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Development and In Vitro Evaluation of Cefuroxime Axetil Pediatric Suppositories

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Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institute.

Signed: ……..

Mohammad Mahareeq

Date: 09/06/2012

Contents

List of Abbreviations

Abs.	Absorbance
Adj.	Adjusted
Anh.	Anhydrous
API	Active Pharmaceutical Ingredient
AR	Analytical Reagent
B.N.	Batch Number
BCS	Biopharmaceutics Classification System
BHT	Butylated hydroxytoluene
BP	British Pharmacopoeia
CFA	Cefuroxime axetil
Conc.	Concentration
CSF	Cerebral spinal fluid
D.V.	Displacement Value
DL	Detection limit
EurP.	European Pharmacopoeia
GI	Gastro Intestinal
HPLC	High Performance Liquid Chromatography
HLB	Hydrophilic Lipophilic Balance
ICH	International Conference of Harmonization
ID	Inside diameter
LOD	Limit of Detection
LOQ	Limit of Quantitation

NMT	Not more than
OD	Outside diameter
PEG	Polyethylene glycol
PVC	Polyvinylchloride
RH	Relative humidity
rpm	Revolution per minute
RSD	Relative standard deviation
RT	Retention time
Sa.	Sample
SD	Standard deviation
SLS	Sodium lauryl sulfate
SOL`N	Solution
St.	Standard
USP	United States Pharmacopoeia
U.V.	Ultraviolet
WH15	Witepsol H15

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Abstract

Cefuroxime axetil (CFA) is a broad spectrum second generation Cephalosporin antibiotic, active against a wide range of common pathogens, including many β-lactamse producing strains. The drug is marketed as powder for oral suspension and tablet dosage forms. CFA extreme bitterness limits its use in a wide spectrum of patients. Administration of CFA in a suppository dosage form may be a useful alternative to oral route and convenient for infants, children and the elderly who find it difficult to swallow tablets or taste the extremes bitterness of the suspension dosage form. This study aims in formulating and evaluating suppositories containing Cefuroxime axetil for pediatric use. CFA suppositories were formulated by the fusion method using two main types of suppository basses; water soluble and fatty bases. PEG bases were used as the water soluble, while Witepsol H15 and Novata (A & BCF) were used as the fatty bases. The PEG water soluble bases were excluded from the study as they were found incompatible with CFA. Witepsol H15 was used in the study formulations as it showed better CFA release and lower melting points when compared with those formulated by using Novata bases. Suppositories were evaluated for physical appearance, uniformity of weight, disintegration time, drug content, in-vitro dissolution study and stability studies.

The rate and extent of CFA release from formulations prepared using fatty bases were influenced by the physicochemical properties such as melting range value. The drug partitioning appeared to favor the lipid phase and had a negative impact on CFA release characteristics.

Sixteen formulations were prepared for the study. Surfactants and melting range modifiers (i.e. Tween 20, Tween 85, SLS, Lanolin Anhydrous and Lecithin S) were added in different percentages and combinations, and as a result they significantly increased CFA release from the formulations they were prepared with.

The mechanism of drug release was evaluated using several mathematical models, including the Higuchi, Korsemeyer-Pappas, zero ordered, first order and Weibull models. CFA release kinetics were best described by the Weibull, Korsemeyer-Peppas and Higuchi model, and the values of the release exponent, n, revealed that the drug release was a consequence of the combined effects

of CFA diffusion, rate of melting of the base and partitioning of the drug which can be considered to be anomalous release.

Stability studies were conducted at three conditions (i.e. 25^{o}C/60%RH, 30^{o}C/60% RH and 2–8^{o}C) for the selected representative formulations; indicated that they are more stable at refrigerator conditions (2-8^{o}C) and most of them showed instability at 25 ^{o}C and 30^{o}C storage conditions.

Part one:

Introduction

1.1 Rectal dosage forms

Suppositories are solid dosage forms of varying weight and shape, intended for the administration of medicines via the rectum, vagina, or urethra for local or systemic drug delivery (David, 2008). They consist of a dispersion of an active ingredient in an inert matrix, which is generally composed of a rigid or semi-rigid base (Lieberman, et al, 1998). These dosage forms melt, soften or dissolve in the relevant body cavity prior to releasing the active ingredient (Abate, et al, 2005). Rectal suppositories are conventionally bullet, torpedo or conically-shaped with a rounded apex. Suppositories can be used to administer drugs for use as protectants or for palliative care of local tissues at the point of introduction or as a carrier for therapeutic agents where they are intended to exert localized or systemic effects (David, 2008).

Suppositories are either used for local action or systemic action. The type of action desired and the type of suppository must be considered when formulating suppositories, as the base exerts a marked influence on the release and action of drug. If systemic actions are desired, the suppository should melt or dissolve rapidly and release the drug readily (Aulton, 2002).

The use of the rectal route for drug administration is certainly not the route of first choice due to poor patient acceptability and psychological biases (Bergogne, Bryskier, 1999). However, the use of rectal delivery is often appropriate in situations where a patient is unwilling or unable to make use of the oral route of drug administration. This may occur in cases where the administration of a drug via the oral route results in intolerance, nausea and vomiting or associated gastric pain (Bolognia, et al, 1996). In addition, in cases where patients are uncooperative, unconscious or lack lucidity or when access to the intravenous route is compromised, as is the case, for example, with children or patients in intensive care units. Rectal dosing may also be of value in achieving appropriate therapeutic outcomes for patients needing multiple drug therapy or continuous intravenous fluid infusion, where treatment is difficult or when there are few undamaged veins available for catheterization (Bergogne, Bryskier, 1999).

The abundant supply of blood vessels and rapid diffusion of drugs through the rectal mucosa permits rapid absorption of many drugs which make the rectum a convenient route for systemic administration of drug. Many classes of medicaments appear to be well absorbed (e.g. antinauseants, tranquilizers, vasodilators, vasoconstrictors, bronchodilators, sedatives, analgesic etc.).

Local medication of the anal region is employed most often in the treatment of hemorrhoids; however these are used for other conditions such as bacterial infection and chronic inflammation (e.g. Local anesthetics, astringents, antiseptics and various anti- bacterial agents) (Aulton, 2002).
The ideal suppository should be easy to administer without pain on insertion and should remain at the administration site for a reasonable period of time. Conventional solid suppositories often give patients a feeling of alien discomfort and subsequently their refusal to use such delivery devices may lead to poor patient compliance. Furthermore, if the solid suppositories lack sufficient muco-adhesivity, they may traverse up the rectal cavity and reach the end of the colon, with the result that the drug delivered in this area may be absorbed into the venous blood system, thereby increasing the potential for the compound to undergo hepatic first-pass metabolism, the avoidance of which is one of the potential advantages of suppository use (De Boer G.A., et al, 1982).
Antibiotics are usually administered either orally or by a parenteral route, the latter being used for drugs that are poorly or not bioavailable by the oral route or when clinical situations require rapid or higher antibiotic concentrations to be achieved in the body. The rectal route of antibiotic administration is seldom mentioned in experimental and clinical pharmacokinetic studies and the characteristics of administration of antibiotics by suppository are poorly documented.
There are significant differences between countries in terms of the acceptability of suppositories by patients, but, in some populations, rectal drug delivery could represent a convenient, alternative route of antibiotic administration when other routes are not available. (Bergogne, Bryskier, 1999)

1.2 Advantages and disadvantages of rectal dosage forms

Rectal dosage forms have the following advantages (David, 2008):

- They may be successfully employed to provide a local effect for the treatment of infection and inflammation, e.g. hemorrhoids, proctitis.
- They are used to promote evacuation of the bowel (by irritating the rectum), to relieve constipation or to cleanse the bowel prior to surgery.
- They may be employed to provide systemic drug absorption in situations where oral drug absorption is not recommended. Examples of such applications include:
  - patients who are unconscious, e.g. in intensive care or who are postoperative
  - patients who are vomiting, e.g. gastrointestinal infection, migraine

  - gastro irritant drugs, e.g. non-steroidal anti-inflammatory agents, particularly in chronic usage
  - drugs that are prone to degradation in the stomach
  - drugs that are erratically absorbed from the upper gastrointestinal tract
- They may be employed to provide local treatment of diseases of the colon, e.g. Crohn's disease, ulcerative colitis.
- Their administration is easily performed by the patient.

Disadvantages of rectal dosage forms include:

- In certain countries, especially the USA and the UK, the rectal dosage forms are generally unpopular, especially for systemic administration of therapeutic agents, whereas the opposite is true in European countries.
- Specialist advice is required concerning the administration of dosage forms.
- The absorption of therapeutic agents from the rectum is slow and prone to large intrasubject and intersubject variability. The presence of feces within the rectum considerably affects both the rate and extent of drug absorption.
- Rectal administration of therapeutic agents may result in the development of local side-effects, in particular proctitis.
- The industrial manufacture of suppositories is more difficult than for other common dosage forms.

1.3 Physiology and anatomy of the rectum

A diagrammatic representation of the gastrointestinal tract, featuring the rectum, is shown in Figure (1.1). The main physiological features of the rectum that are related to drug delivery and hence to the formulation of rectal products are as follows (David, 2008):

- The length of the rectum is about 15–20 cm. The rectum is joined to the sigmoid colon at the top and to the anus.
- The rectum is divided into two sections: (1) the anal canal; and (2) the ampulla. The ampulla is the larger of the two sections (approximately four times larger than the anal canal). Feces are stored in the ampulla and excreted through the anus (a circular muscle) via the anal canal.

- There are three separate veins in the rectum: upper haemorroidal vein that drains into the portal vein, which flows to the liver, middle and lower haemorroidal veins that drain directly into the general circulation (Figure1.2).
- The wall of the rectum is composed of an epithelial layer that is one cell thick. Two cellular types exist: (1) cylindrical cells; and (2) goblet cells – the latter are responsible for the secretion of mucus. There are no villi (or microvilli).
- When empty the rectum contains about 3 ml of mucus, spread over a rectal surface area of approximately 300 cm^2.
- The pH within the rectum is essentially neutral with minimal buffering capacity (approximately 7.5 for mucous layer). Therefore, due to the inability of the fluids within the rectum to alter the degree of ionisation, the salt form of the drug is an important determinant of the resulting local efficacy and/or systemic absorption. The presence of fecal matter will markedly affect both the dissolution of the drug in the rectal fluids and the subsequent absorption of the drug into the systemic circulation.
- The fate of the absorbed drug is dependent on the area of the rectum from which absorption has occurred. Drugs that are absorbed into the inferior and middle haemorrhoidal veins will enter the circulation via the inferior vena cava and will subsequently avoid direct exposure of the drug to, and hence metabolism by, the liver. Absorption into the upper (superior) haemorrhoidal vein will result in entry into the liver (and subsequent metabolism) via the portal vein.
- There are no esterases or peptidases in the rectal fluid.
- Local muscle activity within the rectal wall may influence the rate of dissolution of solid dosage forms within the rectum, i.e. suppositories.

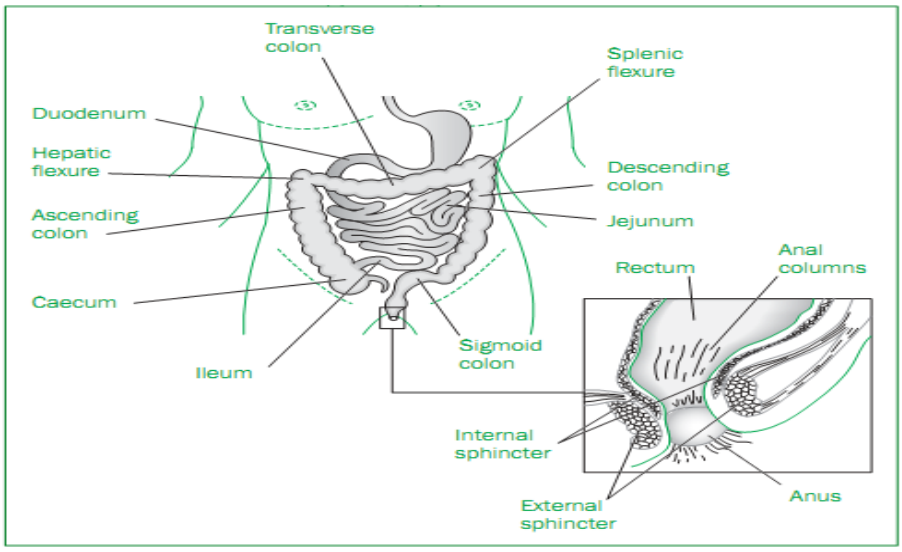


Figure 1.1 Diagrammatic representation of the gastrointestinal tract, with particular emphasis on the rectum (David, 2008).

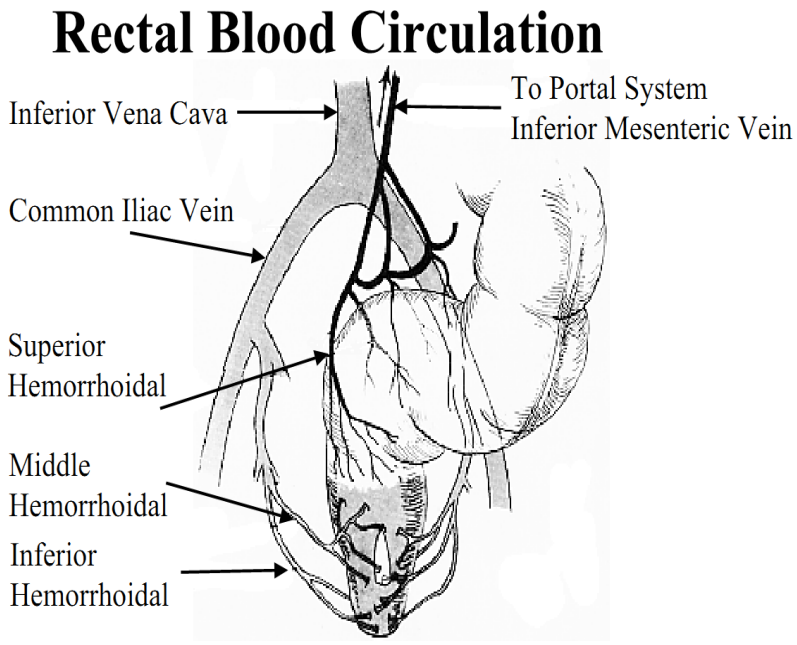


Figure 1.2 Diagrammatic representation of blood flow into and from the rectum (Stephen W. Hoag, 2002).

Since many experimental studies of rectal drug administration are performed in animals, it is necessary to note the differences in structure between human and animal rectums. In most animal

species, histological analysis reveals more goblet cells in the rectal mucosa than in the colon; in rats and rabbits there are many lymph nodes in the lamina propria and submucosa.

The mucosa is also thrown into several longitudinal folds containing large veins: this structure seems favourable to local absorption of drugs. A rapid colorectal cell turnover has also been described, potentially stimulated by chemicals such as ethanol or isoenergetic carbohydrates but such response has not always been discussed in studies of antibiotic administration in rats or rabbits (Bergogne, Bryskier, 1999).

1.4 Rectal absorption

The mechanism of absorption of systemically active drugs from the rectum involves drug release from the suppository into the rectal cavity, diffusion of the drug through rectal fluids to the rectal mucosa, followed by absorption across the rectal tissues and subsequent transport into the general circulation. The mechanism of absorption is similar to that occurs in the gastrointestinal tract, which in turn involves two main routes of penetration, the transcellular and paracellular routes. The transcellular route involves absorption of drugs across epithelial cells whereas the paracellular route involves absorption of drugs via the interconnecting tight junctions between mucosal cells (Toshiaki N., Rytting J.H., 1997). The rectal absorption of drugs is governed largely by the general principles of transfer of drugs. Depending on their chemical structure, drugs may cross the rectal wall either by absorption across the epithelial cell (transcellular) or via the tight junctions interconnecting the mucosal cells (paracellular). (Bergogne, Bryskier, 1999)

Following absorption from the rectum, the therapeutic agent enters the haemorroidal veins. Blood from the upper haemorroidal vein enters the portal vein, which flows into the liver, where drug metabolism occurs. Conversely, blood in the middle and lower haemorroidal veins enters the general circulation (David, 2008).

1.4.1. Factors affecting drug availability from suppositories

Several local factors may influence absorption in the rectum: the mucous layer, the variable volume of rectal fluid, the basal cell membrane, the tight junctions and the intracellular compartments may each constitute local barriers to drug absorption, depending on histological factors and on the molecular structure of the administered drug. The pharmaceutical formulation, therefore, may play a major role in the rectal absorption and consequently in the systemic

distribution and pharmacokinetics of drugs administered via suppository (Bergogne, Bryskier, 1999).

Rectal absorption and systemic distribution of a rectally administered drug may be influenced directly by the formulation composition, in addition to physiological factors. These factors relate specifically to the volume and composition of the rectal fluids and the associated environment, the physicochemical properties of the drug substance in addition to the physicochemical properties of the suppository base from which the drug is to be delivered (De Boer G.A., et al, 1982). The factors affecting rectal absorption of a drug administered in suppository formulations are summarised in Table 1.1

Table 1.1: Summary of the factors affecting drug availability from suppositories

Physiological Factors	**API**	**Formulation Parameters**
Buffer capacity	Solubility	Composition
Rectal fluid volume	Surface properties	Melting behavior
Surface tension	Particle size	Rheological properties
Composition	Drug concentration	Surface tension
Motility of the rectal wall	Partition coefficient pKa and the degree of ionization	

1.4.1.1 Physiological factors

The diffusivity of a drug is influenced by its physicochemical nature, the physiological state of the colon and rectum, including the amount and nature of fluid and solids present.

In the absence of faecal matter, an administered drug will have a greater potential to make contact with the mucosal surfaces of the rectum from which absorption will take place. The membranous wall of the rectum is covered with a continuous relatively viscous mucous blanket, which acts as a mechanical barrier to the free passage of a drug through the epithelial wall (David, 2008).

The rectum has a relatively small surface area available for drug absorption (About 200 to 400cm^2) compared to the small intestine. The rapidity and intensity of the therapeutic effects of suppositories are related to the surface area of the rectal mucous membrane covered by the melted base-drug mixture (the spreading capacity of the suppositories). This spreading capacity may be related to the presence of surfactants in the base (David, 2008).

The positioning of a suppository in the rectum is critical in terms of the potential for exposure of a drug to liver enzymes following absorption and subsequent metabolism. A drug that is absorbed into the systemic circulation via the inferior or middle rectal veins will bypass the liver, resulting in a higher bioavailability than one transported by the superior vein to the liver via the hepatic portal system, prior to its entry into the systemic circulation (De Boer G.A., et al, 1982).

The pH of the rectal fluids also plays a significant role in drug absorption and is often a rate-controlling step in rectal drug absorption. Rectal fluids have virtually no buffering capacity and, as a consequence, the characteristics of dissolved drugs will to a large extent determine the pH that prevails in the anorectal area following administration. It had been demonstrated that the intra-luminal pH of the rat colon can affect the absorption of acidic and basic drugs and that the unionized form of a drug is preferentially permeable. Thus the absorption of basic drug will be more favorable from rectal fluids, since it would be largely unionized and remain unionized at rectal pH, which is approximately 7.2. Therefore, it can be suggested that ionized substances that are lipid-insoluble will be poorly absorbed through rectal tissues (Lachman, et al, 1986).

1.4.1.2 Physicochemical characteristics of the drug and base affecting absorption:

1.4.1.2.1 Drug solubility:

The solubility of an API in the vehicle to be used as the suppository base determines whether the product that is produced is either a solution or suspension formulation and the solubility of a drug in the rectal fluid will determine the maximum attainable concentration possible, in the rectum, and consequently the driving force for the absorption process (Aulton, 2002) .

If a drug has a high oil to water partition coefficient and the base of choice is a fatty material, the API will primarily be in solution in the base. Therefore the ability or tendency of the drug to leave the vehicle will be low and the subsequent release rate into the rectal fluids will be slow (Aulton, 2002).

1.4.1.2.2 Partition coefficient

Drug absorption from the rectum is a consequence of the partitioning of a dissolved drug from a molten base into the rectal fluids and from the rectal fluids to the rectal mucosa, in addition to the rate of solution of the drug in the body fluids. It has been suggested that penetration of a drug through the barrier phase or epidermal mucosa of the rectum is proportional to the permeability constant of the drug, which is a complex constant taking into account factors such as transfer of

drug from the base to the barrier phase and diffusion of a drug through the barrier membrane. The transfer of a medicament from a base is related to the solubility of the medicaments in that base, whereas diffusion through the barrier membrane is related to the lipid/water partition coefficient of the drug between those fluids (Abate, et al, 2005).

1.4.1.2.3 Particle size:

When the formulation is composed of an API that has been dispersed in the appropriate formulation base/vehicle, e.g. a hydrophilic drug dispersed in a lipophilic base or vice versa, the rate of dissolution of the drug is inversely proportional to the particle size of the dispersed active agent (David, 2008). However, size reduction and the use of a smaller particle size does not necessarily ensure higher blood levels, as the drug release process is relatively complex and involves the melting and spreading of the base, in addition to the wetting, sedimentation and dissolution of the drug (Herman, 1995).

1.4.1.2.4 Surface properties:

If wetting of the API by the vehicle or base does not occur, powder particles may agglomerate, which in turn may affect the uniformity of dispersion of the API, due to the increased tendency for the agglomerated powder to sediment prior to the setting of the suppository. In order to reduce the surface effects of poorly wettable API's, the addition of a surfactant to a formulation will more than likely improve the wetting of the API and subsequently the facilitate dissolution of the drug in the suppository and in the rectal fluids (Aulton, 2002).

1.4.1.2.5 Nature of the base:

The base must be capable of melting, softening, or dissolving to release its API for absorption. If the base interacts with the API inhibiting its release, then drug absorption will be impaired or even prevented. Also, if the base is irritating to the mucous membranes of the rectum, then it may initiate a colonic response and a bowel movement that results incomplete API release and absorption (David, 2008).

1.4.2 Enhancement of rectal absorption

The rate at which the drug diffuses into the rectal mucosa is influenced by the physicochemical relationship that exists between a drug, the rectal fluids, the suppository base and the membranes of the rectal cavity. Drug absorption from a suppository formulation can be modulated by the incorporation of absorption or permeation enhancers into the dosage form. The derivatives of amino acids, surfactants, fatty acids derivatives, and carboxylic acid derivatives have been

reported to act as effective absorption or penetration enhancers for rectally administered compounds (Toshiaki, Rytting, 1997).

The addition of adjuvant to a formulation can affect drug absorption by changing the rheological properties of the base at body temperature or by altering the dissolution rate of a drug in the rectal fluids. The safety, efficacy and compatibility of a drug and/or base with absorption enhancers must be established during pre formulation studies since the addition of an absorption enhancer may either reduce or increase drug release rates, depending on the nature of the enhancer, base and drug to be incorporated into a specific formulation (Lachman, et al , 1986).

The promoting effect of sodium salts of saturated straight chain fatty acids on the rectal absorption of ampicillin and of ceftizoxime has been confirmed in mice, rats, rabbits and dogs with bioavailability rates higher in mice and rabbits(76–100%) than in dogs (28.9% and 42% for ampicillin and ceftizoxime, respectively. The fatty acid used in the latter study and in others was sodium caprate, a carboxylic acid sodium salt, which improved the rectal absorption of poorly absorbed drugs such as lactams. Several other fatty acid salts, e.g. sodium capronate, sodium caprylate and sodium palmitate, also improved the absorption of ampicillin but the best absorption-promoting effect was exhibited by sodium caprate, with satisfactory bioavailability of 71.3% and 64.2% for ampicillin and piperacillin, respectively. In the same study, the bioavailability of cephalosporin generally ranged between 60.6% (cefotiam) and 92.4% (cefazolin), with lower bioavailability for cefpiramide (26.2%) and cefoperazone (27.5%). It seems likely that the absorption-promoting effect on lactams is stronger for antibiotics of smaller molecular size. Various other absorption promoters have been used in experiments in animals. For example, Witepsol H-15, a saturated triglyceride, has been used in suppositories of bacampicillin and compared with the same formulation of ampicillin. For the rectal administration of latamoxef in rats, the release rates from suppositories containing Witepsol H-15 only, or with the addition of Tween 80 (1%), with or without diclofenac sodium, a non-steroidal antiinflammatory drug, were compared. It was shown that the latter additions significantly increased the rectal absorption of latamoxef, with bioavailability as high as 72%.

Several other studies in animals of the suppository route of administration of amino glycosides have used similar preparations, with triglycerides (Witepsol H-15 or H-42) for gentamicin11 or with medium-chain glycerides (Capmul) for gentamicin and tobramycin rectal administration,

resulting in enhanced absorption of amino glycosides which are otherwise poorly absorbed (Bergogne, Bryskier, 1999).

1.5 Formulation of suppositories

The typical weight range for suppositories is 1–4 grams, with the 2-gram suppository being the commonly used size. The smallest suppositories are mainly reserved for use in children, whereas the largest size may be administered to adults, e.g. glycerin suppositories that are used to relieve constipation in adults. Suppositories are tapered at one end (to aid insertion) and are frequently wider in the middle before tapering towards the other end (thereby aiding retention in the rectum and enabling the suppository to be pressed forward by the anal sphincter). The drug loading of suppositories ranges from 0.1 to 40% w/w. In general, suppositories are composed of an inert base into which the therapeutic agent is incorporated (dissolved/dispersed) (David, 2008).

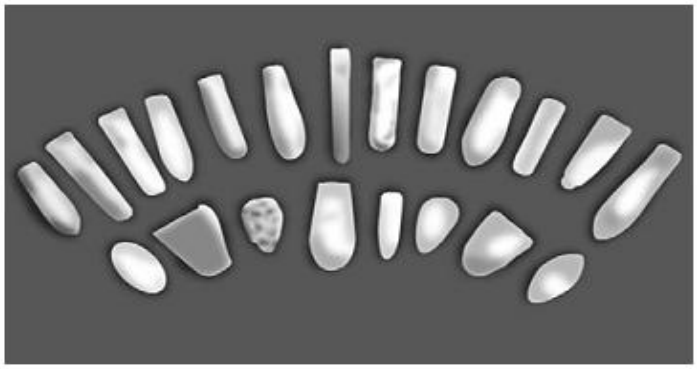


Figure 1.3 Examples of the different shapes and sizes of suppositories (David, 2008).

1.5.1 Suppository bases:

Suppository bases are usually classified, according to their physical and chemical characteristics, into three main classes: **(**David, 2008)

- Fatty or oleaginous bases, such as theobroma oil, synthetic and semi-synthetic fatty bases.
- Water-soluble or water miscible bases that may consist of glycerol, gelatin and/or polyethylene glycol.
- Miscellaneous bases such as hydrophilic or water-dispersible compounds that may include nonionic surfactants mixed with either vegetable oils or waxy solids

1.5.1.1 Fatty bases:

Cocoa butter (Theobroma Oil):

This is a natural material that consists of a mixture of fatty acid esters of glycerol, such as stearic, palmitic and oleic, predominantly triesters, e.g. glyceryl tripalmitate. The presence of unsaturated esters (e.g. oleic acid) contributes to the low melting point of cocoa butter (30–36^{0}C), thereby facilitating cocoa butter melting following insertion within the rectum. The incorporation of lipophilic drugs into cocoa butter has been reported to lower the melting-point range of suppositories produced using this base, which may lead to stability problems and may result in suppositories that are too soft to insert. Cocoa butter is safe, non-toxic and non-irritating.

The major problem with the use of cocoa butter as a base for suppositories is polymorphism, i.e. the ability of this material to exist in different crystalline forms; this is accredited to the high content of triglycerides, which may lead to instability issues (notably poor setting properties or re-melting of the suppositories following manufacture) (David, 2008).

Synthetic and semi-synthetic fatty bases:

Semi-synthetic fats are usually white, brittle, solid, odorless and unctuous to touch and produce suppositories that are white and have an attractive, clean, polished appearance (Raymond, et al, 2006).

Hard fats are available in a variety of grades with different melting ranges, hydroxyl values and other physicochemical characteristics. The hydroxyl value is one of the physicochemical properties of a base that can be used to distinguish fatty bases in terms of their compatibility with an API and an associated extended shelf life. A high hydroxyl value indicates that the base has a greater ability to absorb water relative to a base with a low hydroxyl value and it has been suggested that these bases should not be used to manufacture formulations containing drugs that are readily hydrolyzed (Aulton, 2002). The water absorbing capacity of a suppository base could influence the formation of w/o emulsions *in situ* in the rectum, which must be avoided since drug release rates from these systems have been reported to be very slow (Aulton, 2002). A base with a high hydroxyl value will have a tendency to form hydrogen bonds with components of the formulation and the API, which in turn may result in relatively slow release rates of a drug from the base to the rectal mucosa. Bases with a high hydroxyl value have also been reported to be irritant to the rectal mucosa (Raymond, et al, 2006).

The use of hard fat suppository bases is preferred over the use of cocoa butter, as they do not exhibit polymorphism and their solidification is unaffected by overheating during the manufacturing process (Lachman, et al, 1986). The hard fat bases have a narrow temperature interval between their melting and solidification points, which is generally between 1.5°C and 2°C and seldom over 3°C (Lachman, et al, 1986). The narrow temperature range between melting and solidification aids in the manufacture of uniform suppositories, as the risk of sedimentation of an insoluble drug dispersed in the base is usually low. In addition, hard fat suppository bases contract markedly on cooling thereby reducing the need for the use of a lubricant to facilitate removal of products from moulds following manufacture.

As the presence of unsaturated fatty acids in the semi-synthetic bases is reduced, the bases are relatively resistant to oxidation when compared to cocoa butter, which contains a considerable amount of unsaturated oleic acid (Aulton, 2002). Semi-synthetic fatty bases have low acid and iodine values of < 2 and < 7, respectively, when compared to cocoa butter, which has an acid value of < 5 and an iodine value of 34-38. Low acid and iodine values are essential properties of suppository bases should a long shelf-life be required (Lachman, et al, 1986). The possibility of decomposition by moisture, acids and oxygen, which leads to rancidity in fats, increases with high iodine values (Lachman, et al, 1986).

Examples of commercially available semi-synthetic fatty suppository bases include fractionated palm kernel oil, and hard fats such as Novata with different types, Massa Estarium®, Massupol®, Suppocire® and Witepsol®.

1.5.1.2 Water-soluble and water-miscible bases**:**

There are two main categories of suppository base in this classification: (1) glycerol–gelatin base which dissolves in the rectal fluids; and (2) water-miscible bases, composed of polyethylene glycols (PEGs) (David, 2008):

<u>Glycerol–gelatin:</u>

Glycerol–gelatin bases are mainly used for the formulation of suppositories that contain a water-soluble APIs. These suppository bases are prepared by dissolving gelatin (about20% w/w) in glycerol (70% w/w) with the aid of heating (about100^{o}C); the API is generally dissolved/dispersed in an aqueous phase (<10% w/w) and then combined with the glycerol phase with stirring prior to pouring into the suppository mould.

The use of this type of base is restricted by several disadvantages, including:

- Physiological effect. Glycerol–gelatin suppositories will induce defecation and, hence, are used to relieve constipation or to facilitate bowel evacuation prior to surgery.
- Difficult to manufacture.
- Hygroscopic. Glycerol–gelatin bases will absorb moisture from the atmosphere and therefore must be carefully packaged to prevent moisture uptake and to maintain both the shape and mechanical properties of the suppository. This ability of glycerol–gelatin bases to absorb water will also occur within the rectum, leading to dehydration and irritation of the rectal mucosa. To minimise this phenomenon, the suppository may be moistened with water prior to insertion.
- Potential interactions with APs.

Water-miscible bases:

The melting point of PEGs increases as the molecular weight increases, e.g. the melting points of PEG 1000 and PEG 8000 are 37–40^{o}C and 60–63^{o}C, respectively. Typically the melting point of PEG suppository bases is about 42^{o}C; this is generally achieved and controlled using the appropriate mixtures of grade of this polymer.

There are two concerns regarding the use of PEG-based suppositories. PEG is known to enhance the solubility of therapeutic agents and therefore this interaction between the drug and polymer may affect the subsequent release of the drug from the liquefied base. Secondly, the solubility of the drug in the solid base may change as functions of both storage conditions and time and this may result in crystal growth within the suppository.

Following insertion into the rectum, these suppositories will not melt but, due to their hygroscopic properties, will gradually dissolve (the volume of rectal fluid is too small to allow rapid dissolution) and, in so doing, will enable drug dissolution to occur. This ability to absorb moisture may lead to patient discomfort due to the extraction of water from the rectal mucosa into the suppository; however, this may be minimized by the inclusion of water (> 20% w/w) and by moistening the suppository prior to insertion. PEG-based suppositories will require storage in moisture-resistant packaging.

1.5.2 Criteria for selecting a suitable suppository base

The properties of an ideal suppository base: (Saritha, 2005), (Aulton, 2002)

1- Melts at body temperature or dissolves in body fluids.

2- Non-toxic and non-irritant.

3- Compatible with the APIs.

4- Releases the APIs readily.

5- Easily molded and removed from the mould.

6- Stable to heating above the melting point.

7- Easy to handle.

8- Stable on storage.

9- If the base is fatty, it has the following additional requirements.

- "Acid Value" is below 0.2.
- "Saponification value" ranges from 200 to 245.
- "Iodine value" is less than 7.

The selection of a suitable suppository base depends on a number of physicochemical variables, including, but not limited to the solubility of the drug in the base and rectal fluids, in addition to the intended therapeutic goals following rectal administration. Table 1.2 shows the recommended suppository base in relation to API solubility.

