QUALITY EVALUATION OF CLOBETASOL PROPIONATE AND BETAMETHASONE VALERATE TOPICAL CORTICOSTEROID CREAMS MARKETED IN ADDIS ABABA

A thesis submitted to the school of graduate studies of Addis Ababa University in partial fulfillment of the requirements for degree of master science in pharmaceutics in the department of pharmaceutics, school of pharmacy.

By

Abebayehu Terefe Desalegn

April, 2010

ADDIS ABABA UNIVERSITY

SCHOOL OF GRAGUATE STUDIES

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DEPARTMENT OF PHARMACEUTICS SCHOOL OF PHARMACY

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Acronyms

ACTH	Adrenocorticotropic hormone
APIs	Active pharmaceutical ingredients
BV	Betamethasone valerate
CP	Clobetasol propionate
DACA	Drug administration and control authority
DNA	Deoxyribo nucleic acid
GRE	Glucocorticoid responsive element
GRG	Glucocorticoid responsive
HPA	Hypothalamic-pituitary adrenal
I.D.	Internal diameter
IS	Internal standard
nm	Nanometer
Rf	Retention factor
RP-HPLC	Reversed phase high performance liquid chromatography
Rpm	Revolution per minute
SAA	Surface active agent
SC	Stratum corneum
TLC	Thin layer chromatography
USP	United states pharmacopoeia
UV	Ultra-violet

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Abstract

Topical corticosteroids have revolutionized the practice of dermatology since their introduction in the late 1950s. They have improved the management of many inflammatory skin diseases but like all medications, the drugs are associated with potential adverse (side) effects.

Five brands of cream formulations (GlaxoSmithKline of England and Saudi Arabia), Zygpharma pvt. Ltd (India), Hoe Pharmaceuticals (Malaysia) and Pharma Inkl (Jordan)) containing, 0.05 % w/w clobetasol -17-propionate (CP) and 0.1% w/w betamethasone -17-valerate (BV) as active ingredients were purchased from different retail outlets in Addis Ababa and their qualities were evaluated for microbiological, identification (qualitative) and assay(quantitative) tests using the official USP 2008.

Microbial limit test mannital – salt and cetrimide agar media were used to test the presence or absence of Staphylococcus aureus and Pseudomonas aeruginosa**,** respectively. The specimens were incubated for five days at a temperature of 25 ^{O}C but the growth of the expected microorganisms were not observed (i.e the results were negative).

Identification test was performed using thin layer chromatography; clobetasol propionate (1.0 gm) and betamethasone valerate (2.0 gm) creams were weighed and extracted with a series of steps using appropriate chemicals which are recommended by USP 2008. The dried residues were dissolved in chloroform and equal volumes (10μl) of the standards and the tests were spotted. After the plates were dried in the hood their retention factor (Rf) values were calculated by measuring the distance traveled by the standards and the extracts to that of the solvent front and found to be as 0.56 for clobetasol propionate and 0.33 for betamethasone valerate.

Assay was performed by using RP-HPLC. Clobetasol propionate (2 gm) and betamethasone valerate (2.5 gm) cream were weighed and extracted with methanol. The mixtures were shaken using a shaker and centrifuged at about 3500 rpm for 10 minutes followed by filtration through a 0.45 μm filter paper and the filtrates were added into a vial. Equal volumes of the standard and the sample (10 μl) were injected at a flow rate of 1.0 ml/min for clobetasol propionate and 1.2 ml/min for betamethasone valerate and the amount in mg of each active ingredient were found as 0.908 to 1.149 mg (90.8-114.9%) clobetasol propionate and 2.41 to 2.76 mg (96.4-110.4 %) betamethasone valerate.

The above results showed that all the brands of the two cream products fulfill the USP 2008 specifications interms of physical stability (appearance), microbiological, identification and assay tests.

Key words: corticosteroids, clobetasol 17-propionate, betamethasone-17-valerate, microbial limit, identification, assay and retention factor.

1. INTRODUCTION

1.1 Corticosteroids

Corticosteroids, also referred to as "steroids" or "cortisone", are synthetic derivatives of cortisone which are effective when applied locally to control many types of inflammatory, allergic and pruritic dermatomes (Ramsing and Agner, 1995). Structural formula of cortisone is depicted in Figure 1.

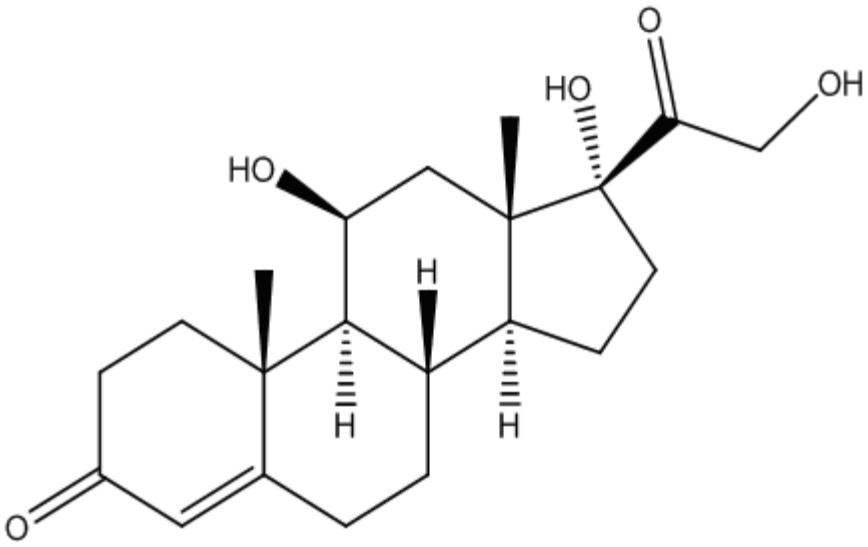

Fig.1.1: Chemical structure of cortisone

1.2 History and structure of corticosteroids

Hydrocortisone acetate, the first topical corticosteroid developed for the treatment of inflammatory skin diseases, was introduced in 1952 and revolutionized the field of dermatology (Witten *et a.,* 1992).

Shortly thereafter, fluorohydrocortisone and prednisone (1955), triamcinolone acetonide (1958), and fluorometholone (1959) entered the market. All of these compounds share the basic 4-ring structure of cholesterol, and many other corticosteroids have been developed through modification of the side chain of the corticosteroid analog (Hughes and Rustin, 1997).

The essential steroid structure consists of seventeen carbon atoms arranged in three six membered rings and one five-membered ring. The steroid nucleus is a rigid structure and various substitutions or alterations in the steric configuration can lead to great changes in the biological activity of the steroid (Elks and Dermato, 1976).

All topical corticosteroids have a basic skeletal structure consisting of a reduced phenanthrene ring system fused to a five-membered ring, giving rise to a cyclopentanoperhydrophenanthrene nucleus, which is comprised of three six-membered and one five-membered ring (Meyer, 1998).

The four rings of the corticosteroid skeleton do not exist in a flat place and the structure has no elements of symmetry with each of the 19 positions being chemically distinct from each of the other positions in the structure. Furthermore, the corticosteroid skeleton is a rigid structure, and it has been suggested that small changes in the position of a substituent usually results in a significant change in the biological activity of the molecule (Brazzini and Pimpinelli, 2002). The basic corticosteroid structure is shown in Figure 1.2.

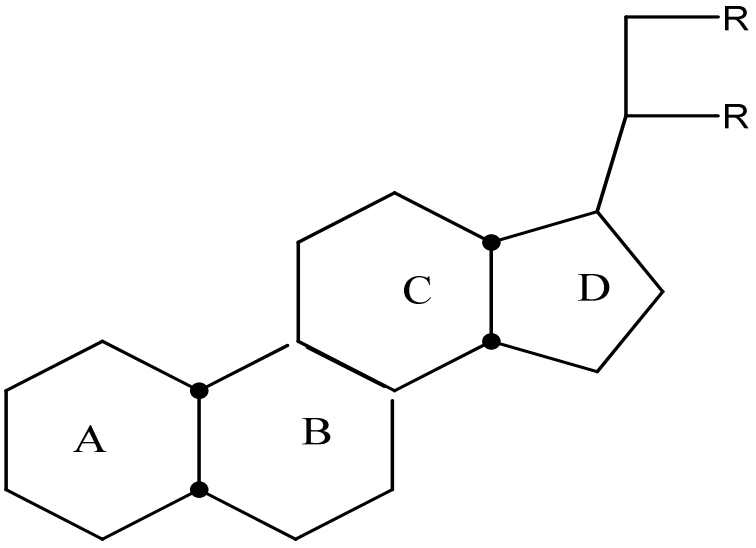

Fig.1.2: Basic corticosteroid structure

Topical corticosteroid products are the most frequently prescribed products used in dermatology but unfortunately their increasing availability has resulted in misuse and abuse. For example, topical corticosteroid products are often used as skin lighteners, particularly in black populations (Tsai, *et al.,* 2004).

1.3 Pharmaceutical creams

Pharmaceutical creams are described as emulsions of a semi-solid consistency or emulsions of a high apparent viscosity with a typical creamy white appearance that are manufactured for topical application (Billany, 2002). However, more recently (Buhse *et al.,* 2005) classified and reported creams as semisolid dosage forms that contain >20% water and volatile ingredients and/or <50% of hydrocarbon, wax or polyethylene glycol constituents as a vehicle and that are intended for external application to the skin.

Similar to liquid emulsions, creams usually contain a third component *viz.,* an emulsion stabilizing system or an emulsifier. However, in contrast to liquid emulsions, creams are reported to contain more emulsifier than that required to form a condensed monomolecular surfactant film at a droplet interface in liquid emulsions. It has been argued that excess emulsifiers in cream formulations interact with other components of these formulations either at the droplet interface or in the bulk phase to produce complex, multiphase structures and these complex multiphase structures are reported to be essential for the formation of creams that are stable for extended periods of time (Barry, 1983).

Pharmaceutical creams may contain one or more APIs dissolved or dispersed in either an o/w or a w/o system. Oil-in-water creams are usually referred to as “vanishing creams” as when rubbed into the skin the formulation disappears without leaving any trace of their presence on the skin (Barry, 1983).

1.4 Instability mechanisms in creams

The physical instability of creams has been reported to occur through various time- and temperature-dependent physicochemical destabilizing mechanisms (Walters and Brain, 2002) and these mechanisms are summarized schematically in Figure 1.3.

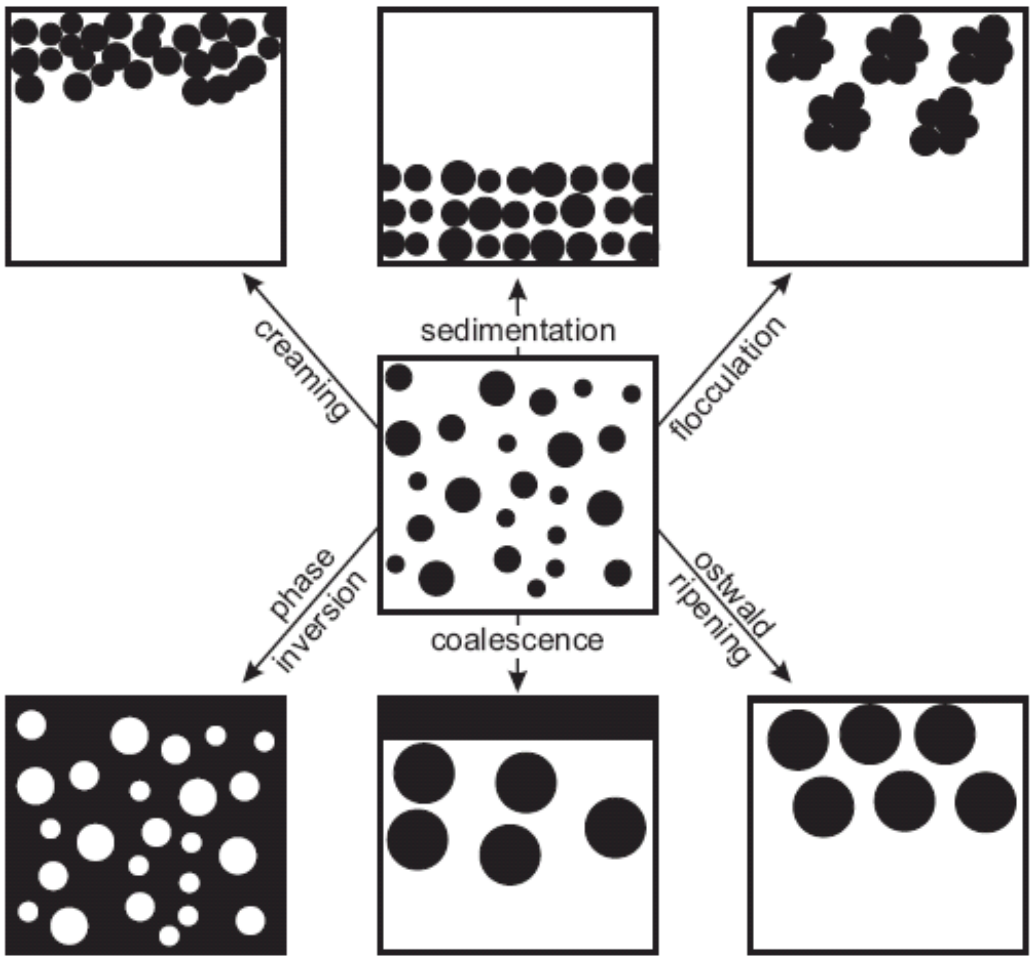

Fig. 1.3**:** Schematic representation of the mechanisms by which creams show instability (adapted from Tadros, 2004)

1.4.1 Flocculation

In order for a cream to form, two immiscible liquid phases must be mechanically agitated in the presence of an emulgent. It has been reported that when agitation occurs in the absence of any form of interfacial stabilization both phases of the cream will form droplets that will rapidly flocculate and separate into two distinct phases. Flocculation may therefore be described as the close accumulation of two

or more individual droplets of a dispersed phase to form loose assemblies or flocs, without loss of the interfacial film. In other words, in flocculated cream systems the single droplets of the dispersed phase become replaced by twin droplets or multiple flocs separated by a thin interfacial film (Tadros, 2004).

It has been reported that flocculation occurs as a result of van der Waal's attractive forces that take place in the absence of adequate repulsion between the droplets of the dispersed phase. Usually, individual dispersed phase droplets move through the external phase due to diffusion or agitation. If resistance between the droplets is not sufficient, flocculation occurs and for example individual droplets may aggregate to form flocs. Flocculation does not lead to an increase in the average size of the emulsion droplets, indicating that individual droplets do not lose their integrity (Taylor, 1998).

1.4.2 Coalescence

The term coalescence is used to describe the aggregation of flocculated droplets into one large droplet. It has been argued that thinning and/or disruption or loss of the interfacial film between approaching droplets in a cream layer is the main driving force for coalescence. Tadros (2004) reported that when two flocculated droplets are in close proximity to each other, the liquid surfaces undergo fluctuations forming what Tadros referred to as "waves". The apices of these fluctuations become the point at which strong van der Waal's forces of attraction are prevalent and when these fluctuations grow in amplitude the distance separating the apices of the interfacial film may reach a critical value that will cause the film to collapse. Subsequently, the two flocculated droplets combine to form a single larger droplet. Unlike flocculation coalescence invariably leads to an increase in the average size of the droplets as seen in Figure 1.3.

