to store in the fridge, where possible to enhance stability.
- It is crucial to collaborate with other institutions involved in paediatric preparations to exchange and improve on the knowledge and experiences on Paediatric formulations.

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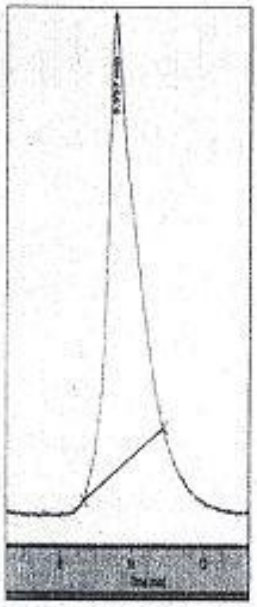
APPENDICES

Appendix I: FUROSEMIDE CHROMATOGRAMS



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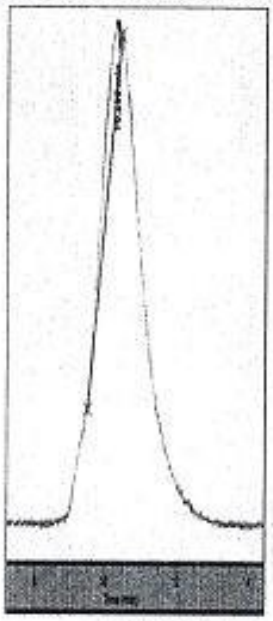
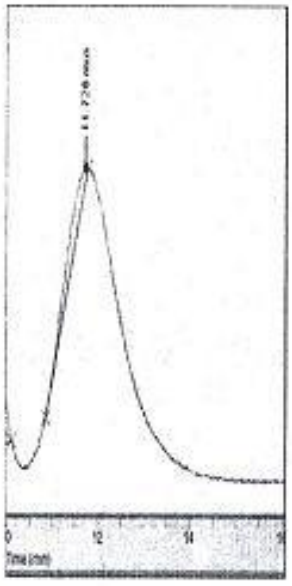


FURO/T0



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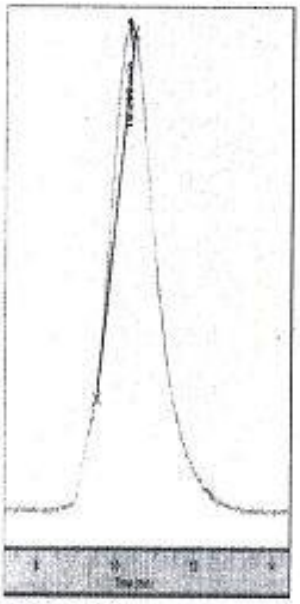
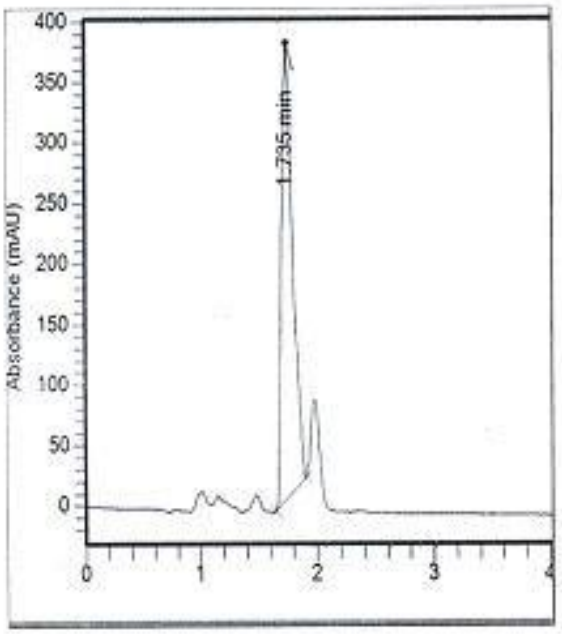