Table 1.2: API solubility and recommended suppository base (Aulton, 2002)

API solubility in		**Choice of base**
Fat	**Water**	
Low	High	Fatty base
High	Low	Aqueous base
Low	Low	Indeterminate

In order to ensure that the maximum amount of drug is released from a base, a principal of opposites may be applied. A water-soluble drug may be incorporated into a fatty base while a fat soluble drug may be best incorporated into a water soluble or miscible base. The selection of a suitable base shall be based on knowledge of the physicochemical properties and intrinsic pharmaceutical or pharmacological activity of the active ingredients to be incorporated into the suppository (Lachman, et al, 1986).

The physical properties of a suppository base that may or may not be affected by the addition of a drug or that can influence drug release rate, as well as the stability of the final product are the melting characteristics, iodine value and the hydroxyl value. These parameters are widely used in the pharmaceutical industry for a range of applications with regard to suppository base selection (Leiberman, et al, 1998).

The following rules are considered: (Leiberman, et al, 1998), (Jayanti, N.D)

1) A narrow interval between the melting point and the solidification point, especially the small scale (say, in a pharmacy).
2) For a drug that can lower the melting point, high melting range bases are used (say 37 to $41^{O}C$). Examples for such drugs are camphor, chloral hydrate, menthol, phenol, thymol, and volatile oils
3) When large amounts of total solids, which can increase the viscosity of the melted suppository, are used, bases with low melting ranges, such as (30 to $34^{O}C$) shall be used.
4) Bases with low acid values (below 3) and iodine values (below 7) give suppositories with long shelf life.
5) For drugs intended for systemic effect, the chosen base must liquefy at or below body temperature, whereas only base softening or dispersion may be adequate for the delivery of compounds intended for local action, sustained and/or modified release of the API.
6) Suppository bases with high melting points may be useful for delivering drugs that tend to lower the melting point of a base after inclusion, or for suppositories intended for use in warm climates. The high molecular weight PEG bases, in combination with low molecular weight PEG, may be appropriate.

7) A suppository base with a low hydroxyl value should be selected in cases where the API(s) to be incorporated in the delivery system is/are sensitive to the presence of the free hydroxyl radicals.

1.5.3 Additives used in the formulation of suppositories

The formulation of successful suppositories, in addition to suppository base, may necessitate the inclusion of other excipients. These include: (David, 2008)

1.5.3.1 Surface-active agents:

Surfactants, such as Sorbian esters and polyoxyethylene sorbitan fatty acid esters, are used to enhance the wetting properties of the suppository base with the rectal fluid, and consequently enhance the drug release or dissolution rate. The use of surfactants is mainly reserved for formulations composed of a lipophilic suppository base and/or a lipophilic drug.

1.5.3.2 Agents to reduce hygroscopicity:

These agents reduce the uptake of water by fatty suppository bases from the atmosphere during storage, and thus enhance the chemical and physical stability of the finished dosage form. Colloidal silicon dioxide is an example for this category.

Water uptake during storage of water-miscible bases will result in changes to the mechanical properties (softening) and shape of these dosage forms. Accordingly, protection against water uptake during storage is also afforded by the use of moisture-resistant packaging.

1.5.3.3 Agents to control the melting point of the base:

The melting point of the base may be manipulated to enhance the mechanical properties and physical stability of the suppository in response to the deleterious effects of storage at higher temperature and/or the presence of a therapeutic agent that is soluble in the suppository base.

Examples of excipients that are commonly used to increase the melting point of suppositories prepared using fatty bases include:

- beeswax (white or yellow wax)
- cetyl esters wax stearic acid
- stearic alcohol
- aluminium mono- or distearate
- colloidal silicon dioxide
- magnesium stearate

- bentonite.

Conversely there may be a requirement to reduce the melting point of the fatty suppository base, e.g. to enable melting within the rectum. Examples of excipients that may be used for this purpose include:

- glyceryl monostearate
- myristyl alcohol
- polysorbate 80
- propylene glycol.

The melting point of PEG-based suppositories may be controlled by using different molecular weights and ratios of PEGs.

1.5.3.4 Lubricants

Lubricating the cavities of the mould is helpful in producing elegant suppositories and free from surface depression. The lubricant must be different in nature from the suppository base; otherwise it will become absorbed and fail to provide a buffer film between the mass and the metal. The water soluble lubricant is useful for fatty bases while the oily lubricant is useful for water soluble bases.

Table 1.3 Lubricants for use with suppository bases:

Base	**Lubricant**
Theobroma oil	Soap spirit
Glycerol-gelatin base	liquid paraffin
Synthetic fats	No lubricant required
Macrogols	No lubricant required

1.5.4 Preparation of suppositories:

Suppositories may be prepared by either cold or fusion/melt molding methods (Lachman, et al, 1986), (Abate, et al, 2005).

1.5.4.1 Cold Methods:

In these methods the API is well mixed with grated suppository base by the aid of water or wool fat, then the suppositories are formed either by hand molding into rods or compression molding through suitable openings. The rods were cut to the suitable length or weight.

These methods are suitable for preparing small numbers of suppositories, and for heat labile APIs. The major disadvantage of these methods is the unavoidable air entrapment, which makes it impossible to control the weight, and increase the possibility for oxidation of both the base and the API.

1.5.4.2 Fusion or Melt Molding:

This is the most commonly used method for producing suppositories on both small and large scale. Suppository molds are available for the preparation of various types and sizes of suppositories. Molds are made of aluminum alloy, brass, or plastic and are available with from six to several hundred cavities.

- In this method the base material is first melted, preferably on a water bath to avoid local overheating.
- The drug is then dispersed or dissolved in the melted suppository base.
- The mixture then is poured into a suppository mold, allowed to cool, and the finished suppositories are removed by opening the mold.

The method of choice for commercial production involves the automated filling of molds or preformed shells by a volumetric dosing pump that meters the melt from a jacketed kettle or mixing tan directly into the molds or shells. Strips of preformed shells pass beneath the dosing pump and are filled successively, passed through cooling chambers, sealed, and then packaged.

1.5.4.3 Problems in formulation:

1- Water in suppositories:

Formulators do not like to use water for dissolving drugs in suppositories for the following reasons:

a. Water causes oxidation of fats.

b. If the suppositories are manufactured at a high temperature, the water evaporates, the drugs crystallize out.

c. Absorption of water soluble drugs is enhanced only if the base is an oil – in – water emulsion with more than 50% of the water in the external phase.

d. Drug excipient interactions are more likely to happen in the presence of water.

e. Bacterial contamination may be a problem

2- Hygroscopicity:

a. Glycerogelatin suppositories lose moisture in dry climates and absorb moisture in humid conditions.
b. The hygroscopicity of polyethylene glycol bases depends on the chain length of the molecule. As the molecular weight of these ethylene oxide polymers increases the hygroscopicity decreases

3- Drug excipient interactions

4- Viscosity:

When the base has low viscosity, sedimentation of the drug is a problem. 2% aluminum monostearate may be added to increase the viscosity of the base. Cetyl and stearyl alcohols or stearic acid are added to improve the consistency of suppositories.

5- Brittleness:

Cocoa butter suppositories are elastic, not brittle. Synthetic fat bases are brittle. This problem can be overcome by keeping the temperature difference between the melted base and the mold as small as possible. Materials that impart plasticity to a fat and make them less brittle are small amounts of Tween 80, castor oil, glycerin or propylene glycol

6-Density:

Density of the base, the drug, the volume of the mould and whether the base is having the property of volume contraction are all important. They all determine the weight of the suppository.

7- Lubrication of moulds:

Some widely used lubricating agents are mineral oil, aqueous solution of SLS, alcohol and tincture of green soap. These are applied by wiping, brushing or spraying.

8- Volume contraction:

On solidification the volume of the suppository decreases. The mass of the suppository pulls away from the sides of the mould. This contraction helps the suppository to easily slip away from the mould, preventing the need for a lubricating agent. Sometimes when the suppository

mass is contracting, a hole forms at the open end. This gives an inelegant appearance to the suppository. Weight variation among suppositories is also likely to occur. This contraction can be minimized by pouring the suppository mass slightly above its congealing temperature into a mould warmed to about the same temperature. Another way to overcome this problem is to overfill the molds, and scrape off the excess mass which contains the contraction hole.

10- Weight and volume control:

Various factors influence the weight of the suppository, the volume of the suppository and the amount of active ingredient in each suppository, they are:

a. Concentration of the drug in the mass

b. Volume of the mould cavity

c. The specific gravity of the base

d. Volume variation between moulds

e. Weight variation between suppositories due to the inconsistencies in the manufacturing process.

11- Rancidity:

The unsaturated fatty acids in the suppository bases undergo auto oxidation and decompose into aldehydes, ketones and acids. These products have strong, unpleasant odors. The lower the content of unsaturated fatty acids in a base, the higher is its resistance to rancidity.

1.5.5 Calculation of the mass of base required:

One concern regarding the manufacture of suppositories is the calculation of the mass of base that is required. The volume of each suppository mould is known and has been calibrated. However, if the drug is dispersed in the molten formulation, the volume of the formulation will be dependent on the mass of drug present (remembering that solids displace an equal volume of base). To ensure that the correct volume of base is used, a calculation is performed based on the displacement value, i.e. the ratio of the weight of the drug to the weight of base displaced by the drug. The displacement factor may be visualised as the weight of drug required to displace unit weight of base (David, 2008).

In practice the displacement value is calculated as follows:

- The average weight of the suppository mould is calculated using the blank suppository base (the molten base is added to the correct volume, allowed to cool and then weighed).

- The weight of drug needed for the total number of suppositories is calculated (weight of drug per suppository.
- Suppositories are then prepared by adding the mass of drug to the notional mass of suppository base, melting and then dispensing into the suppository moulds. The weight of the cooled suppositories is then determined.

Displacement Value:

The volume of a suppository from the particular mould is obviously uniform, but its weight will vary according to the density of medicaments. Consequently, products made from moulds cannot be prepared accurately unless allowance is made for the alteration in density of the mass due to added medicaments. The quantity of medicament which displaces 1 part of cocoa-butter or any other base (called the displacement value) is the most convenient method of making this allowance (Lachman, et al, 1986).

The amount of base that is replaced by active ingredients in the suppository formulation can be calculated. The replacement factor, f, is derived from the following equation:

$$\boldsymbol{f = \frac{100(E-G)}{(G)(X)} + 1} \qquad \text{..... (1.1)}$$

Where *E*=weight of pure base suppositories, *G*=Weight of suppositories with *X*% active ingredient. The appropriate mass of suppository base to be used for a specific batch of product is calculated using the following Equation:

$$\boldsymbol{P = (N \times S) - \frac{ND}{f}} \qquad \text{..... (1.2)}$$

Where,

P = the amount of base required
D = the amount of drug that is required
N = the number of prepared suppositories
f = displacement value
S = the size of the mould used

1.6 Quality Control of Suppositories:

The quality control of suppositories includes the physical, chemical and physio-chemical aspects.

1.6.1 Visual examination

This includes odor, colour, surface condition and shape. It is important to check for the absence of fissuring, pitting, fat blooming, exudation, sedimentation, and the migration of the active ingredients. Suppositories can be observed as an intact unit and also by splitting them longitudinally.

1.6.2 Uniformity of mass

Twenty units are individually weighed and the average mass is determined. Not more than two of the individual masses deviate from the average mass by more than 5.0% and deviate by more than twice that percentage. It is used as indicator to potential problems in manufacturing process. (BP, 2011)

1.6.3 Melting time

Melting time is the time taken by an entire suppository to melt when it is immersed in a constant temperature bath at 37°C.

1.6.4 Melting range (melting point, melting zone)

It indicates the temperature at which the base starts melting and the temperature at which it is completely molten. A number of different techniques are used to study melting behavior, including the open capillary tube, the U-tube, the ascending melting point and the drop point methods (Loyed, 2007).

1.6.5 Content and content uniformity testing

In order to ensure content uniformity, individual suppositories must be analyzed to provide information on dose-to-dose uniformity. Testing is based on the assay of the individual content of drug substance(s) in a number of individual dosage units to determine whether the individual content is within the limits set.

Ten units are assayed individually. The requirements for dosage uniformity are met if the amount of API in each of the 10 dosage units as determined from the Content Uniformity method lies within the range of 85.0% to 115.0% of the label claim, and the acceptance value is not more than 15 (USP32, 2010).

1.6.6 Mechanical strength/crushing test

Suppositories can be classified as brittle or elastic by evaluating the mechanical force required to break them. Tests are used that measure the mass (in kilograms) that a suppository can bear without breaking. A good result is at least 1.8–2 kg pressure. The purpose of the test is to verify that the suppository can be transported under normal conditions, and administered to the patient (Loyed, 2007).

1.6.7 Disintegration test for suppositories

The disintegration test determines whether suppositories soften or disintegrate within a prescribed time when placed in an immersion fluid (BP, 2011), (Loyed, 2007).

Disintegration is considered to be achieved when:

- The components of the suppositories have separated, e.g. melted fatty substances have collected on the surface of the liquid, insoluble powders have fallen to the bottom, and soluble components have dissolved or are distributed in one or more of the ways described in Methods 1 and 2;
- There is softening of the test sample, usually accompanied by an appreciable change of shape without complete separation of the components. The softening process is such that a solid core no longer exists when pressure is applied with a glass rod.
- Rupture of the gelatin shell or rectal capsule occurs resulting in release of the contents.
- Dissolution is complete.

For disintegration testing the Apparatus indicated in Fig.1.4 is used for water-soluble, hydro dispersible and fat-based suppositories, while Apparatus in Fig.1.5 may be used as alternative apparatus for fat-based.

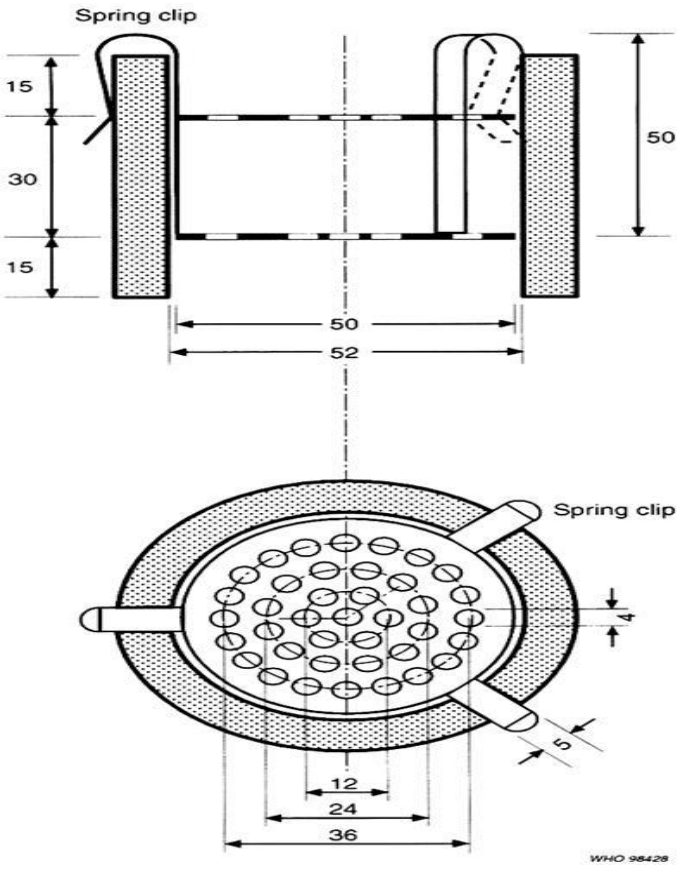


Fig.1.4 Disintegration apparatus for hydro dispersible and fat-based suppositories (BP, 2011)

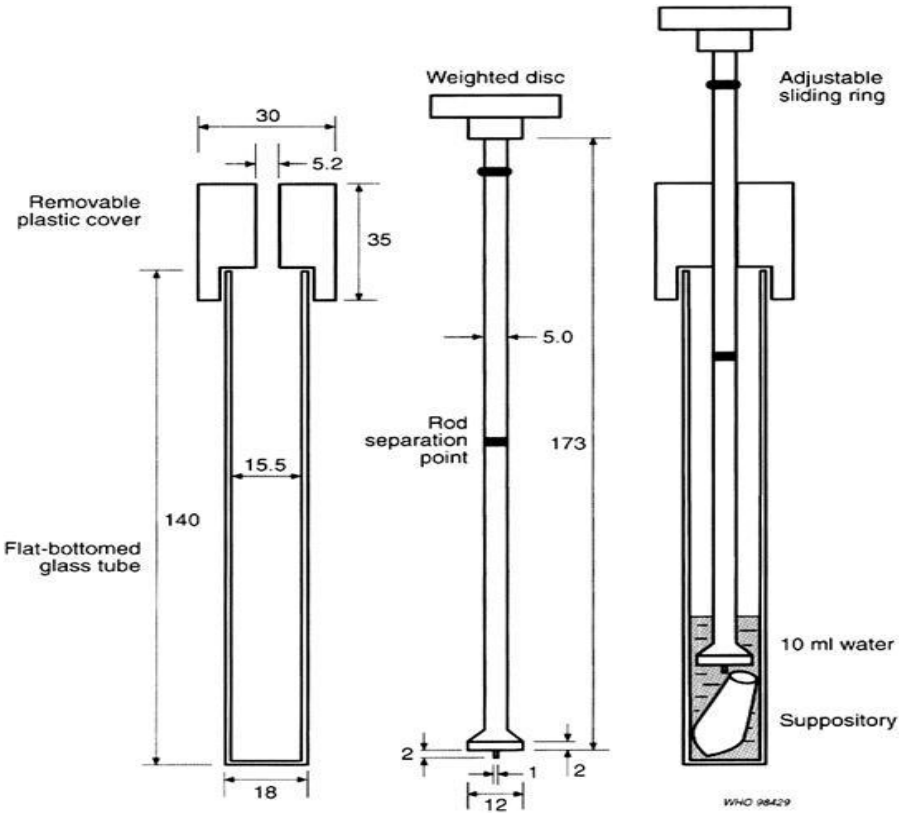


Figure 1.5 Alternative disintegration apparatus for fat based suppositories (Loyed V, 2007)

1.6.8 Dissolution testing

The most frequently used techniques for the measurement of *in vitro* drug release from suppository dosage forms are those used for the assessment of drug release from solid oral dosage forms as described in the USP. The apparatus that has been used includes the USP Apparatus I or basket apparatus, USP Apparatus II or paddle apparatus and USP Apparatus IV or flow-though cell apparatus, or modifications thereof (USP 32, 2010).

No single method of dissolution testing is suitable for all the various suppository formulations and types of suppositories (Loyed, 2007).

The mechanism by which a drug is made available for absorption from suppositories manufactured using hydrophilic bases is quite different from that of suppositories manufactured using lipophilic bases. Drug release from hydrophilic bases such as Polyethylene glycole (PEG) is a result of the progressive dissolution of the base and associated excipients in the intra-rectal

fluids. By contrast, drug release from lipophilic suppository bases is the result of a series of successive steps that involve the melting of the base at or below body temperature (37° C), migration of the drug particles to the interface between the melted excipients and the rectal secretions, diffusion of drug molecules from the molten base to the rectal barrier membranes and subsequent absorption of the drug into general circulation (Happiness, 2006).

A schematic diagram summarizing the aforementioned release processes of a drug from a lipophilic suppository formulation is depicted in Figure 1.6

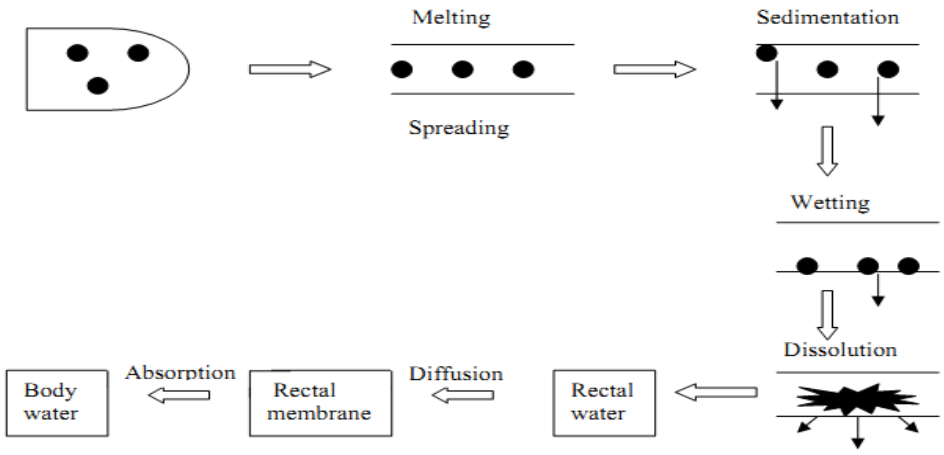


Fig. 1.6 Schematic representation of a drug release process dispersed in a lipophilic suppository base

A number of techniques have been used for the study of in vitro drug release from suppository dosage forms. The techniques that are in use differ mainly in the extent to which they are able to mimic in vivo physiological conditions. Two basic techniques have been employed, those that use membranes in the assessment of drug release and those that do not. Animal studies have also been used in conjunction with in vitro dissolution studies, in an attempt to correlate in vitro-in vivo drug availability.

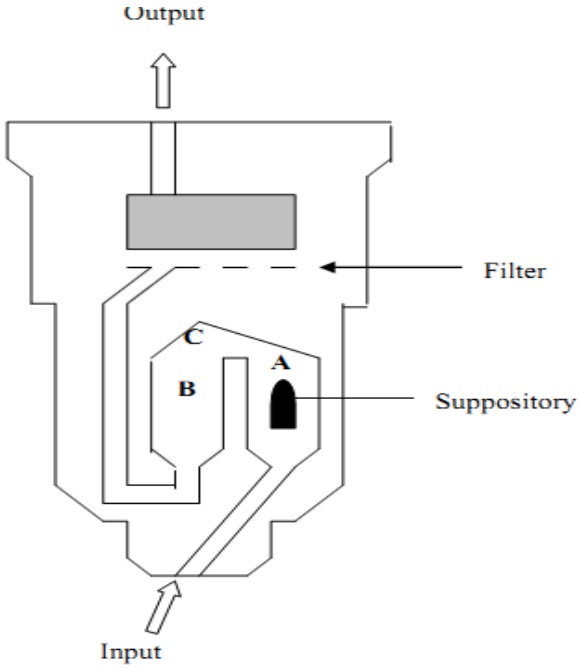


Fig 1.7 Schematic representation of the flow-through dissolution cell (BP, 2011).

The physiological environment in which drug release from a suppository is achieved can only occur in the presence of a small volume of rectal fluid or secretions of approximately 3-5 ml. Subsequently, drug that is released is transferred through a highly viscous mucous barrier to the rectal membranes and following absorption, into the systemic circulation to exert a therapeutic effect.

The use of a membrane method for the assessment of drug release, potentially avoids surface variation effects that may occur between the suppository and a receptor phase, which is one of the major causes of poor reproducibility of the methods that do not use membranes to assess drug release rates from suppositories. In addition, membrane methods facilitate sampling and analysis since a clear filtered solution is sampled for analysis, rather than a complex mixture of dissolution medium and suppository base. These models also take into consideration factors such as type of excipients used, viscosity of molten bases and water solubility of the drug, which might influence the availability of drugs for dissolution and subsequent absorption in vivo, in particular when a drug is administered in combination with lipophilic excipients in the form of suppositories (Loyed, 2007), (Happiness, 2006).

1.7 Mathematical Modeling of Dissolution Rate Profile

In vitro dissolution has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of Bio- equivalence. Several theories /kinetics models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where f_t is a function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. The quantitative interpretation of the values obtained in the dissolution assay is facilitated by the usage of a generic equation that mathematically translates the dissolution curve in function of some parameters related with the pharmaceutical dosage forms. (Paulo C., et al, 2001)

The kind of drug, its polymorphic form, cristallinity, particle size, solubility and amount in the pharmaceutical dosage form can influence the kinetics of release. A water-soluble drug incorporated in a matrix is mainly released by diffusion, while for a low water-soluble drug the self-erosion of the matrix will be the principal release mechanism. To compare dissolution profiles between two drug products model dependent (curve fitting), statistical analysis and model independent methods can be used.

Mathematical models have been used extensively for the parametric representation of drug release kinetics from suppository formulations. Models that have been used include, the zero order, first order, Higuchi, Korsmeyer-Peppas and Weibull models. (Paulo C., et al, 2001)

The major objectives of mathematical modeling are as listed below:

1. Designing the new drug delivery system based on general release expression.
2. Prediction of the exact behavior of drug or drug release rates from and drug diffusion behavior through polymers, thus avoid excessive experimentation.
3. Optimization of the release kinetics.
4. Elucidation of the physical mechanism of drug transport by simply comparing the release data to mathematical models.

1.7.1 Zero order models

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) can be represented by the following equation: (Paulo C., et al, 2001)

$$\mathbf{Q_t = Q_0 + K_0\, t} \quad \text{……. (1.3)}$$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution and K is the zero order release constant.

Ermiş et al, have reported that the zero order release kinetic process from systems such as water-soluble suppository formulations containing polyethylene glycol, in which the drug is released in a controlled manner, is independent of drug concentration (Ermiş, Tarimci, 1995).

1.7.2 First order model

This model was first proposed by Gibald & Feldman (1967) later by Wagner (1969). The pharmaceutical dosage forms containing water-soluble drugs in porous matrices follow first order release kinetics, and can be expressed by the equation:

$$\mathbf{Q_t = Q_0 e^{-kt}} \quad \text{…… (1.4)}$$

Where Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in the solution and k is the 1st order release constant. The above equation in decimal logarithm will take the form,

$$\mathbf{\ln Q_t = \ln Q_0 + kt} \quad \text{…… (1.5)}$$

This equation implies that a graphic of the decimal logarithm of the amount of drug versus time will be linear. The dosage forms that follow this dissolution profile release the drug in a way that is proportional to the amount remaining in the interior of the dosage form, in such a way that the amount of drug released by unit of time diminishes. Thus any system obeying this model releases the drug in such a way that the remaining amount in the system governs the rate of release of drugs (Paulo C., et al, 2001).

1.7.3 Higuchi Model

In 1961 Higuchi introduced the most famous and often used mathematical equation to describe the release rate of drugs from matrix system initially; it was valid only for planar systems. It was later modified and extended to consider different Geometries and matrix characteristics including porous structure. Higuchi developed an equation for the release of a drug from an ointment base and later applied it to diffusion of solid drugs dispersed in homogeneous and granular matrix dosage system. In this model, it is assumed that solid drug dissolves from the surface layer of the device first; when this layer becomes exhausted of drug, the next layer begins to be depleted by dissolution through the matrix to the external solution. In this way the interface between the regions containing dissolved drug and that containing dispersed drug moves into the interior as a front (Paulo C., et al, 2001).

In a general way it is possible to resume the Higuchi model to the following expression (generally known as the simplified Higuchi model):

$$\mathbf{Q_t = K_H t^{0.5}} \quad \textbf{…… (1.6)}$$

Where, K_H is the Higuchi dissolution constant. Higuchi describes drug release as a diffusion process based on the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs. This modified Higuchi relationship has been used to describe drug release from various types of modified release pharmaceutical dosage forms and for lipophilic suppository formulations containing the sparingly soluble drug acetaminophen (Paulo C., et al, 2001), (Toshihito, et al, 2004).

1.7.5 Korsmeyer- Peppas Model (The Power Law)

Power law equation is more comprehensive very simple and semi-empirical equation developed by Korsmeyer- Peppas which can be used to analyse data of drug release from polymers. The equation implies that; the fractional release of drug is exponentially related to release time.

$$\frac{M_t}{M_\infty} = kt^n \quad \text{…….. (1.7)}$$

Where, M_t & $M\infty$ are the absolute cumulative amounts of drug released at time t and infinity respectively, k is a constant incorporating structural and geometrical characteristics of the device, the k value is experimentally determined, and n is the exponent, indicative of the mechanism of drug release. The numerical value of the release exponent, n, is characteristic of the mechanism of diffusion release from delivery system. Peppas used the n value to characterise different release mechanisms from non-eroding polymers and the data are summarised in Table (1.4) (Paulo C., et al, 2001).

Table 1.4 Exponent n of the power law and drug release mechanism from polymeric controlled delivery systems of cylindrical and spherical geometry.

Exponent, n			
Thin Film	Cylinder	Sphere	Drug Release Mechanism
0.5	0.45	0.43	Fickian diffusion
0.5<n<1.0	0.45<n<0.89	0.43<n<0.85	Anomalous transport
1.0	0.89	0.85	Case II transport

When the release mechanism is not well known or when more than one type of release phenomena could be involved, this model can be used to analyze the release of poly-metric dosage form. This equation was later modified to accommodate the lag time (L) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_{\infty}} = \mathbf{a\,(t-l)^{n.}} \qquad \ldots\ldots\ldots\ldots\ldots (1.8)$$

And when there is possibility of burst effect (b),

$$\frac{M_{l}}{M_{\infty}} = \mathbf{at^{n.} + b} \qquad \ldots\ldots\ldots\ldots\ldots (1.9)$$

Whenever there is absence of lag time and burst effect 1 and b value would be zero and only Kt^n is used. This mathematical model has been frequently used to describe the drug release from different modified release dosage forms (Paulo C., et al, 2001). The Korsmeyer-Peppas model has been used to characterise diclofenac sodium release from poloxomer based solid suppositories and the dissolution rate of the API was found to be independent of the time, the exponent n approached 1.0 (Yong , et al, 2005) .

1.7.6 Weibull Model

Weibull introduced a general empirical equation which is highly applied to drug dissolution or release from pharmaceutical dosage forms (Paulo C., et al, 2001). The accumulated fraction of the drug m in solution at time t is given by Weibull equation:

$$m = 1 - \exp\left[\frac{-(t - T_i)^b}{a}\right] \quad \ldots\ldots\ldots (1.10)$$

In this equation a, defines the time scale of the process. The location parameter, Ti, represents the lag time before the onset of the dissolution or release process and in most cases will be zero. The shape parameter, b, characterizes the curve as either exponential (b = 1), sigmoid, S- shaped, with upward curvature followed by a turning point (b>1). This equation may be rearranged into:

$$\mathbf{Log\ [-In\ (1-m)] = b\ log\ (t - T_t) - log\ a} \quad \ldots\ldots\ldots (1.11)$$

From this equation a linear relation can be obtained for a log-log plot of – Ln (1 – m) versus time t. the shape parameter (b) is obtained from the ordinate value (1/a) at time t = 1. The parameter a can be replaced by the more informative dissolution time T_d that is defined by $a = (T_d)^b$ and is read from the graph as the time value corresponding to the ordinate – In (1 – m) = 1.

Since –In (1 – m) =1 is equivalent to m = 0.632, T_d represents the time interval necessary to dissolve or release 63.2% of the drug present. In the pharmaceuticals systems following this model, the logarithm of the dissolved amount of drug versus the logarithm of time plot will be linear (Paulo C., et al, 2001).

Limitations:-

i. There is not any kinetic fundament and could only describe, but doesn't adequately characterize, the dissolution kinetic properties of the drug.

ii. There is not any single parameter related with the intrinsic dissolution rate of the drug and

iii. It is of limited use for establishing in vivo/in vitro correlation.

Drug release from lipophilic suppository formulations is often accompanied by a more or less long-lasting lag phase that occurs as a result of the need for the base to melt prior to drug release and therefore the melting rate of the base is a factor that contributes to the lag time (Loth, Bosche, 1996).

1.7.7 Selection of Best Model:

The selection of the appropriate model in the drug release studies is critical to ensure the effectiveness of the study. There are various criteria for the selection of the mathematical models which are based on the statistical treatments. The most widely used method employs the coefficient of determination, R^2, to assess the fit of the model equation. This method can be used when the parameters of the model equations are similar. But when the parameters of the comparing equations increased; a modification is incorporated in this technique where an adjusted coefficient of determination (R^2 adjusted) given by:

$$R^2_{adjusted} = 1 - \frac{n-1}{n-p}(1-R^2) \qquad \text{..... (1.12)}$$

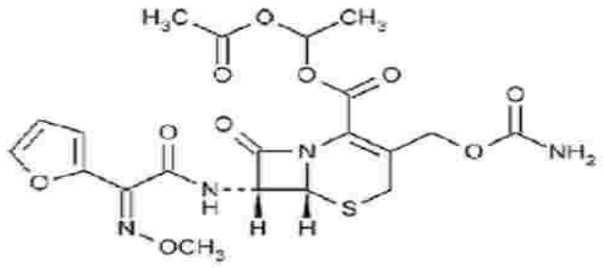
Where n is the number of dissolution data points and p is the number of parameters in the model. Hence, the best model is the one with the highest adjusted coefficient of determination. A value for R^2 adjusted > 0.950 is considered acceptable for the purposes of comparison of modeling dissolution profiles generated.

Similarly other statistical tools like correlation coefficient (R), Analysis of Variance (ANOVA) and Multivariate analysis of variance (MANOVA) are used for the comparison and selection of the models (Paulo C., et al, 2001).

1.8 Background Information on Cefuroxime Axetil

1.8.1 Description:

Structural formula:



Chemical name: (1*RS*)-1-(acetyloxy) ethyl (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-[[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

Molecular formula: $C_{20}H_{22}N_4O_{10}S$

Relative molecular mass: 510.48 CAS: *64544-07-6*

Content:

It contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of a mixture of the 2 diastereoisomers of Cefuroxime Axetil, calculated with reference to the anhydrous and acetone-free substance. It contains the equivalent of not less than 745 µg and not more than 875 µg of cefuroxime (C16H16N4O8S) per mg, calculated on the anhydrous basis. (USP32, 2010)

1.8.2 General properties:

Appearance: White to cream powder

Melting point: Cefuroxime Axetil decomposes below its melting point

Solubility at 20 ^{0}C**:** Cefuroxime Axetil is soluble in dimethyl sulphoxide, dimethylformamide, 1,4-dioxan, chloroform, acetone, glacial acetic acid, ethyl acetate and methanol, soluble with decomposition in alkali and slightly soluble in diethyl ether, 95% ethanol and toluene. It is insoluble (i.e. less than 0.1 % w/v soluble) in 2M hydrochloric acid.