1.4.3 Creaming or sedimentation

Creaming refers to the process by which buoyant droplets of a dispersed phase rise to the top of a container and sedimentation takes place when dispersed droplets sink to the bottom of a container. Creaming or sedimentation occurs due

to differences in the densities of the dispersed and continuous phases. The dispersed phase is usually less dense than the continuous phase and therefore droplets will cream or sediment in a gravitational field. Creaming or sedimentation does not involve an increase in the average size of the dispersed droplets, although both creaming and sedimentation may occur prior to coalescence, since coalescence requires the droplets of the dispersed phase to be in close proximity (Taylor, 1998).

1.4.4 Ostwald ripening

Another relatively important but often neglected cream or emulsion instability is known as Ostwald ripening. Ostwald ripening has been described as the growth of large emulsion droplets at the expense of smaller ones due to differences in the solubility or the chemical potential of small and large droplets. The difference in chemical potential arises from the difference in the radius of curvature of the droplets. Ostwald ripening may also be described as a mass transfer between drops of different curvatures through a surrounding continuous phase. The chemical potential of a droplet is reported to increase with a decreasing radius of curvature and, as a consequence, the solubility of the dispersed material at the surface of the droplet also increases (Taylor, 1995).

It follows that materials at the surface of smaller droplets tend to dissolve and subsequently diffuse through the continuous phase down a concentration gradient and are deposited on the surface of larger droplets. As a result of mass transfer from small droplets to large droplets the small droplets shrink and ultimately disappear, whereas large droplets grow eventually leading to the formation of a cracked or separated cream. Ostwald ripening does not require the droplets to be in close proximity, since the process takes place by transport of dissolved matter from one droplet to another, through the external phase. Ostwald ripening generally proceeds with the cube of the average radius of a droplet varying linearly with time and this is one of the reasons why Ostwald ripening is usually not considered as being an important phenomenon when considering macroemulsions, since droplets in macroemulsions have radii in excess of

between 1-2 μm. Nevertheless, Ostwald ripening is an important cause of instability in creams (Taylor, 1995).

1.4.5 Phase inversion

Phase inversion is a phenomenon that occurs when one of the two phases of a cream, for example the internal phase, becomes the external phase as the droplets of the internal phase coalesce faster than the droplets of the external phase. Phase inversion may also be caused by an increase in the volume fraction of a dispersed phase or by a transition produced by changing temperature and/or the addition of an electrolyte to a previously stable cream formulation (Tadros, 2004).

1.5 Stabilization of pharmaceutical creams

1.5.1 Surfactants

The physical instability of creams invariably occurs due to the tendency of formulations to revert back to the original distinct two phase systems that have a minimum interfacial free energy (Taylor, 1998). Since interfacial free energy is the driving force for the irreversible fusion of droplets as seen with flocculation and coalescence creams may be stabilized by the inclusion of an appropriate emulsion-stabilizing system that will concentrate at the oil-water interface (Barry, 1983).The use of emulsion stabilization results in a lowering of the interfacial tension between oil and water phases (Walters and Brain, 2002). The stabilizing systems used in most creams consist of surfactants or surface active agents (SAAs) or amphiphiles (Buskirk *et al.,* 1994).

SAAs tend to settle at the boundary between two immiscible phases due to their chemical structure. SAAs are characterized by having two distinct regions in their chemical structure *viz.,* a hydrophilic and hydrophobic region. The hydrophilic portions may be ionic or non-ionic, whereas hydrophobic regions are invariably saturated or unsaturated hydrocarbon chains or, less commonly, heterocyclic or aromatic ring structures. Generally, SAAs are classified according to the nature of the hydrophilic group, therefore SAAs can be anionic, such as for example sodium

alkyl sulphates, cationic, such as for example alkylammonium halides, or non-ionic, such as polyoxyethylene alkyl ethers or polysorbates (Billany, 2002).

Non-ionic SAAs are reported to be the most widely used group of surfactants in pharmaceutical creams. Non-ionic surfactants do not possess a charged group in their hydrophilic polar group, and therefore have a greater degree of compatibility with other components of cream formulations than do the ionic surfactants. In addition non-ionic surfactants are reportedly less sensitive to changes in pH or to the addition of electrolytes, and when used in topical cream formulations non-ionic surfactants tend to cause less skin irritation than ionic surfactants. However, it has been suggested that the main disadvantage of using non-ionic surfactants is that they tend to be more expensive than ionic surfactants (Billany, 2002).

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When incorporated into a cream formulation, the hydrophilic region of a SAA is orientated towards the aqueous phase and the hydrophobic portion towards the oil phase. The resultant cream that is formed is either an o/w or a w/o emulsion and the specific configuration that is formed depends on the properties of the emulgent system used to stabilize the interface between the dispersed droplets and the continuous phase. The emulsification capacity of SAA is reported to be determined by the relative difference in the size and strength of the polar and non-polar groups that make up the molecule (Buskirk *et al.,* 1994).

Oil in water creams are prepared if the hydrophilic characteristics of a SAA is slightly more dominant than the hydrophobic characteristics since the SAA molecule will orientate at the interface in such a way that the hydrophobic portion is forced to the centre of the unit. Similarly, it has been reported that w/o creams are prepared if the hydrophobic properties of an amphiphile are slightly more dominant than the hydrophilic characteristics of that molecule (Walters and Brain, 2002).

1.5.2 Mixed emulgents

In the preparation of simple liquid emulsions, it has been suggested that surfactants alone can stabilize such formulations and mixtures of surfactants have the ability to form more stable emulsions than individual surfactants. However the manufacture of a consistent cream product with a realistic shelf-life may only be achieved by incorporating a specific mixed emulsifying system into the cream formulation. By definition, a mixed emulsifier is an emulsifying system that consists of a combination of an ionic or non-ionic SAAs and a fatty amphiphile, such as a fatty alcohol, fatty acid or monoglyceride (Eccleston, 1997).

The combination of a surfactant with a fatty amphiphile in the correct ratio is able to produce a powerful emulsifying system of the o/w type with excellent stabilization and thickening properties. It has been suggested that nine (9) parts by weight of fatty alcohol to one (1) part by weight of an ionic surfactant (12:1 molar ratio) or four (4) parts by weight of a fatty alcohol to one (1) part by weight of a non-ionic surfactant (20:1 molar ratio) may also be used to prepare appropriate emulsifying blends for use in o/w creams. The components of mixed emulsifying systems may be added to a cream formulation separately during the manufacturing process or alternatively as a previously blended mixture of emulsifying wax (Barry, 1983).

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In its simplest form a cream consists of an oil phase, a water phase and a mixed emulsifying system. Commercial pharmaceutical o/w creams, however, are complex polydispersed systems usually manufactured with several emulgents which complement the properties of each other. Mixed emulgents that are used in o/w creams are usually water-soluble and may consist of anionic or cationic SAA, such as sodium lauryl sulphate or cetrimide respectively, and/or a non-ionic SAA, such as cetomacrogol 1000 in combination with fatty amphiphiles (Eccleston, 1997).

The amphiphiles are usually higher fatty alcohols having chain lengths of between fourteen (14) and eighteen (18) carbon atoms (C_{14} to C_{18}) and may include

substances such as cetyl, stearyl and cetostearyl alcohols, glycerol monosterate and stearic acid (Eccleston, 1997).

1.6 Chromatography

Chromatography is a multi stage separation technique based on differences between compounds in adsorbing on a surface or dissolving in a thin film of liquid. The separation is accomplished by the distribution of components of the mixture between two phases: one that is stationary and another one that is moving. Chromatography works on the principle that different compounds will have different solubilities and adsorption to the two phases. The more common chromatographic techniques are paper, thin layer (TLC), high performance (HPLC), gas, and gel permeation (Clifton, 1999). According to USP 2008, the two chromatographic methods recommended for qualitative and quantitative analysis are TLC and HPLC.

1.6.1 Principles of thin layer chromatography

Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. TLC is a sensitive technique – microgram quantities can be analyzed by TLC - and it takes little time for an analysis (about 5-10 minutes) (Hahn-Deinstrop, 2000).

TLC consists of three steps - spotting, development, and visualization. First the sample to be analyzed is dissolved in a volatile (easily evaporated) solvent to produce a very dilute solution (Hahn-Deinstrop, 2000).

Spotting consists of using a micro pipette to transfer a small amount of this dilute solution to one end of a TLC plate, in this case a thin layer of powdered silica gel that has been coated onto a plastic sheet. The spotting solvent quickly evaporates and leaves behind a small spot of the material.

Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the silica gel plate and the development solvent for the spotted material. The very polar silica gel tries to hold the spot in its original place and the solvent tries to move the spot along with it as it travels up the plate. The outcome depends upon a balance among three polarities - that of the plate, the development solvent and the spot material. If the development solvent is polar enough, the spot will move some distance from its original location. Different components in the original spot, having different polarities, will move different distances from the original spot location and show up as separate spots. When the solvent has traveled almost to the top of the plate, the plate is removed, the solvent front marked with a pencil, and the solvent allowed to evaporate.

Visualization of colored compounds is simple – the spots can be directly observed after development. Because most compounds are colorless however, a visualization method is needed. The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light. A spot will interfere with the fluorescence and appear as a dark spot on a glowing background. While under the UV light, the spots can be outlined with a pencil to mark their locations. A second method of visualization is accomplished by placing the plate into iodine vapors for a few minutes. Most organic compounds will form a dark-colored complex with iodine. It is a good practice to use at least two visualization techniques in case a compound does not show up with one particular method. The Rf value is used to quantify the movement of the materials along the plate. Rf is equal to the distance traveled by the substance divided by the distance traveled by the solvent. It's value is always between zero and one.

If a development solvent of too high a polarity is used, all components in the mixture will move along with the solvent and no separation will be observed (Rf's will be too large). If the solvent is of too low a polarity the components will not move enough, and again separation will not occur (Rf's will be too small).

The TLC evaluation depends on the purpose of a chromatographic analysis. For qualitative determination often localization of substances is sufficient. This can be easily achieved by parallel runs with reference substances. A parameter often used for qualitative evaluation is the Rf value (retention factor). The Rf values are between 0 and 1, best between 0.1 and 0.8 (Hahn-Deinstrop *et al.,* 2000).

1.6 .2 Principles of HPLC

RP-HPLC is a commonly used, powerful and reliable analytical tool that can be used for the *in vitro* analysis of formulations such as creams, ointments and gels that are of a complex nature, since HPLC not only provides separation and quantitative data but also has the ability to eliminate almost all interference problems (Garcia *et al.,* 2003).

Liquid chromatography (LC) is a method of chromatographic separation based on the difference in distribution of an analyte between two immiscible phases, in which the mobile phase is a liquid and percolates through a stationary phase, usually contained in a column. Although various terms, including high-speed LC, high-efficiency LC and high-pressure LC, have been used to describe LC, high-performance liquid chromatography (HPLC) is now the generally accepted terminology (Hamilton and Sewell, 1981).

In HPLC, a stationary phase is either coated onto a finely divided inert support or chemically bonded to a support material, contained within a stainless steel tube over which the mobile phase flows, thereby affecting the separation of individual components of a mixture (Raghavan and Joseph, 2002).

In HPLC, the sample to be analysed is dispersed into a mobile phase and the analyte(s) of interest pass through the stationary phase by pumping the mobile phase through the stationary phase using a solvent delivery module. As the analyte molecules pass through the column, there is constant interaction between the solute molecules, the stationary phase and the mobile phase. Separation of the various components of a mixture is reported to occur as a result of differences in

equilibrium of distribution of different solute molecules in the sample undergoing analysis (Snyder and Kirkland, 1979).

1.6.2.1 Principles of RP-HPLC

Two principle modes of HPLC, *viz.,* normal-phase HPLC (NP-HPLC) and reversed-phase HPLC (RP-HPLC) can be distinguished by the relative differences in the nature of the stationary phases used to effect a separation in addition to the corresponding mobile phase composition and differences in the nature of the interaction of functional groups present in solute molecules with these phases. The nature of the functional groups of a molecule dictate the selectivity and specificity of an interaction between an analyte and the column support material or mobile phase, leading to the selectivity and specificity of a separation (Raghavan and Joseph, 2002).

RP-HPLC entails the use of a hydrophobic bonded stationary phase with a mobile phase that consists of polar solvents such as water, with or without buffers or mixtures of water and water-miscible organic solvents such as methanol and acetonitrile. RP-HPLC is usually the first choice of method for use in most pharmaceutical applications and especially for the analysis of neutral or non-polar compounds that dissolve in water-organic solvent mixtures. Since the majority of pharmaceutical compounds of interest are relatively non-polar, most HPLC analyses in pharmaceutical research are carried out using RP-HPLC techniques (Pollack, 1987).

RP-HPLC is used extensively in various scientific fields *viz.,* pharmaceutical, agricultural and medical sciences, in addition to fundamental studies in the separation sciences. However, despite its extensive application, the retention mechanism of molecules in RP chromatographic process has not yet been fully elucidated. This is more than likely due to the complexity of RP-HPLC systems, the properties of which have been reported to change dynamically with the composition of the mobile phase and type(s) of the stationary phase used in separations. Nevertheless, some investigators have alluded to the fact that

separation in RP-HPLC may be due to either adsorption effects or partitioning of a solute between a stationary phase and a mobile phase, or combinations thereof (Snyder and Kirkland, 1979).

In HPLC, an internal standard (IS) is a compound added in equal amounts to all standards and test samples to be analyzed and is used to improve the accuracy of an analytical method by compensating for varying injection volumes and day-to-day instrumental changes. The physicochemical and analytical property of an ideal IS should be similar to those of the analyte of interest (Riley, 1996).

1.7 Clobetasol -17- propionate

Clobetasol propionate is has a chemical name of 21-chloro-9α-fluoro-11β,17α-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione-17-propionate (Sweetman, 2002). Has a molecular formula of $C_{25}H_{32}ClFO_5$ and its structural formula is depicted in the Fig 1.4.