FURO/ T60

AppendixII: PHENOBARBITONE CHROMATOGRAMS



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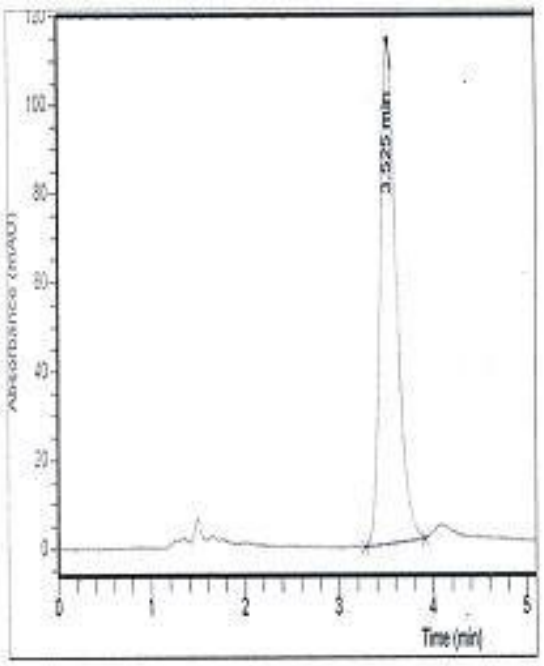

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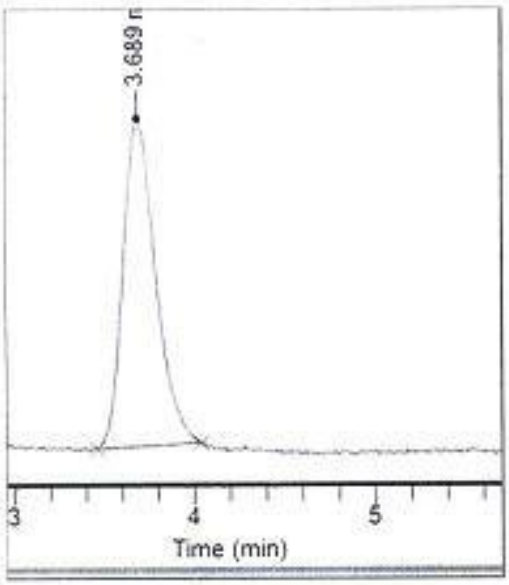

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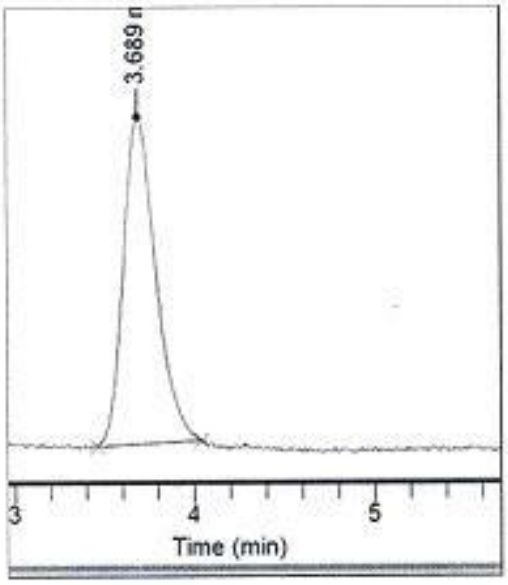

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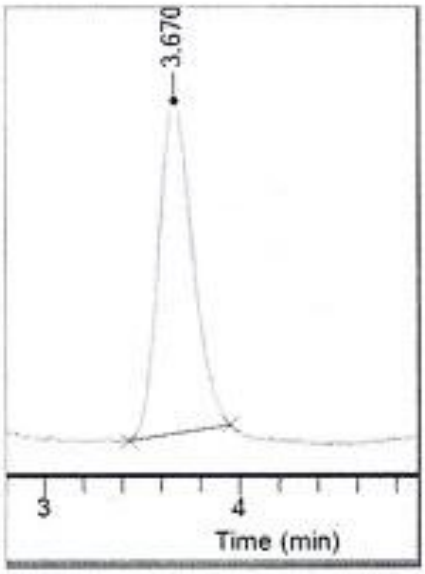