The solubility of the amorphous material in aqueous solution at 20°C is approximately 0.12% that on standing converts to mainly crystalline material which has solubility in aqueous solution of about 0.03 %. (BP 2011, USP32, 2010)

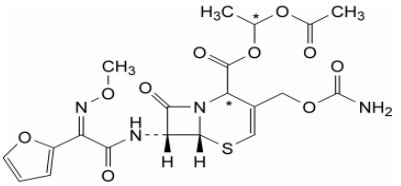
1.8.3 Impurities:

Specified impurities: A, B, E.

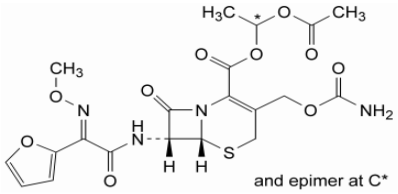
Other detectable impurities: C, D.

Chemical Structure

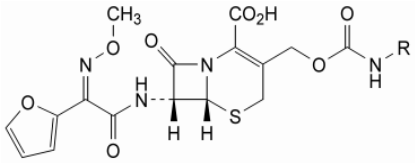
A. 1-(acetyloxy) ethyl (6R, 7R)-3-[(carbamoyloxy) methyl] - 7-[[(Z)-2-(furan-2-yl)-2-(methoxyimino) acetyl] amino]-8- oxo-5-thia-1-azabicyclo [4.2.0] oct-3-ene-2-carboxylate (Δ^3-isomers).



B. (1RS)-1-(acetyloxy) ethyl (6R, 7R)-3-[(carbamoyloxy) methyl]-7-[[(E)-2-(furan-2-yl)-2-(methoxyimino) acetyl] amino]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate (E-isomers),

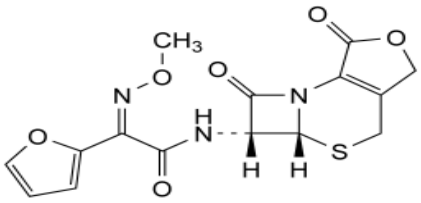


C. R = CO-CCl3 :(6R, 7R)-7-[[(Z)-2-(furan-2-yl)-2-(methoxyimino) acetyl] amino]-8-oxo-3-[[[(trichloroacetyl) carbamoyl] oxy] methyl]-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid,



D. R = H: cefuroxime

E. (5aR,6R)-6-[[(2Z)-2-(furan-2-yl)-2-(methoxy-imino)acetyl]amino]-5a,6-dihydro-3H,7H-azeto[2,1-b]furo[3,4-d][1,3]thiazine-1,7(4H)-dione (descarbamoylcefuroxime lactone). (EuroP., 2002)



1.8.4 Pharmacokinetics

Absorption and Metabolism: Cefuroxime axetil is a broad spectrum second-generation cephalosporin antibiotic active against β-lactamase producing strains. It belongs to class IV drug according to Biopharmaceutical Classification (BPC). It is an ester prodrug of cefuroxime. Its activity depends upon *in-vivo* hydrolysis by nonspecific esterases in the intestinal mucosa and blood and release of cefuroxime. Cefuroxime is rendered more lipophilic by esterification of the C4 carboxyl group of the molecule by the racemic 1-acetoxyethyl bromides, thus enhancing oral absorption.

Cefuroxime axetil is an orally active drug though its absorption is incomplete. Its bioavailability ranges between 25 to 52%. It is the axetil form of cefuroxime that is absorbed but when it is hydrolysed to cefuroxime its permeation is low. The axetil moiety is metabolized to acetaldehyde and acetic acid (Sambhakar, et al, 2011).

Peak plasma concentration is reported about 2 to 3 hours after an oral dose. Up to 50% of cefuroxime in the circulation is bound to plasma proteins. The plasma half life is about 70 minutes and is prolonged in patients with renal impairments and in neonates. Cefuroxime axetil is widely distributed in the body including plural fluid, sputum bone synovial fluid, and aqueous humour, but only achieves therapeutic concentration in the CSF when the meninges are inflamed. It crosses the placenta and has been detected in breast milk. Cefuroxime is excreted unchanged, by glomerular filtration and renal tubular secretion, and high concentration is achieved in urine. Probenecid competes for renal tubular secretion with cefuroxime resulting in higher and more prolonged plasma concentration of cefuroxime. Small amounts of cefuroxime are excreted in bile (Zinat® Tablets, 2011), (Cefuroxime Axetil monograph, 2009), (Cefuroxime Axetil information, 2012).

1.8.5 Indications and Clinical Uses:

It is used for the treatment of patients with mild to moderately severe infections caused by susceptible strains of the designated organisms in the following diseases: (Cefuroxime Axetil monograph, 2009)

- Upper Respiratory Tract Infections: Pharyngitis and tonsillitis caused by S. pyogenes. Otitis Media caused by S. pneumoniae, S. pyogenes (group A beta-hemolytic

streptococci), H. influenzae (beta-lactamase negative and beta-lactamase positive strains) or M. catarrhalis.

- Sinusitis caused by M. catarrhalis, S. pneumoniae or H. influenzae (including ampicillin-resistant strains).
- Lower Respiratory Tract Infections: Pneumonia or bronchitis caused by S. pneumoniae, H. influenzae (including ampicillin-resistant strains), H. parainfluenzae, K. pneumoniae or M. catarrhalis.
- Skin Structure Infections: Skin structure infections caused by S. aureus, S. pyogenes or S. agalactiae.
- Gonorrhea: Acute uncomplicated urethritis and cervicitis caused by N. gonorrhea.

1.8.6 Stability

The effect of temperature and relative air humidity on the degradation of diastereomers A and B of CFA was estimated by studying the stability of CFA in solid state. Changes in the concentration of the two diastereomers (A and B) of CFA were recorded by means of HPLC with UV detection. It was concluded that the kinetic mechanism of CFA decomposition depends on the storage conditions of the respective substance. In a dry ambient atmosphere the decomposition is the result of a reversible process and follows the kinetics of a pseudo-first order reaction. When stored in a humid environment (RH/50%), the degradation of CFA is of an autocatalytic nature. Environmental humidity is a paramount factor determining the decomposition of CFA, especially at high temperatures. The B diastereomer of CFA is more stable than the A one, both in a dry and in a humid ambient atmosphere (Marianna, 2003).

The degradation of amorphous CFA yields three main products: Δ^3-isomers, E-isomers of cefuroxime axetil and cefuroxime regardless of relative humidity. All three products except cefuroxime at RH= 0% underwent further decomposition in the consecutive reactions (Fig1.8) (Anna, 2006).

The hydrolysis kinetics follows a first-order reaction in a pH range 1-9. The pH-rate profile for the total isomeric mixture shows a maximum stability in the pH range 3.5-5.5 and different hydrolysis rate constants for the two isomers. Isomer A is always more reactive than isomer B with a maximum difference in reactivity about 27% being observed at pH=1. Acetate or phosphate buffer catalyzes the degradation, but ionic strength does not have a significant effect

on the kinetics. The hydrolysis proceeds through different routes, yielding the Δ^2-isomer, cefuroxime, and small quantities of sulfoxides (Fabre, 1994). The photoisomerization kinetics of cefuroxime axetil revealed competition between the isomerization and photolysis of the β-lactam ring, with the two diastereoisomers reacting at different rates. The fact that photoisomerisation occurs on exposure to UV radiation at 254 nm confirms the need for photo protection from light (Glass, 2004).

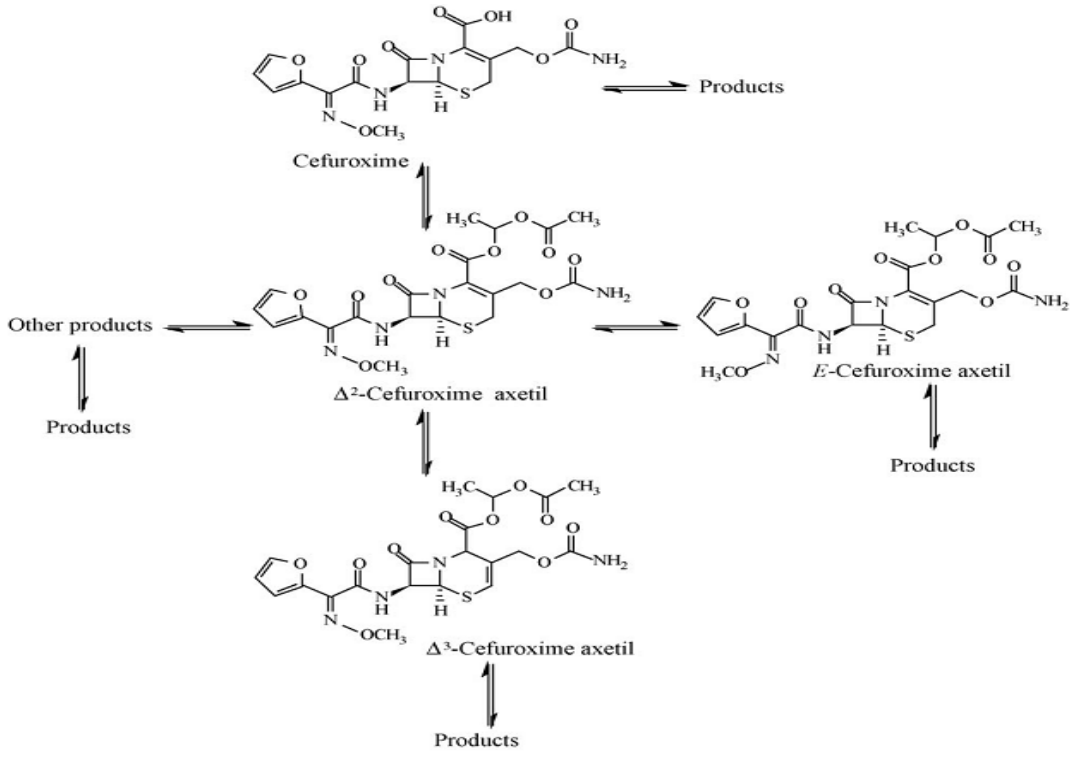


Figure 1.8 Degradation products of the amorphous form of cefuroxime axetil in solid state (Anna, 2006).

Part Two:

Objectives

2.1 Needs for the study

The rectal route is commonly used as an alternative when oral administration is inconvenient because of inability to swallow or because of gastro - intestinal side effects such as nausea, vomiting and irritation.

More important, rectal drug administration has the advantage of minimizing or avoiding hepatic first pass metabolism.

It's well known that the rectal route can deliver 60-70% of the administered drug directly into systemic circulation. The lymphatic circulation helps also in absorbing a rectally administered drug from liver. The most common dosage form used for drug administration via rectal route is solid suppositories (Saritha, 2005).

Antibiotics are usually administered either orally or by a parenteral route, the latter being used for drugs that are poorly or not bioavailable by the oral route or when clinical situations require rapid or higher antibiotic concentrations to be achieved in the body.

In humans the rectum comprises the last 12–19 cm of the large intestine and the rectal epithelium is formed by a single layer of columnar or cubical cells and goblet cells; its surface area is about 200–400 cm^2. The absorbing surface area of the rectum is considerably smaller than that of the small intestine, as the former lacks villi and microvilli. However, the epithelia in the rectum and the upper intestinal tract are histologically similar, giving them comparable abilities to absorb drugs.

The rectal mucosa is richly vascularized: this important blood supply comprises the inferior and middle veins, which are directly connected to the systemic circulation, and the superior rectal vein, which is connected to the portal system. This ensures that drugs in suppository form which are absorbed in the upper rectum will not by-pass the hepatic 'first-pass' elimination, responsible for the metabolism and rapid clearance of many orally administered drugs.

Cefuroxime axetil (CFA) is abroad spectrum ß-lactamase cephalosporin that has well defined pharmacokinetics after intramuscular and intravenous administration in the form of sodium salt (Kar, et al, 2010). CFA is a Biopharmaceutics Classification System (BCS) Class IV drug due to its poor aqueous solubility (Sambhakar, et al, 2011).

It is available for oral administration as tablet dosage form in 250mg and 500mg strengths, and as powder for suspension dosage form in 125mg/5ml and 250mg/5ml strengths. In humans, gastrointestinal absorption of cefuroxime is negligible. Cefuroxime (Cefuroxime axetil) an oral prodrug shows a bioavailablity of 30% to 40% when taken on fasting and 5% to 60% when taken after food (Kar, et al, 2010).

The in vivo bactericidal activity of Cefuroxime Axetil is due to cefuroxime binding to essential target proteins and the resultant inhibition of cell wall synthesis. Cefuroxime has bactericidal activity against a wide range of common pathogens, including many beta-lactamase-producing strains. Cefuroxime is stable to many bacterial beta-lactamases, especially plasmid-mediated enzymes that are commonly found in enterobacteriaceae. Cefuroxime has been demonstrated to be active against gram-positive (Staphylococcus aureus, Streptococcus pneumoniae, and Streptococcus pyogenes) and gram-negative (Moraxella catarrhalis, Escherichia coli, Haemophilus influenzae, Haemophilus parainfluenzae, Klebsiella pneumoniae, and Neisseria gonorrhea) organisms (Ceftin®, 2010).

Vomiting, abdominal pain, and gastrointestinal irritation are often reported adverse reactions for Cefuroxime Axetil tablet and powder for suspension dosage forms.

Cefuroxime Axetil has very bitter taste, and problems are encountered in patient acceptability, especially for children and pediatrics.

The advent of Cefuroxime Axetil suppository dosage form may be the solution for the above mentioned problems. Since suppositories avoid any gastrointestinal irritation, and can be used in unconscious patients, for systemic absorption to avoid first-pass metabolism, for babies or old people who cannot swallow oral medication and for people suffering from severe nausea or vomiting.

2.2 Objectives of the study:

- To develop a 125 mg Cefuroxime Axetil pediatric suppositories in both water soluble and fatty bases.
- To assess and evaluate the rate and extent of Cefuroxime Axetil release from the suppositories, using an appropriate dissolution method.
- To develop and validate a suitable method of analysis to measure Cefuroxime Axetil in suppository dosage forms.
- To determine the effects of aging of selected suppository formulations on Cefuroxime Axetil release.
- To study the dissolution kinetics and release mechanism for selected Cefuroxime Axetil suppository formulations manufactured using fatty bases.

Part Three:

Experimental part

3.1 Work Strategy

- Choosing the API and the excipients with suitable properties for formulation of the different types of suppositories
- Preformulation trials and evaluation
- Formulation of CFA Suppositories with the selected base(s) and additives
- Test methods development and analytical test method validation
- Studying the stability of CFA suppositories
- Analyzing data and dissolution profile modeling of CFA suppositories

3.2 Materials and Reagents

All materials used in the formulation of CFA suppositories are of Pharmacopoeia grade, the materials and reagents used in the preparation of CFA suppositories are listed in tables 3.1 &3.2.

Table 3.1: Reagents used in the study

No.	**Item**	**Grade**
1.	Monobasic ammonium phosphate	HPLC grade
2.	Methanol	HPLC grade
3.	Sodium dihydrogen phosphate	AR
4.	Disodium hydrogen phosphate	AR
5.	Distilled water	HPLC grade
6.	Acetonitrile	HPLC grade

Table 3.2: Materials used in the study

No.	Item	Manufacturer	Donated by
1.	Cefuroxime Axetil	Orchid Chemicals & pharm., India	Bir Zeit Pharm. Co
2.	Witepsol H15		Jerusalem Pharm. Co
3.	Novata A	Cognis Gmbh, dusseldorf, Germany	Bir Zeit Pharm. Co
4.	Novata BCF	Cognis Gmbh, dusseldorf, Germany	Bir Zeit Pharm. Co
5.	Paraffin Oil	Sonneborn Refined product.	Bir Zeit Pharm. Co
6.	Sodium Lauryl Sulfate	Samkeal Pharmachem Ltd.	Bir Zeit Pharm. Co
7.	Tween 80	Sabo	Jerusalem Pharm. Co
8.	Tween 85	Seppic	Bir Zeit Pharm. Co
9.	Tween 20	Polaquim, S.A. DE C.V. /Mexico	Bir Zeit Pharm. Co
10.	Polaxomer 188	Croda	Pharmacare Pharm. Co
11.	Lecithin		Sinokrot foods Co
12.	Lanolin anhydrous	Stella	Jerusalem Pharm. Co
13.	Aerosil	Evorik Degussa	Jerusalem Pharm. Co
14.	BHT	Lanxess AG, Leverkusen, Germany	Al Raed Cosmetics Co
15.	Cremophor A6	BASF	Jerusalem Pharm. Co
16.	Span 80	Polaquim, S.A. DE C.V. /Mexico	Jerusalem Pharm. Co
17.	PEG 4000	BASF	Jerusalem Pharm. Co
18.	PEG 400	BASF	Jerusalem Pharm. Co
19.	PEG 6000	BASF	Jerusalem Pharm. Co
20.	PEG 1500	BASF	Jerusalem Pharm. Co
21	Empty PVC Suppository Shells	Sarong s.p.a	Bir Zeit Pharm. Co

3.3 Tools and Equipment

Syringes, vials, pipette, glassware, stands and tubes were supplied by Jerusalem pharmaceuticals

Table 3.3 illustrates the tools and equipment used in the study.

Table 3.3 Tools and Equipment used in the study.

Equipment	**Source/Model**
HPLC 1 (method validation)	Lachrom Elite, HPLC system equipped with: L2130, 4 channels gradient pump, L2200 auto sampler, L2300 column oven and L2400 U.V detector.
HPLC 2 (Stability studies)	Ultimate 3000, HPLC system equipped with: ultimate 3000 variable wave length detector, column compartment, auto sampler, 4 channel gradient pump.
U.V. Spectrophotometer	Merck Hitachi: U2900, U.V visible spectrophotometer.
pH meter	Metrohm
Balance	XT 220 A, Percisia analytical Balance
Magnetic Stirrer	Fried Electronic
Incubator 25C°	Advantec CL-310
Incubator 30C°	WTB binder
Submersible water pump	Minjiang, NS 160
Water bath	Tuttnauer Co. LTD
Sonicator	Elmasonic
Refrigerator	L.G.
Dissolution tester, apparatus 1, Modified Teflon Basket	Erweka, Type DT 820
Suppository disintegration tester	Erweka, model ST 30, Serial NO. 1086191069
Melting point tester	Mettler Toledo, type: FP 62, Serial No. 5117084333
Aluminum Metal Suppository Mold	-
Flow through Cell	Home-made

3.4 Methodology:

3.4.1 Choosing the API and the excipients:

Cefuroxime is an optically active molecule containing two chiral centers. The biological origin of 7-aminocephalosporanic acid (7-ACA) (the origin of the cephalosporin nucleus) ensures that only one optical isomer is produced.

Cefuroxime as sodium salt is not appreciably absorbed from the GI tract. The esterification of cefuroxime with 1-acetoxyethyl bromide to produce cefuroxime axetil results in the addition of another chiral center to the molecule and results in two diastereoisomers of cefuroxime axetil about this optically active center in the ester group.

The 1-acetoxyethyl ester group in position 4 of CFA ensures its lipophilicity and promotes the intestinal absorption of cefuroxime.

For the preparation of pharmaceutical formulations only the amorphous form is used. It has better physicochemical and biological properties than the crystalline form, e.g. significantly higher solubility and bulk density as well as higher degree of absorption after oral administration (Ceftin® prescribing information, 2010).

Accordingly, amorphous CFA will be used during the course of this study.

3.4.2 Formulation of Cefuroxime Axetil Suppositories:

3.4.2.1 Suppository bases:

The suppository formulations in this study were prepared from either water –soluble bases or semisynthetic fatty bases. Polyethylene glycol (PEG) in different grades was used for the preparation of water-soluble base suppositories, while, witepsol® and Novata® were used as fatty bases, for their characteristics and availability. The characteristics of the fatty and water- soluble bases are shown in tables 3.4 and 3.5 respectively.

Table 3.4 Characteristics of Fatty bases used (Lachman, 1986)

Parameters	**Novata A**	**Novata BCF**	**Witepsol H15**
Iodine value	< 3	< 3	< 7
Melting range ^{o}C	33.5-35.5	35-37	33.5-35.5
Saponification value	225-240	225-240	230-240
Solidification point ^{o}C	29-31	30-32	32.5-34.5
Hydroxyl value	20-40	20-40	5-15

Table 3.5: Characteristics of the water-soluble bases (Raymond, 2006)

PEG	Mean Molecular weight	Melting ranges (°C)	Hydroxyl value
400	400	< 10	264-300
1000	1000	33.3-33.4	107-118
1540	1450	43.1-43.3	70-86
4000	3400	57.4-57.6	30-36
6000	6750	60.7-61.0	-

3.4.2.2 Determination of Displacement Value

The volume of the suppository shells where the melt is filled in is uniform, but the weight of the suppository may vary, due to the difference in densities between the API_S, adjuvants and the base. Therefore, in order to prepare products accurately, allowance was made for the differences in density of the suppository base, owing to the presence of the added API and other adjuvants. The factor used to account for these differences is termed the displacement value (D.V.), which is the amount of API by weight that displaces one part by weight of a specific base being used for the manufacture of the suppositories.

To determine the Displacement Value the suppository shells are calibrated with the specific base alone to obtain an accurate weight for each no medicated suppository; after that ten suppositories containing 12.5% w/w of CFA are prepared by the fusion method of manufacture and weighed.

The D.V. is then calculated using the following Equation:

$$\boldsymbol{f = \frac{100(E - G)}{(G)(X)} + 1}$$

Where *E*=weight of pure base suppositories, *G*=Weight of suppositories with *X*% active ingredient. The appropriate mass of suppository base to be used for a specific batch of product is calculated using the following Equation:

$$\boldsymbol{P = (N \times S) - \frac{ND}{f}}$$

Where,

P = the amount of base required
N = the number of prepared suppositories
S = the size of the mould used
D = the amount of drug that is required
f = displacement value

3.4.2.3 Method of Preparation:

The fusion or melting method was used for the manufacture of the CFA suppositories. Each suppository was manufactured so as to contain an equivalent amount of 125mg of CFA in each suppository. The quantity of bases was weighed accurately. The suppository base is melted at 45-50°C, by using a water bath. Any other additive is added at this stage of preparation. The molten mixture was cooled to approximately 40^{o}C, and then the CFA powder was incorporated into the melted base while mixing. The mixture was filled manually into an appropriate suppository shells using a-20 ml syringe and left to cool at room temperature. The filled suppository shells are finally sealed thermally.

3.4.2.4 Development of the formulation:

To decide on the best and suitable combinations of the CFA, the bases, and the modifying additives, some preformulations as illustrated in tables 3.6, 3.7, and 3.8 were prepared in small quantities (i.e. 20 suppositories/ formulation), using different combinations of the bases and additives. The quality attributes, including colour, appearance, surface texture, melting range, dissolution rate, and CFA content and impurities of the formulations were assessed. From the assessment results of the preformulations, Witepsol® fatty base was found to be the best base; therefore it was selected for the final study formulations along with the additives shown in table 3.9. Sixteen formulations in larger quantities (i.e. 200 suppositories/ formulation) were prepared from the selected Witepsol® H15 fatty-base with the additives in different combinations and different percentages as illustrated in table 3.10.The quality attributes, including colour, appearance, surface texture, melting range, dissolution rate, disintegration time, and CFA content and impurities of the formulations were assessed. The stability of the formulations was evaluated at three storage conditions ($30^{o}C$, $25^{o}C$, and ($2\text{-}8^{O}C$)) for a period of three months.

Table 3.6: Summary of preformulations using Witepsol H15 trials

	Formula B.N (quantities in grams for 20 suppositories)													
Ingredients	PW1	PW2	PW3	PW4	PW5	PW6	PW7	PW8	PW9	PW10	PW11	PW12	PW13	PW14
CFA	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Witepsol H15	21	17.4	18.43	18.31	18.37	18	18.64	18.65	20.87	18	20.76	20.64	20.52	20.52
Paraffin oil	0	2.4	2.4	2.4	0.24	0.24	0.24	0.24	0	0	0	0	0	0
Lanolin anh.	0	0	0	0	0	0	0	0	0	0	0.24	0.24	0.24	0.24
Aerosil	0	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0	0	0	0	0	0
Tween 20	0	0	0	0	0	0	0	0	0	0	0	0.12	0.12	0
Tween 80	0	0	0	0	0	0	0	0	0	0	0	0	0	0.24
Polaxomer 188	0	1.2	0	0	0	0	0	0	0	0	0	0	0	0
SLS	0	0	0	0.24	0	0	0.024	0.05	0	0	0	0	0	0
Cremophor A6	0	0	0.12	0	0.33	0.66	0	0	0	0	0	0	0	0
Cremophor RH40	0	0	0	0	0	0	0	0	0.11	0	0	0	0	0
Span 80	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0
Lecithin (soya bean)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BHT	0	0	0	0	0.024	0.024	0.024	0.024	0	0	0	0	0	0

Table 3.6: Summary of preformulations using Witepsol H15 trials (continued)

	Formula B.N(quantities in grams for 20 suppositories)								
Ingredients	PW15	PW16	PW17	PW18	PW19	PW20	PW21	PW22	PW23
CFA	3	3	3	3	3	3	3	3	3
Witepsol H15	20.28	18.1	17.35	18.55	20.76	19.56	20.52	18.6	20.28
Parraffin oil	0	2.4	2.4	2.4	0	0	0	0	0
Lanolin anh.	0.24	0	0	0	0.24	0.24	0.24	0	0
Aerosil	0	0	0.05	0.05	0.05	0	0	0	0
Tween 20	0.48	0.48	0	0	0	0	0	0	0
Tween 80	0.24	0	0	0	0	0	0	0	0
SLS	0	0	0	0	0	0	0.24	0	0
Cremophor A6	0	0	1.2	0	0	1.2	0	0	0
Lecithin (soya bean)	0	0	0	0	0	0	0	2.4	0.24

Table 3.7: Summary of preformulations trials using Novata A & BCF

	Formula B.N (quantities in grams for 20 suppositories)														
Ingredients	PN1	PN2	PN3	PN4	PN5	PN6	PN7	PN8	PN9	PN10	PN11	PN12	PN13	PN14	PN15
CFA	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Novata A	0	20.76	20.76	20.28	20.52	19.56	6.3	6	5.22	5.22	5.42	5.22	5.22	5.41	5.41
Novata BCF	21	0	0	0	0	0	14.7	14	12.18	12.18	12.58	12.18	12.18	12.63	12.63
Paraffin oil	0	0.24	0	0	0	0	0	0	2.4	2.4	2.4	2.4	2.4	0.24	0.24
Lanolin anh.	0	0	0.24	0.24	0.24	0.24	0	0	0	0	0	0	0	0	0
Aerosil	0	0	0	0	0	0	0	0	0.05	0.05	0.05	0.05	0	0.05	0.05
Tween 20	0	0	0	0.48	0	0	0	0	0	0	0	0	0	0	0
Polaxomer 188	0	0	0	0	0	0	0	0.5	1.2	0	0	0	0	0	0
SLS	0	0	0	0	0.24	0	0	0	0	0	0	0.24	0	0	0
Cremophor A6	0	0	0	0	0	1.2	0	0	0	1.2	0.48	0	0	0.33	0.66
Span 80	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0
BHT	0	0	0	0	0	0	0	0	0	0	0	0	0	0.024	0.024

Table 3.8: Summary of preformulations trials using PEG bases

	Batch No. (quantities in gm for 20 suppositories)	
Ingredients	PEG 1	PEG 2
CFA	3	3
PEG 400	4.2	0
PEG 1500	7.35	13.65
PEG 6000	9.45	0
PEG 4000	0	7.35

Table 3.9 Composition and functions of materials used in formulation

Ingredients	**% (w/w)**	**Function**
CFA	12.5	Active Ingredient
Witepsol H15	Q.S	Suppository Base
Paraffin Oil	5.0	Melting point modifier
Lanolin anhydrous	2.0	Emulsifying agent
Lecithin soya bean	1.0	Emulsifying and solubilizing agent
Tween 20	1.0 & 2.0	Emulsifying and solubilizing agent
SLS	0.5 & 1.0	Emulsifying and solubilizing agent
BHT	0.02	Anti oxidant
Tween 85	1.0	Emulsifying and solubilizing agent
Aerosil	0.1	Emulsion stabilizer and suspending agent

Table 3.10: Summary of selected study formulation

Quantities required in grams per 200 suppositories										
Ingred. B. N	CFA	WH15	Paraf. Oil	Lanolin Anhyd.	Aerosil	Lecithin	Tween® 20	SLS	BHT	Tween® 85
F01	30	209.7	0	0	0.24	0	0	0	0.048	0
F02	30	196.8	12	0	0.24	0	0	1.2	0.048	0
F03	30	195.7	12	0	0.24	0	0	2.4	0.048	0
F04	30	195.7	12	0	0.25	0	2.4	0	0.048	0
F05	30	193.2	12	0	0.24	0	4.8	0	0.048	0
F06	30	203.9	0	4.8	0	0	0	1.2	0.048	0
F07	30	202.8	0	4.8	0	0	0	2.4	0.048	0
F08	30	202.8	0	4.8	0	0	2.4	0	0.048	0
F09	30	200.4	0	4.8	0	0	4.8	0	0.048	0
F10	30	206.4	0	0	0	2.4	0	1.2	0.048	0
F11	30	205.2	0	0	0	2.4	0	2.4	0.048	0
F12	30	205.2	0	0	0	2.4	2.4	0	0.048	0
F13	30	202.8	0	0	0	2.4	4.8	0	0.048	0
F14	30	207.6	0	0	0	0	0	0	0	2.4
F15	30	205.2	0	2.4	0	0	0	0	0	2.4
F16	30	205.2	0	0	0	2.4	0	0	0	2.4

Anhyd: Anhydrous

Ingred: Ingredient

Paraf: Paraffin

3.4.3 Test methods development

3.4.3.1 Assay test method of CFA suppositories:

The assay test method was adapted from the USP 34th edition monograph for Cefuroxime Axetil tablets, and validated for testing the amount of CFA present in suppository dosage form.

Reagents used: 0.2 M Monobasic ammonium phosphate (Dissolve 23.0 g of monobasic ammonium phosphate in water to obtain 1000 ml of solution, methanol and distilled water). All reagents are of HPLC grade.

Equipment: Analytical balance, sonicator, hot plate magnetic stirrer and HPLC (EZ Chrom Elite).

Mobile phase: A filtered and degassed mixture of 0.2 M Monobasic ammonium phosphate and methanol (620: 380).

Standard preparation: A quantity of CFA working standard accurately weighed (equivalent to 250 mg Cefuroxime base) is transferred to a 100-ml volumetric flask, dissolved in methanol, diluted with methanol to volume, and mixed. Promptly 5.0 ml of this solution is transferred to a 50-mL volumetric flask, 3.8 ml of methanol is added, and the volume is completed with 0.2 M Monobasic ammonium phosphate and mixed.

Assay preparation: Tow suppositories are transferred to a 100-ml volumetric flask, dissolved in methanol with the aid of gentle heat, the volume is completed with methanol, and mixed. 5-ml of this solution is transferred to a 50-mL volumetric flask, 3.8 ml of methanol are added, and the volume is completed with 0.2 M Monobasic ammonium phosphate and mixed.

Note: All solutions containing CFA shall be used promptly, or stored in a refrigerator and used in the same day.

Chromatographic system:

- Detection wavelength: 278 nm
- Column: 25 cm length and 4.6 mm internal diameter, packed with Octylsilane chemically bonded to porous silica.
- Flow rate: 1.0 ml/min

Procedure: Equal volumes (10 µL) from both the standard preparation and the assay preparation are injected into the Liquid chromatography. The chromatograms are recorded and the responses are measured for the major peaks.

The percentage of Cefuroxime in the product is calculated using the following formula:

$$\% \mathbf{CFA} = \frac{\boldsymbol{Average\ area\ of\ sample}}{\boldsymbol{Average\ area\ of\ standard}} \times \mathbf{100}\%$$

The average areas are taken as the sum of the peak responses of the cefuroxime axetil diastereoisomers A and B for both standard preparation and sample preparation.

System suitability:

- The relative retention times: are about 0.8 for cefuroxime axetil diastereoisomer B, 0.9 for cefuroxime axetil diastereoisomer A, and 1.0 for cefuroxime axetil delta-3 isomers.
- The resolution, *R*, between cefuroxime axetil diastereoisomer A and B is not less than 1.5; and the resolution, *R*, between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers is not less than 1.5.
- The column efficiency: not less than 3000 theoretical plates when measured using the cefuroxime axetil diastereoisomer "A" peak.
- The relative standard deviation for replicate injections is not more than 2.0%.

3.4.3.2 Dissolution Test method using (USP apparatus 1):

Medium: 0.07 M pH 7.0 phosphate buffer, prepared by dissolving 3.7 g of monobasic sodium phosphate and 5.7 g of anhydrous dibasic sodium phosphate in 1000 ml of water. Dissolution vessels are filled with 900 ml.

Tools: 100-mL volumetric flask, seven 50- ml volumetric flask, 1-ml volumetric pipette, 5-ml volumetric pipette, 1-Lt graduated cylinder, six 20-ml test tubes and 0.2 micron filters.

Equipment: Dissolution tester (Erweka, Type DT 820), USP apparatus I, with modified baskets, UV-Visible spectrophotometer and analytical balance.

Standard preparation: An amount of CFA, equivalent to 138 mg Cefuroxime base is weighed and transferred into a 100-ml volumetric flask and dissolved in methanol, the volume is completed

with methanol, a 1.0-ml from the prepared solution is transferred into a 100-mlvolumetric flask and the volume is completed with the dissolution media.