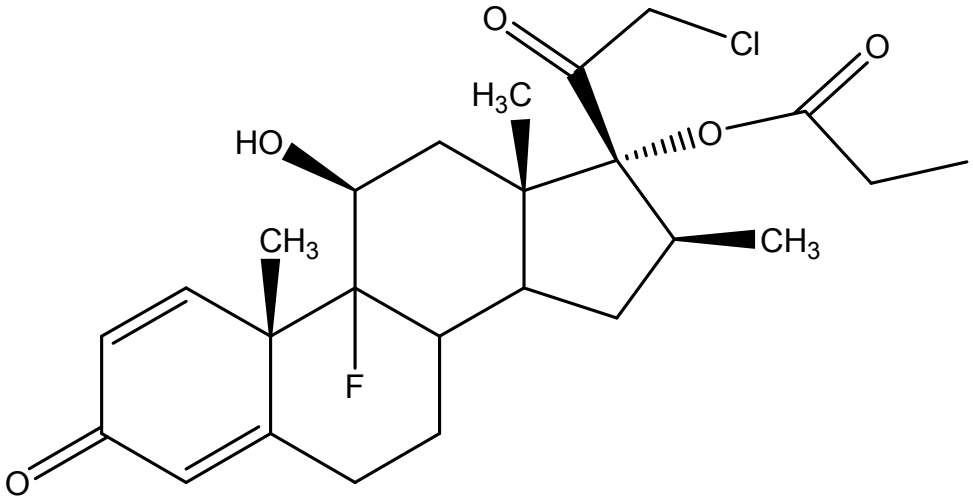


Fig.1.4: Chemical structure of Clobetasol 17-propionate

It is a class I or super-potent synthetic di-halogenated analogue of prednisolone and is 1800 times more potent than hydrocortisone when potency is measured using the human skin blanching assay. It is currently the most potent topical corticosteroid available on the market. Since 1973, clobetasol propionate has been used for the short-term treatment of patients with inflammatory and pruritic

manifestations of moderate-to-severe glucocorticoid-responsive dermatoses (Dyderski *et al.,* 2001). Clobetasol propionate occurs as a white, almost white or cream-colored, crystalline powder and is odorless (Dyderski *et al.,* 2001). The United States Pharmacopoeia (USP 2008) specifies that clobetasol propionate cream formulations should contain not less than 90.0% and not more than 115.0% of the labelled amount of clobetasol propionate.

1.8 Betamethasone -17- valerate

Betamethasone valerate is a white to practically white, odorless powder. It melts at 190 ^{0}C with decomposition. It is practically insoluble in water, freely soluble in acetone and in chloroform, soluble in alcohol, and slightly soluble in benzene and in ether.

It is a potent topical corticosteroid which exhibits anti-inflammatory and anti-allergic properties when applied to the skin and mucosa. The mechanism of action is related to causing vasoconstriction, stabilizing lysosomal membranes, suppressing cell division and the immune response.

It is chemically designated as: 9-fluoro-11β,21-dihydroxy-16β-methyl-3, 20-dioxop-regna-1,4-dien-17-pentanoate. It has a molecular formula of $C_{27}H_{37}FO_6$ and its structural formula is depicted in the Fig 1.5.

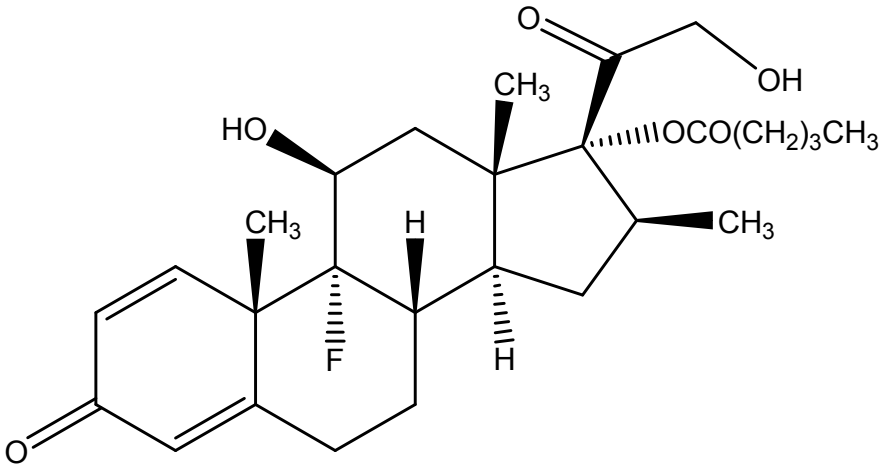


Fig. 1.5 Chemical structure of betamethasone -17-valerate

According to USP 2008 each gram of betamethasone valerate cream contains 1.2 mg betamethasone (equivalent to 1.0 mg betamethasone valerate) with mineral oil, white petrolatum, cetyl alcohol, stearyl alcohol, propylene glycol, phosphoric acid, sodium phosphate monobasic, sodium hydroxide, purified water and 4-chloro-m-cresol as a preservative.

The United States Pharmacopoeia (USP 2008) specifies that betamethasone valerate cream formulations should contain not less than 90.0% and not more than 110.0% of the labelled amount of betamethasone valerate.

1.9 Objectives

1.9.1 General Objective

The aim of this study was to investigate the quality of clobetasol-17- propionate and betamethasone -17- valerate topical corticosteroid cream products marketed in Addis Ababa depending on USP and BP specifications.

1.9.2 Specific Objectives

- To examine the presence or absence of the expected contaminant micro-organisms in the cream products.

- To identify the specified active ingredients in the dosage forms using thin layer chromatography (TLC) technique.

- To quantify the extract of the creams using reversed phase high performance liquid chromatography (RP-HPLC) technique.

2. EXPERIMENTAL

2.1 Materials and methods

2.1.1 Materials

2.1.1.1 Tested Samples

Products (clobetasol-17-propionate and betamethasone-17-valerate topical corticosteroid creams) sampled for the study were those having a remaining shelf life of at least one year (as claimed by the manufacturers) at the time of sampling. The drug products were sampled from different retail outlets in Addis Ababa.

2.1.1.2 Chemicals and reagents

USP reference standards of clobetasol -17-propionate (% purity: 98.85%, A.R. No. R/503/2008, manufacturing date 06/2008 and expiry date 05/2010), betamethasone-17-valerate (B.No. / Q.C Ref. No. 2109VMOOO80422; assay on as 99.05% and expiry date 04/06/2010) and beclomethasone dipropionate (Batch No. Y8072001; Potency 99.54%; P.R. China, manufacturing date October 22, 2008) were obtained from Drug Administration and Control Authority (DACA) of Ethiopia and were used as received.

Acetonitril (Shcarlau Chemie S.A., Spain) methanol and ethanol (Fischer Scientific UK), dihydrate sodium biphosphate (Reagent Chemical Service, USA), hydrochloric acid (Sigma Aldrich Laboratory, England), acetone, chloroform, anhydrous sodium sulphate, sodium hydroxide, toluene, ethyl acetate, sodium chloride and distilled water (in-house supply: EPHARM), were used as received. All reagents used for the assay were of HPLC grade.

2.1.1.3 Equipment and instruments

Analytical balance [SCAALTEC®, SBC31, Germany], pH meter [Metteler Toledo inlab® expert PLC, Switzerland], thermostatic water bath [GFL1092, West Germany], and HPLC with ultraviolet-visible detector (Shimadzu, Class VP, Japan) equipped with degasser unit (DGU-20As), pumping system (LC-20AT), auto sampler unit (SIL-20A), Ultraviolet-visible detector (SPD-20AV), communication

bus module (CBM-20A), personal computer installed with class VP software for data integration and analysis were used. Table-2.1 and Table-2.2 provide the list of the drug products included in the survey.

Table 2.1: List of clobetasol propionate (CP) products studied. The claimed amount of each tube of the products was 25 g cream.

Batch No	Expiratory date (Month/year)	Manufacturer
7608	07/10	GlaxoSmithKline (England)
9048	08/10	GlaxoSmithKline (England)
083564	10/10	GlaxoSmithKline (England)
0398	09/10	GlaxoSmithKline (England)
388851	12/10	GlaxoSmithKline (England)
0028	11/10	GlaxoSmithKline (Saudi Arabia)
392946	12/10	GlaxoSmithKline (Saudi Arabia)
409560	03/10	GlaxoSmithKline (Saudi Arabia)
7678	07/10	GlaxoSmithKline (Saudi Arabia)
393961	12/10	GlaxoSmithKline (Saudi Arabia)
2543	19/10	Zygpharma Pvt.Ltd (India)
0167	10/10	Zygpharma Pvt.Ltd (India)
1490	21/10	Zygpharma Pvt.Ltd (India)
1117	05/10	Hoe Pharmaceuticals (Malaysia)
0043	02/10	Hoe Pharmaceuticals (Malaysia)
8709	11/10	Hoe Pharmaceuticals (Malaysia)
5541	24/10	Pharma Inkl (Jordan)
0189	27/10	Pharma Inkl (Jordan)
2233	29/10	Pharma Inkl (Jordan)

Table 2.2: List of betamethasone valerate (BV) products studied. The claimed amount of each tube of the products was 30 g cream.

Batch No	Expiratory date (Month/year)	Manufacturer
7668	07/10	GlaxoSmithKline (England)
7668	07/10	GlaxoSmithKline (England)
0029	09/10	GlaxoSmithKline (England)
5789	10/10	GlaxoSmithKline (England)
276842	11/10	GlaxoSmithKline (England)
345687	10/10	GlaxoSmithKline (Saudi Arabia)
239946	09/10	GlaxoSmithKline (Saudi Arabia)
4395	07/10	GlaxoSmithKline (Saudi Arabia)
490321	08/10	GlaxoSmithKline (Saudi Arabia)
15340	08/10	GlaxoSmithKline (Saudi Arabia)
5243	09/10	Zygpharma Pvt.Ltd (India)
6701	16/10	Zygpharma Pvt.Ltd (India)
1220	30/10	Zygpharma Pvt.Ltd (India)
5110	15/10	Hoe Pharmaceuticals (Malaysia)
6673	25/10	Hoe Pharmaceuticals (Malaysia)
781	14/10	Hoe Pharmaceuticals (Malaysia)
111541	20/10	Pharma Inkl (Jordan)
01844	26/10	Pharma Inkl (Jordan)
2003	28/10	Pharma Inkl (Jordan)

2.1.2 Methods

2.1.2.1 Study strategy

A total of thirty eight cream tubes (clobetasol propionate and betamethasone valerate) were included in the study. All products were subjected to the quality testing recommended by the USP 2008 guideline for microbiological, identification (qualitative) and assay (quantitative) tests. Samples of the creams were kept at room temperature and all the tests were carried out before their expiry dates. In

this study the TLC plate consisted of a thin plastic sheet coated with a thin layer of silica gel as an adsorbent which were received from EPHARM.

2.1.2.2 Preparation of HPLC mobile phase

Mobile phase for clobetasol propionate analysis was prepared by adding (95:85:20) parts by volume of 0.05M $NaH_2PO_4.H_2O$ and methanol. It was filtered through a 0.45 µm millipore filters and degassed under vacuum before use.

The mobile phase for betamethasone valerate analysis was prepared by adding (3:2) parts by volume of HPLC-grade acetonitril and distilled water. It was filtered through a 0.45 µm millipore filter and degassed under vacuum before use.
Table 2.3 displays the chromatographic conditions.

Table 2.3 Chromatographic conditions for the analysis of clobetasol propionate (CP) and betamethasone valerate (BV)

	Clobetasol propionate	**Betamethasone valerate**
Column	Uv-vis detector (Shimadzu)	Uv-vis detector(Shimadzu)
	4.6 mm x 15 cm ID	4 mm x 30 cm ID
Mobile phase	$NaH_2PO_4.H_2O$:NaOH:CH_3OH	CH_3CN:H_2O (3:2) % V/V
	(95:85:20) % V/V/V	
Flow rate	1.0 ml/min	1.2 ml/min
Column pressure	1300 psi	1300 psi
Column temperature	Ambient (25°)	Ambient (25°)
Injection volume	10 µl	10 µl
Wave length	240 nm	254 nm

ID: Internal Diameter; psi: pound per square inch.

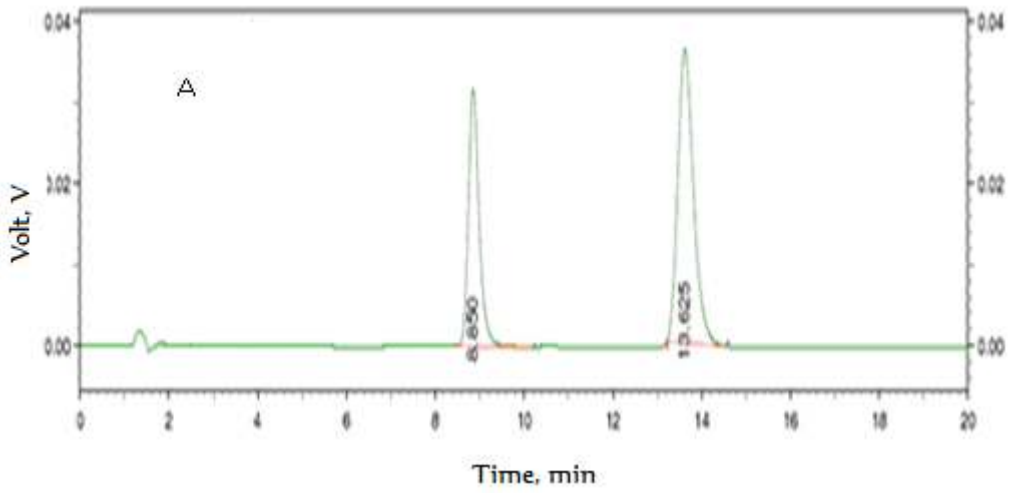
2.1.2.3 Construction of calibration curves

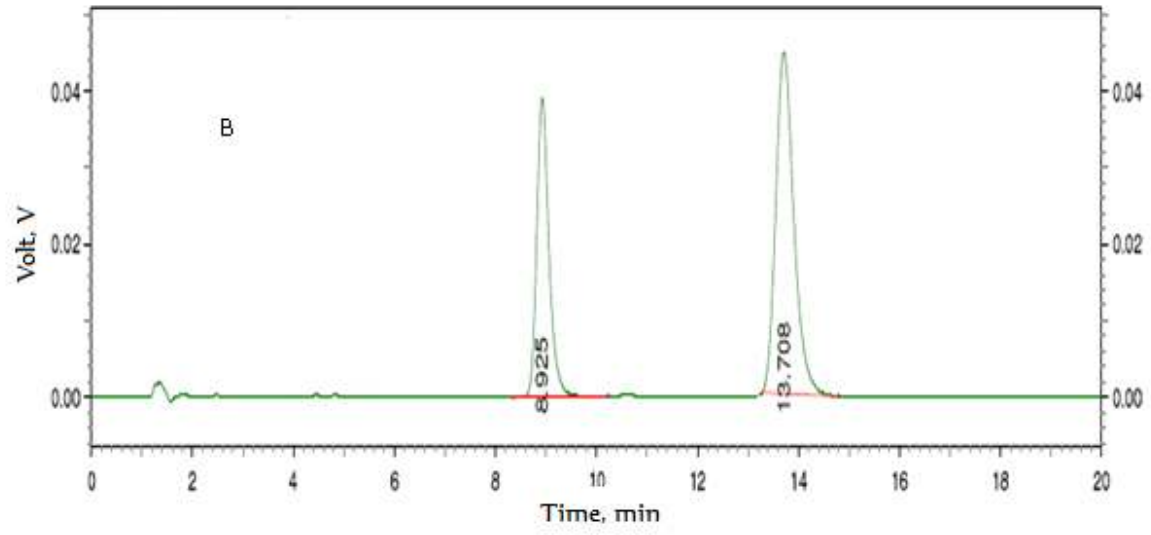
2.1.2.3.1 Clobetasol propionate

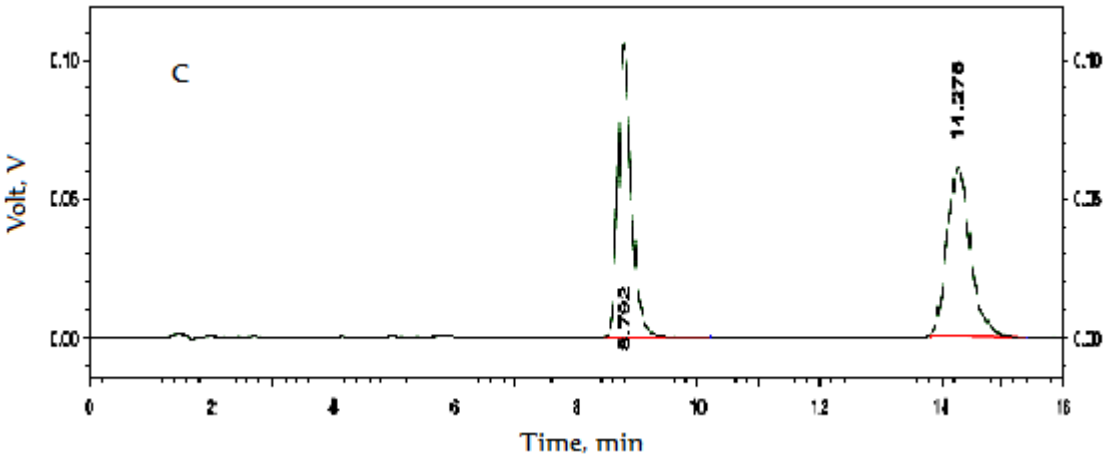
Standard stock solution of clobetasol propionate (0.048 mg/ml) and beclomethasone dipropionate (0.096 mg/ml) were prepared by accurately weighing and mixing 24 mg of clobetasol propionate and 48 mg beclomethasone