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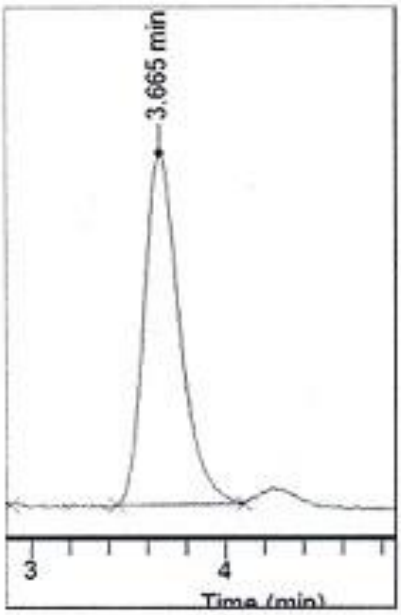
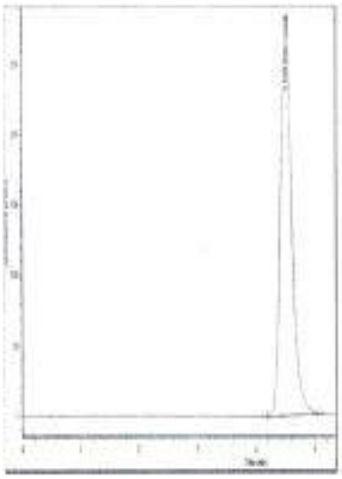


PHENO/ T120

Appendix III: **LAMIVUDINE CHROMATOGRAMS**



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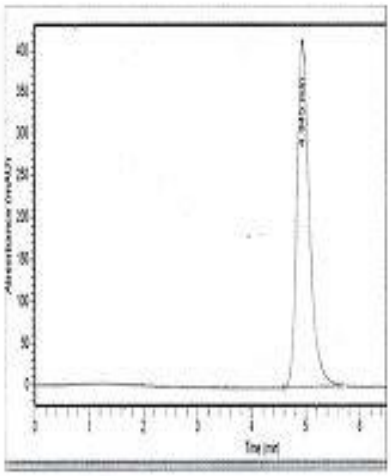
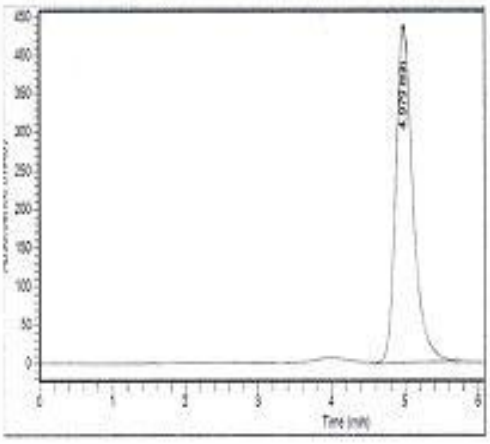


LAM/T0



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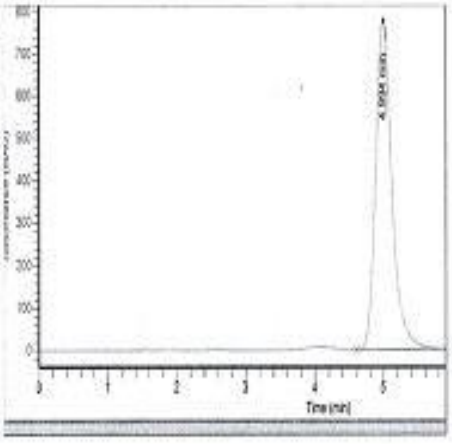

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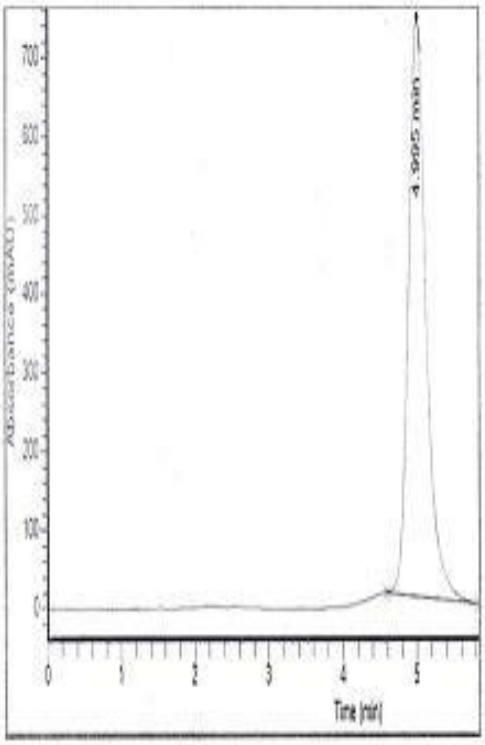