Test conditions:

Dissolution apparatus:	USP apparatus I
Dissolution medium:	0.07 M pH 7.0 phosphate buffer
Temperature:	37± 0.5°C
Initial volume:	900 ml
Basket speed:	100 rpm
Filter size:	0.2 µm
Volume withdrawn:	10 ml
Volume replaced:	5 ml
Sampling times:	0, 15, 30, 45, 60, 120, 180 minutes

Test preparation: Using a 10-ml syringe a 10- ml portion from each dissolution vessel is withdrawn into six separate test tubes. A 5-ml portion from each test tube is transferred into a 50-ml volumetric flask and the volume is completed with the dissolution media. The remaining portions in the test tubes are returned to the vessels and 5-ml from dissolution media is added to each vessel.

Procedure: The absorbance at $\lambda = 278$ nm of the standard preparation and the test preparations is determined for all different bathes of the product.

The percentage of Cefuroxime released at each time interval is calculated using the following equation:

$$\% \mathbf{CFA} = \frac{\boldsymbol{Absorbance\ of\ test\ preparation}}{\boldsymbol{Absorbance\ of\ standard\ preparation}} \times \mathbf{100}\%$$

3.4.3.3 Dissolution Test method using (Flow through cell):

In order to study the drug release from the CFA suppositories using the flow through cell device as recommended by the US and the European Pharmacopoeias, a flow through cell was proposed and designed by us and was shaped in Bir Zeit University as a generous donation. The design of the cell was derived from a release cell for ointments (Wen-Di Ma, et al, 2008). It was different from the design of both USP apparatus IV and the EurP.

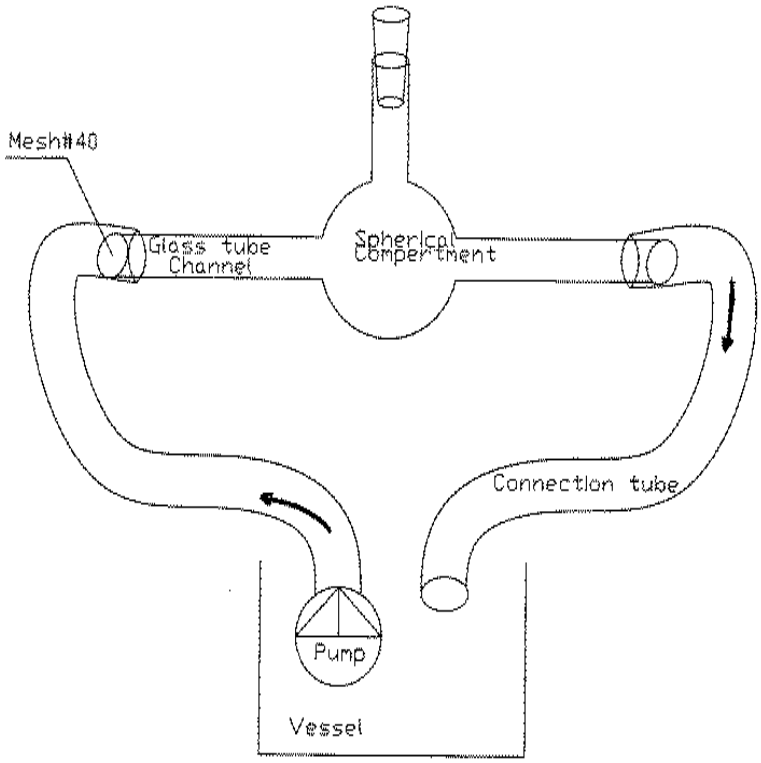


Figure 3.1: schematic drawing of the modified flow-through cell apparatus

The modified apparatus is made of glass and it is consisted of the following parts as shown in figure 3.1

- A spherical compartment having a capacity of approximately 25cm^{3}with an inlet and outlet I.D 6.77 mm and O.D 10.03 mm.
- Three tube channels connected to the compartment; two of them are used as inlet and outlet channels with an I.D 7.3 mm. The third channel is used to insert the suppositories into the compartment. It is 13.9 mm I.D
- A low flow rate pump.
- Water bath maintained at 37.5^{o}C
- One liter glass or plastic vessel
- A # 40 mesh screen fixed at the outlet of the cell to retain disintegrated portions from the suppositories

Standard preparation: An amount of CFA, equivalent to 138 mg Cefuroxime base is weighed and transferred into a 100-ml volumetric flask and dissolved in methanol, the volume is completed with methanol, a 1.0-ml from the prepared solution is transferred into a 100-ml volumetric flask and the volume is completed with the dissolution media.

Test conditions:

Dissolution apparatus:	Flow through cell
Dissolution medium:	0.07 M pH 7.0 phosphate buffer
Temperature:	37± 0.5^{o}C
Initial volume:	900 ml
Pump flow rate:	30 ml/min
Filter size:	0.2 µm
Volume withdrawn:	5 ml

Volume replaced: 5 ml

Sampling times: 5, 10, 15, 30, 45, 60 minutes

Procedure: The system was connected as shown in figure 2.1, the pump was operated; using a 10-ml syringe 10- ml portions were withdrawn into a test tube. A 5-mL portion from the test tube is transferred into a 50-ml volumetric flask and the volume is completed with the dissolution media. The remaining portion in the test tube was returned to the vessels and 5-mL from dissolution media was added to each vessel. The absorbance at λ = 278 nm of the standard preparation and the test preparations was determined for all different bathes of the product.

The percentage of Cefuroxime released at each time interval is calculated using the following equation:

$$\%\ \mathbf{CFA} = \frac{\boldsymbol{Absorbance\ of\ test\ preparation}}{\boldsymbol{Absorbance\ of\ standard\ preparation}} \times \mathbf{100}\%$$

3.4.3.4 Weight variation

Twenty suppositories are individually weighed using an analytical balance and the average weight is determined.

Acceptance criteria:

The maximum percentage deviation from the average is 5%.

3.4.3.5 Disintegration test:

The apparatus used for conducting the test is similar to the one described previously in method I and figure 1.4 with the difference that the disintegration is determined for three suppositories simultaneously instead of one (Erweka, model ST 30, Serial NO. 1086191069).

Procedure:

Water maintained at a temperature of 36-37°C was used as the immersion fluid. The samples were placed on the lower disc of the metal device and then inserted into the cylinder. The apparatus was placed into the beaker and inverted every 10 minutes without removing it from the liquid. The time required for the disintegration of the suppositories was recorded.

Acceptance criteria:

The state of each of the three suppositories shall be examined after 30 minutes for fat-based suppositories.

Disintegration is considered to be achieved when:

- Dissolution is complete
- The components of the suppositories have separated.
- There is softening of the test sample, usually accompanied by an appreciable change of shape without complete separation of the components. The softening process is such that a solid core no longer exists when pressure is applied with a glass rod.

3.4.3.6 Melting range:

The ascending melting point method was used for the determination of the melting point of all formulations. Capillary tubes of approximately 10 cm in length were sealed at one end and were filled with the formulation.

Procedure: Tow suppositories from each batch were crushed into small pieces and mixed; the tubes were filled to a height of 3-6 mm. Following filling, the tubes were placed in an automated melting point test apparatus (Mettler Toledo, type: FP 62, Serial No. 5117084333).

The start and end temperature of the apparatus were set at 32^{o}C and 40^{o}C respectively, the heating rate was set at 2.0 ^{o}C/min, the tube was observed every minute after the apparatus reached the start temperature and the melting temperature was recorded after melting was observed.

3.4.4 Test methods validation

3.4.4.1 HPLC Method validation

3.4.4.1.1 Introduction

"The object of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose" determined by means of well-documented experimental studies. Accuracy and reliability of the analytical results is crucial for ensuring quality, safety and efficacy of pharmaceuticals.

The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) was initiated in 1990, as a forum for a constructive dialogue between regulatory authorities and industry, in order to harmonize the submission requirements for new pharmaceuticals between Europe, the United States of America and Japan. One of the first topics within the Quality section was analytical validation and the ICH was very helpful in harmonizing terms and definitions as well as determining the basic requirements.

The ICH guidelines require that accuracy, precision, specificity; linearity, range, limit of quantitation (LOQ) and limit of detection (LOD) are assessed for assay and impurities determination analytical methods. The efficiency and long term reliability of an analytical method is dependent on establishing whether or not the analyte of interest is stable in an aqueous solution during the entire period of sample collection, processing, storage and analysis. Therefore, the stability of CFA in the mobile phase solution was also determined (USP32, 2010), (ICH Q2 (R1), 2005).

3.4.4.1.2 Linearity

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Range is the interval between the upper and lower levels of analyte (inclusive) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results obtained by the method. A minimum of five concentration levels, along with certain minimum

specified ranges are done. For assay, the minimum specified range is from 80-120% of the target concentration. For content uniformity testing, the minimum range is from 70-130% of the test or target concentration.

Acceptance Criteria: The correlation coefficient (R^2) is not less than 0.999 for the least squares method of analysis of the line.

Procedure:

A standard stock solution with concentration of 2.5 mg/ml was prepared by dissolving an amount of CFA standard material equivalent to 250 mg cefuroxim base in 100.0 ml methanol, then ten separate standards with different concentrations were prepared by diluting proportions from the stock solution according to the following table 3.11, the standards were analysed in according to the HPLC analytical method.

Data Analysis:

The response of each concentration was plot versus standard concentrations prepared for linearity and Range. The least squares linear regression analysis, the slope, and Y-intercept of the data were performed. The results are shown in the results section.

Table 3.11: Standard solutions preparation for linearity determination

Solution No.	Conc. %	Conc. (mg/ml)	Volume Pipetted from Stock St Solution (ml)	Final Volume
1	10	0.025	1.0	100.0
2	20	0.05	1.0	50.0
3	30	0.075	3.0	100.0
4	40	0.1	2.0	50.0
5	60	0.15	3.0	50.0
6	80	0.2	2.0	25.0
7	100	0.25	5.0	50.0
8	120	0.3	3.0	25.0
9	140	0.35	7.0	50.0
10	160	0.4	4.0	25.0

3.4.4.1.3 Range

The range is the interval between the upper and lower concentrations of analyte in the sample that have been demonstrated to have a suitable level of precision, accuracy, and linearity. The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

The following minimum specified ranges should be considered:

- For the assay of an active substance or a finished product: normally from 80 to 120 percent of the test concentration;
- For content uniformity testing, the minimum range is from 70-130% of the test or target concentration.

3.4.4.1.4 Accuracy:

The accuracy of an analytical procedure measures the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and value found (i.e. accuracy is a measure of exactness of an analytical method). Accuracy is evaluated by analyzing synthetic mixtures spiked with known quantities of active pharmaceutical ingredient.

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations /3 replicates each of the total analytical procedure).

Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

Acceptance Criteria: The mean recovery of the assay should be within 100±2.0% at each concentration over the range of 80 – 120% of nominal concentration.

Procedure:

A placebo formulation of cefuroxime axetil suppositories was prepared according to the formulation procedure of the CFA suppositories. The weight of tow suppositories was transferred to a 100-ml volumetric flask and dissolved with methanol with the aid of gentle heat, the volume was completed to mark with methanol, then three separate samples with different concentrations were prepared by diluting proportions from the stock solution according to table 3.12, the samples were analysed according to the HPLC analytical method

Table 3.12 Accuracy determination standard solution

Conc. (%)	**Concentration (mg/ml)**	**Pipetted Volume of sample (ml)**	**Flask Volume (ml)**
80	0.2	2.0	25.0
100	0.25	5.0	50.0
120	0.3	3.0	25.0

Data Analysis:

- Calculate the recovery data for each determination; calculate the average of recovery data and the RSD for each level.
- Verify that the mean recovery of the assay should be within 100±2.0% at each concentration over the range of 80 – 120% of nominal concentration.

3.4.4.1.5 Specificity/ Selectivity

Specificity is the ability to assess unequivocally the analyte in the presence of components which are expected to be present. Typically these might include impurities, degradants, matrix, etc. It is a measure of the degree of interference from such things and is measured and documented in a separation by the resolution, plate count (efficiency), and tailing factor.

Procedure:

A. No interference from excipients:

This was conducted by preparing synthetic mixture of the product excipients, prepared as sample preparation and measured. Standard of 100 % nominal concentration (0.25 mg/ ml) and Sample of 100 % nominal concentration were injected in triplicate and measured.

B. No interference from degradation products:

A stock solution was prepared by dissolving an amount of cefuroxime axetil equivalent to 250 mg cefuroxime base in methanol, and then a-100% standard solution of a final concentration of 0.25 mg/ml was prepared for forced degradation conditions as follows:

Alkali degradation studies (0.5 N NaOH):

From the stock solution, 5.0 ml solution was transferred into a-50.0 ml volumetric flask, a quantity of the mobile phase approximately equivalent to 25 ml was added to the flask, a-5.0 ml of 0.5 N NaOH was added to the mixture and volume was completed with mobile phase. The solution was allowed to stand for three hours and measured in triplicates.

Acid degradation studies (0.5 N HCl):

It was preceded as in alkali degradation except with the HCL solution was added instead of NaOH solution.

Oxidation with Hydrogen Peroxide (10% H_2O_2):

It was preceded as in alkali degradation except with the H_2O_2 solution was added instead of NaOH solution.

Light:

From the stock solution 5.0 ml solution was transferred into a-50.0 ml volumetric flask, the volume was completed with mobile phase solution, mixed and left under U.V. light for 24 hours and the response was measured in triplicates.

Heat (heat on a boiling water bath for one hour):

It was preceded as in light exposure except that instead of exposure to U.V. light the solution was heated in a boiling water bath for one hour and the response was measured in triplicates.

C. solution Stability:

Tow nominal concentration (0.25 mg/ ml) solutions were stored in refregirator (2-8^{o}C) and at room temperature for 24 houres and the responses were measured in triplicates.

3.4.4.1.6 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Procedure:Tow levels of precision were considered for this study, repeatability and intermediate preecision.

a) Repeatability:

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability was assessed using:

- 9 injections covering the specified range for the procedure (3 concentrations (0.2, 0.25, 0.3 mg/ ml) / 3 replicates each) and
- 6 injections at 100% (0.25 mg/ ml) of the test concentration for both sample and standard solutions. All were prepared as mentioned for the accuracy.

Acceptance Criteria: Relative Standard Deviation shall not be greater than 1.5%.

b) Intermediate precision:

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

It was assessed by repeating accuracy in different day by different analyst and different instrument.

3.4.4.1.7 Limit of Detection and Limit of Quantitation Test:

Limit of Detection (LOD):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental:

- Based on Visual Evaluation: The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.
- Based on Signal-to-Noise: Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.
- Based on the Standard Deviation of the Response and the Slope: The detection limit (DL) may be expressed as:

  $$\mathbf{DL = 3.3\ \sigma / S}$$

  where σ = the standard deviation of the response

  S = the slope of the calibration curve

Limit of Quantitation (LOQ):

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

- Based on Visual Evaluation: The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.
- Based on Signal-to-Noise Approach: This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.
- Based on the Standard Deviation of the Response and the Slope: The quantitation limit (QL) may be expressed:

$$\mathbf{QL = 10\ \sigma / S}$$

where σ = the standard deviation of the response

S = the slope of the calibration curve

Procedure:

From the standard solution having the concentration of 0.25 mg/ ml, 5-ml solution were transferred to a-50ml volumetric flask, the volume was completed with buffur solution to get a nominal concentration of 0.025mg/ml, and the following dilutions were prepared from this solution as shown in the table below by transferring the mentioned volumes and dilution with the mobile phase:

Table 3.13: standard solutions for LOD & LOQ determination

Solution #	Conc. Of cefuroxime (mg/ml)	Volume Pipetted from Stock Solution (ml)	Final Volume (ml)
1	0.01	10.0	25.0
2	0.001	2.0	50.0
3	0.0005	1.0	50.0
4	0.00025	5.0	250.0
5*	0.0001	5.0	50.0

*5ml from solution 2 were transferred to 50.0 ml vulumetric flask

3.4.4.2 Dissolution Method Validation

3.4.4.2.1 Introduction:

Dissolution testing is one of the most common analytical techniques performed in a pharmaceutical analytical laboratory. An ideal dissolution test should deliver information in three key areas. First, the dissolution test should be able to detect changes in the physicochemical properties of the drug product from the effect of these changes on the rate or amount of the drug substance released. Second, dissolution testing should be able to distinguish drug products that have been manufactured using different processes and/or formulations during the development phase. Finally, when in vitro in vivo correlation is established, dissolution should also reflect release and absorption rates in humans.

The role of an analytical method validation is to demonstrate that the method is capable of measuring an analyte accurately (accuracy, which includes specificity) and reliably (precision, which includes repeatability and reproducibility). In addition, if the analyte is expected to be in a wider range e.g. zero to 100 %, which is usually the case in dissolution testing, then it has to be

established that concentrations and responses have a linear relationship (linearity), by measuring responses at different concentrations.

For the purpose of drug dissolution testing, it has to demonstrate that the analytical method is capable of measuring it accurately and reliably. Therefore, for validation of such methods, one needs to add the drug ("spiking") in solution form to a dissolution testing apparatus i.e. vessel containing required volume of medium maintained at 37 ºC and spindle rotating. Samples are withdrawn and processed exactly as if these were from a product (filtration, dilution, extraction etc) and responses are measured accordingly. If responses and concentrations are as one would expect (as explained above), then that dissolution method has been validated.

3.4.4.2.2 Linearity and Range:

Linearity and range are typically established by preparing solutions of the drug, ranging in concentration from below the lowest expected concentration to above the highest concentration during release (USP32, 2010), (ICH Q2(R1), 2005).

Acceptance criteria:

Linearity is calculated by using an appropriate least-squares regression program. A square of the correlation coefficient ($R^2 \geq 0.98$) demonstrates linearity. The *y*-intercept must not be significantly different from zero.

Procedure:

A standard stock solution with concentration of 0.1388 mg/ml cefuroxime base was prepared by dissolving an amount of CFA standard material equivalent to 138.8 mg cefuroxime base in 100.0 ml methanol, then ten ml of the solution were transferred to a-100 ml volumetric flask and the volume was completed with the dissolution media solution. From this stock solution separate standards with different concentrations were prepared by diluting proportions from the stock solution according to the following table 3.14 the standards were analysed in according to the U.V analytical method.

Data Analysis:

The absorbance of each concentration was plot versus standard concentrations prepared for linearity and Range. The least squares linear regression analysis, the slope, and Y-intercept of the data were performed.

Table 3.14: standard solutions preparation for dissolution method linearity determination

No.	Conc. %	Conc. (mg/ml)	Volume Pipetted from Stock St	Final Volume (ml)
1	25	0.00347	5.0	200.0
2	50	0.00694	5.0	100
3	75	0.01041	15.0	200.0
4	100	0.01388	5.0	50.0
5	120	0.016656	3.0	25.0
6	150	0.02082	15.0	100.0

3.4.4.2.3 Accuracy/Recovery

Accuracy/recovery are typically established by preparing multiple samples containing the drug and any other constituents present in the dosage form ranging in concentration from below the lowest expected concentration to above the highest concentration during release.

Acceptance criteria:

The measured recovery is typically 95% to 105% of the amount added.

Procedure:

The formulation F06 was selected for the accuracy study. A placebo containing all the excipients in the formulation F06, as shown in table 3.15 was prepared in a quantity equivalent to 10 suppositories. From the prepared placebo an amount equivalent to the weight of one suppository

(1.2gm/supp.) was weighed and completely dissolved in 900 ml dissolution buffer solution at 37^{o}C. An amount of cefuroxime axetil (approximately 173.32 mg) equivalent to 138.8 mg cefuroxime base was weighed, transferred into a 100-ml volumetric flask and dissolved in methanol, the volume was completed with methanol, then 10.0.ml from this solution were transferred into a-100.0 ml volumetric flask and volume was completed with buffer solution (stock solution with 0.1388 mg/ml concentration). From the stock solution, three different solutions (50%, 100%, and 150%) were prepared as in table 3.16. The absorbance of these solutions was measured in triplicate for each concentration.

Table 3.15 Placebo suppository preparations (20 supp. Each)

Excipients	**Weight**
WH15	23.52gm
Lanolin 1%	0.24gm
SLS 1%	0.24gm

Table 3.16 Solution preparations for dissolution method accuracy determination

No.	**% Concentration**	**Conc. Mg/ml**	**Volume from stock (ml)**	**Final volume (ml)**
1	50	0.00694	5.0	100.0
2	100	0.01388	5.0	50.0
3	150	0.02082	15.0	100.0

3.4.4.2.4 Precision

Repeatability is determined by replicate measurements of standard and/or sample solutions. It can be measured by calculating the RSD of the multiple spectrophotometric readings for each standard solution, or from the accuracy or linearity data.

Acceptance criteria:

The relative standard deviation (RSD) of recoveries should be ≤ 4%

Procedure:

The formula F06 was chosen for the study, since it showed the best release rate. The dissolution of this batch was repeated six times and samples were withdrawn at two time intervals (30, 60 min.) and the absorbance was measured.

3.4.4.2.5 Specificity/Placebo Interference:

It is the demonstration that the results are not affected by placebo constituents, other active drugs, or degradation materials. The placebo consists of all the excipients without the active ingredient.

Acceptance criteria:

The placebo interference must not exceed 2%. The % recovery is 100% ±5%.

Procedure:

Six placebo formulations were prepared without CFA as shown in table 3.17, then the weight equivalent to one suppository (1.2 gm) from each formula was dissolved in 900 ml dissolution media solution at 37^{o}C, from this solution 5.0 ml were transferred into 50.0 ml volumetric flask and volume was completed with the media solution, then the absorbance for each of these formulations was measured at 278 nm and Scand at the λ range of 240-340 nm. Six suppository formulations were prepared as shown in table 3.18, then one suppository from each formulation was dissolved in 900 ml dissolution buffer at 37^{o}C, from this solution 5.0 ml were transferred into a 50.0 ml volumetric flask, a portion was filtered through 0.22µ filter and absorbance was measured for each formulation and the percentage recovery was determined.

Calculations:

$$Absorbance = \frac{\text{Abs. of stand.} + \text{placebo Abs.}}{\text{Abs. of stand.}}$$

Table 3.17 Placebo formulations for dissolution specificity determination (quantity for 10 supp.)

Excipients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
WH 15	11.88 gm	11.76 gm	11.76 gm	11.88 gm	11.76 gm	11.76
Lecithin 1	0	0	0.12 gm	0	0	0.12
Lanolin	0	0.12 gm	0	0	0.12	0
SLS	0.12 gm	0.12 gm	0.12	0	0	0
Tween 85	0	0	0	0.12	0.12	0.12

Table 3.18 Sample formulations for dissolution specificity determination (quantity for 10 supp.)

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
CFA	1.561	1.561	1.561	1.561	1.561	1.561
WH 14	10.32 gm	10.2 gm	10.2 gm	10.2 gm	10.2 gm	10.2 gm
Lecithin	0	0	0.12 gm	0	0	0.12 gm
Lanolin	0	0.12 gm	0	0	0.12 gm	0
SLS	0.12 gm	0.12 gm	0.12 gm	0.12 gm	0	0
Tween 85	0	0	0	0.12 gm	0.12 gm	0.12m

3.5 Stability studies of selected formulations

3.5.1 Introduction:

To provide an adequate shelf-life for products, active ingredients and dosage forms must show chemical and physical stabilities for long periods. The USP description of suppository dosage form instability is summarised by excessive softening, although some suppositories may dry out and harden or shrivel. Evidence of oil stains on packaging material should warn the pharmacist to examine individual suppositories more closely by removing any foil covering. As a general rule, the USP recommends storage in a refrigerator, unless otherwise indicated.

The bioavailability of chemically stable rectal drugs is influenced by the physical stability of suppositories during storage. The so-called hardening effect occurs during storage of suppositories. It results in an increase in the melting time of suppositories. Considerable changes in melting times arise only with bases of higher melting ranges (e.g. Witepsol H 37, 36-38°C). Bases with the lowest melting points (e.g., Witepsol H 32, 31-32 ° C) are subject only to minor changes (Hermann, 1995)

It was found that any little hardening resulting in little or no suppository melting, can cause local irritation, a defecator reflex or bowel obstruction and therefore it is important to consider this during formulation development (Cohen, Lordi, 1980).

Hardening of suppositories in storage may be due to of polymorphic phase transitions, increased crystallinity and/or increased transesterification of the bases (Cohen, Lordi, 1980).

Long term storage of suppositories manufactured using semi synthetic fatty suppository bases may result in a reduction in drug release from these dosage forms (Webster, et al, 1998).

3.5.2 Procedure:

From the selected final formulations only six batches (F01, F07, F11, F14, F15 and F16) were selected for the stability testing as representative formulations. Samples from these formulations were stored at different conditions as required by the ICH Q1A R2 Guidelines for three months

period. The storage conditions were as follows:

25°C ± 2°C/60% RH ± 5% RH, 30°C ± 2°C/65% RH ± 5% RH, and 2-8^{o}C (refrigerator)

The suppositories were evaluated at initial time and every month thereafter. The following parameters were subjected for reevaluation, i.e. colour, surface texture, disintegration time, dissolution profile, assay, melting range, and degradation products.

3.5.3 Stability acceptance criteria:

- 5% significant change in the % assay
- Physical changes in colour, texture, appearance of sediments
- Significant change in the dissolution rate
- Impurities:
  - The sum of the areas for the pair of peaks corresponding to E-isomers is NMT 1.5% by normalisation
  - The sum of the areas of any peaks corresponding to delta isomers corresponding to delta isomers is NMT 2.0% by normalisation
  - The area of any other secondary peak is NMT 1.0% by normalization

Part Four:

Results and Discussion

4.1 Preformulations Studies:

4.1.1 Displacement Value:

The calculated displacement value results for the different fatty bases used in preformulations are illustrated in table 4.1. From the results it is observed that the values of D.V are less than one which indicates very minor effect on the amount of suppository bases to be used for formulation.

Table 4.1 displacement value of CFA in different suppository bases

Base	**Displacement value**	**Weigh of base/suppository [g]**
Novata BCF	0.936	1.040
Novata A	0.811	1.015
Novata BCF/Novata A (30:70%)	0.935	1.0396
Novata BCF/Novata A (70:30%)	0.936	1.040
Whitepsol H15	0.936	1.040

The value for PEG bases was not calculated since these bases were excluded from the study in the early stages for their incompatibility with the CFA.

4.1.2 Organoleptic and melting range test results for preformulations batches:

The physical appearance results for suppository formulations containing Novata A/BCF are illustrated in table 4.2., while the results for suppository formulations containing Whitepsol H15 are illustrated in table 4.3.

Table 4.2 Physical appearance data for the formulations using Novata A/BCF

Formulation Code	**Colour**	**Appearance**	**Surface Texture**	**Melting range ^{o}C**
PN1	White	Opaque	Smooth	38-39
PN2	Off White	Opaque	Smooth	36.5-37.4
PN3	White	Opaque	Smooth	38.4-39
PN4	White	Opaque	Smooth	36.8-37.3
PN5	White	Opaque	Smooth	38.5-39.2
PN6	White	Opaque	Smooth	38.3-39
PN7	White	Opaque	Smooth	37.5-38
PN8	White	Opaque	Smooth	38.2-38.8
PN9	White	Opaque	Smooth	37.4-38
PN10	White	Opaque	Smooth	37.8-39
PN11	White	Opaque	Smooth	38.6-39.3
PN12	White	Opaque	Smooth	37-37.4
PN13	White	Opaque	Smooth	36.7-37.3
PN14	White	Opaque	Smooth	38-38.5
PN15	White	Opaque	Smooth	38.4-39

Table 4.3 Physical appearance results for the formulations using Witepsol H15

Formulation	Colour	Appearance	Surface Texture	Melting range ^{o}C
PW1	White	Opaque	Smooth	37.7-38.8
PW2	White	Opaque	Smooth	37-37.6
PW3	White	Opaque	Smooth	36.7-38
PW4	White	Opaque	Smooth	38.3-39.1
PW5	White	Opaque	Smooth	37.4-38.1
PW6	White	Opaque	Smooth	37.7-38.4
PW7	White	Opaque	Smooth	36.8-37.5
PW8	White	Opaque	Smooth	37-37.4
PW9	Yellowish	Opaque	Smooth	38.3-38.8
PW10	White	Opaque	Smooth with precipitates	39-39.4
PW11	Off White	Opaque	Smooth	-
PW12	Off White	Opaque	Smooth	36.6-37.3
PW13	Off White	Opaque	Smooth	35.7-36.2
PW14	Off White	Opaque	Smooth	35.4-36.1
PW15	Off White	Opaque	Smooth	36-37
PW16	White	Opaque	Smooth	36.2-37.3
PW17	White	Opaque	Smooth	35.8-36.8
PW18	White	Opaque	Smooth	37.7-38.5
PW19	White	Opaque	Smooth	36.8-37.9
PW20	White	Opaque	Smooth	37.4-38.2
PW21	White	Opaque	Smooth	35.6-36.7
PW22	Pale yellow	Opaque	Smooth	35.6-36.8
PW23	Pale yellow	Opaque	Smooth	35.2-36.5

When Witepsol H15 was used without additives it was found that the melting point of the prepared suppositories is above 37.5^{o}C, while using Novata BCF instead, the melting point exceeded 38^{o}C. The addition of melting point modifiers (e.g. paraffin oil, lecithin, and lanolin) to Novata bases did not reduce the melting points significantly, while their addition to Witepsol H15 base significantly reduced the melting points.

It was observed that some additives changed the colour of suppositories to off white/pale yellow; the other physical parameters were almost identical for both bases.

4.1.3 Dissolution results:

The results of dissolution profile test for the preformulation trials are listed in tables 4.4 and 4.5.

From the results it clearly observed that the dissolution results of CFA from suppositories compounded using Witepsol H15 were found better than those compounded with Novatas, although both were relatively low. The addition of surfactants and melting point modifiers had improved the dissolution rate significantly.

Table 4.4 summary of preformulations dissolution results using Novata A/BCF bases indicated as percentage release [%] of CFA.

Formula	15 min	30 min	45 min	60 min	120 min	180 min	Diss.
PN1	2	5	6	5	8	-	D1
PN2	53	-	-	-	-	-	D3
PN3	5	11	11	12	11	17	D2
PN4	10	15	21	24	38	53	D2
PN5	5	4	6	7	8	8	D2
PN6	9	4	9	12	13	17	D2
PN7	4	8	10	13	-	-	D1
PN8	7	10	15	24	41	47	D1
PN9	11	17	28	29	41	47	D2
PN10	68	76	79	82	79	-	D2
PN11	44	55	-	42	-	-	D2
PN12	42	69	63	65	77	-	D2
PN13	3	6	14	13	16	-	D2
PN14	3	4	5	6	8	10	D2
PN15	8	11	14	15	20	20	D2

-: Not measured

Table 4.5 Summary of preformulations dissolution results using Witepsol H15 base indicated as percentage release [%] of CFA

Formula	15 min	30 min	45 min	60 min	120 min	180 min	Diss.
PW1	-	3	-	11	19	-	D2
PW2	-	7	-	18	27	-	D2
PW3	-	5	-	7	-	15	D2
PW4	-	105	-	100	-	100	D2
PW5	3	5	4	7	9	-	D2
PW6	8	12	16	21	32	-	D2
PW7	25	42	47	53	63	-	D2
PW8	70	85	87	92	96	-	D2
PW9	16	17	15	34	35	-	D2
PW10	15	19	22	29	37	-	D2
PW11	30	51	-	65	79	-	D5
PW12	9	18	-	30	41	-	D2
PW13	12	20	-	36	45	53	D2
PW14	13	23	-	35	49	56	D2
PW18	6	9	13	17	28	35	D2
PW19	7	10	14	17	28	35	D2
PW20	7	11	13	17	27	35	D2
PW21	31	41	45	47	53	61	D2
PW22	3	8	11	15	18	40	D2
PW23	17	35	54	65	78	83	D2

-: Not measured

Diss. = Dissolution conditions

D1= paddle, 0.2M phosphate buffer, pH 7.0, speed = 50 rpm

D2=modified basket, 0.2 M phosphate buffer, pH 7.0, speed = 100 rpm

D3= modified basket, 0.2M phosphate buffer +1%SLS in media, pH 7, speed = 100 rpm

D4=modified basket, 0.2M phosphate buffer + 0.1%SLS in media, PH 7, speed = 100rpm

D5=modified basket, 0.2M phosphate buffer +0.5% Tween 20 in media, PH 7, speed = 100rpm

4.1.4 Evaluation of CFA assay and impurities:

Representative formulations containing most of the additives were analysed for their CFA and impurities contents. The results are illustrated in Table 4.6.

By comparing the analysis results with the proposed specification mentioned in section 4.2.1, it is obvious that CFA is compatible with the used fatty bases and with the majority of the used additives, i.e. Paraffin oil, Lanolin, Aerosil, SLS, Cremophor A6. However, it was very clear from the number and the percentage of impurities that there was an incompatibility between the PEG base and CFA.

Table 4.6 Drug content evaluation data for some preformulation batches

Formulation code	**%Assay**	**%Free Cefuroxime**	**%Δ^3 Isomer**	**%E1 Enantiomer**	**%E2 Enantiomer**
CFA	100	0.28	N.D	N.D	N.D
PW5	97	0.46	1.89	0.09	0.05
PW6	97.5	0.47	1.54	0.10	0.06
PW7	103	0.48	0.79	0.10	0.07
PW8	99	0.63	0.74	0.09	0.06
PN14	98	0.75	0.96	0.10	0.07
PN15	99.8	0.97	1.54	0.09	0.06
PW14	98	0.84	0.73	0.10	0.07
PW15	98	0.86	0.81	0.11	0.06
PN7	101	0.75	1.13	0.12	0.09
PEG+CFA*	54	0.27	49	0.13	N.D

* Three more unidentified impurities were observed at RT 2.97, 8.7, and 9.14 min.

4.2 Formulation of Cefuroxime Axetil Suppositories:

4.2.1 Proposed drug product specifications:

The following specifications are prevailed for the new CFA suppositories dosage form depending on the results obtained in this study and on the specifications of the tablets dosage form mentioned in the US Pharmacopoeia.