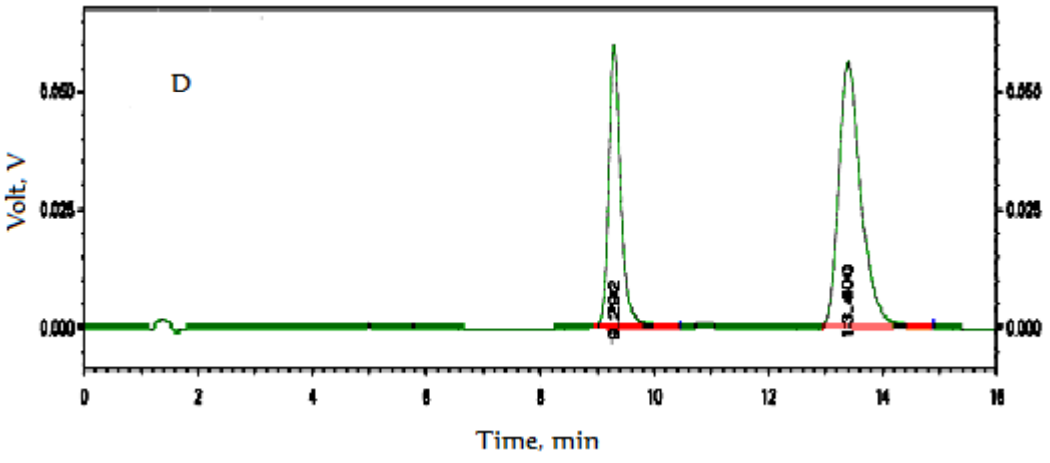
dipropionate in 200 ml of methanol in a 500 ml volumetric flask. The stock solution was placed in an ultrasonic bath for 10 min in order to ensure complete dissolution of the drug after which it was made up to volume with methanol. Five calibration standards of clobetasl propionate (0.034, 0.037, 0.04, 0.044, and 0.048) and beclomethasone dipropionate (0.069, 0.075, 0.08, 0.089, and 0.098) were prepared by serial dilution of the stock solution by methanol. Then the mixtures were filtered through a millipore filter (0.45 µm) before injection.

Triplicate injections of five concentrations of clobetasol propionate (0.034 -0.048) mg /ml and beclomethasone dipropionate (0.069 - 0.098) mg/ml, were used to construct the calibration curve. Representative chromatograms are depicted in the Figure 2.1 A-E.




B
Volt, V
0.04
0.02
0.00
8.925
13.708
0
2
4
6
8
10
12
14
16
18
20
Time, min


C
Volt, V
8.792
11.276
Time, min


D
Volt, V
8.292
13.400
Time, min

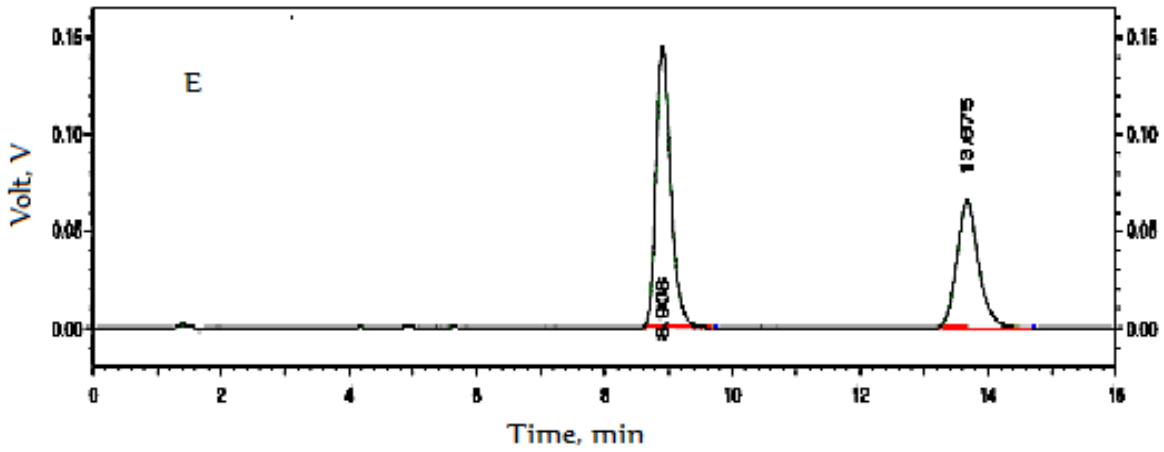


Fig 2.1 A-E Representative chromatograms of clobetasol propionate reference standard

The calibration curve (Figure 2.2) shown below shows a high degree of linearity as demonstrated by the r^2 values that were obtained, 0.9993. The equation for the regression line was found to be *Y* = 2E + 07X – 34002, where Y is the absorbance and X is the concentration in mg/ml. Such linearity confirmed the suitability of the method for the study (i.e., the calibration curve can be used directly to ascertain the mass in mg of the analyte; clobetasol propionate in the drug products).

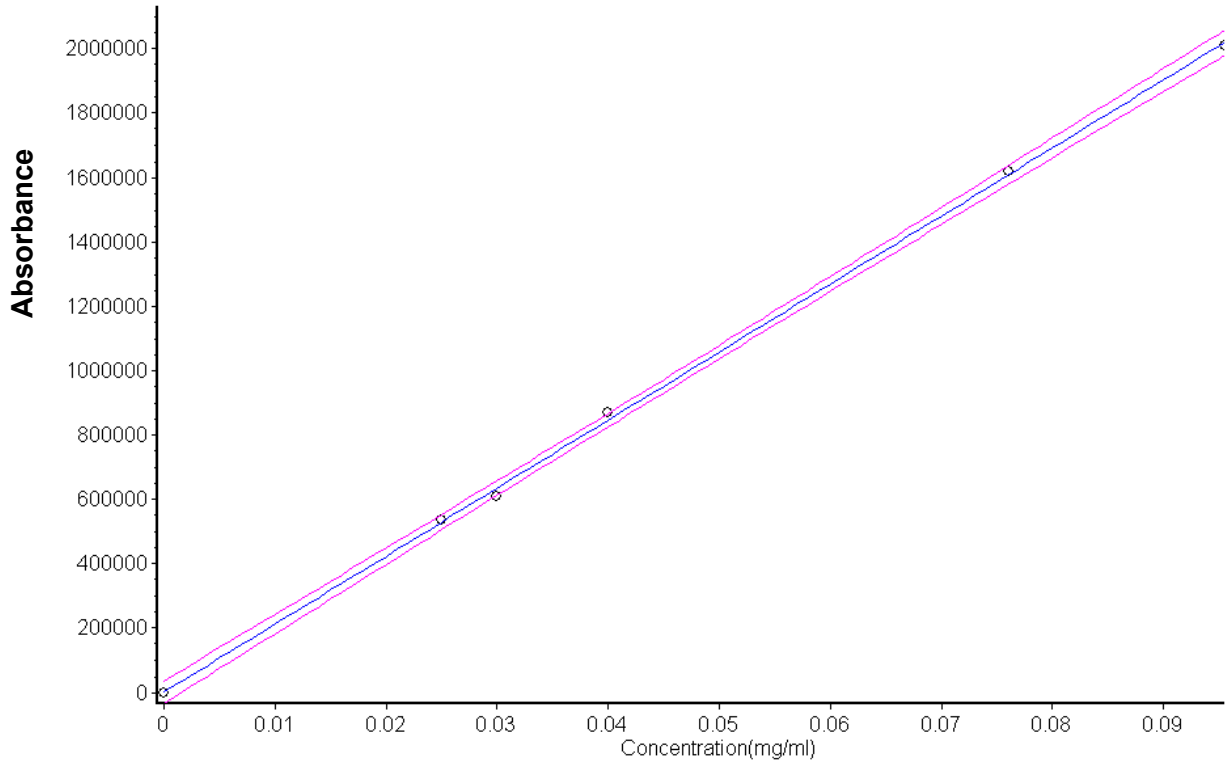
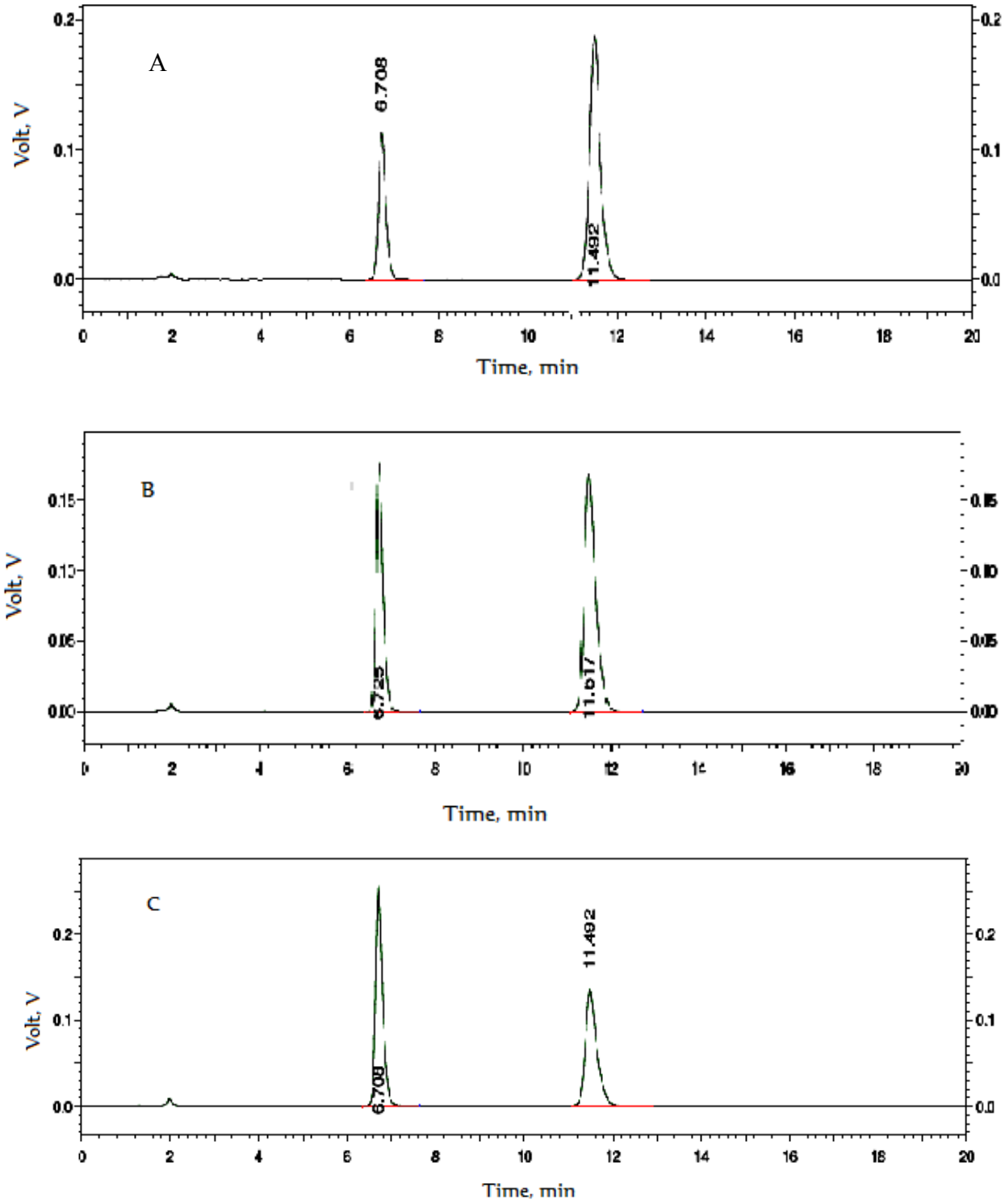


Fig.2.2 Calibration curve of clobetasol propionate reference standard in methanol in the range of 0.034 to 0.048 mg/ml at 240 nm.

2.1.2.3.2 Betamethasone valerate

Standard stock solution of betamethasone-17-valerate (0.24 mg/ml) and beclomethasone dipropionate (0.64 mg/ml) were prepared by accurately weighing and mixing 36 mg of the reference standard and 48 mg of the internal standard in a 50 ml of 1 in 1000 glacial acetic acid in methanol solution. Five different concentrations of betamethasone valerate (0.17, 0.18, 0.2, 0.22, 0.24) and beclomethasone dipropionate (0.46, 0.49, 0.53, 0.58, 0.64) were prepared by serial dilution of the stock solution with methanol. Each solution was placed in ultrasonic water bath for 15 minutes to ensure complete dissolution of the drug.

Triplicate injections of the five concentrations of betamethasone valerate (0.17-0.24) mg/ml and beclomethasone dipropionate (0.46-0.64) mg/ml were used to construct the calibration curve. Representative chromatograms are indicated in the Figure 2.3 A-E.