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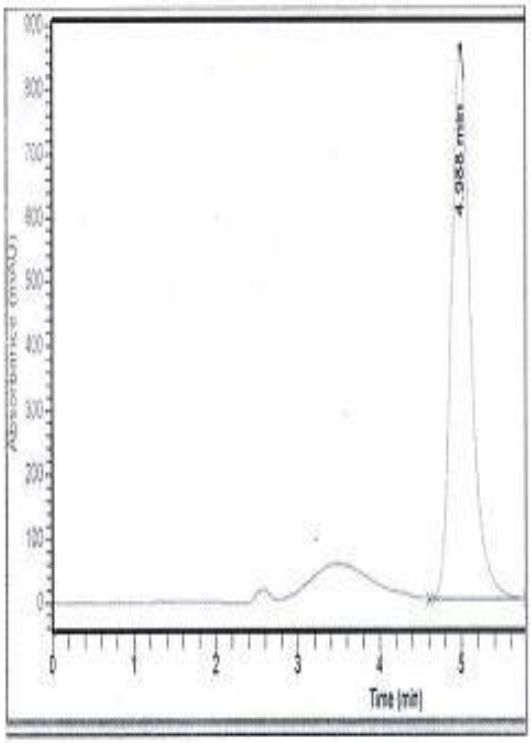
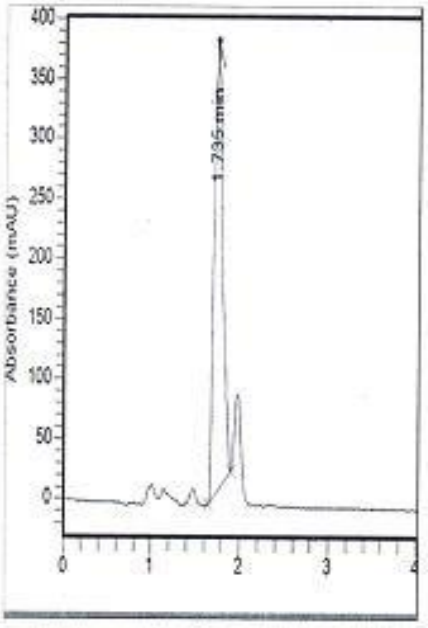


LAM/T120

Appendix IV: **PROPRANOLO CHROMATOGRAMS**



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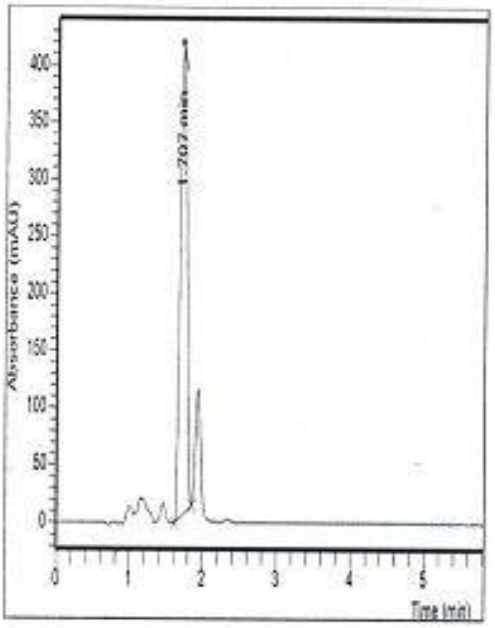

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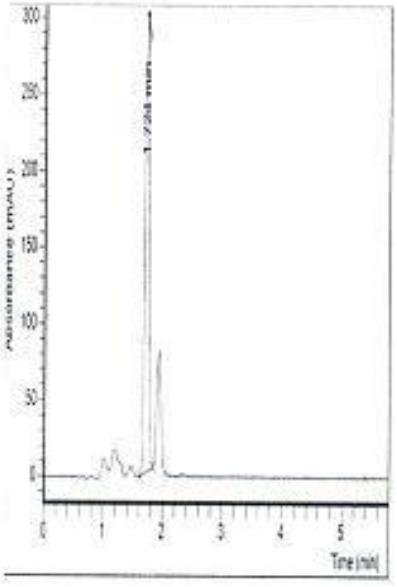

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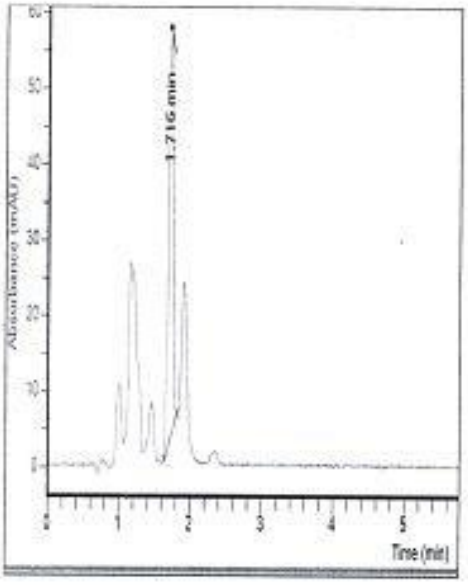