Table 4.7 Proposed drug product specifications

Tests	**Specifications**
Appearance	Smooth, opaque with no precipitates
Melting range	36-37.5^{o}C
Disintegration Time	Not more than 30 minutes.
Assay	The CFA content should be 90-110% of the label claim
Related substances: -Delta-3-Isomer: -E Isomers: -Free Cefuroxime: -Total unknown impurities:	 Not more than 2.5%, calculated by normalization method Not more than 1.5%, calculated by normalization method Not more than 2.0%, calculated by normalization method Not more than 1.0%, calculated by normalization method

4.2.2 Selected Formulae Evaluations:

4.2.2.1 Organoleptic test results:

All the formulated suppositories were evaluated for their shape, color, size and surface texture. The physical appearance of the formulations were checked and compared visually. The suppositories of all the formulations were all conical or bullet shaped.

Table 4.8 Organoleptic test results for the selected formulations

Formulation Code	Colour	Appearance	Surface Texture
F01	White	Opaque	Smooth
F02	White	Opaque	Smooth
F03	White	Opaque	Smooth
F04	White	Opaque	Smooth
F05	White	Opaque	Smooth
F06	Off White	Opaque	Smooth
F07	Off White	Opaque	Smooth
F08	Off White	Opaque	Smooth
F09	Off White	Opaque	Smooth
F10	Pale yellow	Opaque	Smooth
F11	Pale yellow	Opaque	Smooth
F12	Pale yellow	Opaque	Smooth
F13	Pale yellow	Opaque	Smooth
F14	White	Opaque	Smooth
F15	Off White	Opaque	Smooth
F16	Pale yellow	Opaque	Smooth

4.2.3 Uniformity of Weight:

The results are illustrated in table 4.9. The average weight, standard deviation and relative standard deviation were calculated.

From the results it was found that all the batches comply with the requirements for weight uniformity of suppositories, as described in the BP, which recommends a maximum percentage deviation of 5.0%.

Table 4.9 Evaluation of uniformity of weight data (weights are in gram unit)

No.	F01	F02	F03	F04	F05	F06	F07	F08
1	1.239	1.270	1.340	1.281	1.246	1.240	1.284	1.194
2	1.168	1.240	1.220	1.230	1.236	1.257	1.290	1.219
3	1.310	1.262	1.188	1.195	1.207	1.277	1.310	1.199
4	1.350	1.242	1.183	1.274	1.220	1.245	1.350	1.211
5	1.290	1.160	1.218	1.232	1.200	1.262	1.274	1.203
6	1.168	1.170	1.238	1.320	1.198	1.210	1.230	1.204
7	1.238	1.216	1.220	1.207	1.187	1.270	1.382	1.265
8	1.175	1.223	1.182	1.183	1.201	1.230	1.318	1.230
9	1.287	1.224	1.207	1.204	1.239	1.310	1.252	1.244
10	1.260	1.234	1.220	1.270	1.249	1.279	1.170	1.230
11	1.310	1.221	1.208	1.242	1.370	1.214	1.320	1.200
12	1.277	1.227	1.205	1.197	1.286	1.206	1.220	1.216
13	1.293	1.224	1.197	1.200	1.260	1.216	1.255	1.227
14	1.263	1.253	1.305	1.179	1.213	1.213	1.270	1.234
15	1.215	1.188	1.276	1.224	1.205	1.231	1.245	1.256
16	1.175	1.198	1.228	1.277	1.216	1.241	1.287	1.244
17	1.385	1.270	1.210	1.232	1.282	1.214	1.343	1.260
18	1.213	1.246	1.250	1.213	1.257	1.222	1.307	1.187
19	1.213	1.210	1.236	1.331	1.290	1.192	1.221	1.253
20	1.221	1.235	1.211	1.220	1.312	1.198	1.218	1.245
Average	**1.253**	**1.226**	**1.227**	**1.236**	**1.244**	**1.236**	**1.277**	**1.2261**
SD	**0.0611**	**0.0299**	**0.0399**	**0.0434**	**0.0463**	**0.0312**	**0.0524**	**0.0232**
%RSD	**4.9**	**2.4**	**3.1**	**3.5**	**3.7**	**2.5**	**4.1**	**1.9**

Table 4.9 Evaluation of uniformity of weight data (continued)

No.	F09	F10	F11	F12	F13	F14	F15	F16
1	1.207	1.186	1.325	1.160	1.254	1.274	1.395	1.272
2	1.238	1.218	1.235	1.217	1.209	1.212	1.264	1.283
3	1.165	1.197	1.230	1.210	1.240	1.284	1.239	1.227
4	1.239	1.221	1.219	1.195	1.158	1.255	1.264	1.263
5	1.223	1.219	1.220	1.166	1.254	1.279	1.276	1.233
6	1.221	1.200	1.360	1.177	1.255	1.254	1.250	1.235
7	1.186	1.205	1.240	1.230	1.230	1.240	1.268	1.237
8	1.240	1.249	1.220	1.199	1.243	1.234	1.220	1.243
9	1.212	1.190	1.176	1.201	1.233	1.232	1.254	1.240
10	1.230	1.270	1.227	1.220	1.202	1.219	1.237	1.266
11	1.263	1.233	1.290	1.200	1.187	1.239	1.247	1.239
12	1.229	1.228	1.241	1.197	1.203	1.297	1.245	1.255
13	1.254	1.192	1.242	1.236	1.189	1.232	1.260	1.201
14	1.214	1.222	1.249	1.216	1.201	1.212	1.248	1.224
15	1.236	1.214	1.313	1.218	1.243	1.250	1.243	1.173
16	1.232	1.242	1.235	1.201	1.231	1.250	1.246	1.268
17	1.222	1.221	1.259	1.260	1.223	1.200	1.235	1.227
18	1.201	1.219	1.248	1.188	1.216	1.219	1.194	1.209
19	1.219	1.276	1.189	1.219	1.204	1.220	1.205	1.210
20	1.193	1.210	1.221	1.211	1.266	1.240	1.221	1.189
Average	**1.221**	**1.220**	**1.247**	**1.206**	**1.222**	**1.242**	**1.251**	**1.235**
SD	**0.0232**	**0.0247**	**0.0445**	**0.0234**	**0.0278**	**0.0262**	**0.0398**	**0.0287**
%RSD	**1.9**	**2.0**	**3.6**	**1.9**	**2.3**	**2.1**	**3.2**	**2.3**

4.2.4 Disintegration Time and Melting Range:

The data for the formulated suppository are shown in the Table 4.10. The disintegration time for all formulations was found to be within the limits (< 30 min), however the disintegration time was lower in the formulations containing emulsifiers and solubilisers.

The melting points for formulations containing Aerosil as a suspending agent (F01, F02, F03, F04, and F05) were found to be higher than the target temperature (NMT 37.5^{o}C). All other formulations, except formula F09 which contains 2% lanolin and 2% Tween 20, had melting points ranging from (35.9-37.8^{o}C). Suppositories containing Lecithin, Lanolin and Tween 85 showed melting disintegration, however others showed softening disintegration.

Table 4.10 Disintegration time and melting temperature data for selected formulations

Formulation B.N	Disintegration time (min)	Melting range (^{o}C)	Observation
F01	10	38.5-39	Softened
F02	10	38.3-38.8	Softened
F03	10	38.7-39.2	Softened
F04	10	38.6-39.3	Softened
F05	10	37.7-38.2	Softened
F06	10	37.4-37.8	Softened
F07	10	36.5-37.1	Melted
F08	10	37.2-38	Softened
F09	10	38.1-39.2	Softened
F10	10	36.3-37.2	Melted
F11	10	36.5-37	Melted
F12	10	36.2-36.5	Melted
F13	10	36.7-37.3	Melted
F14	7	36.4-37	Melted
F15	7	36-36.8	Melted
F16	7	35.9-36.5	Melted

4.2.5 Drug content and impurities evaluation:

The amount of drug and the amount of degradation products/impurities present in each formulation have been evaluated according to the validated analytical test method. The calculated values are given in the Table 4.11. From the results in the table, the formulations containing the surfactant "Tween 20" (F04, F05, F08, F09, F12, and F13) did not conform with the proposed product specifications. It was found that CFA content was significantly below the acceptable limits due to the hydrolysis effect of Tween 20. The identified degradation materials were found to be free cefuroxim and the Δ^{3}Isomer. This could be due to the presence of hydroxyl groups in the surfactant, which promoted hydrolysis reactions.

Table 4.11 Drug Content Evaluation Data

Formulation Code	**% Assay**	**% Free CF**	**% Δ^3Isomer**	**% E_1 Enantiomer**	**% E_2 Enantiomer**	**% Unidentified Impurities**
CFA as material	100	0.28	ND	ND	ND	ND
F01	100.7	0.455	0.84	ND	ND	ND
F02	99	0.527	0.852	ND	ND	ND
F03	106	0.727	0.981	0.095	0.046	ND
F04	93	1.625	6.28	ND	ND	ND
F05	82	3.06	11.35	ND	ND	0.303
F06	101.4	0.705	0.935	ND	ND	ND
F07	103.6	0.747	0.952	ND	ND	ND
F08	87	2.717	8.03	ND	ND	0.280
F09	80.4	3.825	10.76	ND	ND	0.418
F10	102	0.897	1.069	ND	ND	ND
F11	100.5	0.903	1.00	0.101	0.072	ND
F12	92.3	2.42	5.21	ND	ND	0.367
F13	80.5	4.34	9.99	ND	ND	0.538
F14	101.2	0.424	1.958	ND	ND	ND
F15	101	0.495	2.186	ND	ND	ND
F16	101	0.598	2.29	ND	ND	ND

ND Not detected.

4.2.6 In vitro release studies of Cefuroxime Axetil from suppositories:

4.2.6.1 In vitro release using USP apparatus I (modified basket):

The dissolution profiles of CFA from the selected formulations are illustrated in table 4.12 and depicted in figure 4.1. These results represent the average value of 6 suppositories. It is clearly evident that the release profile of CFA from the formulation containing the active material and the suppository base alone (F01) was found to be very slow (i.e. not more than 8% in 180 minutes).

This result could be due to many factors including:

- The use of a suppository base (WHI5) with a low hydroxyl value of 13.6. As the hydroxyl value of the suppository base increases the water sorption of the suppository increases.
- The melting point of F01 was high (i.e. 39^{o}C), therefore the suppositories melted slowly and incompletely during the dissolution testing, since melting plays a great role in release rates and a prerequisite for drug liberation..
- The CFA is a very lipophilic material, so the partitioning of CFA between the aqueous dissolution medium and the lipoid suppository base phase, appears to favor the lipid phase.

When surfactants, solubilizers and melting point modifiers were added to the formulations, the release rate was increased. From the results illustrated in table 3.12 the following were observed:

- The CFA release rate in formulations F02 and F03 was not significantly increased, although SLS (HLB ≈ 40) was added. This could be due to the high melting point of these batches (about 39 C).
- The addition of (1% and 2%) of Tween 20 to batches F04 and F05 didn't reduce the melting points significantly, however the percentage release rate was increased moderately (i.e. 45% and 49% respectively at 180min).
- The addition of 1% lanolin anhydrous to the formulations (F06 and F07) containing 0.5% and 1% SLS reduced the melting points from 39 to 37^{o}C, but surprisingly it increased the release rate of F06 which had the lower SLS concentration more than F07. This could be a consequence of exceeding the critical micelles concentration (CMC). According to published literature, the presence of surfactants in formulations at concentrations higher than their CMC value generally retards the drug release, as a result of micelles entrapment of the drug (Aulton, 2002).
- The addition of 2% lanolin to batches containing Tween 20 (F08 and F09) increased the release rate to approximately 67% and 51% for F09 and F08 in 180minutes, respectively.
- The addition of Lecithin in 1% (w/w) to the formulations containing SLS and Tween 20 (i.e. F10, F11, F12, and F13) reduced the melting points; however the release rate was not

improved significantly as was expected. This could be a consequence of micelles entrapment of the CFA.

- Tween 85 1% (w/w) (HLB ≈ 11) was used as a nonionic surfactant in the production of batches F14, F15, and F16. Lanolin 1% was added to batch no. F15 and lecithin 1% was added to batch no. F16. The results showed a significant decrease in the melting point (36 – 37^{o}C) and a significantly increase in the percentage of CFA release especially in the case of batch F15 containing lanolin anhydrous showed the higher release rate (about 91% at 180min), however the batch containing lecithin didn't show a high release rate as was expected although the suppositories were completely melted at the first stages of the dissolution testing and micelles were clearly observed at the top of dissolution vessels. The inadequacy in the release rate of CFA from this batch could be a consequence of micelles entrapment of the CFA.

Table 4.12 Cumulative percentage release of CFA from all formulations using (USP apparatus I, modified basket):

Formulation	**15 min**	**30 min**	**45 min**	**60 min**	**120 min**	**180 min**
F01	2	3	3	4	6	8
F02	4	5	6	7	9	10
F03	8	11	12	14	16	18
F04	11	18	24	28	40	45
F05	19	19	24	29	43	49
F06	55	71	78	78	82	82
F07	9	18	22	28	44	48
F08	15	24	29	33	44	51
F09	15	30	32	38	58	67
F10	27	40	45	50	64	75
F11	29	46	53	56	63	78
F12	7	12	17	21	31	38
F13	7	15	26	33	54	64
F14	20	40	41	52	74	81
F15	22	54	71	80	91	91
F16	15	27	35	37	56	68

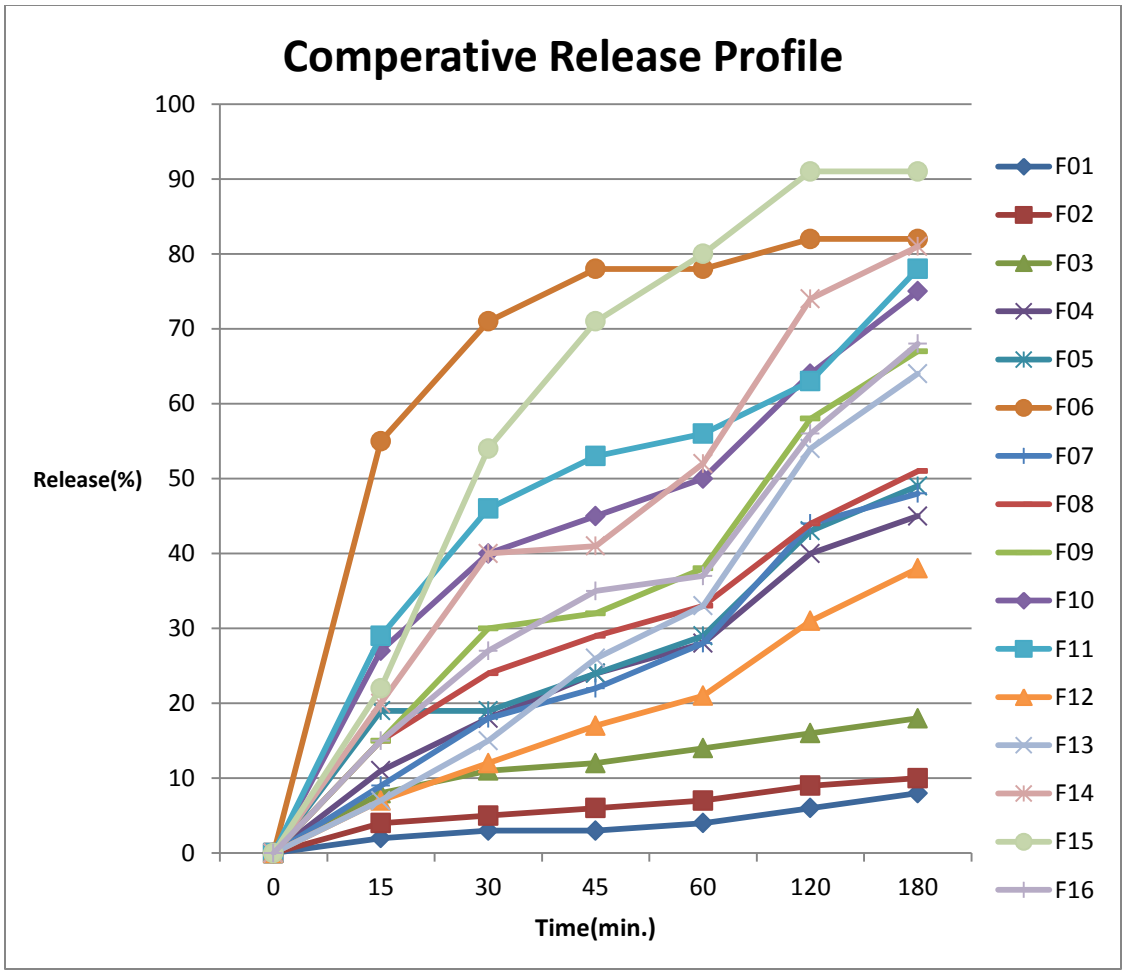


Figure 4.1 Comparative dissolution profiles of all formulations (USP apparatus I, modified basket)

Figures 4.2 to 4.6 represent comparative dissolution profiles between similar formulations with different surfactant concentration.

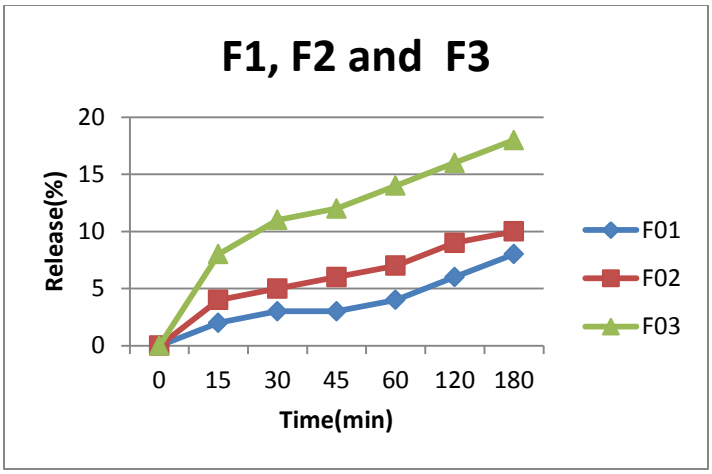


Figure 4.2 Comparative dissolution profiles between F1, F2 and F3

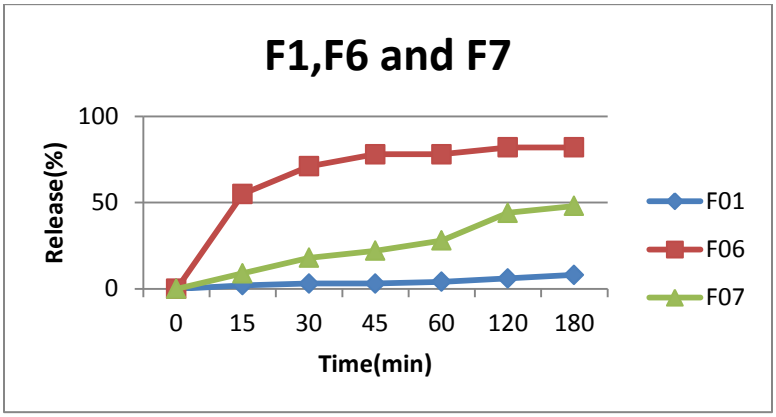


Figure 4.3 Comparative dissolution profiles between F1, F6 and F7

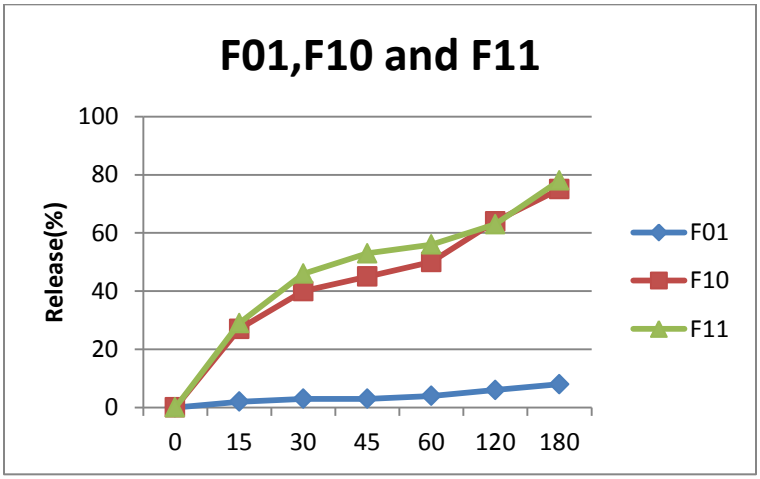


Figure 4.4 Comparative dissolution profiles between F1, F10 and F11

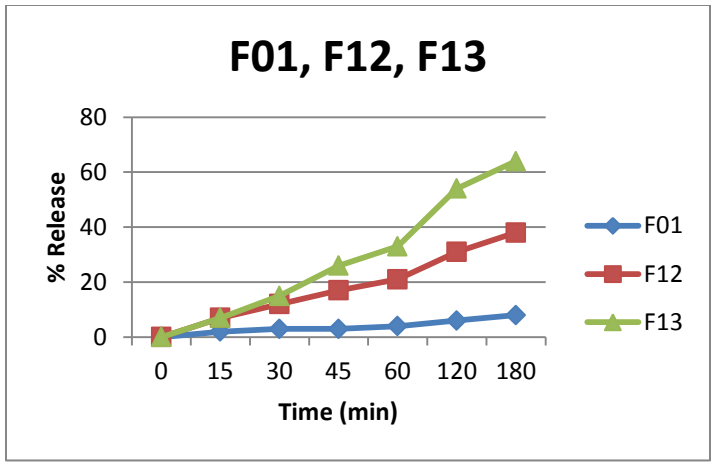


Figure 4.5 Comparative dissolution profiles between F1, F12 and F13

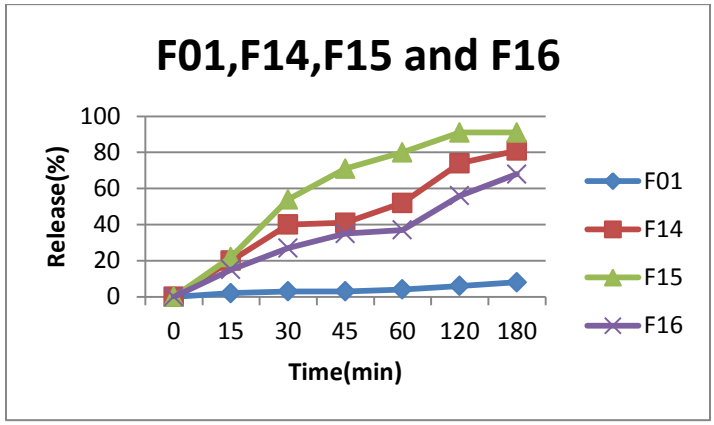


Figure 4.6 Comparative dissolution profiles between F1, F14 and F15, F16

4.2.6.2 In-vitro release using flow through cell:

Six batches (F01, F07, F11, F14, F15, and F16) were tested for dissolution release rate using the modified flow through cell for a time period of 60 minutes. The results obtained are illustrated in table 4.13 and depicted in figure 4.7. The addition of the surfactants and the solubilizers increased the release rate, where it approached the maximum (98% at 60min) in batch F16 with Tween 85 and lecithin. All batches showed a significant increase in the percentage release rate. These results could be explained as a consequence of the continuous flow of fresh dissolution medium through the molten suppository mass as compared to the constant exposure that prevails with the modified USP apparatus I. In this case drug exchange at the lipid/water interface becomes the rate-limiting step of drug release from suppository formulations apart from the solubility of the drug contained in the formulation. The continuous flow of dissolution medium

over the product maintained a concentration gradient between the saturation solubility concentration at the solid/liquid interface and the solute concentration in the bulk of the system, therefore there is a potential for mass transfer.

The objective of the flow through cell design is to expose the product to a homogeneous, non-turbulent, laminar flow to avoid the problems associated with a stirring mechanism, which are:

- When the stirring is fast, eddies are formed and hence the dissolved particles resides in these eddies and as a result the dissolution rate is low.
- When stirring is very slow, eddies are not formed; however the dissolved particles are not homogeneously distributed in the vessel.

Table 4.13 Cumulative percentage release of CFA from selected formulations (flow through cell):

Formulation	5 min	10 min	15 min	30 min	45 min	60 min
F01	23	39	48	59	64	68
F07	29	51	57	59	65	68
F11	81	96	93	92	90	85
F14	5	17	31	68	83	88
F15	51	63	78	72	85	87
F16	73	87	92	92	94	98

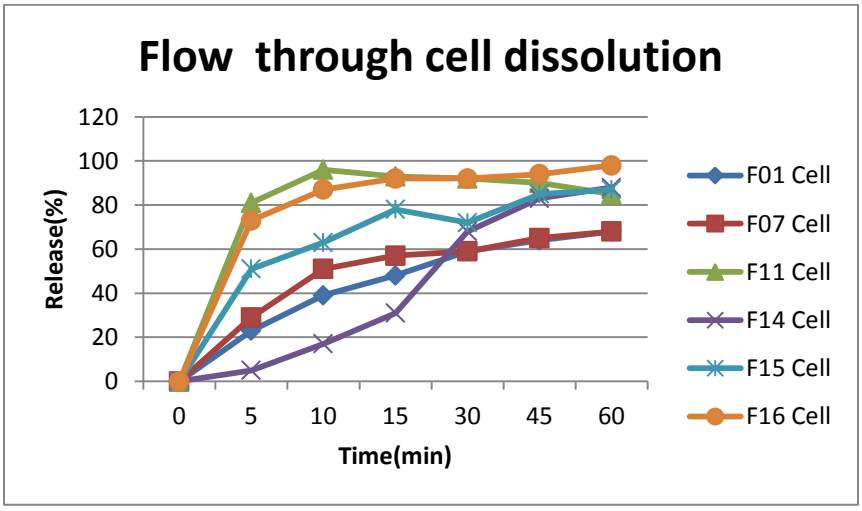


Figure 4.7 Comparative dissolution profiles **(**flow-through cell)

4.3 Mathematical Modeling of dissolution rate profile:

To determine the mechanism by which CFA is released from suppositories manufactured using fatty bases, dissolution data were fitted to selected mathematical models. The Korsmeyer-Peppas model was used to characterize drug release behavior from all batches, in the absence and presence of additives. The data were also fitted to the Higuchi, Zero order, First order and Weibull mathematical models to determine which model best described the release kinetics of CFA from these formulations using DD solver program (Yong Zhang, et al, 2010).

4.3.1 Application of the Korsmeyer-Peppas model:

An analysis of the fitting of experimental data to the Korsmeyer-Peppas model, as described in Equation 1.7, in addition to the interpretation of the corresponding release exponent values (n) were used to characterize and understand the mechanism by which CFA was released from these products.

The best-fit model parameters obtained following fitting of experimental data obtained from these formulations are listed in Table 4.14.

The release exponent n was found to be 0.6000, 0.5840, and 0.5410 for batches F01, F07, and F16 indicating that the release mechanism from these dosage forms was controlled by non-Fickian diffusion, whereas the n value determined for batch F14 was found to be 0.4800, suggesting that the release mechanism of CFA from this batch was controlled by Fickian diffusion as $n \approx 0.50$. The release mechanism elucidated for batches F02, F03, F06, F10, and F15, was not able to be explained by the Korsmeyer-Peppas model, since the resultant n values ($n<0.5$) did not fall within the specified range. The inability to ascribe the mechanism of release to these batches of suppositories may in part be explained by the change in geometry of the suppositories dosage forms on melting, since n is affected by the change in shape of the product.

It was observed that as the suppositories melt, they acquire the shape of the base of the basket in which they are placed during dissolution testing. These suppositories changed from a cone-like shape, in the solid state, to a flattened circular-disk shape after melting.

The kinetic constants (k) calculated are summarized in Table 4.14. Since the Korsmeyer-Peppas kinetic constant incorporates the structural and geometric characteristics of dosage forms, the

change in matrix geometry, as implicated by the change in n value, affected the k value directly. It is clear that there is a direct relationship between the total percentage CFA released and the kinetic rate constant in batches F01, F02, and F03, where SLS surfactant was added to formulations F02, and F03. However after the addition of Lanolin anhydrous and Lecithin S to the formulations containing the SLS and Tween 85, it was clear that there is no relationship between the total percentage CFA released and the kinetic rate constant. This implies that the melting rate of the formulations affected the structural and geometric characteristics of the suppository formulations, which in turn affects the resultant n and k values.

Table 4.14 Summary of Korsemeyer-Peppas best-fit parameters

Batch NO.	**n**	**Kp**	**R^2Adjusted**
F01	0.6	0.348	0.9890
F02	0.297	1.451	0.9963
F03	0.297	3.895	0.9921
F06	0.13	43.98	0.9711
F07	0.584	2.446	0.978
F10	0.379	10.508	0.9968
F11	0.323	14.4	0.9757
F14	0.48	6.98	0.9773
F15	0.361	15.5	0.8842
F16	0.541	4.14	0.9933

4.3.2 Application of other mathematical models:

To establish the kinetics of drug release in a more comprehensive way, dissolution data generated during the study were fitted to various drug release kinetic models, including the Higuchi, Zero order, First order and Weibull models.

The selection criterion for the best-fit model was based on the adjusted coefficient of determination, R^2 adjusted. The R^2 adjusted value was used to compare the results of fitting data to kinetic models with different numbers of parameters. The results of fitting the dissolution data to selected mathematical models are summarized in Table 4.15

When comparing the results of model fitting using the R^2 adjusted selection criteria, the Weibull model was found as the model that best fitted the dissolution data for CFA release from suppository formulations. When model fitting was conducted R^2 adjusted values for these studies

ranged between 0.9830 and 0.9970. This result is in agreement with the nature of drug release from lipophilic suppository formulations which is often accompanied by long-lasting lag phase, that occurs as a result of the need for the base to melt prior to drug release and therefore the melting rate of the base is a factor that contributes to the lag time. The values of α, which is considered as the interval necessary for the process to reach 63.2% of the drug present in the product to be dissolved or to be released, was observed to be proportional to the percentage release of the different batches in the study.

None of the formulations was observed to fit the Zero Order model. This indicates that the release from all formulations is concentration dependent and hence the results came in agreement with the lipophilic nature of the drug substance and the suppository base which are not soluble in the aqueous media.

Three formulations (F14, F15, and F16) which contain Tween 85, Lanoline and Lecithin as surfactant and solubilisers were observed fitting the First Order model. The result could be a consequence to the presence of the surfactant and the solubilisers which helped in making emulsion during the dissolution testing.

Six batches were observed fitting Higuchi model (F01, F02, F07, F10, F14, F16) indicating that diffusion was the predominant factor that controlled the CFA release from these bathes. The K_H values were observed to be a function of the surfactant type and percentage and also to the type of solubilisers used. The highest value was observed for the bathes that contained Tween 85.

Table 4.15 Results of model parameters obtained following fitting CFA dissolution data:

Model Type	**Zero Order**		**First Order**		**Higuchi Model**		**Weibull Model**			
Parameter	**R^2 Adj**	K_0	**R^2 Adj**	K_1	**R^2 Adj**	K_H	**R^2 Adj**	**α**	**β**	**T_i**
Formula										
F01	0.846	0.05	0.859	0.001	0.977	0.552	0.986	298.3	0.614	-0.001
F02	0.3399	0.071	0.4465	0.001	0.9610	0.822	0.9961	62.97	0.369	9.282
F03	0.0977	0.131	0.2119	0.001	0.8800	1.549	0.9966	18.67	0.254	9.282
F06	-0.865	0.668	0.828	0.038	0.4678	8.284	0.997	1.113	0.135	14.85
F07	0.8109	0.322	0.9298	0.005	0.9711	3.609	0.9886	32.638	0.605	8.825
F10	0.4129	0.519	0.8542	0.011	0.9635	6.045	0.9965	14.589	0.572	14.589
F11	0.204	0.545	0.793	0.013	0.899	6.423	0.983	5.31	0.383	10.04
F14	0.551	0.559	0.965	0.012	0.980	6.382	0.985	24.16	0.719	3.69
F15	0.267	0.670	0.976	0.025	0.858	8.210	0.993	5.79	0.558	13.13
F16	0.444	0.770	0.952	0.008	0.992	5.000	0.995	35.7	0.711	2.276

4.4 Stability studies of selected formulations

The stability data for the selected batches (F01, F07, F11, F14, F15, and F16) are illustrated in Tables (3.16-3.21). The rest of formulations (F04, F05, F08, F09, F12, and F13) which contain Tween 20 as a surfactant were excluded from the study as they were out of specifications at initial time. The following discussion is a summary of the stability study observations:

Formulation batch no.F01:

- An increase in the disintegration time (10-18 min) which could be a consequence to the hardening effect in storage.
- An assay decrease (≈5%) at the storage conditions (30°C / 60%RH), and a slight increase in the degradation materials (free cefuroxime and the Δ^3 isomer) quantities at both storage conditions (25°C / 60%RH), and (30°C / 60%RH) as a consequence of the temperature and humidity effects on CFA which is heat and humidity sensitive.

Formulation batch no. F07 and F11:

- A slight change in colour and sediments were observed at 30°C during the three months storage period, and the third month at 25°C. This could be due to polymorphism formation or increased crystallinity of the active ingredient.
- The suppositories were softened during the disintegration time testing and didn't disintegrate without pressing on them. This could be due to the hardening, polymorphism or increased crystallinity of the suppository base and the active ingredient.
- A slight increase in the degradation materials (free cefuroxime and the Δ^3 isomer) quantities at (30°C / 60%RH) as a consequence of the temperature and humidity effects on CFA which is heat and humidity sensitive.
- A significant decrease in the dissolution release rate at 30°C and slight decrease at 25°C due to the hardening and polymorphism effects.