A
6.708
11.492
Volt, V
Time, min
B
6.725
11.617
Volt, V
Time, min
C
6.708
11.492
Volt, V
Time, min

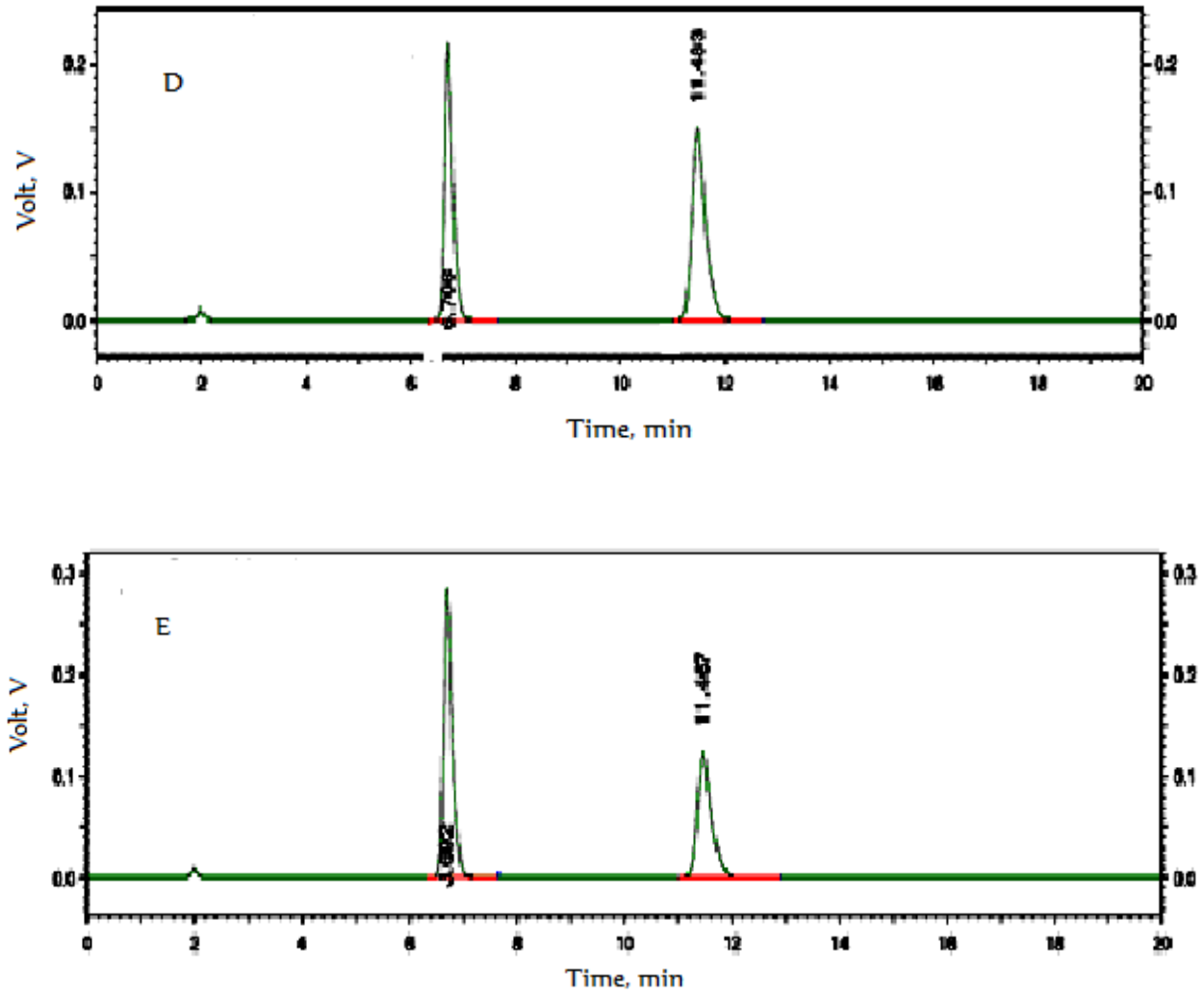


Fig 2.3 A-E Representative chromatograms of betamethasone valrate reference standard

The calibration curve (Figure 2.4) shown below has again a high degree of linearity as demonstrated by the r^2 values that were obtained, 0.9996. The equation for the regression line was found to be *Y* = E + 07X + 22482, where Y is the absorbance and X is the concentration in mg/ml. Such linearity confirmed the suitability of the method to ascertain the mass in mg of the analyte (betamethasone valerate) in the cream formulations.

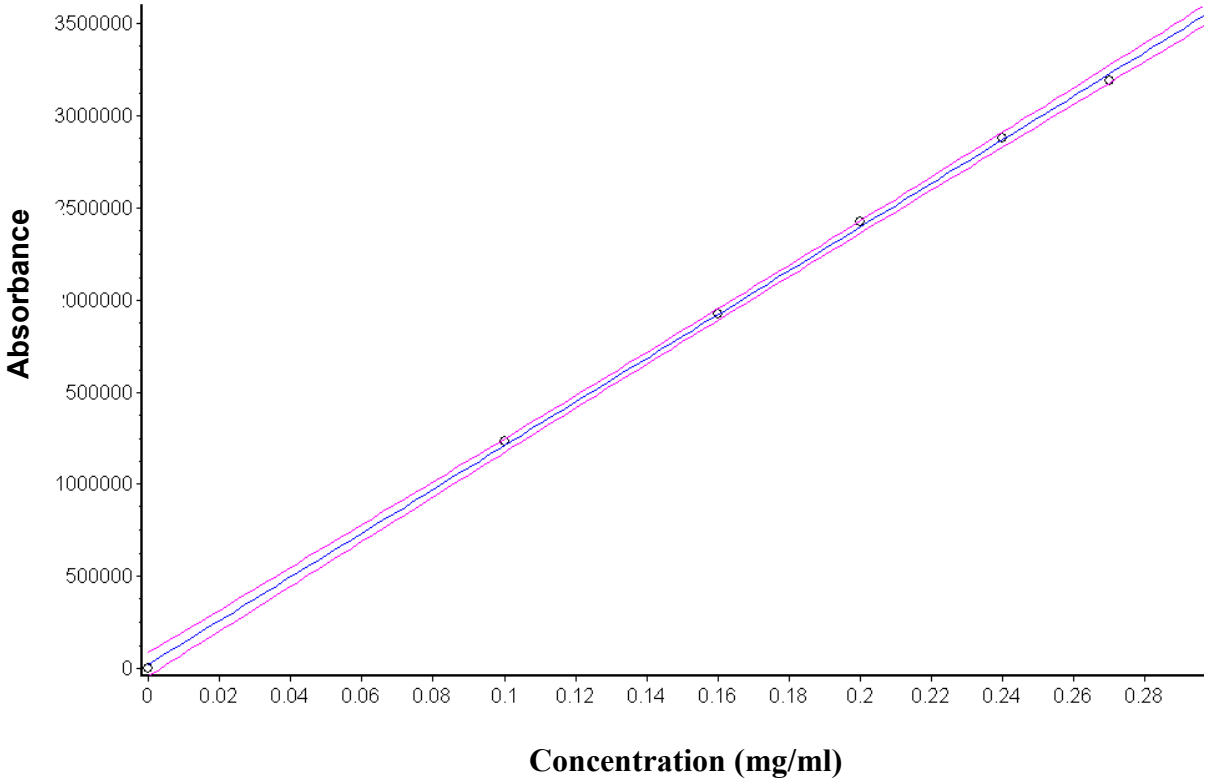


Fig. 2.4 Standard calibration curve of betamethasone valerate reference standard in methanol in the range of 0.17 to 0.24 mg/ml at 254 nm

2.1.2.4 Microbiological limit test: *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Microbial contamination of cream was determined by thinly spreading a loopful of material withdrawn from the depths of the bulk product. From the sealed tubes of clobetasol propionate and betamethasone valerate creams 10 g of each was taken. The fluid soybean-casein digest medium (100 ml) was taken and autoclaved at a temperature of 121 ^{O}C for 15 minutes. Cetrimide agar (4.47 g) and mannitol agar (10.8 g) were taken and melted on a hot plate above a temperature of 45 ^{O}C followed by the inoculation of the broth and the agar mediums on a sterilized petridish using an inoculating tube and incubated at a temperature between 30-35 ^{O}C. The growth of anticipated microorganisms was checked after 24, 48, 72, 96 and 120 hrs.

2.1.2.5 Physical stability

Clobetasol propionate and betamethasone valerate

Any discoloration or other physical change (if any) was noted by viewing against a white background in normal day light. The odor and clarity of the creams were also examined.

2.1.2.6 Identification test using thin-layer chromatography

2.1.2.6.1 Identification test for clobetasol propionate

The mobile phase used was a mixture of toluene - ethyl acetate (1:1) v/v which was filtered, degassed and sonicated before use.

1.5 gm clobetasol propionate cream (or equivalent to 0.75 mg CP accurately weighed) was transferred to a centrifuge tube in which 10 ml CH_3OH was added and heated in a water bath (60 ^{0}C) for 4 min.

The plastic-stoppered centrifuge tube was removed from the water bath and repeatedly heated and shaken vigorously; then, 3.5 ml water was added and centrifuged at 3500 rpm for about 10 min followed by the addition of 5 ml of the supernatant into a 100 ml separator. Then 1 gm NaCl and 10 ml H_2O were added and mixed.

The samples were dissolved in 5 ml of chloroform and shaken for one min followed by evaporation of the samples to dryness then the residues were dissolved in 0.5 ml chloroform to provide a concentration of 1.5 mg/ml. The standard was also prepared by dissolving 0.75 mg of clobetasol propionate in 0.5 ml chloroform to provide a concentration of (1.5 mg/ml).

The standard and the test with the same concentration (1.5 mg/ml) were spotted on TLC plate and kept in the chamber until the solvent front moved 3/4th (13.5 cm) of the length of the plate (18 cm) and then removed from the developing chamber, dried in a hood and the spots were observed under UV radiation and photographs was taken by a digital camera.

2.1.2.6.2 Identification test for betamethasone valerate

The mobile phase used was a mixture of chloroform–acetone–ethanol (100:10:5) v/v/v in which it was degassed and sonicated before use.

2.0 gm betamethasone valerate cream (or equivalent to 2.0 mg betamethasone valerate accurately weighed) was transferred to a separator; 20 ml of water and 2 ml dilute HCl (1 in 120) were added and mixed.

The prepared samples were extracted with four 50 ml portions of chloroform and the extracts were combined; filtered through a cotton pledget previously layered with anhydrous sodium sulphate (Na_2SO_4) and the filtrates were evaporated on a boiling water bath. The residues were dissolved in 2 ml of ethanol (96%) v/v in which the standard and the test solutions having the same concentration (1 mg/ml) in the same solvent were prepared.

The standard and test with the same concentration (1.0 mg/ml) were spotted and kept in the developing chamber until the solvent front moved $3/4^{th}$ (13.5 cm) of the length of the plate (18 cm) thereby removed from the developing chamber and dried in a hood and the spots were observed under UV radiation.

2.1.2.7 Assay

2.1.2.7.1 Assay of clobetasol propionate

2 g of cream (or equivalent to 1 mg of CP) was weighed and transferred to a 50 ml volumetric flask; 10 ml of beclomethasone dipropionate; internal standard solution and 15 ml methanol (CH_3OH) were added and shaken vigorously to disperse the cream followed by centrifuging at about 3500 rpm for 10 min. The standard and the supernatant were filtered through a 0.45 µm filter and injected with 10 µl volumes.

The quantity in mg of CP was calculated using the formula: 25C (Ru/Rs)
Where:

C= concentration of clobetasol propionate reference standard = 0.04 mg/ml

Rs = peak area ratio of clobetasol propionate to beclomethasone dipropionate in the standard preparations

Ru = peak area ratio of clobetasol propionate to beclomethasone dipropionate in the assay preparations

2.1.2.7.2 Assay of betamethasone valerate

2.5 g of cream (or equivalent to 2.5 mg of betamethasone valerate) was weighed and transferred to a 50 ml centrifuge tube; 10 ml of internal standard solution (0.4 mg/ml) and 5 ml of glacial acetic acid in methanol (1 in 1000) were added. The tube was placed in a water bath (60 ^{0}C) until the specimen melts and shaken repeatedly to have more active ingredient from the cream.

The tube was placed in an ice- methanol bath for 20 min. and centrifuged; the supernatant was transferred in to a vial and injected with an injection volume of 10 μl.

The quantity in mg of betamethasone valerate was calculated using the formula:

(392.46/476.59)(15C)(Ru/Rs)

Where:

C = concentration of betamethasone valerate reference standard (0.2 mg/ml)

Rs = peak area ratio of (betamethasone valerate to beclomethasone dipropionate in the standard preparations

Ru = peak area ratio of (betamethasone valerate to beclomethasone dipropionate in the assay preparations 392.46 and 476.59 are the molecular weights of betamethasone valerate and beclomethasone dipropionate, respectively.

3. RESULTS AND DISCUSSION

3.1. Microbiological limit test: Bacterial *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Contamination from micro-organisms is a big problem for all formulations containing moisture. *Pseudomonas aeruginosa* and *Staphylococcus aureus*, are one of the highly toxic to mammalian cells and are the most potent modulators of the immune system (Grandics, 2000).

The warm and rather humid climatic conditions that prevail in most tropical countries would tend to support the survival and growth of many microorganisms. In a situation whereby nutritionally rich pharmaceutical cream products are severely contaminated, rapid growth and multiplication would be expected. This could lead to biodegradation of the product and hence the risk of infection to consumers of the product (Bos *et al.* 1989).

Considering the above reasons, the absence or presence of the mentioned micro-organisms has to be checked and was performed in accordance with the USP 2008 procedures mentioned above.

The contents of each petridish containing clobetasol propionate and betamethasone valerate creams was examined after 24, 48, 72, 96 and 120 hrs and neither of them (Figure 3.1) formed greenish *Pseudomonas aeruginosa* on a centrimide agar medium nor yellowish *Staphylococcus aureus* on mannitol-salt agar medium.

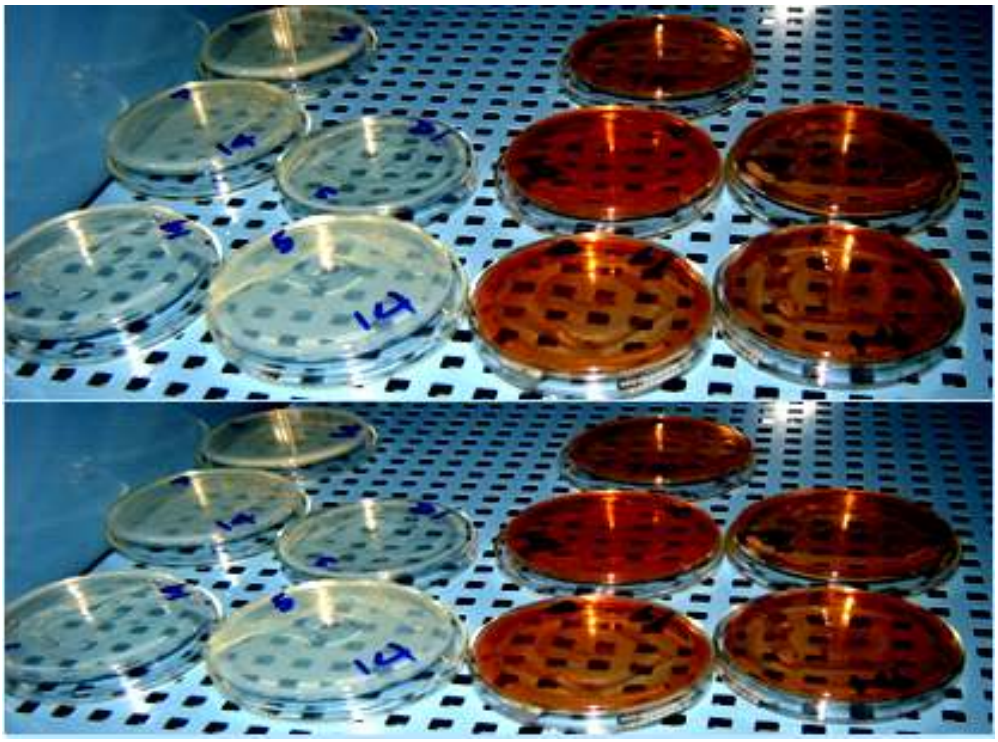


Fig.3.1 Representative microbiological limit test results for *Staphylococcus aureus* (white) and *Pseudomonas aeruginosa* (red) incubated for five days

The qualitative tests showed that all the surveyed cream products fulfilled the USP 2008 microbiological limit test. These results may be because of the tubes are tightly closed and the packaging materials are strong enough to prevent the passage moisture into the finished cream products.