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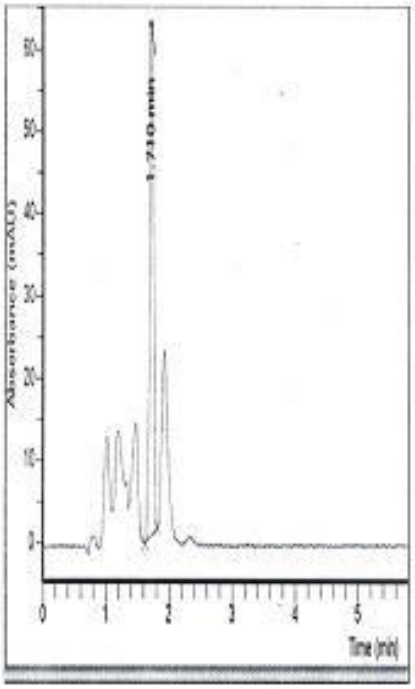
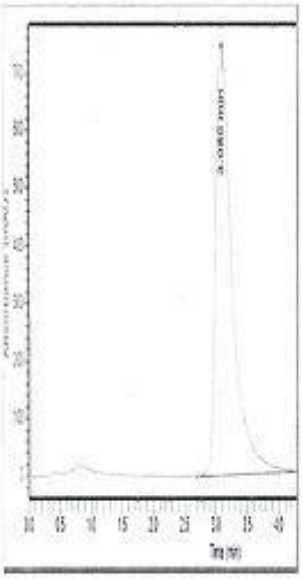


PROPRAN/T120

Appendix V: **ACETAZOLAMIDE CHROMATOGRAMS**



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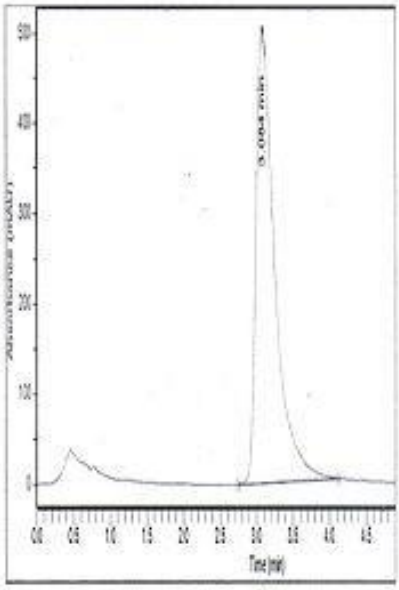

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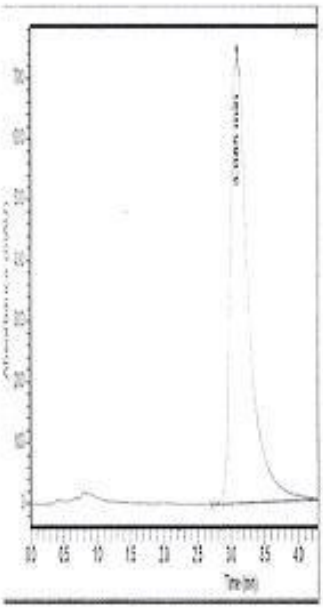

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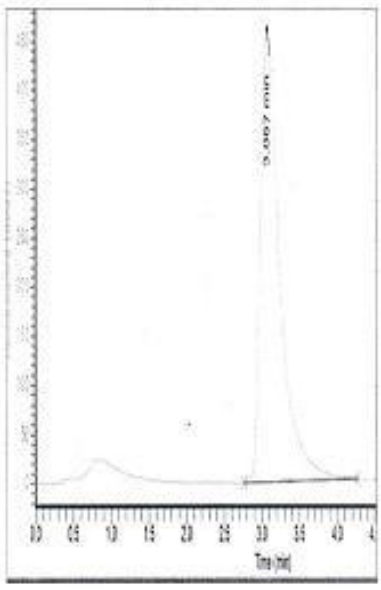