Formulation batch no. F14, F15 and F16:

- A clear change in colour and sediments were observed at 30°C during the three months storage period.
- A significant decrease (more than 5%) in the CFA assay at the 25 & 30°C storage conditions.
- A significant increase in degradation materials quantities (free cefuroxime and the Δ^3 isomer) at 25 & 30°C storage conditions as a consequence of the temperature and humidity effects on CFA which is heat and humidity sensitive and also the presence of Tween 85 increased this effect.
- A significant decrease in the dissolution release rate at 25 & 30°C was observed in batch no. F14 due to the hardening and polymorphism effects. However, the decrease was very minor in batches F15 and F16 at 25°C, which could be due to the presence of lanoline and lecithin in the two bathes.

Table 4.16 Stability results, batch no. F01

B.N. F-01	Zero Time	25°C/60% RH			30°C/60% RH			2 - 8°C		
Time/month Test		1st	2nd month	3rd month	1st	2nd month	3rd month	1st	2nd month	3rd month
Description	White, smooth, no sediments.	No change	No change	No change	No change	No change	No change	No change	No change	No change
Av. Wt/gm	1.234	1.233	1.251	1.280	1.282	1.27	1.159	1.215	1.195	1.180
M.P (°C)	37 – 39	37.1 – 38	36.7-38	37-38	37.2 – 39	36.7-38	38-38.7	36.5-37.5	37-38	37.5-38.2
Disintegration	10 min	14 min	11 min	13 min	13 min	12 min	13 min	18 min	10 min	11 min
% Assay	100.67%	103%	100.8	99	94	95	101	99.7	98	98
Degradation:										
% Free Cefuroxime	0.455%	0.298	0.42	0.59	0.333	0.69	0.71	0.364	0.18	0.39
% Delta Isomer	0.84%	1.106	1.28	1.26	1.02	1.655	1.67	0.807	0.69	0.57
% E1 Enantiomer	N.D	0.064	N.D	N.D	0.071	N.D	N.D	0.089	N.D	N.D
% E2 Enantiomer	N.D	0.044	N.D	N.D	0.049	N.D	N.D	0.057	N.D	N.D
Dissolution:	% Release									
15 min	2	2	2	3	2	1	2	4	3	3
30 min	3	2	3	3	2	2	3	6	6	3
45 min	3	3	4	8	1	2	4	7	8	4
60 min	4	4	6	9	1	2	5	8	10	9
120 min	6	10	6	11	3	4	7	10	11	11
180 min	8	15	8	12	5	5	9	14	13	12

Table 4.17 Stability results, batch no. F07

B.N. F-07	**Zero Time**	**25°C/60% RH**			**30°C/60% RH**			**2 - 8°C**		
Time/month / **Test**		**1st month**	**2nd month**	**3rd month**	**1st month**	**2nd month**	**3rd month**	**1st month**	**2nd month**	**3rd month**
Description	White, smooth, no sediments.	No change	No change	Creamy-white, little sediment	Creamy-white, little sediment	Creamy ,with sediments	Creamy, with sediments	No change	No change	No change
Av. Wt	1.246	1.255	1.189	1.123	1.277	1.246	1.214	1.245	1.265	1.315
M.P/°C	37-37.5	36.9-37.2	36.6-37.8	37-37.7	37.6-38.2	38-38.5	38.7-39	37.5-38	36.9	37.4
Disintegration time/min	10	10	8	10	10*	10*	13*	11	7	10
% Assay	103.6	101.6	98	97	102	95	98	100	99.8	97
Degradation:										
% Free Cefuroxime	0.747	0.454	0.69	0.90	0.627	1.03	1.34	0.479	0.46	0.59
% Delta Isomer	0.952	1.14	1.32	1.41	1.96	2.39	2.56	0.888	0.75	0.70
% E1 Enantiomer	N.D**	0.092	N.D	N.D	0.099	N.D	N.D	0.097	N.D	N.D
% E2 Enantiomer	N.D	0.056	N.D	N.D	0.069	N.D	N.D	0.057	N.D	N.D
% Impurities unidentified	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Dissolution:										
15 min	9	10	10	9	1	3	1	21	15	16
30 min	18	15	13	14	1	4	2	30	21	24
45 min	22	22	16	15	3	4	2	34	25	28
60 min	28	25	18	19	3	5	2	41	30	32
120 min	44	48	19	21	9	6	3	47	38	38
180 min	48	52	21	23	12	7	4	50	40	38

*Suppositories softened only.

Table 4.18 Stability results, batch no. F11

B.N. F-11	Zero Time	25°C/60% RH			30°C/60% RH			2 - 8°C		
Time/month / Test		1st	2nd month	3rd month	1st	2nd month	3rd month	1st	2nd month	3rd month
Description	White, smooth, no sediments.	No change	No change	No change	No change	No change	Creamy-white, little sediment	No change	No change	No change
Av. Wt	1.197	1.237	1.242	1.293	1.24	1.180	1.245	1.23	1.253	1.277
M.P/°C	37-37.5	37.3-37.8	36.9-37.6	37.1-37.9	36.8-37.2	37-37.7	38-38.5	36.8-37.5	37.1-37.8	37.4-38
Disintegration time/min	10	10	10	10	7	7	11	10	7	10
% Assay	100.5	100.8	97	99.3	100	96	97.5	101	99.5	100
Degradation:										
% Free Cefuroxime	0.903	0.699	1.0616	1.14	0.667	1.13	1.58	0.627	0.59	0.87
% Delta Isomer	1.00	1.44	1.208	1.06	0.944	1.83	2.16	0.828	0.89	0.77
% E1 Enantiomer	0.101	0.100	N.D	N.D	0.099	N.D	N.D	0.117	N.D	N.D
% E2 Enantiomer	0.072	0.069	N.D	N.D	0.055	N.D	N.D	0.066	N.D	N.D
% Impurities unidentified	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Dissolution:										
15 min	29	25	15	17	23	8	5	17	26	25
30 min	46	34	29	26	31	18	5	30	38	40
45 min	53	37	36	33	34	23	6	40	43	45
60 min	56	40	41	35	36	27	8	43	47	52
120 min	63	47	50	40	41	33	12	53	56	59
180 min	68	50	56	44	43	39	15	57	62	62

Table 4.19 Stability results, batch no. F14

B.N. F-14	Zero Time	25°C/60% RH			30°C/60% RH			2 - 8°C		
Time/month / Test		1st	2nd month	3rd month	1st	2nd month	3rd month	1st	2nd month	3rd month
Description	White, smooth, no sediments.	No change	No change	No change	Creamy-white, little sediment	Creamy, with sediments	Creamy with sediments	No change	No change	No change
Av. Wt	1.236	1.247	1.219	1.226	1.229	1.268	1.275	1.225	1.233	1.250
M.P/°C	36.5-37.4	37-37.5	37.2-37.8	36.8-37.5	37.2-37.7	37.5-38	37.4- 38.3	37.1-37.7	37-38	37.5-38.6
Disintegration time/min	7	8	12	13	12*	14*	18*	7	10	10
% Assay	105	93	89.5	92.5	92	88.6	85	100	98	95
Degradation:										
% Free Cefuroxime	0.42	2.156	3.10	5.10	2.54	4.06	7.12	1.24	1.65	1.16
% Delta Isomer	1.96	4.25	4.03	4.46	4.94	5.25	5.91	2.37	2.29	2.47
% E1 Enantiomer	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
% E2 Enantiomer	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
% Impurities unidentified	N.D	N.D	0.41	0.695	0.32	0.50	0.83	N.D	N.D	N.D
Dissolution:										
15 min	20	4	3	2	2	1	2	16	18	12
30 min	40	9	10	17	2	2	4	34	29	30
45 min	41	13	14	20	3	2	4	51	45	42
60 min	52	16	20	21	3	2	4	75	59	62
120 min	74	41	27	24	6	3	5	83	77	78
180 min	81	54	31	26	7	5	8	85	81	83

*Suppositories softened only.

Table 4.20 Stability results, batch no. F15

B.N. F-15	Zero Time	25°C/60% RH			30°C/60% RH			2 - 8°C		
Time/month / Test		1st	2nd month	3rd month	1st	2nd month	3rd month	1st	2nd month	3rd month
Description	Creamy, smooth, no sediments.	No change	No change	No change	Creamy to brown with sediments	Creamy with sediments	Creamy with sediments	No change	No change	No change
Av. Wt	1.297	1.250	1.226	1.259	1.237	1.207	1.229	1.245	1.219	1.219
M.P/°C	36.8-37.6	36.7-37.3	36.7-37.5	37.9 38.2	36.7-37.5	37.5-38	38.1-38.6	37.1-38	37.8-38.2	38.2- 38.5
Disintegration time/min	7	8	12	10	11*	11*	13*	7	10	9
% Assay	101	87	88	87.8	86.5	85	90.6	100.5	97.7	95.5
Degradation:										
% Free Cefuroxime	0.495	2.5	3.77	5.77	2.96	5.24	7.67	1.69	2.12	1.22
% Delta Isomer	2.18	3.93	4.20	5.10	5.27	5.77	6.1	2.51	2.12	2.61
% E1 Enantiomer	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
% E2 Enantiomer	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
% Impurities unidentified	N.D	0.30	0.44	0.67	0.44	0.64	0.90	0.17	0.18	N.D
Dissolution:										
15 min	43	17	24	10	3	16	3	21	21	19
30 min	56	40	31	22	4	8	4	52	40	71
45 min	78	66	38	23	5	6	4	79	68	75
60 min	83	78	54	25	6	3	4	91	80	80
120 min	91	95	81	74	12	8	6	99	93	91
180 min	91	94	92	92	17	9	8	97	95	95

*Suppositories softened only.

Table 4.21 Stability results, batch no. F16

B.N. F-16	Zero Time	25°C/60% RH			30°C/60% RH			2 - 8°C		
Time/month / Test		1st	2nd month	3rd month	1st	2nd month	3rd month	1st	2nd month	3rd month
Description	Faint yellow, smooth, no sediments.	Faint brown, smooth, no sediments.	Faint brown, smooth, no sediments.	Faint brown, smooth, no sediments.	Faint brown, smooth, with sediments.	Faint brown, smooth with sediments.	Faint brown smooth with sediments.	No change	No change	No change
Av. Wt	1.230	1.243	1.234	1.237	1.260	1.232	1.233	1.246	1.214	1.197
M.P/°C	36-36.5	35.8-36.7	36.3-37	35.7-36.5	37.3-37.7	37.5-38	37.5-38.2	36-37.2	36.3-37.3	36-37.1
Disintegration time/min	7	7	10	10	10	13	12	6	10	9
% Assay	100.6	91	83	90	88	86	84	98.8	98.7	95
Degradation:										
% Free Cefuroxime	0.598	3.4	5.26	7.01	4.2	6.93	10.06	1.94	2.37	2.84
% Delta Isomer	2.3	4.6	4.63	5.45	5.9	6.15	6.85	2.47	1.87	2.44
% E1 Enantiomer	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
% E2 Enantiomer	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
% Impurities unidentified	N.D	0.42	0.73	0.76	0.60	0.82	1.09	0.21	0.19	0.24
Dissolution:										
15 min	15	8	5	7	10	4	5	24	10	8
30 min	27	15	14	12	14	5	6	24	13	12
45 min	35	22	26	16	21	4	8	29	19	17
60 min	37	30	36	21	18	9	12	37	24	29
120 min	56	54	39	36	40	12	8	49	41	48
180 min	68	61	54	47	47	15	16	65	63	61

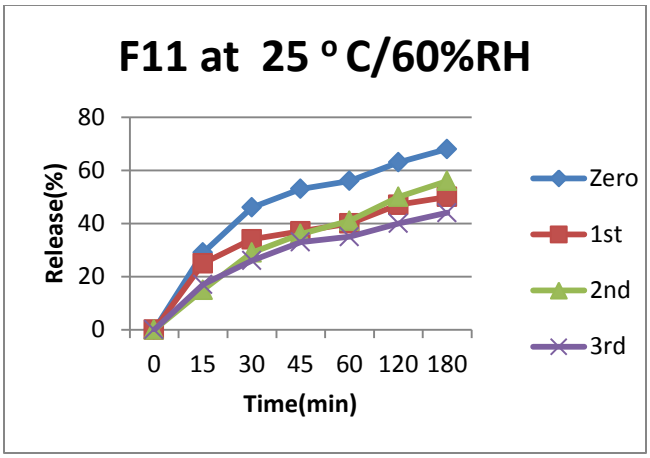


Figure 4.8 Comparative dissolution profiles for batch F11 stored at 25°C for 3 months

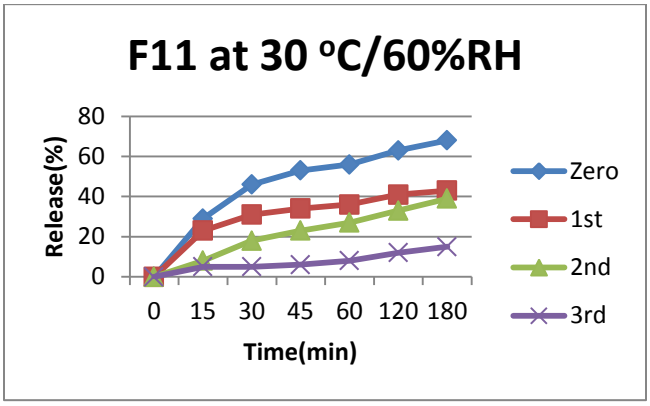


Figure 4.9 Comparative dissolution profiles of batch F11stored at 30°C for 3 months

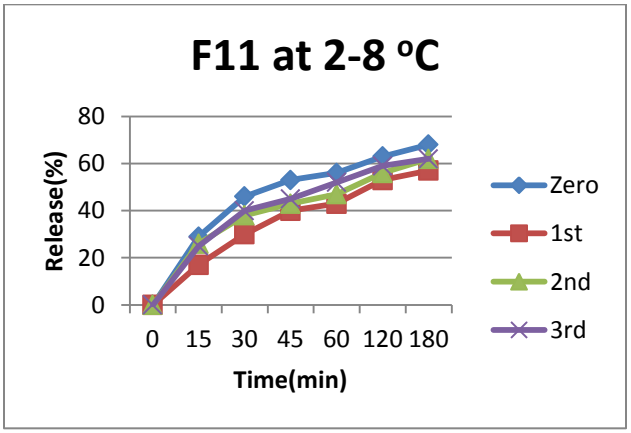


Figure 4.10 Comparative dissolution profiles of batch F11 stored at 2-8°C for 3 months

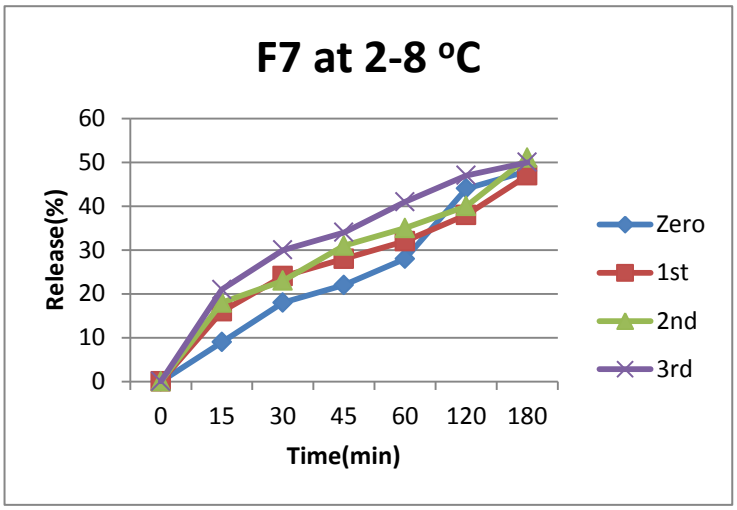


Figure 4.11 Comparative dissolution profiles of batch F7 stored at 2-8°C for 3 months

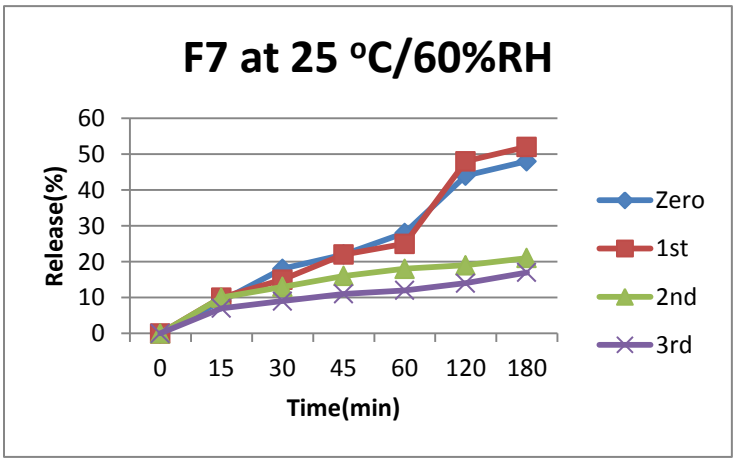


Figure 4.12 Comparative dissolution profiles of batch F7 stored at 25°C for 3 months

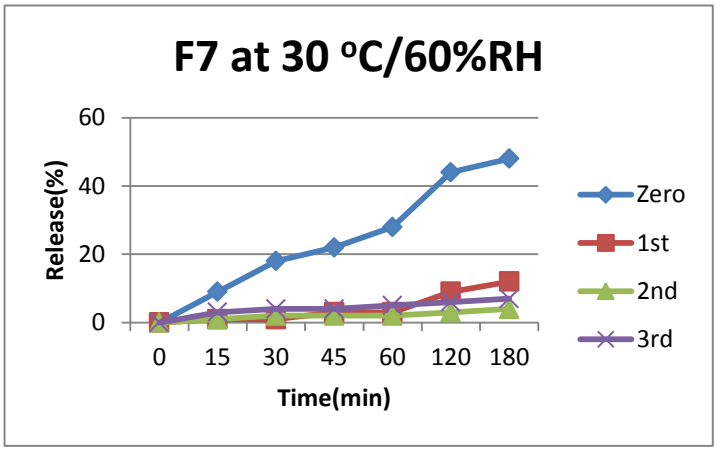


Figure 4.13 Comparative dissolution profiles of batch F7 stored at 30°C for 3 months

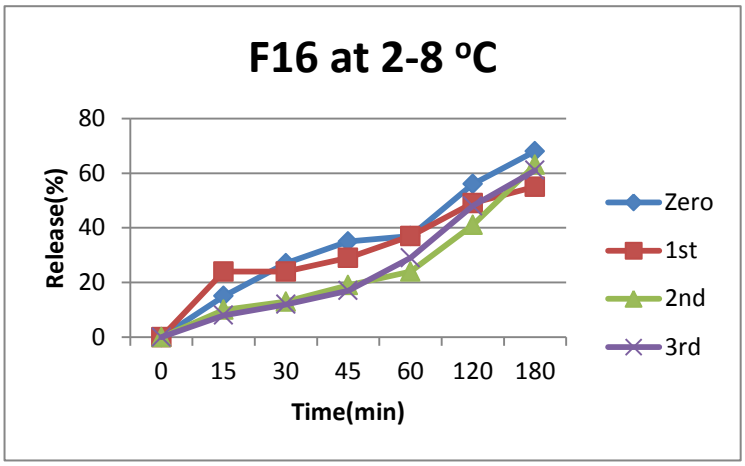


Figure 4.14 Comparative dissolution profiles of batch F16 stored at 2-8°C for 3 months

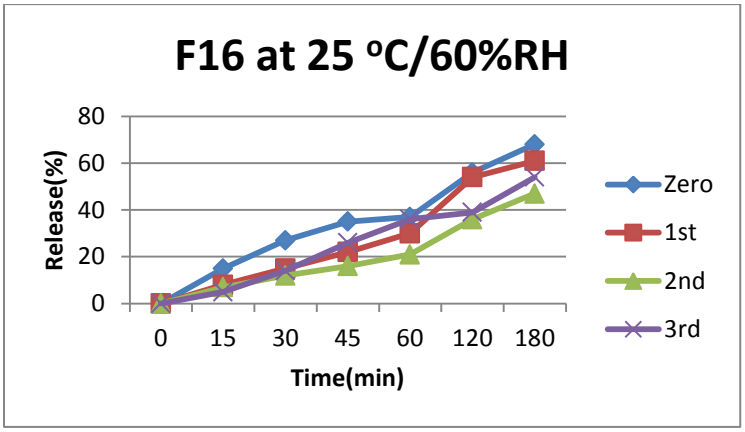


Figure 4.15 Comparative dissolution profiles of batch F16 stored at 25°C for 3 months

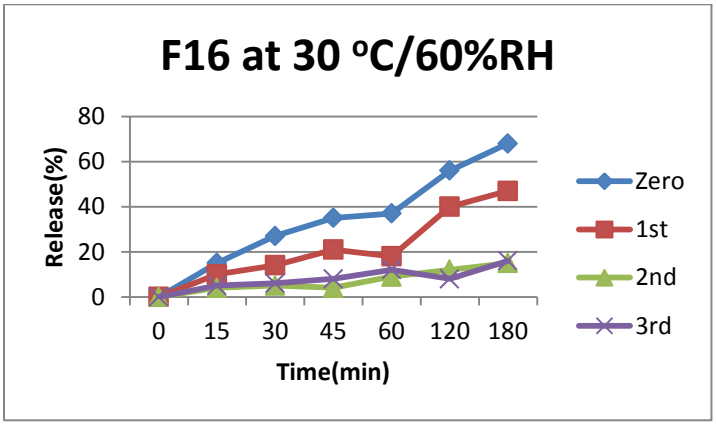


Figure 4.16 Comparative dissolution profiles of batch F16 stored at 30°C for 3 months

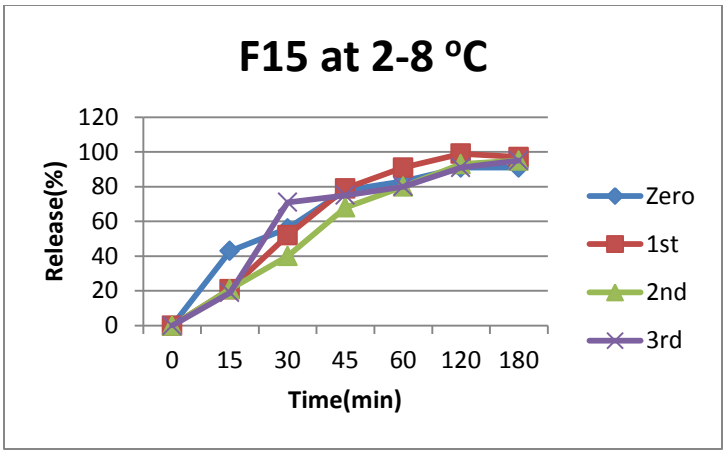


Figure 4.17 Comparative dissolution profiles of batch F15 stored at 2-8°C for 3 months

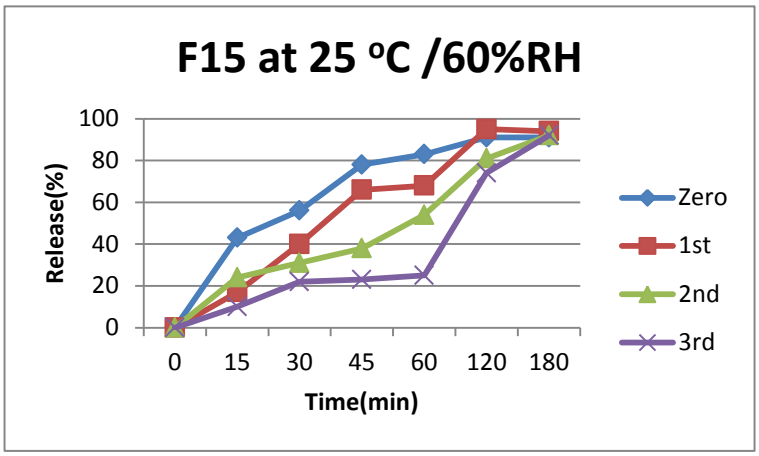


Figure 4.18 Comparative dissolution profiles of batch F15 stored at 25°C for 3 months

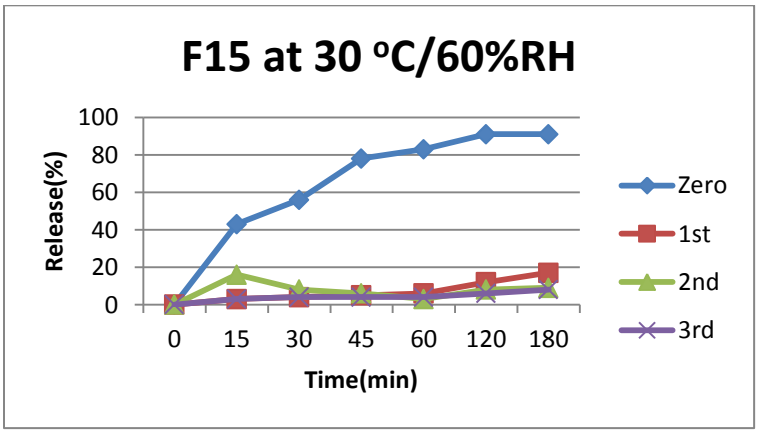


Figure 4.19 Comparative dissolution profiles of batch F15 stored at 30°C for 3 months

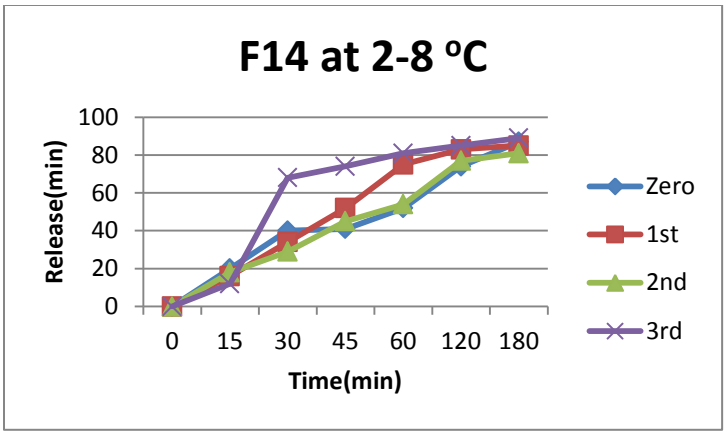


Figure 4.20 Comparative dissolution profiles of batch F14 stored at 2-8^{o}C for 3 months

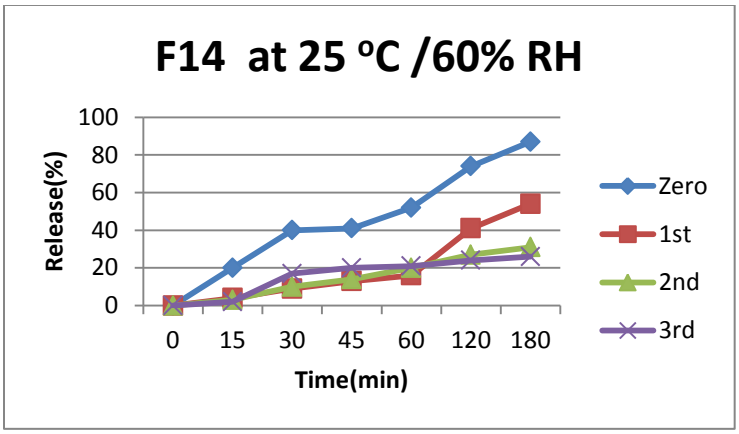


Figure 4.21 Comparative dissolution profiles of batch F14 stored at 25^{o}C for 3 months

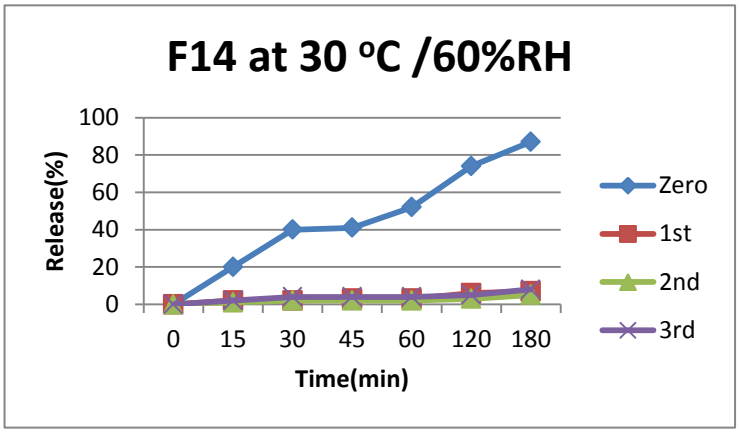


Figure 4.22 Comparative dissolution profiles of batch F14 stored at 30^{o}C for 3 months

4.5 Analytical Method validation Results

4.5.1 Assay method validation:

The method was validated according to USP category I and the ICH Q2 (R1) guidelines for the quantitation of drug substance in dosage forms. As the guidelines require; the accuracy, precision, specificity, linearity and range are assessed in order to ensure that the method is reliable. In addition the limit of quantitation (LOQ) and limit of detection (LOD) were also determined. The stability of the CFA in an aqueous solution was also determined. The results are clarified in the following tables and figures.

4.5.1.1 Linearity

Linearity was assessed by analyzing ten standard sample solutions of different concentrations. The calibration curve was plotted in order to establish whether a correlation between response and analyte concentration existed. A typical calibration curve obtained for these studies is depicted in Figure 4.23. The linearity of the method was established from the correlation coefficient (R^2) of the best fit least squares linear regression curve, which was obtained by plotting peak areas versus known CFA concentrations. For these studies, an R^2 value of > 0.990 was considered appropriate to demonstrate the linearity of the analytical method. The calibration curve was found to be linear over the concentration range stated, with an R^2 of 0.9999 and the equation for the line of $y = 4544.3x + 4347.1$.

Table 4.22 linearity results of HPLC assay method validation

Conc. %	St. Conc. mg/ml	Peak Area 1	Peak Area 2	Peak Area 3	Average	%RSD
10%	0.025	2366472	2353809	2364787	2361689.33	0.29
20%	0.05	4690796	4688138	4658794	4679242.66	0.38
30%	0.075	6783518	6820667	6849720	6817968.33	0.49
40%	0.1	9005470	9035022	9050428	9030306.67	0.25
60%	0.15	13253757	13270062	13303616	13275811.67	0.19
80%	0.2	17957105	17824477	18137927	17973169.67	0.88
100%	0.25	22468536	22503691	22547026	22506417.66	0.17
120%	0.3	27135946	26984281	27086790	27069005.66	0.29
140%	0.35	31665006	31532767	31775682	31657818.33	0.38
160%	0.4	35807222	36016087	35872141	35898483.33	0.30

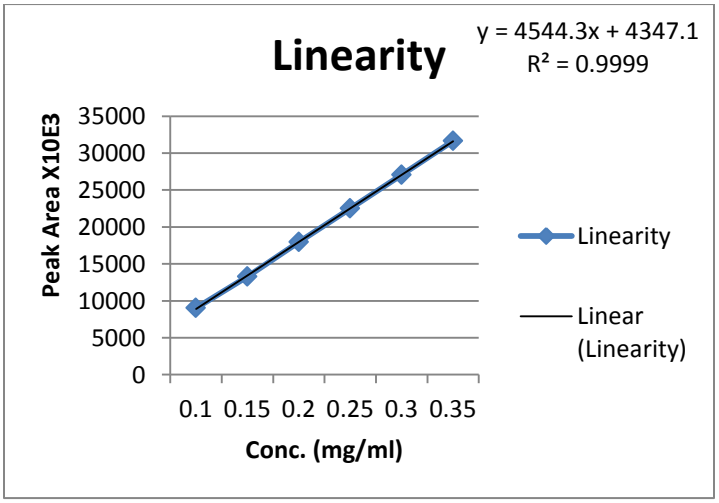


Figure 4.23 Linearity graph for HPLC method validation

4.5.1.2 Accuracy

The percentage CFA recovered from spiked placebo samples for three concentrations, i.e. 80, 100, 120 % respectively was calculated in addition to the % RSD of the three readings of each spiked sample. An acceptance criteria for accuracy was considered to be % RSD<2.0% and a recovery of 100±2.0%.

The results obtained are clarified in table 4.23 and figure 4.24. The resultant values for % RSD and recovery satisfied the criteria and the plot of peak areas vs. concentration were found linear with an R^2 value of 0.9991.

Table 4.23 Accuracy results of HPLC method validation

No. of injection	Target Conc. **(%)**	**Theo. Conc. (mg / ml)**	**Conc. after Spiking (mg/ml)**	**Spiked Sample Response**	**Recovery (%)**	**Mean (%)**	**RSD (%)**
1.1	80%	0.2	0.2005	18026135	100.25	100.33	0.06
1.2			0.2008	18047464	100.4		
1.3			0.2007	18037957	100.35		
2.1	100%	0.25	0.247	22216157	98.8	99.2	0.41
2.2			0.249	22390118	99.6		
2.3			0.248	22348501	99.2		
3.1	120%	0.3	0.3007	27135946	100.2	99.99	0.29
3.2			0.2991	26984281	99.7		
3.3			0.3002	27086790	100.06		

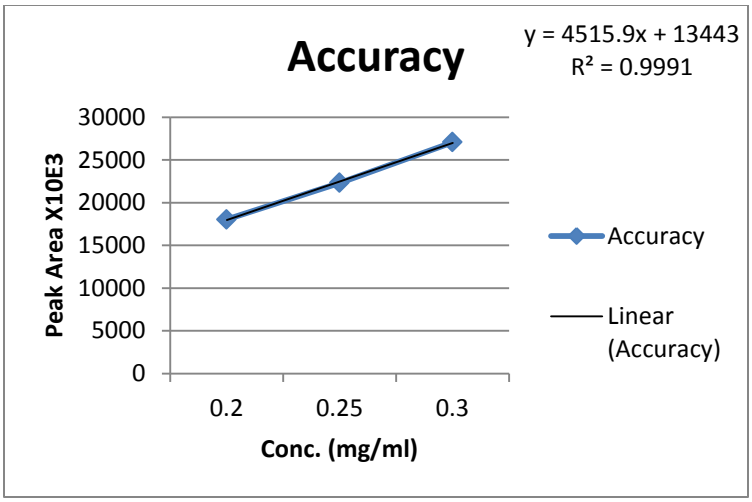


Figure 4.24 HPLC method validation accuracy regression line

4.4.1.3 Precision:

The precision is the ability of a method to produce precise analytical results from a series of measurements of the same homogenous sample under prescribed assay conditions. The standard deviation (SD) or percentage relative standard deviation (% RSD) of a series of measurements is usually used to assess the precision of an analytical method. The % RSD is calculated using the following equation.

$$RSD = \frac{\sigma}{X}$$

Where,

σ = Standard deviation around the mean of a set number of samples (calculated using nonbiased or n-1 method)

X = Mean of the peak height ratio responses for a set number of samples

The precision of the method was considered at two levels, repeatability and intermediate precision. A value for % RSD of < 1.5% was set as an acceptable limit

Repeatability:

The repeatability was determined by the analysis of six determinations at 100% of the test concentration. The repeatability results obtained are shown in Table 4.24.The results reveal that % RSD values were within the acceptable limits thus the method is repeatable for the analysis of CFA suppositories.