3.2 Physical stability test: Evaluation physical appearance

The appearance and lack of any change in a physical sense are important as the patient only observes the exterior and any change from the original position may frighten or bother him/her. Some physical changes can have deleterious effects too. So, it is absolutely essential to check and test the physical stability of all formulations in which instability is likely to occur (Lippicot *et al*, 2005).

Emulsions are thermodynamically unstable since the two immiscible phases that exist in close conjunction impart a positive interfacial free energy to the emulsion

system. As a consequence of the positive interfacial free energy, the two immiscible liquids will always tend to divide into their separate components in an attempt to achieve thermodynamic equilibrium (Eccleston, 1997).

In this study, the orgnoleptic properties (appearance, color and odor) of the cream products was performed and the surveyed products retained their original organoleptic properties thereby complied with the USP 2008 specifications.

Quality problems in the mentioned property were not observed may be because of the emulsifying agents used overcome the interfacial free energy between the phases so that the products become stable. The other contributors may be an optimum temperature in their storage areas and the packaging materials may also an important role by protecting the entrance of moisture, which may cause the degradation of the cream products.

3.3 Clobetasol propionate

3.3.1 Identification test

As the USP 2008 addresses, a qualitative test for the presence or absence of the claimed active ingredient in pharmaceutical cream has to be checked.

After the TLC-plate was developed in the developing chamber, the eluted spots on the plate (Figure 3.2) were examined under short- wave length (UV) radiation at 254 nm and almost all the primary spots of the extracts (which were encircled with a pencil) have same retention factor (Rf) value with the reference standard.

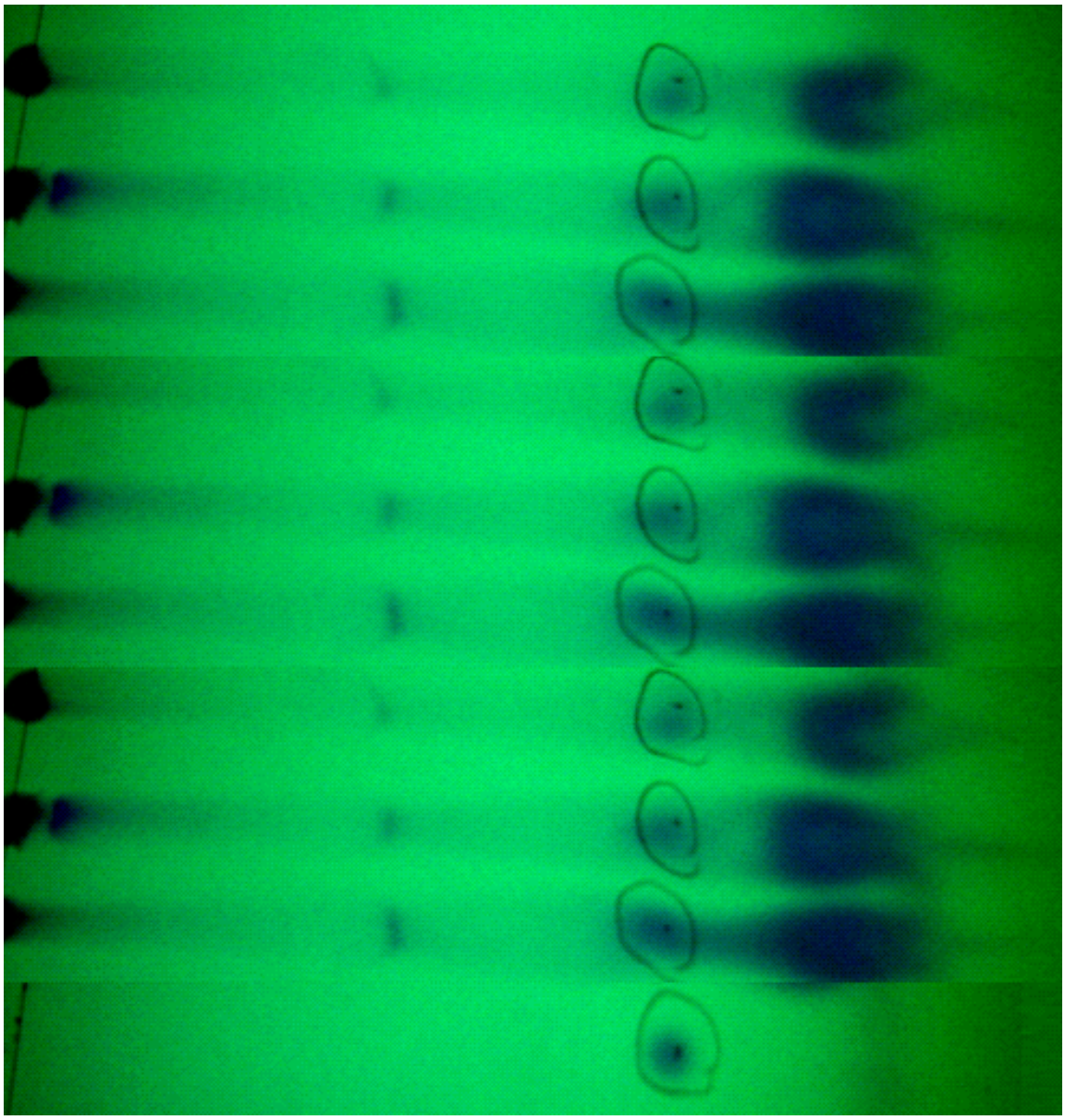


Fig.3.2 Representative TLC identification test results of clobetasol propionate cream extracts observed under UV (254 nm) radiation

3.3.2 Retention factor (Rf) values

The retention factor (Rf) values of clobetasol propionate was determined by measuring the distance moved by the analyte and compared to that of the distance moved by the solvent front and the obtained results are indicated in the Table 3.1 below.

Table 3.1 Identification test for clobetasol propionate cream

Manufacturer	Distance moved by the sample (cm)	Rf-Value	Retention factor (%)
GlaxoSmithKline (England)	7.5	0.560	56.0
GlaxoSmithKline (England)	7.5	0.560	56.0
GlaxoSmithKline (England)	7.5	0.560	56.0
GlaxoSmithKline (England)	7.5	0.560	56.0
GlaxoSmithKline (England)	7.5	0.560	56.0
GlaxoSmithKline (Saudi Arabia)	7.5	0.560	56.0
GlaxoSmithKline (Saudi Arabia)	7.5	0.560	56.0
GlaxoSmithKline (Saudi Arabia)	7.5	0.560	56.0
GlaxoSmithKline (Saudi Arabia)	7.5	0.560	56.0
GlaxoSmithKline (Saudi Arabia)	7.5	0.560	56.0
Zygpharma Pvt.Ltd (India)	7.4	0.550	55.0
Zygpharma Pvt.Ltd (India)	7.4	0.550	55.0
Zygpharma Pvt.Ltd (India)	7.4	0.550	55.0
Hoe Pharmaceuticals (Malaysia)	7.5	0.560	56.0
Hoe Pharmaceuticals (Malaysia)	7.5	0.560	56.0
Hoe Pharmaceuticals (Malaysia)	7.5	0.560	56.0
Pharma Inkl (Jordan)	7.6	0.563	56.3
Pharma Inkl (Jordan)	7.6	0.563	56.3
Pharma Inkl (Jordan)	7.6	0.563	56.3

As can be seen from the above table (Table 3.1) the retention factor for the standard was found 0.560 and those of the extracts were 0.550, 0.560 and 0.563, which closer to the reference standard. The Rf values of the extracts showed that the surveyed tubes contain the expected active ingredient, clobetasol propionate.

3.3.3 Chemical assay

The USP 2008 specifies that CP cream formulations should contain not less than 90.0% and not more than 115.0% of the labeled amount of CP. As a consequence, it was considered essential to assay CP content in all formulations in order to

ensure that the formulations manufactured contained CP within this specified range.

Using USP 2008 procedures mentioned above; the expected amounts of residue (1 mg) of clobetasol propionate were dissolved in 25 ml of methanol to have identical concentrations of the standard and the extracts (0.04 mg/ml). Triplicate injections of the reference standard and each extracts were applied and chromatograms were obtained. Representative reference standard chromatogram (Figure 3.3) and that of the extracts (Figure 3.4 A-E) are shown below.

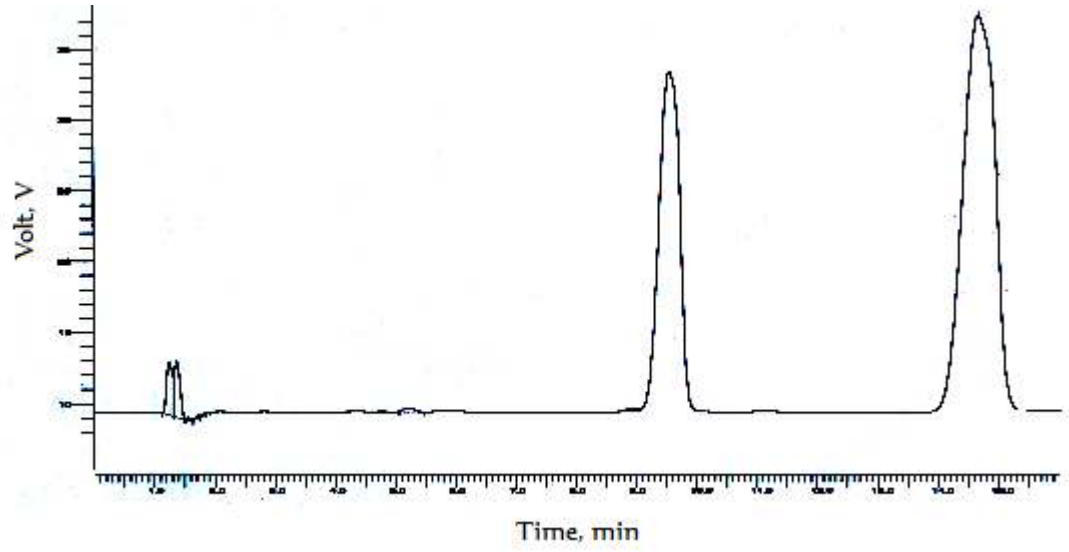
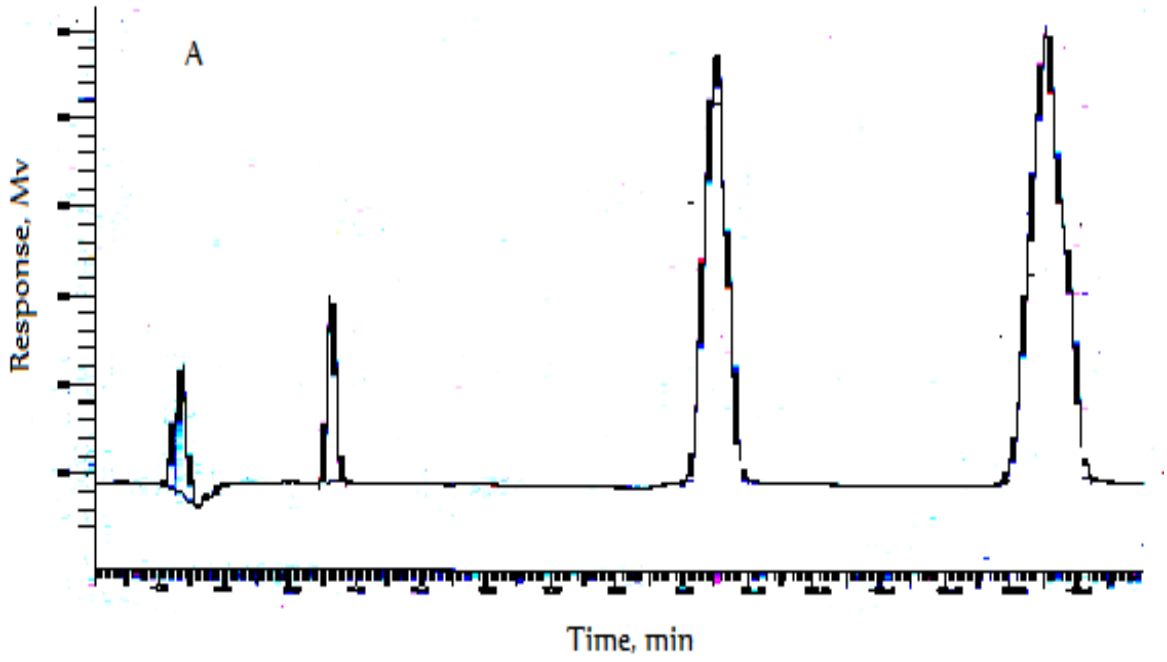
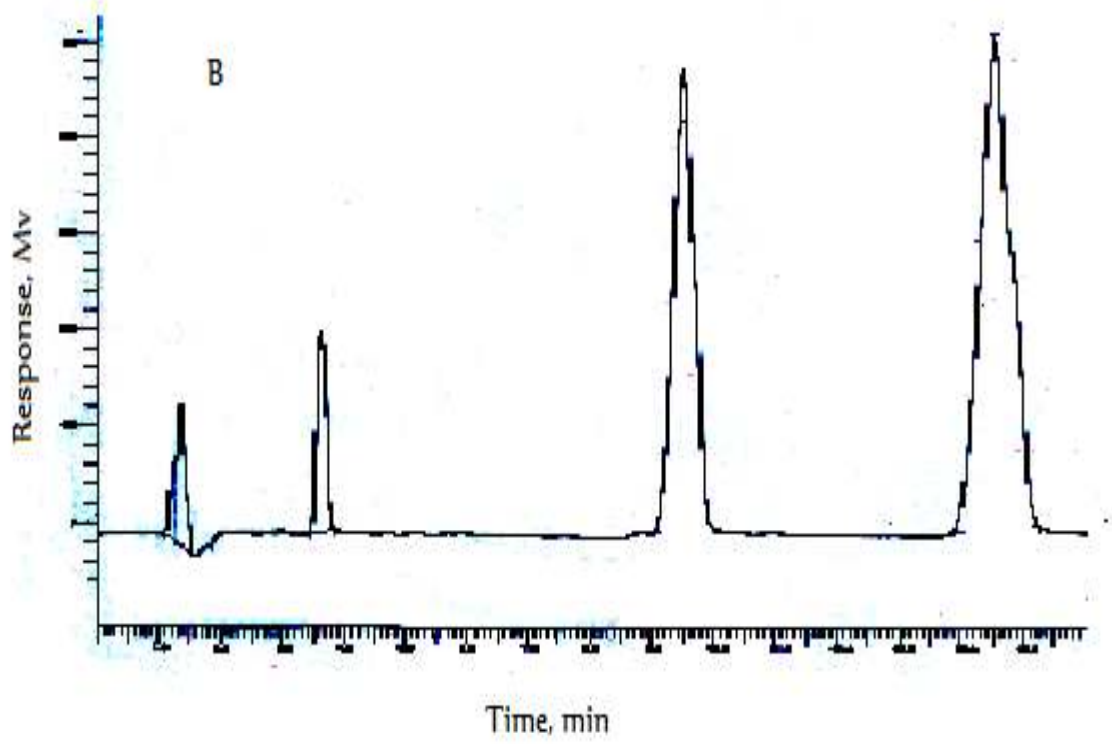
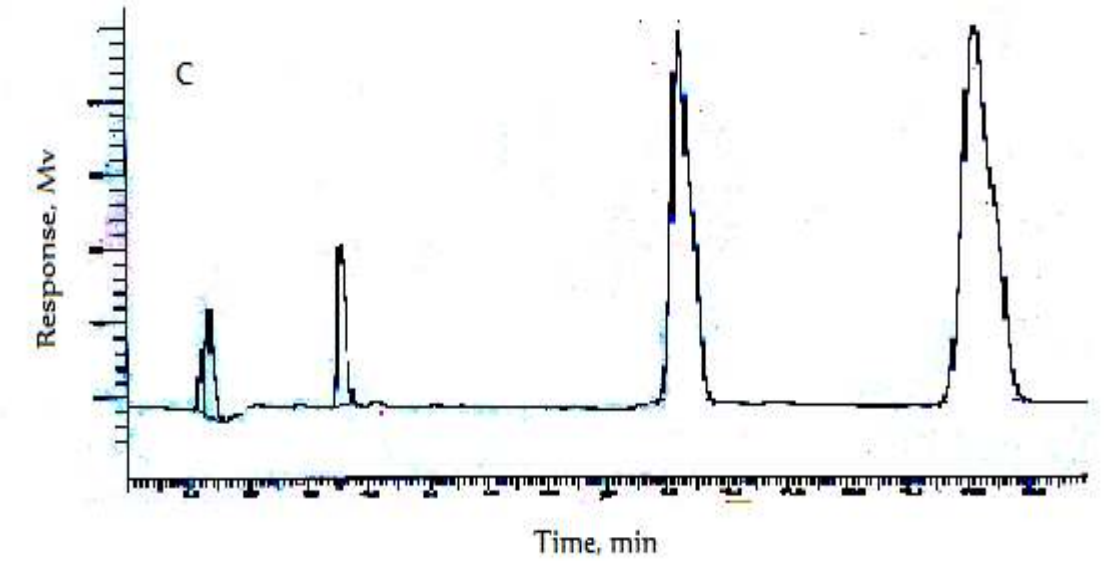
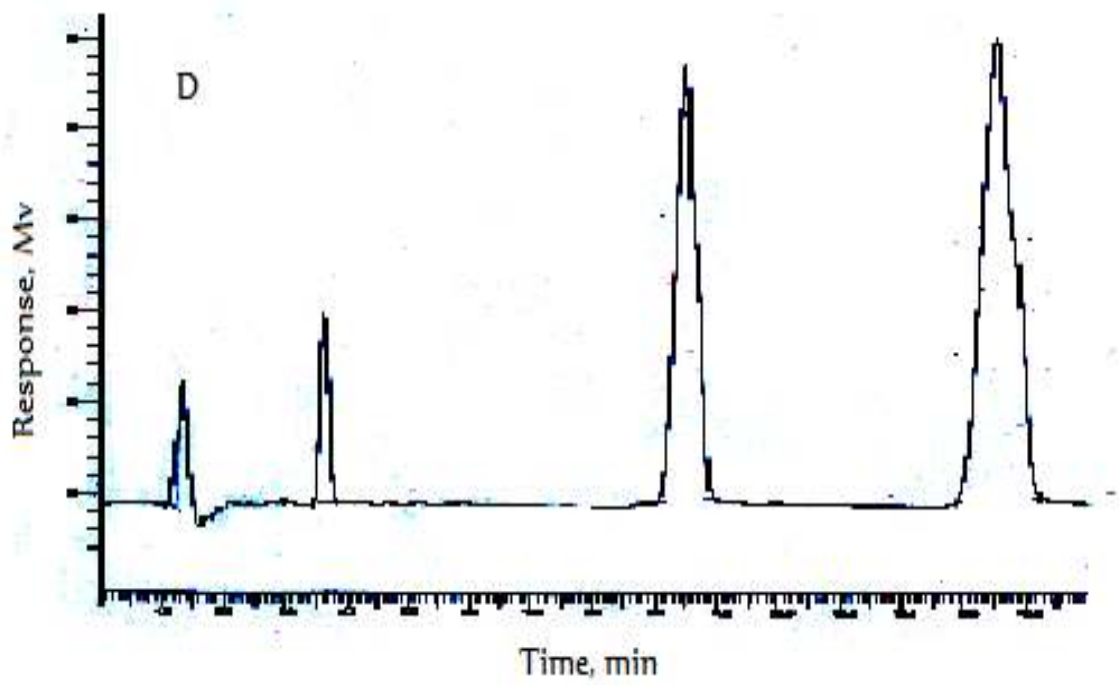