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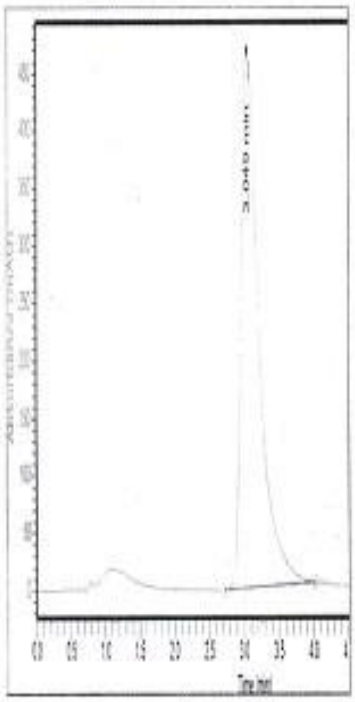
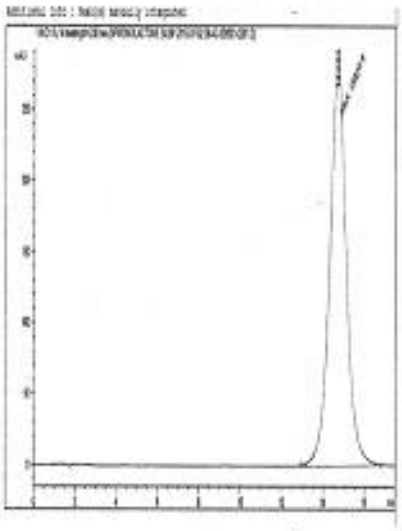


ACETA/T90

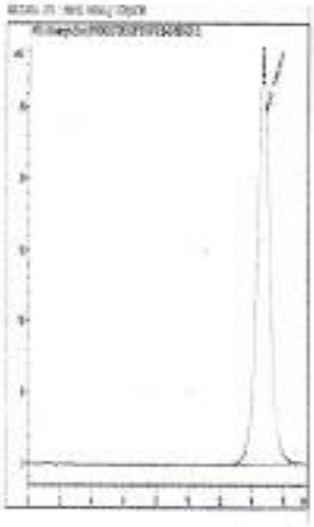
ACETA/T120

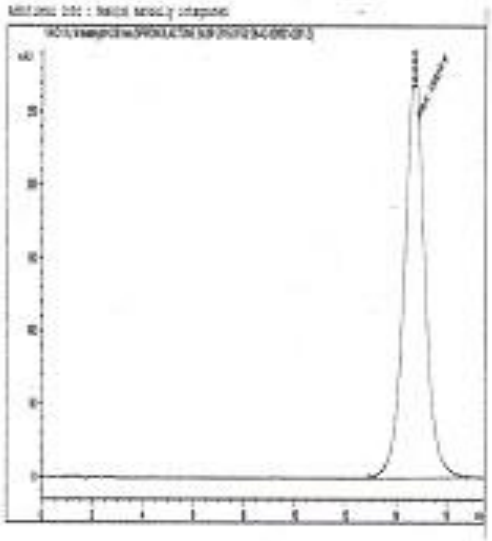
Appendix VI: SPIRONOLACTONE CHROMATOGRAMS



STANDARD

Appendix VI: SPIRONOLACTONE CHROMATOGRAMS





STANDARD

Appendix VII: PAEDTRIC FORMULATIONS FORMULATION PROTOCOL SHEET AT KATH

The formulae and procedure of preparing CMC compatible drugs is as below: Table2.

4 **Formulae for preparing suspension of CMC compatible drugs**

Dosage	**X**mg daily x 30			
Raw materials	Quantity	Quantity weighed	Weighed by	Checked by
NO. drug/dosage form	---------			
CMC	q.s.			
Sodium benzoate	0.2%			
Aspartame	q.s.			
Flavour	q.s.			
Purified water to	**Y**mL			

METHOD

……..Tablets/caps of (drug) …….. were crushed and triturated with measured amount of water. …….of Sodium Benzoate added as a preservative. Sufficient amount of Aspartame and CMC were added to the mixture respectively. Sufficient quantity flavor was then added to the mixture. Distilled water was then added sufficiently to the required volume of ……..ml.

The preparation was labeled and dispensed.

Prepared by: ……. Signature: …… Receipt Number: …….

Place label here…….

The above formula was standardized and modified to enable the preparation of 1000mL (1L) formulations.