Intermediate precision:

Intermediate precision or inter-day variability expresses the within laboratory variation. The accuracy testing was repeated by different analyst and using different instrument. The results of these studies are listed in Tables 4.25 and 4.26. The results show that all % RSD values fell below 1.5%, which is within the limits and therefore the method is precise.

Table 4.24 Repeatability results of HPLC method validation

	N	Concentration (mg/ ml)	Response	Average Response	SD of Response	% RSD
Standard	1	0.25	32494885	318687559	422009	1.3
	2		32089680			
	3		31988964			
	4		31707647			
	5		31827885			
	6		31103493			
Sample	1	0.25	32674912	33238609	404851	1.2
	2		32776548			
	3		33188534			
	4		33666096			
	5		33394346			
	6		33731217			

Table 4.25 Intermediate precision results using Elite HPLC Instrument

No. of injection	Target Conc. **(%)**	**Theo. Conc. (mg / ml)**	**Conc. after Spiking (mg/ml)**	**Spiked Sample Response**	**Recovery (%)**	**Mean (%)**	**RSD (%)**
1.1	80%	0.2	0.2005	18026135	100.25	100.33	0.06
1.2			0.2008	18047464	100.4		
1.3			0.2007	18037957	100.35		
2.1	100%	0.25	0.247	22216157	98.8	99.2	0.41
2.2			0.249	22390118	99.6		
2.3			0.248	22348501	99.2		
3.1	120%	0.3	0.3007	27135946	100.2	99.99	0.29
3.2			0.2991	26984281	99.7		
3.3			0.3002	27086790	100.06		

Table 4.26 Intermediate precision results using Ultimate 3000 HPLC Instrument

No. of injection	Target Conc. **(%)**	**Theo. Conc. (mg / ml)**	**Conc. after Spiking (mg/ml)**	**Spiked Sample Response**	**Recovery (%)**	**Mean (%)**	**RSD (%)**
1.1	80%	0.2	0.202	90.88	101	101.17	0.283
1.2			0.203	91.51	101.5		
1.3			0.202	91.12	101		
2.1	100%	0.25	0.254	114.00	101.6	101.3	0.228
2.2			0.253	113.91	101.2		
2.3			0.253	113.89	101.2		
3.1	120%	0.3	0.303	134.74	101	101.8	0.839
3.2			0.305	136.00	101.7		
3.3			0.308	136.97	102.7		

4.5.1.4 Specificity / Selectivity Test:

It is a measure of the degree of interference from materials other than active material, such as excipients, impurities, and degradation products. It should be ensuring that the peak response is due to a single component only. To validate for specificity, the interference from excipients and the Interference with degradants were determined.

The interference with excipients was determined by finding the response of the excipients alone, the response of the active material (100%) alone and the response of a spiked sample, and then the percentage recovery was calculated. From the results in table 4.25, it is observed that there is no interference with the excipients. The interference with degradation products was determined by performing forced degradation studies on solutions containing CFA (0.25 mg / ml), and the resolution and % recovery of CFA were determined. The results are summarized in table 4.28,

from which the resolutions of all degradation products were found to be more than the minimum accepted limits (> 1.5). The results of the forced degradation studies indicate that the method has a high degree of selectivity for the determination of CFA in the presence of degradation products.

Following exposure of CFA API to basic conditions, a golden yellow solution resulted when compared to the colorless control solution. The resultant chromatogram following exposure of CFA API to basic conditions is depicted in Figure 4.26. It is evident that the degradation of CFA API is significant with four degradation products under basic conditions. One of the major degradation products reported to be found after base degradation of CFA is the free cefuroxime, which is known to be produced after a hydrolysis process.

CFA was found to be relatively stable when stored under Acidic conditions at room temperature. When CFA was treated with a 0.5 M HCl solution, no degradation products were observed after three hours storage.

Following exposure of CFA to a solution of 10% v/v H_2O_2 at ambient temperature, it was evident as shown in figure 4.27 that the degradation of CFA is significant. Four degradation products were observed; from the peaks of these products the free cefuroxime product was identified.

The exposure of CFA solution to heat at 60^{o}C for a period of one hour lead to the appearance of four degradation products; free cefuroxime, Δ^3Isomer, and unidentified two products (degradants 1 and 2) as shown in figure 4.30.

The exposure of CFA solution to U.V light for a period of twenty hours lead to the appearance of four degradation products; free cefuroxime, Δ^3Isomer, and cefuroxime E_1 & E_2 Enantiomer as shown in figure 4.29.

The CFA solution was kept in the refrigerator (2-8^{o}C) for a period of 24 hours and it was observed that the quantities of free cefuroxime and of Δ^3Isomer were slightly increased.

The CFA solution was kept at room temperature for a period of 24 hours and it was observed that the quantities of free cefuroxime and of Δ^3Isomer were increased and the unidentified degradation product (3) appeared.

Table 4.27: No interference from excipients

	Average Response X 10^3	**Theoretical conc. (mg/ml)**	**Recovery %**	**Retention time (min.)**
Synthetic mixture	No response	0.00	0.00	N.A
Diluent	No response	0.00	0.00	N.A
100% TC Standard	22506.4	0.25	100	Isomer B 8.84 Isomer A 10.19
100% Sample	22318.3	0.25	99.2	Isomer B 9.14 Isomer A 10.61

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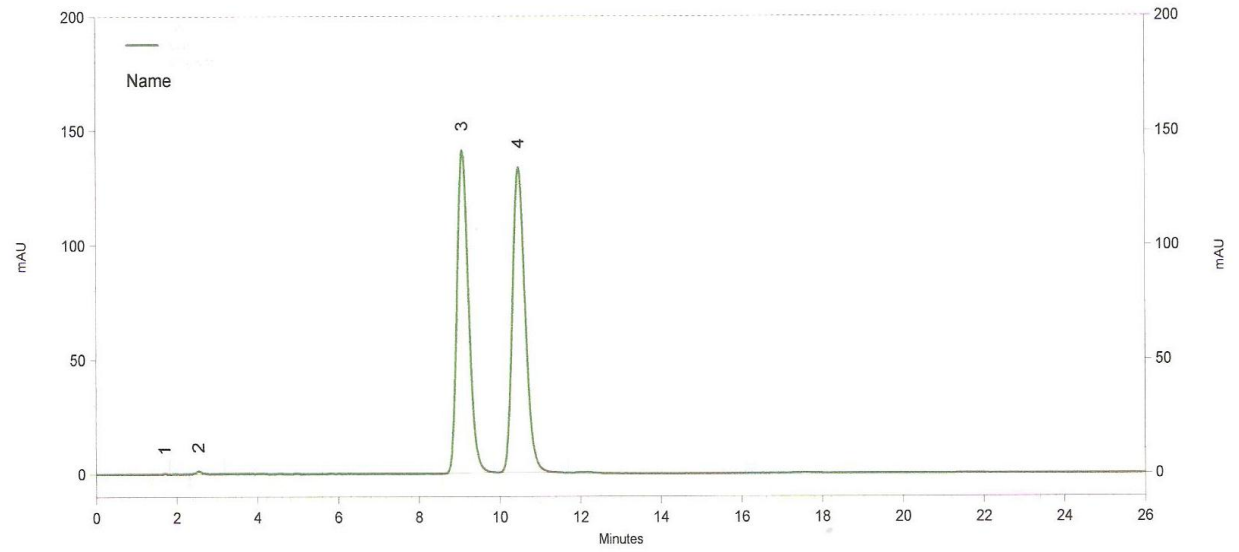


Figure 4.25: typical chromatogram obtained for CFA obtained for CFA at normal conditions. (1) unretained, (2) free cefuroxime, (3) cefuroxime axetil diastereoisomer B, (4) cefuroxime axetil diastereoisomer A.

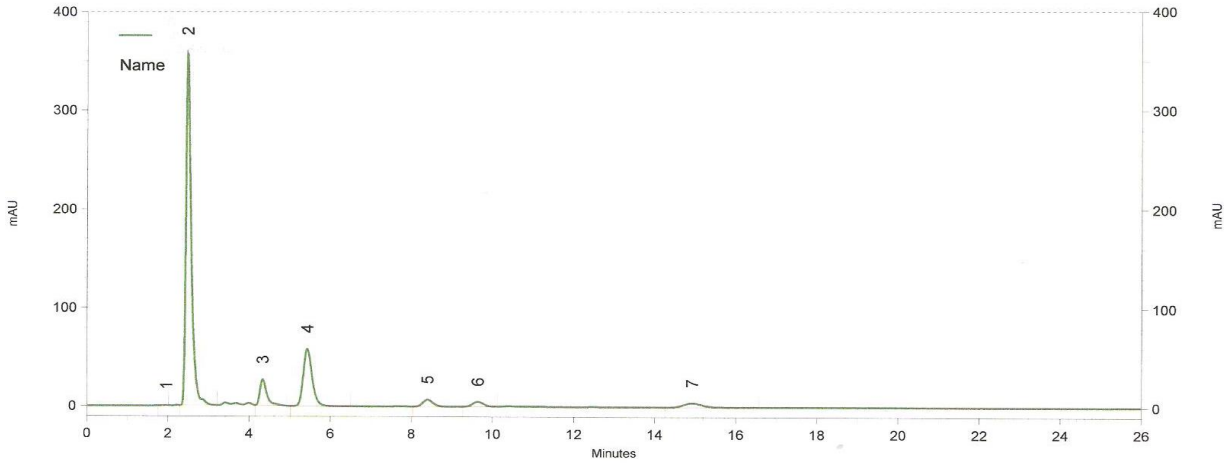


Figure 4.26 typical chromatogram obtained for CFA following base degradation. (1) unretained, (2) free cefuroxime, (3) unidentified degradant1, (4) unidentified degradant 2, (5) cefuroxime axetil diastereoisomer B, (6) cefuroxime axetil diastereoisomer A, (7) unidentified degradant 3

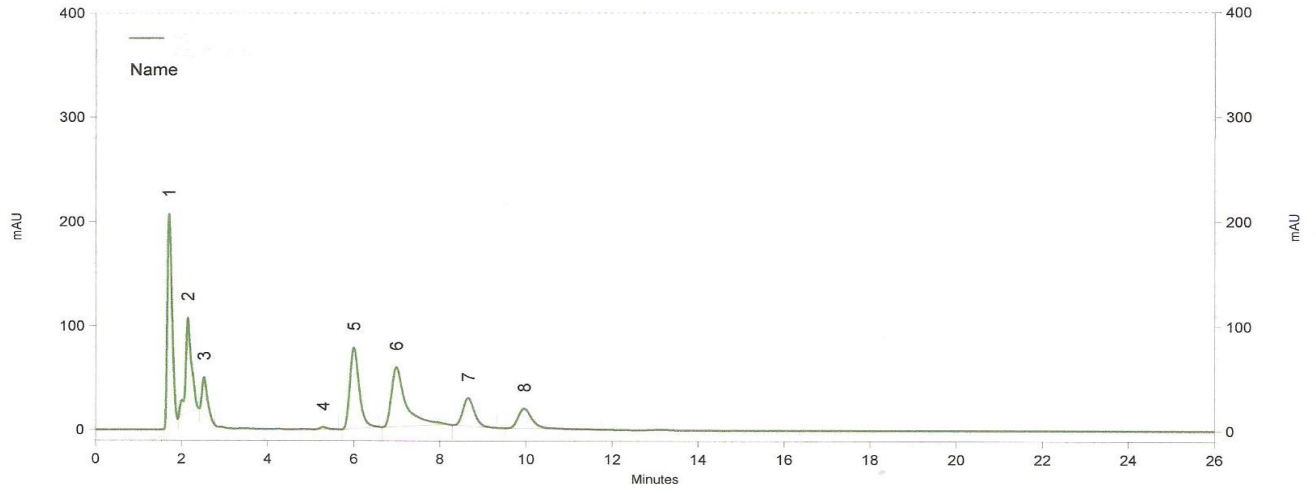


Figure 4.27: typical chromatogram obtained for CFA following exposure to 10% H_2O_2. (1) unretained , (2) unidentified degradant1, (3) free cefuroxime, (4) unidentified degradant2, (5) unidentified degradant3, (6) unidentified degradant4, (7) cefuroxime axetil diastereoisomer B, (8) cefuroxime axetil diastereoisomer A

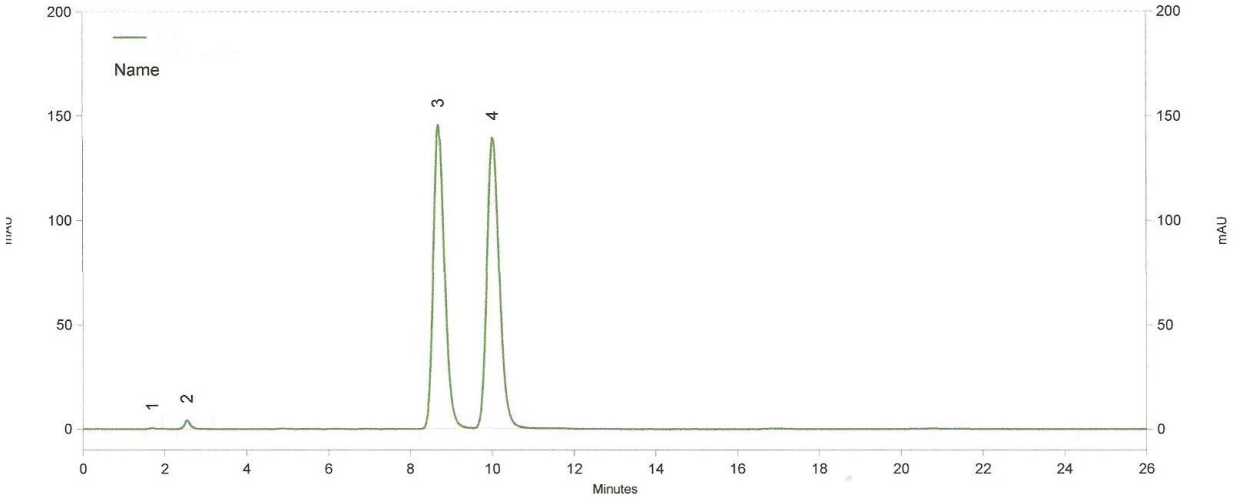


Figure 4.28: typical chromatogram obtained for CFA following exposure to 0.5 M HCl. (1) unretained, (2) free cefuroxime, (3) cefuroxime axetil diastereoisomer B, (4) cefuroxime axetil diastereoisomer A

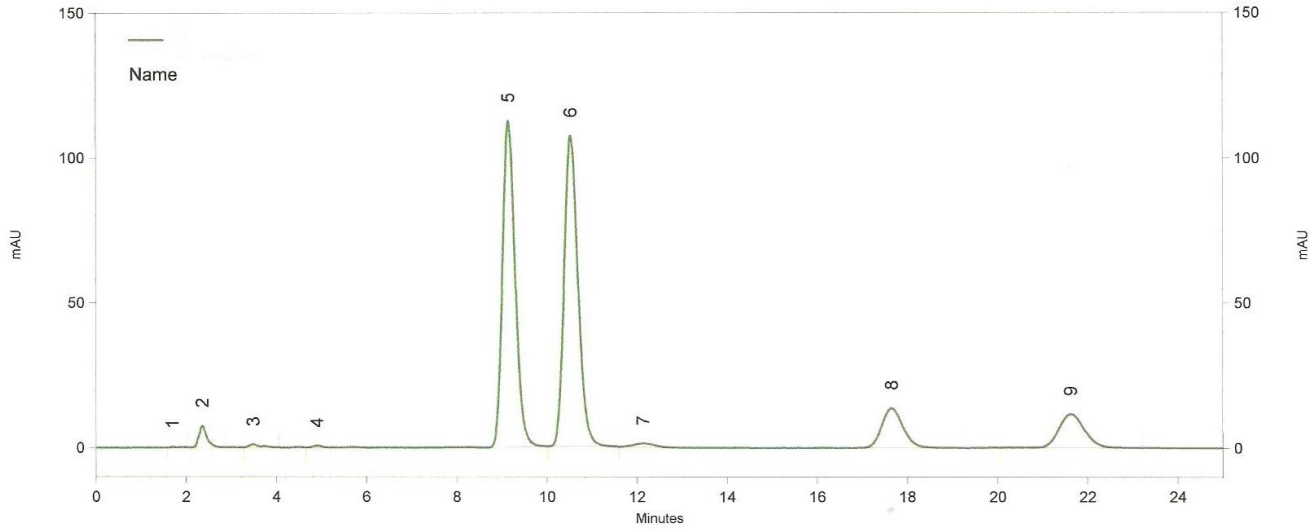


Figure 4.29: typical chromatogram obtained for CFA obtained following exposure to U.V light. (1) Unretained, (2) free cefuroxime, (3) unidentified degradant1, (4) unidentified degradant2, (5) cefuroxime axetil diastereoisomer B, (6) cefuroxime axetil diastereoisomer A, (7) cefuroxime axetil delta-3 isomer, (8) cefuroxime axetil E1 enantiomer, (9) cefuroxime axetil E2 enantiomer

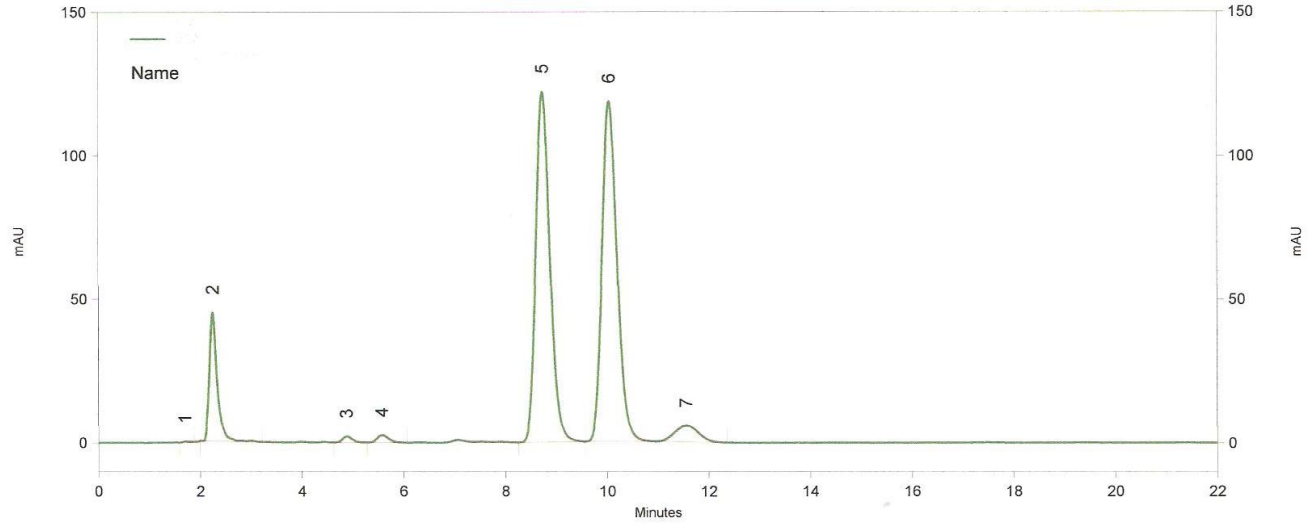


Figure 4.30: typical chromatogram obtained for CFA obtained following exposure to heat at 60^{o}C for 1 hr. (1) unretained, (2) free cefuroxime, (3) unidentified degradant1, (4) unidentified degradant2, (5) cefuroxime axetil diastereoisomer B, (6) cefuroxime axetil diastereoisomer A, (7) cefuroxime axetil delta-3 isomer.

Table 4.28: CFA Interference with degradation products

						Secondary Peaks Detected																
						Free Cefuroxime					Δ^3 Isomer					E Isomers (E_1+E_2)						
Sample	Conc. Mg/ml	% Recov.	Area A&B X10^6	# of* peaks	Res. B&A	Area X10^3	Rt	%	RRT	Res.	Area X10^3	Rt	%	RRT	Res.	Area X10^5	Rt E_1	Rt E_2	%	RRT E_1	RRT E_2	Res.
Non-stressed standard	0.25	100.00	23.01	1	2.5	50.06	2.56	0.2	0.24	4.1	ND	-	-	-	-	ND	-		-	-		-
Base	0.25	3.87	0.89	4	2.5	13057	2.5	67	0.25	2.4	ND	-	-	-	-	ND	-		-	-		-
Acid	0.25	99.60	22.92	1	2.5	ND	-	-	-	-	ND	-	-	-	-	ND	-		-	-		-
Heat	0.25	83.65	19.25	4	2.5	2040	2.24	9.2	0.22	2.1	743	11.6	3.4	1.15	2.1	ND	-		-	-		-
Light	0.25	78	18.01	4	2.5	367	2.37	1.6	0.22	2.7	144	12	0.64	1.15	2.2	37.6	17.7	21.6	16.8	1.67	2.05	4.1
Refrigerator 24 hrs.	0.25	101	23.3	2	2.5	220.8	2.4	0.93	0.22	3.8	166.4	12.2	0.7	1.15	2.2	ND	-	-	-	-	-	-
Hydrogen Peroxide	0.25	20	4.6	4	2.46	1374	2.5	8.7	0.25	3.4	ND	-	-	-	-	ND	-	-	-	-	-	-
Room temp. 24 hrs.	0.25	96	22.16	2	2.5	729.52	2.37	3.12	0.23	3.6	333	12.1	1.43	1.15	2.2	N.D	-	-	-	-	-	-

Table 4.28: CFA Interference with degradation products (continued)

	Secondary Peaks Detected																			
	Degradant 1					Degradant 2					Degradant 3					Degradant 4				
Sample	Area X10^3	Rt	%	RRT	Res.	Area X10^3	Rt	%	RRT	Res.	Area X10^3	Rt	%	RRT	Res.	Area X10^3	Rt	%	RRT	Res.
Base	1249.4	4.3	6.5	0.45	7.2	3541.7	5.42	18.3	0.563	3.16	565.6**	15	2.9	1.54	8.1	-	-	-	-	-
Acid	ND	-	-	-	-	ND	-	-	-	-	ND	-	-	-	-	ND	-	-	-	-
Heat	118	4.9	0.52	0.48	8.5	162.5	5.57	0.73	0.554	1.78	ND	-	-	-	-	ND	-	-	-	-
Light	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Refrigerator 24 hrs.	ND	-	-	-	-	ND	-	-	-	-	ND	-	-	-	-	ND	-	-	-	-
H_2O_2	*4404.4	6.9*	28*	0.7*	2.3*	ND	-	-	-	-	134	5.3	0.85	0.54	10	4521	6	28.5	0.61	1.95
Room temp. 24hrs	N.D	-	-	-	-	ND	-	-	-	-	87.74	4.99	0.37	0.48	8.9	ND	-	-	-	-

*Degradation product 5

**Degradation product 6

4.5.1.5 Limit of Detection and Limit of Quantitation:

LOD and LOQ for the analytical method were determined based on finding the Standard Deviation of the Response and the Slope and calculating the limits using the following equations:

LOD = 3.3 σ / S

LO Q = 10 σ / S

The SD was found to be 460.81 and the slope was found to be 120375000. The LOD was calculated to be 1.3 X 10^{-5} and LOQ was found to be 3.8 X 10^{-5}.

Table 4.29: Summary results of dilutions and response for LOD & LOQ determination

Concentration (mg CFA/ml)	**Average Peak Area (A & B)**	**S/N (A)**	**S/N (B)**
0.0001	35376	4.2	0.97
0.00025	29737	5.87	4.15
0.0005	82006	4.86	5.15
0.001	149019	14	15.3
0.01	1221087	117.7	124.3
SD	**460.81**		

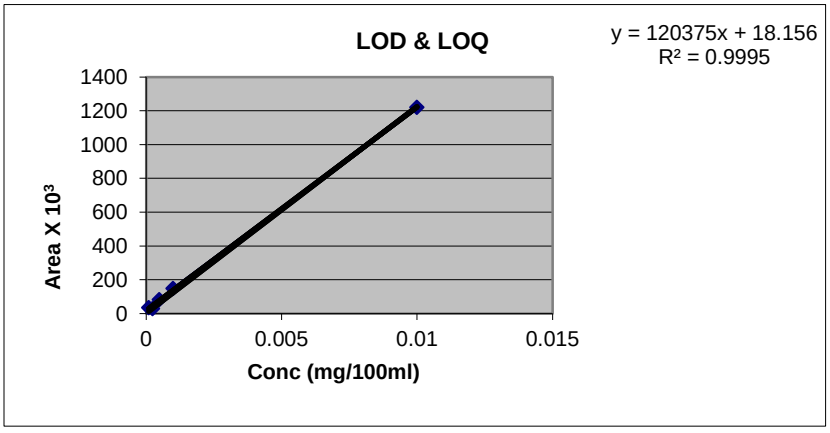


Figure 4.31: Calibration curve obtained for LOD & LOQ determination

4.5.2 Dissolution method validation

The method was validated according to USP category IV guidelines for. As the guidelines require; the accuracy, precision, specificity, and linearity are assessed in order to ensure that the method is reliable.

4.5.2.1 Linearity

Linearity was assessed by analyzing six standard sample solutions of different concentrations. The calibration curve was plotted in order to establish whether a correlation between response and analyte concentration existed. A typical calibration curve obtained for these studies is depicted in Figure 4.32. The linearity of the method was established from the correlation coefficient (R^2) of the best fit least squares linear regression curve, which was obtained by plotting peak areas versus known CFA concentrations. For these studies, an R^2 value of > 0.980 was considered appropriate to demonstrate the linearity of the analytical method. The calibration curve was found to be linear over the concentration range stated, with an R^2 of 0.9993 and the equation for the line of y = 156.7x + 2.293.

Table 4.30: Linearity results of dissolution method validation

Conc. %	St. Conc.	Absorbance	Absorbance2	Absorbance3	Average	RSD
25%	0.00347	159.1	158.8	158.7	158.8	0.11
50%	0.00694	314.9	315.2	317.3	315.8	0.34
75%	0.01041	478	480.1	475.5	477.8	0.39
100%	0.01388	629.2	629.5	631.3	630	0.15
120%	0.016656	770.6	773.7	769.9	771.4	0.21
150%	0.02082	951.5	952.1	950.7	951.4	0.06

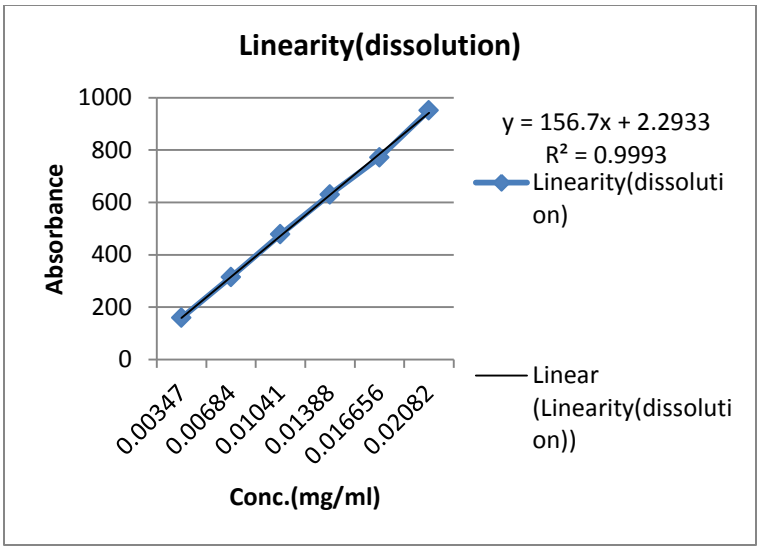


Figure 4.32: Linearity graph for dissolution method validation

4.5.2.2 Accuracy

The percentage CFA recovered from spiked placebo samples for three concentrations, i.e. 50, 100, 150 % respectively was calculated in addition to the % RSD of the three readings of each spiked sample. An acceptance criterion for accuracy was considered to be a recovery of 100±5.0% and % RSD ≤2. The results obtained are clarified in table 4.31 and figure 4.33. The resultant values for % RSD and recovery satisfied the criteria and the plot of absorbance vs. concentration were found linear with an R^2 value of 0.9999.

Table 4.31: Accuracy results of dissolution method validation

No. of injection	Target Conc. (%)	Theo. Conc. (mg / ml)	Conc. after Spiking (mg/ml)	Spiked Sample Response	Recovery (%)	Mean (%)	RSD (%)
1.1	50%	0.00694	0.007088	312.4	102	101.5	0.42
1.2			0.007054	310.9	101.6		
1.3			0.007157	309.2	101		
2.1	100%	0.01388	0.01399	616.7	100.8	101.3	0.37
2.2			0.01409	621	101.5		
2.3			0.01411	622.1	101.7		
3.1	150%	0.02082	0.02127	937.7	102	102	0.07
3.2			0.02127	937.7	102		
3.3			0.02131	936.4	102		

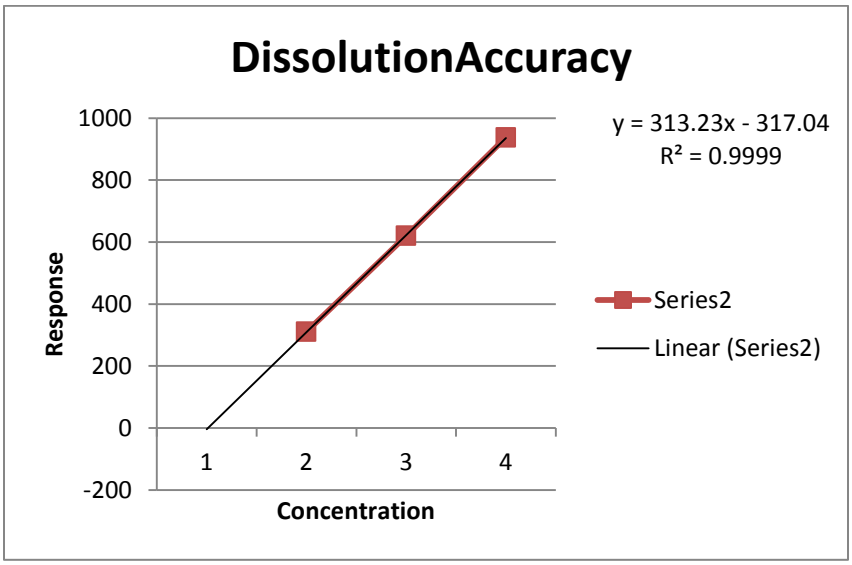


Figure 4.33: Accuracy regression curve for dissolution method validation

4.5.2.3 Precision:

The precision is the ability of a method to produce precise analytical results from a series of measurements of the same homogenous sample under prescribed conditions.

The standard deviation (SD) or percentage relative standard deviation (% RSD) of a series of measurements is usually used to assess the precision of an analytical method.

The precision of the method was considered at repeatability level. A value for % RSD of < 4% was set as an acceptable limit

The repeatability was determined by the analysis batch no. F06 for six times and samples were withdrawn at 30 and 60 min. The repeatability results obtained are shown in Table 4.32.The results reveal that % RSD values were within the acceptable limits thus the method is repeatable for the dissolution analysis of CFA suppositories.

Table 4.32: Dissolution method validation repeatability results

Lot No. F06									
No.	**Time (min)**	**% Dissolution**						**Average**	**RSD,%**
1	30	387	379	359	370	358	389	374	3.3
	60	449	446	456	453	455	459	453	0.9
2	30	335	367	372	357	369	388	365	4.4
	60	439	456	447	438	463	457	450	2.0
3	30	360	372	375	362	377	386	371	2.1
	60	460	455	442	459	446	456	453	1.5
4	30	369	361	380	359	388	392	374	3.4
	60	437	457	446	463	449	459	452	1.9
5	30	372	365	378	387	367	358	371	2.5
	60	441	453	462	445	470	448	453	2.2
6	30	356	382	360	364	353	370	364	2.7
	60	432	457	443	442	450	461	448	2.2
Avg.	30								**369.8**
	60								**451.5**
RSD,%	30								**3.1**
	60								**1.8**

4.5.2.4 Specificity:

Six placebo formulations containing all the excipients used in the formulation were prepared, and the absorbance of these formulations was scanned over the wave length range 240-340 nm. The resultant scanning showed no absorbance for any of the placebo formulations which indicates that there isn't any interference from the excipients in the dissolution results.

Six sample suppository formulations were prepared, and the absorbance of these formulations was measured at λ = 278 nm. The percentage recovery was calculated for each formulation and the results were within the acceptable limits (i.e. 100 ±5%) as shown in table 4.33.