Fig.3.3 Representative chromatogram of clobetasol propionate reference standard at 0.04mg /ml concentration


A
Response, Mv
Time, min


B
Response, Mv
Time, min


C
Response, Mv
Time, min


D
Response, Mv
Time, min

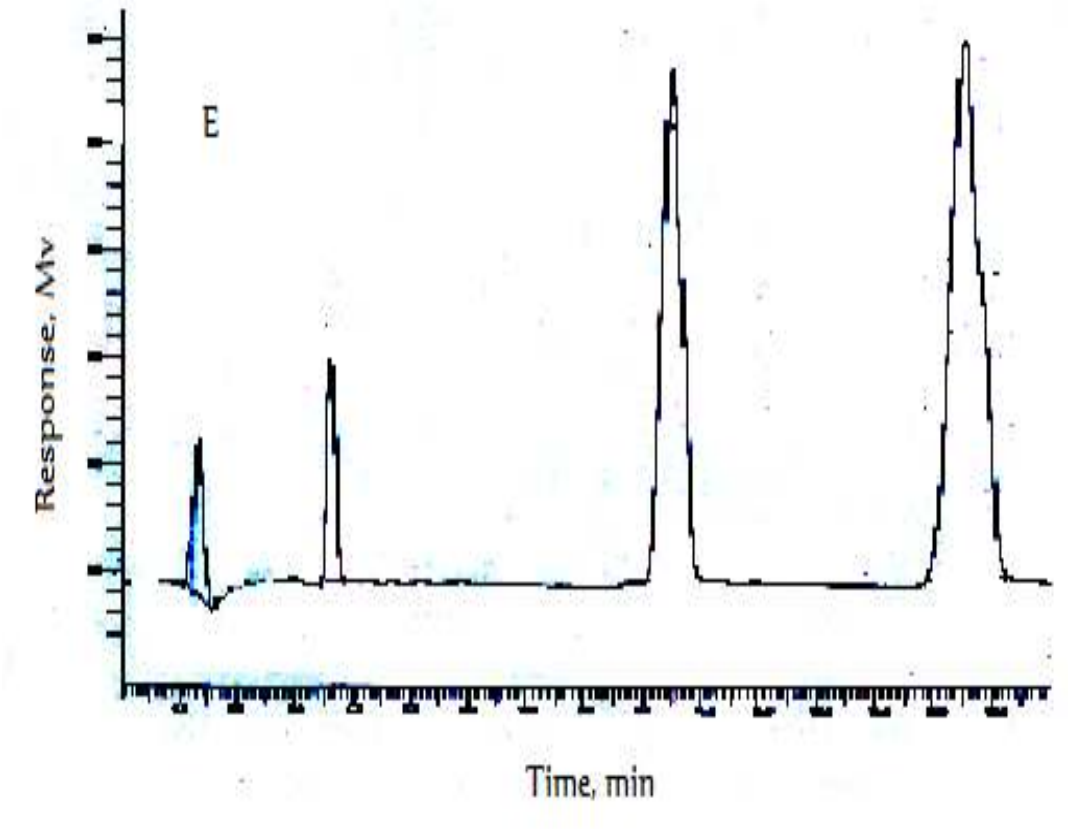


Fig. 3.4 A-E Representative chromatograms of clobetasol propionate extract at 0.04 mg /ml concentrations

As can be seen from the above representative chromatograms of the cream extracts (Fig.3.4 A-E), they comprised an additional peak (first peak) which was not observed in the reference standard (Fig.3.3).The additional peak in the chromatograms of the extracts with the smallest retention time is due to an excipient in the creams. This by itself is an indication that creams contain excipients for stabilizing and retaining organoleptic properties.

Average peak areas of clobetasol propionate and beclomethasone dipropionate standards were 1069001.64 and 1927226.6 respectively and the Rs (peak area ratio of clobetasol propionate to beclomethasone dipropionate in the reference standard was calculated as: Rs =1069001.64 / 1927226.66 = 0.5547. The concentration of clobetasol propionate was 0.04 mg/ml.

The quantity in mg of clobetasol propionate was calculated by using the formula: 25C (Ru/Rs) = 25(0.04) (Ru/0.5547) = 1.80Ru.Where Ru is peak area ratio of clobetasol propionate to beclomethasone dipropionate in the assay preparation.

Table 3.2 Assay of clobetasol propionate cream

Manufacturer	Peak area of		Ru	Mass of Cp (mg)	Mass of Cp (%)
	clobetasol propionate	Beclomethason dipropionate			
GlaxoSmithKline (England)	1081778.49	1742414.60	0.6208	1.117	111.70
GlaxoSmithKline (England)	1098947.57	1800962.90	0.6102	1.098	109.80
GlaxoSmithKline (England)	1036274.80	1622270.32	0.6388	1.149	114.90
GlaxoSmithKline (England)	1045264.12	2070650.01	0.5048	0.908	90.80
GlaxoSmithKline (England)	1058943.97	2052614.21	0.5159	0.929	92.90
GlaxoSmithKline (Saudi Arabia)	1067549.37	1718250.44	0.6213	1.118	111.80
GlaxoSmithKline (Saudi Arabia)	1073612.23	1681945.85	0.6383	1.149	114.90
GlaxoSmithKline (Saudi Arabia)	1074862.48	1937736.58	0.5547	0.998	99.80
GlaxoSmithKline (Saudi Arabia)	1048259.90	1702549.78	0.6157	1.108	110.80
GlaxoSmithKline (Saudi Arabia)	1050271.52	2014329.73	0.5214	0.938	93.80
Zygpharma Pvt.Ltd (India)	1175895.85	1990335.19	0.5908	1.063	106.30
Zygpharma Pvt.Ltd (India)	1159183.28	1953165.36	0.5935	1.068	106.80
Zygpharma Pvt.Ltd (India)	1150955.16	1932493.39	0.5956	1.072	107.20
Hoe Pharmaceuticals (Malaysia)	1163327.78	1962503.73	0.5928	1.067	106.70
Hoe Pharmaceuticals (Malaysia)	1158864.89	1962300.24	0.5906	1.063	106.30
Hoe Pharmaceuticals (Malaysia)	1158864.89	1962300.24	0.5906	1.063	106.30
Pharma Inkl (Jordan)	1156328.84	1915820.52	0.6036	1.086	108.60
Pharma Inkl (Jordan)	1169665.96	1916146.31	0.6104	1.099	109.90
Pharma Inkl (Jordan)	1160292.83	1947259.45	0.5959	1.072	107.20

As shown in Table 3.2 above, the products tested complied with the compendial quality in terms of content of active ingredient.

3.4 Betamethasone valerate

3.4.1 Identification test

After the TLC-plate was eluted; the spots of betamethasone valerate reference standard and those of the extracts were examined under short- wave length (UV) radiation at 254 nm and as shown in Figure 3.5 below.

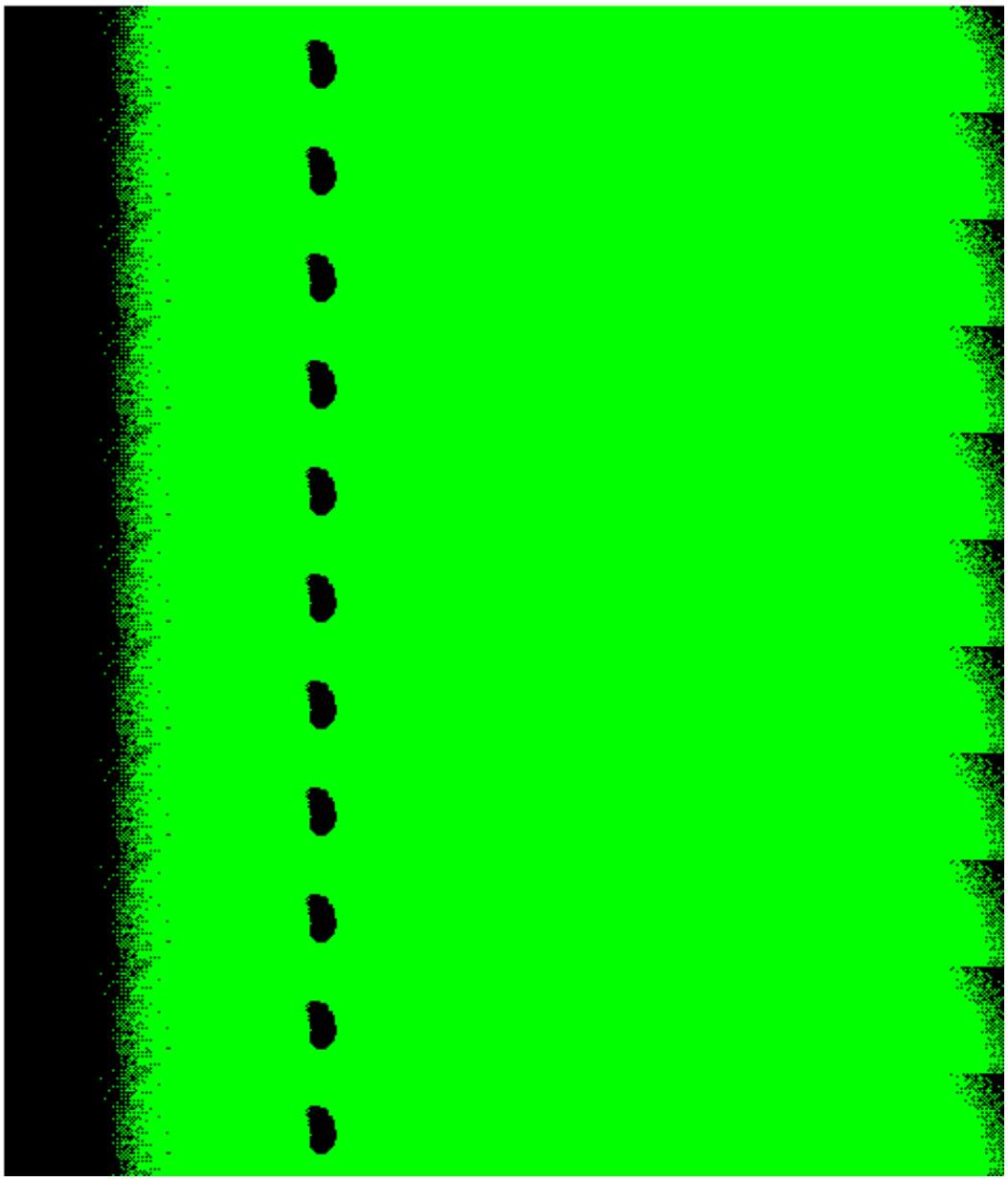


Fig.3.5 Representative TLC primary spots of betamethasone valerate cream extracts observed under UV (254) nm radiation

3.4.2 Retention factor (Rf) values

The retention factor (Rf) values of betamethasone valerate and those of the extracted from the creams were determined by measuring the distance moved by the analyte (betamethasone valerate) and the distance moved by the solvent front (according to USP 2008) and the results are indicated in the Table 3.3 below.