Table 4.33 percentage recovery from sample suppositories containing the different excipients used in suppository preparations

Formula	**Abs. 1**	**Abs. 2**	**Abs. 3**	**Average**	**% recovery**	**% RSD**
F1	707	704	694	702	100.7	0.79
F2	668	665	671	668	95.8	0.37
F3	663	663	665	664	95.2	0.14
F4	672	680	671	674	97	0.59
F5	700	698	696	698	100.1	0.23
F6	675	669	672	696.4	96.4	0.36

Abs = Absorbance

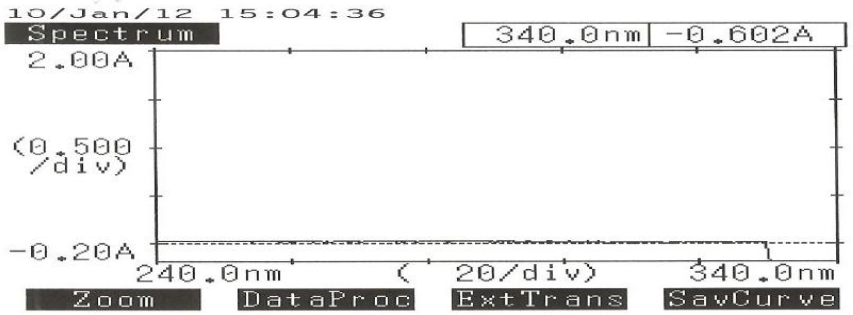


Figure (4.34) placebo F1 scanning spectrum:

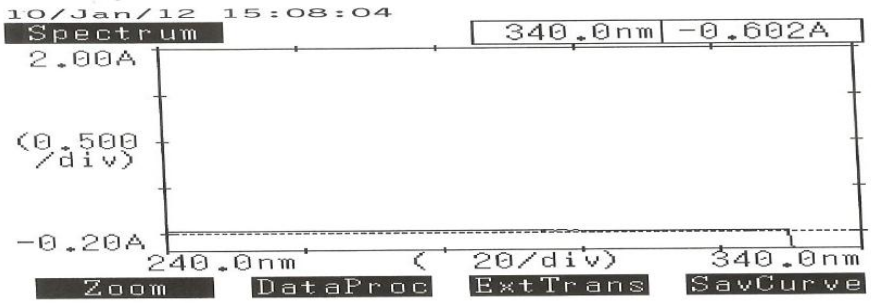


Figure (4.35) placebo F2 scanning spectrum:

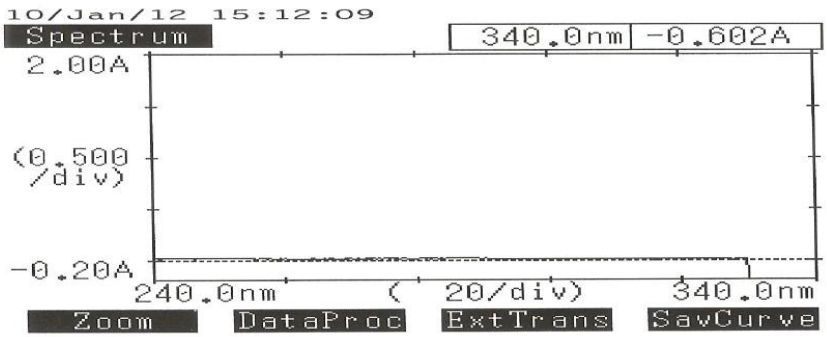


Figure (4.36) placebo F3 scanning spectrum:

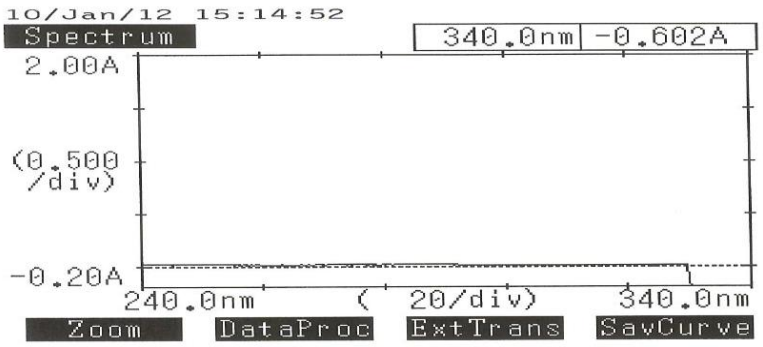


Figure (4.37) placebo F4 scanning spectrum:

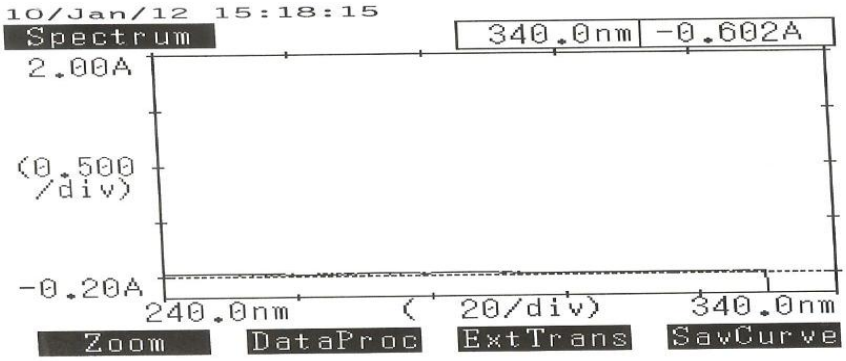


Figure (4.38) placebo F5 scanning spectrum:

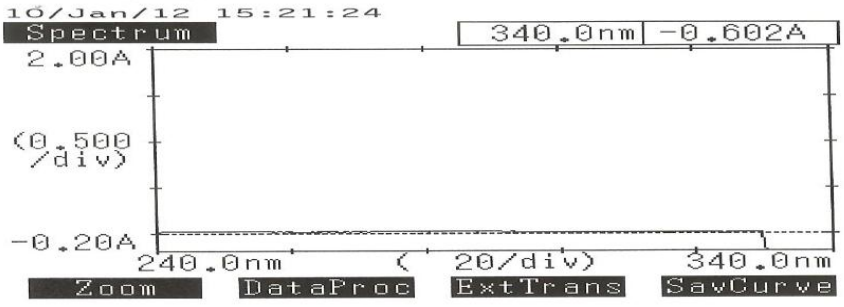


Figure (4.39) placebo F6 scanning spectrum:

Part Five:

Summary and Conclusions

CFA is a broad spectrum second generation cephalosporin antibiotic, active against β-lactamase producing bacterial strains. It is demonstrated to be active against gram-positive and gram-negative organisms. CFA is marketed as a powder for oral suspension in 125mg and 250 mg per 5ml strengths, and as tablet dosage form in 125mg, 250mg, and 500mg strengths. However CFA extreme bitterness limits its use to a wide spectrum of patients. Administration of CFA in a suppository dosage form may be a useful alternative for the treatment of the aforementioned diseases when patients, especially children are unwilling or unable to take oral medications.

We attempted to formulate CFA suppositories in two main types of suppository bases, i.e. water soluble and fatty bases. PEG bases were used as the water-soluble bases, while Witepsol H15 and Novata (A and BCF) were used as fatty bases. The suppositories were prepared by the fusion method of manufacture and were assessed in terms of their physical appearance, weight uniformity, melting range, disintegration time, CFA content, and dissolution behavior. The PEG water soluble bases were excluded from the study at the preformulation stage as they showed incompatibilities with the CFA active material.

The displacement values for Witepsol H15 and Novata A and BCF suppository bases were found to be less than one (0.811-0.936), therefore the API quantity (125 mg cefuroxim base) has no significant effect on the quantity of fatty base required for one suppository.

For the quantitation of CFA in suppositories dosage form an HPLC method was adapted from the USP monograph for CFA tablets and was validated according to USP category I and the ICH Q2 (R1) guidelines. The method was found to be linear over the concentration range of 25 μg / ml to 400 μg / ml with a correlation coefficient (R^2) of 0.9999. The resultant % RSD values for the method precision at the two levels; repeatability and intermediate precision were found to be ≤ 1.5% RSD for all concentrations studies. The method was also found to be accurate with %RSD values of ≤ 2% and recovery of 100 ± 2%. In addition the method was considered selective for the detection and quantitation of CFA in the presence of formulation excipients and degradation products, thus the method can also be considered as stability indicating.

In vitro dissolution testing was performed on all batches using USP apparatus I with modified baskets. The method was validated according to USP category III guidelines. The method was found to be linear over the concentration range of 3.5 μg / ml to 21 μg / ml with a correlation

coefficient (R^2) of 0.9998. The resultant % RSD values for the method repeatability were found to be ≤ 4% RSD for all concentrations studied. The method was also found to be accurate with %RSD values of ≤ 2% and recovery of 100 ± 2%. The method was found to be selective for the detection of CFA in the presence of all formulation excipients.

In the preformulations stage CFA suppositories were compounded with WH15 and Novata A/BCF fatty bases alone and with surfactants and physical properties modifiers (i.e. Tween 20, Tween 80, Tween 85, SLS, lanolin anhydrous and lecithin S, Poloxamer 188, Cremophore A6, Cremophore RH 40, and Span 80). Tween 80, Cremophore A6, Cremophore RH 40 were found to be incompatible with CFA. Poloxamer 188 and Span 80 were also excluded as they had no significant added value to the performance of CFA suppositories.

The fatty base, Witepsol H15 was selected as the base for further studies, since it showed better viscosity during compounding, relatively low melting range temperatures and lead to finished suppositories with low disintegration times compared to Novata bases..

The suppository formulations were assessed initially for their performance: Physical appearance, weight uniformity, melting ranges, disintegration time, dissolution, and CFA content and impurities. All suppositories had a smooth and opaque appearance; however the colour ranged between white, off white to pale yellow as per the additive type. The weight uniformity was found to comply with the BP requirements for suppositories (i.e. %RSD <5.0). The melting points for formulations containing Aerosil in 0.1% concentration, as a suspending agent were found to be higher than the target temperature (NMT 37.5^{o}C), while the other additives except SLS were found to reduce the melting points below 37^{o}C.

The disintegration times of suppositories complied with BP requirements. The addition of Lecithin, Lanolin and/or Tween 85 decreased the melting point and as a result the disintegration time decreased accordingly.

All formulations exhibited an acceptable CFA content except those containing Tween 20 which underwent hydrolysis to form free cefuroxim and the Δ^3Isomer due to the presence of hydroxyl groups in the surfactant.

The release rate of CFA from the formulation containing the active material and the suppository base alone was found to be very slow (i.e. not more than 10% in 180 minutes). This is due to the high lipophilicity of CFA, to the low hydroxyl value of WH 15 base and to the relatively high melting point. The CFA release was modified by adding surfactants and physical properties modifiers (i.e. Tween 20, SLS, lanolin anhydrous, Aerosil and lecithin S) in different concentrations and combinations.

The use of Flow through Cell for dissolution testing instead of the USP apparatus I modified basket, increased the percentage release rate significantly for all formulations tested in 60 minutes duration time. As a comparison, the maximum release from the formulation containing the suppository base (WH 15) only approached 68% in 60 minutes compared to 8% in 180 minutes using USP apparatus I. These results ascertain that the flow through cell apparatus is more suitable for the use in poorly soluble drugs than the conventional static method (Farrugia, 2002)

The release data obtained from in vitro release studies were fitted to various mathematical models, such as the Zero order, First order, Higuchi, and Weibull models. In addition, the mechanism of CFA release from fatty suppositories was evaluated using the Korsmeyer- Peppas model. The drug release mechanism can be considered to occur primarily by means of anomalous transport kinetics, which is an indication of the presence of more than one type of release phenomenon. These findings were not entirely unexpected, due to the complexity of the drug release process from suppositories, which involves a series of consecutive steps, such as melting, drug partitioning and diffusion through the molten base to the hydrophilic dissolution medium. For most of the formulations tested, the data were best fitted to the Weibull model, This result is in agreement with the nature of drug release from lipophilic suppository formulations which is often accompanied by long-lasting lag phase, that occurs as a result of the need for the base to melt prior to drug release and therefore the melting rate of the base is a factor that contributes to the lag time. Six formulations fitted to the Higuchi model indicating that diffusion is one of the primary mechanisms governing drug release from the lipophilic suppository formulations tested.

The results from the preliminary stability studies for the selected formulations revealed that there was a significant decrease in the dissolution at 30^{o}C/60%RH. All formulations containing Tween 20 failed the stability acceptance criteria for assay and degradation impurities. The Δ^{3}Isomer increased significantly at 30^{o}C/60%RH and slightly at 25^{o}C/60%RH. Change in color was observed at 30^{o}C/60%RH and slightly changed at 25^{o}C/60%RH. Sediments were observed at 30^{o}C/60%RH and at 25^{o}C/60%RH for formulations containing Tween85. Storage at 2-8^{o}C revealed a stability of all parameters for three months. Long-term stability studies are crucial to ensure that effective antimicrobial activity is retained in such products when stored under specified storage conditions. Therefore, CFA suppositories should be stored in refrigerator.

Despite the apparent complexity of suppository formulations, these studies have shown the applicability of using fatty bases for the formulation of CFA suppository dosage forms for pediatric use. It has been observed that the use of surfactant in combination with fatty base can improve the release of CFA from such suppositories. Further studies should be conducted to elucidate any potential interactions between CFA and the specific excipients used. Analytical methods, such as DSC, can be used to investigate drug/excipient incompatibility and would be of value when undertaking these investigations. Also further studies must be conducted on the basis of determining drug partitioning in the presence of suppository base-rectal fluid systems, to further elucidate and/or predict the process of drug release. It would be necessary to determine the in vivo bioavailability of CFA, of the suppository dosage forms prior to determining whether an in vitro-in vivo correlation exists for CFA following administration of a rectal suppository formulation.

Part Six:

Appendix

6. Excipients profile

6.1 Witepsol H15 (Raymond C., et al, 2006), (EurP., 2002)

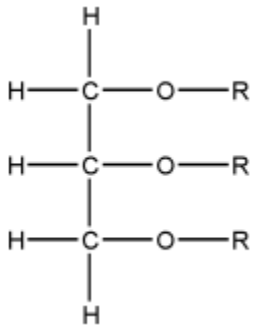
Definition:

Hard Fats are mixture of triglycerides, diglycerides and monoglycerides, which may be obtained either by esterification of fatty acids of natural origin with glycerol or by transesterification of natural fats. It contains no added substances.

Empirical Formula and Molecular Weight:

Hard fat suppository bases consist mainly of mixtures of the triglyceride esters of the higher saturated fatty acids ($C_8H_{17}COOH$ to $C_{18}H_{37}COOH$) along with varying proportions of mono- and diglycerides. Special grades may contain additives such as beeswax, lecithin, polysorbates, ethoxylated fatty alcohols, and ethoxylated partial fatty glycerides.

Structural Formula:



Where R = H or OC- $(CH_2)n\text{-}CH_3$; n = 7–17

Not all Rs can be H at the same time.

Applications in Pharmaceutical Formulation:

The primary application of hard fat suppository bases, or semisynthetic glycerides, is as a vehicle for the rectal or vaginal administration of a variety of drugs, either to exert local effects or to achieve systemic absorption.

Characters:

Appearance: white or almost white, waxy, brittle mass.

Solubility: practically insoluble in water, slightly soluble in anhydrous ethanol.

When heated to 50 °C, it melts giving a colourless or slightly yellowish liquid.

Typical properties:

Characteristic values	Limits
Ascending melting point ^{o}C	33.5-35.5
Hydroxyl value mg KoH/g	5-15
Acid value mg KoH/g	<0.2
Iodine value g I_2/100g	<3.0
Peroxide value meq./kg	<1.0
Saponification value mg KoH/g	230-245
Alkaline impurities ml HCL/2g	<0.15
Heavy metals ppm	<10
Ash	<0.05
Unsaponifiable matter	<0.3

Safety

Suppository bases are generally regarded as nontoxic and nonirritant materials when used in rectal formulations. However, animal studies have suggested that some bases, particularly those types with a high hydroxyl value, may be irritant to the rectal mucosa.

Handling and Storage:

Dry, protected from light, in original containers and at temperatures below 25°C, shelf life is at least three years.

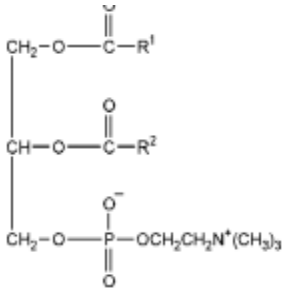
6.2 Lecithin (Raymond C., et al, 2006), (BP, 2011)

Lecithin is a complex mixture of acetone-insoluble phosphatides, which consist chiefly of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl inositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates, as separated from the crude vegetable oil source. It contains not less than 50.0 percent of acetone-insoluble matter.

Empirical Formula:

The composition of lecithin (and hence also its physical properties) varies enormously depending upon the source of the lecithin and the degree of purification. Egg lecithin, for example, contains 69% phosphatidylcholine and 24% phosphatidylethanolamine, while soybean lecithin contains 21% phosphatidylcholine, 22% phosphatidylethanolamine, and19% phosphatidylinositol, along with other components.

Structural Formula:



α-Phosphatidylcholine

R^1 and R^2 are fatty acids, which may be different or identical.

The structure above shows phosphatidylcholine, the principal component of egg lecithin, in its a-form. In the b-form, the phosphorus-containing group and the R^2 group exchange positions.

Functional Category:

Lecithin is mainly used as emollient, emulsifying agent and solubilizing agent.

Description:

Lecithins vary greatly in their physical form, from viscous semi liquids to powders, depending upon the free fatty acid content. They may also vary in color from brown to light yellow, depending upon whether they are bleached or unbleached or on the degree of purity. When they are exposed to air, rapid oxidation occurs, also resulting in a dark yellow or brown color.

Lecithins have practically no odor. Those derived from vegetable sources have a bland or nutlike taste, similar to that of soybean oil.

Pharmacopoeia Specifications:

Test	USPNF 23
Water	⩽1.5%
Lead	⩽0.001%
Heavy metals	⩽20 μg/g
Acid value	+
Hexane-insoluble matter	⩽0.3%
Acetone-insoluble matter	–
Organic volatile impurities	+

Incompatibilities:

Lecithin is incompatible with esterases owing to hydrolysis.

Applications in Pharmaceutical Formulation:

- Lecithins are used in a wide variety of pharmaceutical applications. They are also used in cosmetics and food products.
- Lecithins are mainly used in pharmaceutical products as dispersing, emulsifying, and stabilizing agents and are included in intramuscular and intravenous injections, parenteral nutrition formulations, and topical products such as creams and ointments.
- Lecithins are also used in suppository bases, to reduce the brittleness of suppositories, and have been investigated for their absorption-enhancing properties in an intranasal insulin formulation.
- Lecithins are also commonly used as a component of enteral and parenteral nutrition formulations.
- Liposomes in which lecithin is included as a component of the bilayer have been used to encapsulate drug substances; their potential as novel delivery systems has been investigated.
- Therapeutically, lecithin and derivatives have been used as a pulmonary surfactant in the treatment of neonatal respiratory distress syndrome.

Stability and Storage Conditions:

Lecithins decompose at extreme pH. They are also hygroscopic and subject to microbial degradation. When heated, lecithins oxidize, darken, and decompose. Temperatures of 160–1808 ^{o}C will cause degradation within 24 hours.

Packaging and storage**:** Preserve in well-closed, light-resistant containers. Store at the temperature indicated on the label. Protect from excess heat and moisture.

6.3 Lanolin (EurP., 2002), (Raymond C., et al, 2006), (USP34, 2010)

Definition:

Purified, anhydrous, waxy substance obtained from the wool of sheep (Ovis Aries). It may contain no more than 200 ppm of butylhydroxytoluene.

Characteristics:

Appearance: yellow, unctuous substance. When melted, it is a clear or almost clear, yellow liquid. A solution in light petroleum is opalescent.

Solubility: freely soluble in benzene, chloroform, ether, and petroleum spirit; sparingly soluble in cold ethanol (95%), more soluble in boiling ethanol (95%); practically insoluble in water.

It has a characteristic odour.

Empirical Formula:

It contains not more than 0.25% w/w of water and may contain up to 0.02% w/w of a suitable antioxidant; the PhEur 2005 specifies up to 200 ppm of butylated hydroxytoluene as an antioxidant.

Functional Category:

Lanolin is used as emulsifying agent; ointment base.

Applications in Pharmaceutical Formulation:

- Lanolin is widely used in topical pharmaceutical formulations and cosmetics.
- Lanolin may be used as a hydrophobic vehicle and in the preparation of water-in-oil creams and ointments.
- When mixed with suitable vegetable oils or with soft paraffin, it produces emollient creams that penetrate the skin and hence facilitate the absorption of drugs.

- Lanolin mixes with about twice its own weight of water, without separation, to produce stable emulsions that do not readily become rancid on storage.

Pharmacopoeia Specifications:

Test	Specifications
Melting range	38-44^{o}C
Loss on drying	≤0.5%
Sulfated ash	≤0.15%
Chloride	≤150 ppm
Acid value	≤1.0
Iodine value	18-36
Peroxide value	≤20
Saponification value	90-105
Paraffin	≤1.0
Butylated hydroxytoluene	≤200 ppm

Stability and Storage Conditions:

Lanolin may gradually undergo autoxidation during storage. To inhibit this process, the inclusion of butylated hydroxytoluene is permitted as an antioxidant. Exposure to excessive or prolonged heating may cause anhydrous lanolin to darken in color and develop a strong rancid like odor. However, lanolin may be sterilized by dry heat at 1508^{o}C. Ophthalmic ointments containing lanolin may be sterilized by filtration or by exposure to gamma irradiation.

Lanolin should be stored in a well-filled, well-closed container protected from light, in a cool, dry place. Normal storage life is 2 years.

Incompatibilities:

Lanolin may contain prooxidants, which may affect the stability of certain active drugs.

6.4 Polysorbate 85 (Tween 85) (EurP., 2002), (Raymond C., et al, 2006)

Chemical name: Polyoxyethylene 20 sorbitan trioleate. CAS number [9005-5-70-3]

Definition:

Mixture of partial esters of fatty acids, mainly Oleic acid (0799), with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

Characters:

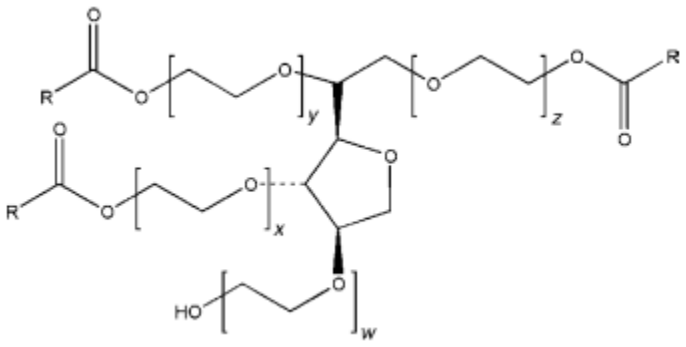
Appearance: oily, yellowish or brownish-yellow, clear or slightly opalescent liquid.

Solubility: dispersible in water, in anhydrous ethanol, in ethyl acetate and in methanol, practically insoluble in fatty oils and in liquid paraffin.

Empirical Formula and Molecular Weight:

Polysorbate 85, Formula $C_{100}H_{188}O_{28,}$ Molecular weight= 1839

Structural Formula:



Polyoxyethylene sorbitan triester

w + x + y + z = 20 (Polysorbates 20, 40, 60, 65, 80, and 85)

R = fatty acid

Functional Category:

Polysorbate 85 is used as emulsifying agent; nonionic surfactant; solubilizing agent; wetting, dispersing/suspending agent.

Applications in Pharmaceutical Formulation:

Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical

emulsions. Polysorbate 85 is used as an emulsifier in combination with a variety of oil in water, and water in oil emulsion systems.

Individually, it is an excellent solubilizer of vegetable oils and fragrances, a wetting agent, viscosity modifier, stabilizer and dispersing agent. It is useful for oil-in-water emulsions and to make anhydrous ointments water soluble washable.

Polysorbates are also widely used in cosmetics and food products.

Typical properties:

Physical form at 25°C: Yellow liquid

HLB: 11

Solubility: Vegetable oil, water and mineral oils

Specific Gravity at 25°C: 1.03

Specifications:

Saponification value: 80-90

Hydroxyl value: 39-52

Acid value: <2.0

Water: <0.5%

Surface tension at 208C (mN/m): 41.0

Incompatibilities:

Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tarlike materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates.

Stability and Storage Conditions:

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides. Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

6.5 Sodium Lauryl Sulfate (Raymond C., et al, 2006)

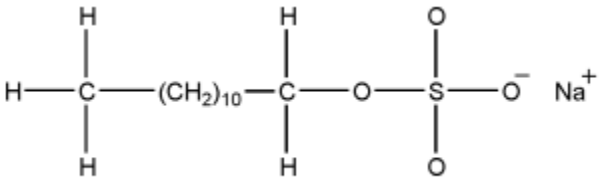
Chemical Name and CAS Registry Number:

Sulfuric acid monododecyl ester sodium salt [151-21-3]

Empirical Formula and Molecular Weight: $C_{12}H_{25}NaO_4S$, 288.38

The USPNF describes sodium lauryl sulfate as a mixture of sodium alkyl sulfates consisting chiefly of sodium lauryl sulfate (C12H25NaO4S). The PhEur states that sodium lauryl sulfate should contain not less than 85%of sodium alkyl sulfates calculated as $C_{12}H_{25}NaO_4S$.

Structural Formula:



Functional Category:

Used as anionic surfactant; detergent; emulsifying agent; skin penetrant; tablet and capsule lubricant; wetting agent.

Applications in Pharmaceutical Formulation:

Sodium lauryl sulfate is an anionic surfactant employed in a wide range of nonparenteral pharmaceutical formulations and cosmetics. It is a detergent and wetting agent effective in both alkaline and acidic conditions. In recent years it has found application in analytical electrophoretic techniques: SDS (sodium dodecyl sulfate) polyacrylamide gel electrophoresis is one of the more widely used techniques for the analysis of proteins; and sodium lauryl sulfate has been used to enhance the selectivity of micellar electrokinetic chromatography.

Uses of sodium lauryl sulfate: (Raymond, 2006)

Use	Concentration %
Anionic emulsifier, forms self-emulsifying bases with fatty alcohols	0.5–2.5
Detergent in medicated shampoos	≈ 10
Skin cleanser in topical applications	1
Solubilizer in concentrations greater than critical micelle concentration	> 0.0025
Tablet lubricant	1.0-2.0
Wetting agent in dentifrices	1.0-2.0

Description:

Sodium lauryl sulfate consists of white or cream to pale yellow colored crystals, flakes, or powder having a smooth feel, a soapy, bitter taste, and a faint odor of fatty substances.

Typical Properties:

Acidity/alkalinity: pH = 7.0–9.5 (1% w/v aqueous solution)

Acid value: 0

Antimicrobial activity: sodium lauryl sulfate has some bacteriostatic action against Gram-positive bacteria but is ineffective against many Gram-negative microorganisms. It potentiates the fungicidal activity of certain substances such as sulfanilamide and sulfathiazole.

Critical micelle concentration: 8.2mmol/L (0.23 g/L) at 20^{o}C

Density: 1.07 g/cm3 at 20 ^{o}C

HLB value: ≈ 40

Interfacial tension: 11.8mN/m (11.8 dynes/cm) for a 0.05% w/v solution (unspecified non aqueous liquid) at 30^{o}C.

Melting point: 204–207^{o}C (for pure substance)

Moisture content: 45%; sodium lauryl sulfate is not hygroscopic.

Solubility: freely soluble in water, giving an opalescent solution; practically insoluble in chloroform and ether.

Surface tension: 25.2mN/m (25.2 dynes/cm) for a 0.05% w/v aqueous solution at 30^{o}C

Stability and Storage Conditions:

Sodium lauryl sulfate is stable under normal storage conditions. However, in solution, under extreme conditions, i.e., pH 2.5 or below, it undergoes hydrolysis to lauryl alcohol and sodium bisulfate.

The bulk material should be stored in a well-closed container away from strong oxidizing agents in a cool, dry place.

Safety:

Sodium lauryl sulfate is widely used in cosmetics and oral and topical pharmaceutical formulations. It is a moderately toxic material with acute toxic effects including irritation to the skin, eyes, mucous membranes, upper respiratory tract, and stomach. Repeated, prolonged exposure to dilute solutions may cause drying and cracking of the skin; contact dermatitis may develop.

Prolonged inhalation of sodium lauryl sulfate will damage the lungs. Pulmonary sensitization is possible, resulting in hyperactive airway dysfunction and pulmonary allergy.

Sodium lauryl sulfate should not be used in intravenous preparations for humans. The probable human lethal oral dose is 0.5–5.0 g/kg.

Incompatibilities:

Sodium lauryl sulfate reacts with cationic surfactants, causing loss of activity even in concentrations too low to cause precipitation. Unlike soaps, it is compatible with dilute acids and calcium and magnesium ions.

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تطوير وتقييم مخبري لمستحضر السيفيوروكسيم آكسيتل على شكل تحاميل شرجية للاطفال

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المشرف: د. نعمان مالكية

المشرف المشارك: د. طارق الجعبة

الملخص

سيفوروكسيم آكسيتل مضاد حيوي واسع الطيف من الجيل الثاني من السيفالوسبورينات، فعال ضد طائفة واسعة من مسببات الامراض الشائعة، بما في ذلك منتجات "البيتالاكتميز". يتم تسويق الدواء على شكل مسحوق صلب معلق واقراص تعطى للمرضى عن طريق الفم. مادة السيفوروكسيم آكسيتل شديدة المرارة مما يحد من استخدامها من قبل مجموعة واسعة من المرضى وعليه فان إعطاء الدواء على شكل تحاميل قد يكون بديلاً مقبولاً لدى المرضى أكثر من الاشكال الصيدلانيه عن طريق الفم خصوصاً لدى الاطفال وكبار السن الذين يجدون صعوبة في ابتلاع الاقراص او تذوق المرارة الشديدة عن طريق تناول المستحضر على شكل معلق.

لقد تم تشكيل تحاميل سيفوروكسيم أكسيتل باستخدام طريقة الانصهار، حيث استخدم في ذلك نوعان من قواعد تشكيل التحاميل أحدهما تذوب في الماء والاخرى دهنية لا تذوب في الماء. حيث تم استخدام مادة "البولي أيثيلين جلايكول" بتدرجات مختلفة كقاعدة تذوب في الماء واستخدمت مادتي " وايتبسول H15" و "نوفاتا A ونوفاتا BCF" كقواعد دهنية. تم استبعاد مادة البولي ايثيلين جلايكول منذ بداية الدراسة وذلك لظهور تعارض بينها وبين مادة السيفوروكسيم آكسيتل.

وكنتيجة للفحوصات التي اجريت للمستحضر في المراحل الاوليه من الدراسة تبين وجود أفضلية لمادة "وايتبسول H15" على مادة نوفاتا من حيث درجة الانصهار ومعدل تحرر المادة الفعالة, لذا استخدمت مادة "وايتبسول H15" في الدراسة كقاعدة لتشكيل التحاميل بتراكيبها المختلفة. تم تقييم التحاميل المصنعة من خلال عدة فحوصات تم اجراؤها على هذه التحاميل, حيث اشتملت على الفحص الحسي، فحص تجانس الوزن، فحص زمن التفتت، تركيز المادة الفعالة، دراسة الذوبان الرطب ودراسة الثباتية. لقد وجد من نتائج الفحوصات بأن معدل تحرر المادة الفعالة من المستحضر تتأثر بشكل مباشر بالخواص الفيزيوكيميائية مثل درجة انصهار التحاميل. ولقلة ذائبية المستحضر في المحاليل المائية وميله للطبقة الزيتية فإن توزعه بين الجزء المائي والزيتي كان يميل بشكل كبير وواضح نحو الطبقة الزيتية مما كان له الاثر الكبير على تأخير تحرر المادة الفعالة من المستحضر الى الجزء المائي.

تم تحضير ستة عشر تركيبة مختلفة لاجراء الدراسة عليها، حيث تم استخدام مخفضات التوتر السطحي ومحسنات الانصهار (مثل توين 20 ، توين 85، صوديوم لوريل سلفات، لانولين وليسيتين الصويا) بنسب مختلفة وكنتيجة لذلك طرأ زيادة بشكل ملحوظ على تحرر المادة الفعالة من التراكيب التي استخدمت فيها , علما بأن نسبة التحرر لم تتعدى 8% من دون استخدام هذه الاضافات. تم دراسة وتقييم آلية تحرر المادة الفعالة من المستحضر باستخدام نماذج رياضية عدة منها، هيجونشي، كورس ماير–باباس، معادلة من الدرجة الصفرية، معادلة من الدرجة الاولى ونموذج ويبول. لوحظ من هذا التطبيق بأن آلية تحرر المادة الفعالة من المستحضر تخضع بشكل واضح ومميز الى نموذج ويبل، كورسماير باباس ونموذج هيجوتشي، وأن قيم "n" التي تم حسابها تفيد بأن تحرر الدواء كان نتاجاً لخاصية الانتشار ولمعدل انصهار المستحضر ولتجزأة المادة الفعالة بين الوجه الزيتي والوجه المائي، وعليه فإن تحرر المادة الفعالة يمكن ان يوصف بالشاذ

تم اجراء دراسة ثباتية للمستحض لمدة ثلاثة شهور، حيث وضع عدد مختار من التشغيلات في ثلاثة ظروف تخزين (وهي درجة حرارة 25°م/60% رطوبة نسبية، درجة حرارة 30°م/60% رطوبة نسبية، 2 – 8°م). أفادت النتائج بأن غالبية التركيبات من المستحضر وجدت ثابتة على درجة حرارة الثلاجة (2 – 8°م) ومعظمها كان غير ثابتاً على درجة حرارة 25°م و 30°م.