The retention factor for the standard was 0.330 and that of the extracts was 0.326, 0.330 and 0.340, which are very close to the reference standard and these results implied that all the surveyed creams contained the expected active ingredient, betamethasone valerate.

Table 3.3 Identification test for betamethasone valerate cream

Manufacturer	Distance moved by the sample (cm)	Rf-Value	Retention factor (%)
GlaxoSmithKline (England)	4.5	0.330	33.0
GlaxoSmithKline (England)	4.5	0.330	33.0
GlaxoSmithKline (England)	4.5	0.330	33.0
GlaxoSmithKline (England)	4.5	0.330	33.0
GlaxoSmithKline (England)	4.5	0.330	33.0
GlaxoSmithKline (Saudi Arabia)	4.5	0.330	33.0
GlaxoSmithKline (Saudi Arabia)	4.5	0.330	33.0
GlaxoSmithKline (Saudi Arabia)	4.5	0.330	33.0
GlaxoSmithKline (Saudi Arabia)	4.5	0.330	33.0
GlaxoSmithKline (Saudi Arabia)	4.5	0.330	33.0
Zygpharma Pvt.Ltd (India)	4.4	0.326	32.6
Zygpharma Pvt.Ltd (India)	4.4	0.326	32.6
Zygpharma Pvt.Ltd (India)	4.4	0.326	32.6
Hoe Pharmaceuticals (Malaysia)	4.6	0.340	34.0
Hoe Pharmaceuticals (Malaysia)	4.6	0.340	34.0
Hoe Pharmaceuticals (Malaysia)	4.6	0.340	34.0
Pharma Inkl (Jordan)	4.5	0.330	33.0
Pharma Inkl (Jordan)	4.5	0.330	33.0
Pharma Inkl (Jordan)	4.5	0.330	33.0

As seen from the above table (table 3.3), the Rf values of the extracts are almost the same with the Rf values in the reference standard so all the surveyed betamethasone valerate cream products contained the expected active ingredient, betamethasone valerate.

3.4.3 Chemical Assay

The USP 2008 specifies that BV cream formulations should contain not less than 90.0% and not more than 110.0% of the labelled amount of BV. As a result, it was considered essential to assay BV content in all formulations in order to ensure that the marketed products contained BV within this specified range.

Using the USP 2008 procedures mentioned above; specific amount of residue (2.5 mg) betamethasone valerate was dissolved in 12.5 ml of methanol to provide equal concentration of the standard and the extracts (0.2 mg/ml). Then triplicate injections of the reference standard and the extracts were applied. Chromatograms were obtained in which a representative of reference standard (Figure 3.6) and that of the cream extracts (Figures 3.7 (A-E)) are shown below.

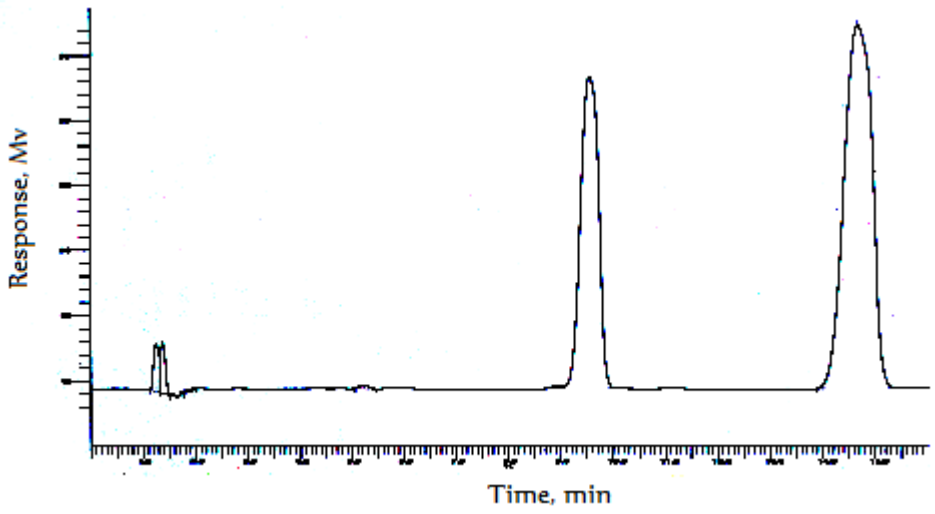
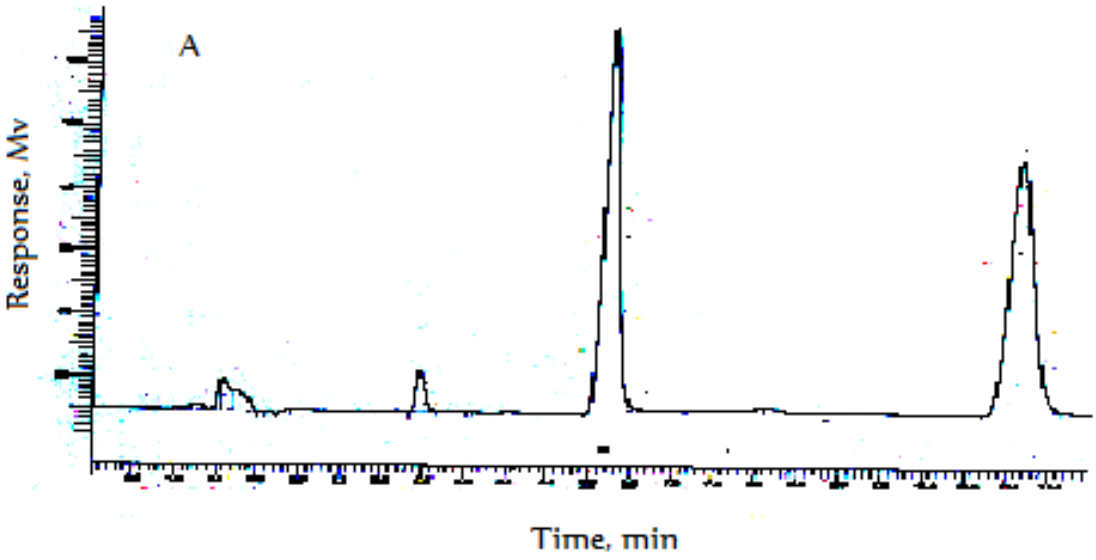
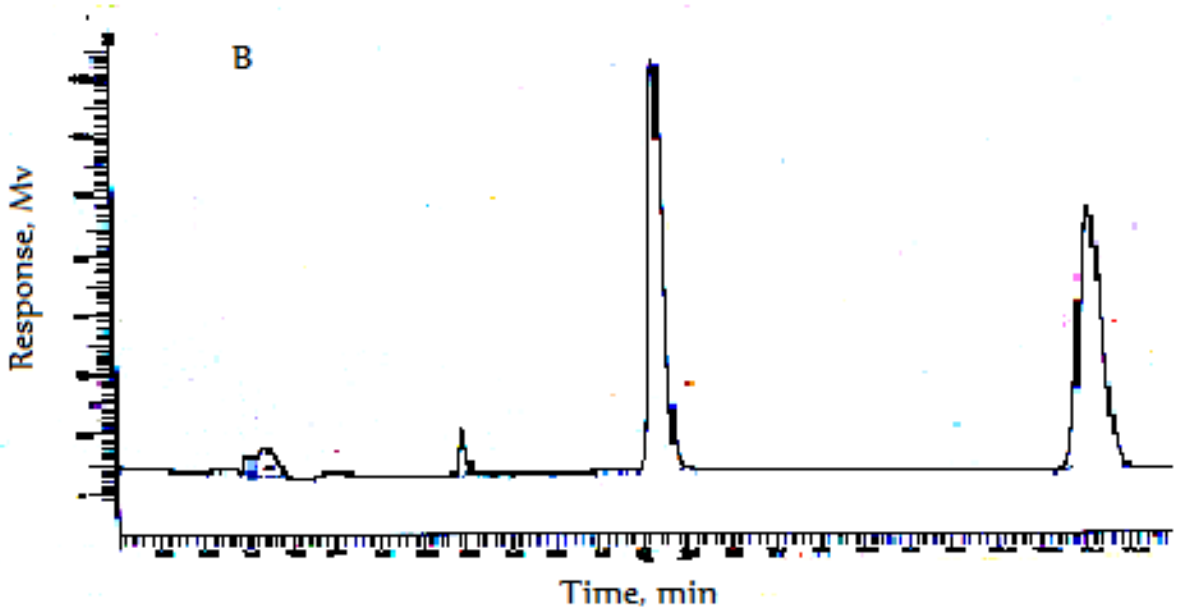
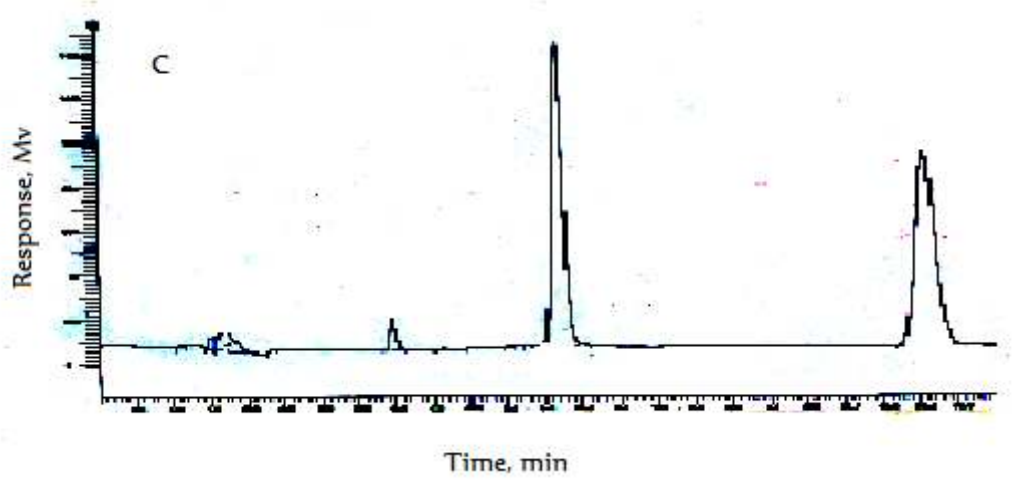
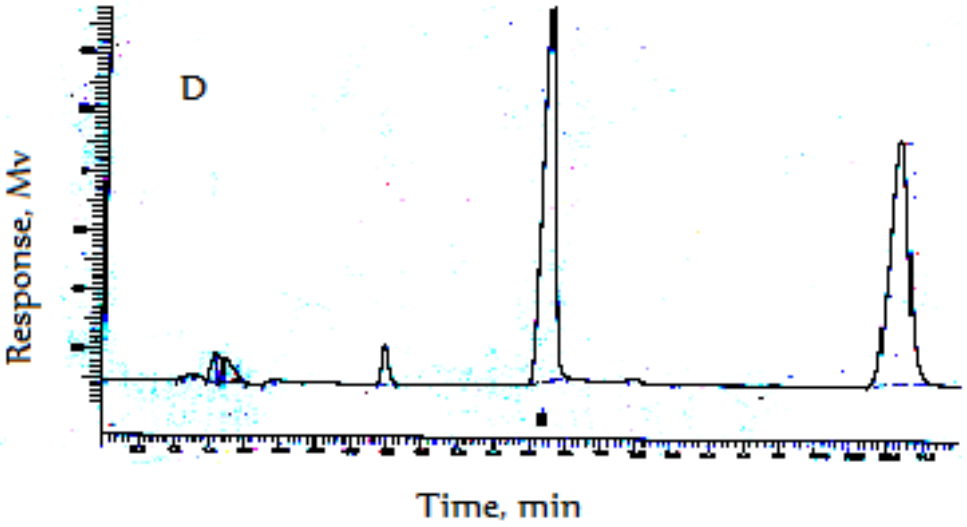


Fig 3.6 Representative chromatogram of betametasone valerate reference standard at 0.2 mg /ml concentration


A
Response, Mv
Time, min


B
Response, Mv
Time, min


C
Response, Mv
Time, min


D
Response, Mv
Time, min

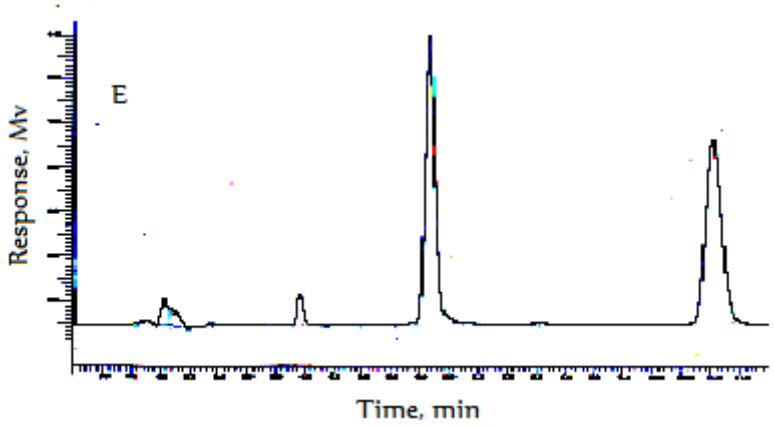


Fig. 3.7 A-E Representative chromatograms of betamethasone valerate cream extracts at 0.2 mg /ml concentrations

As seen from the above representative chromatograms of the cream extracts of betamethasone valerate (Fig. 3.7 A-E), an additional peak was observed, which indicated that the excipients in the dosage form absorb energy in the given range and displayed an additional peak.

Average peak areas of betamethasone valerate and beclomethasone dipropionate in the reference standard are 3112581.647 and 3532482.573, respectively, and the Rs (peak area ratio of betamethasone valerate to beclomethasone dipropionate in the reference standard is calculated as: 3112581.647/3532482.573=0.881. The concentration of betamethasone valerate is 0.2 mg/ml.

The quantity in mg of betamethasone valerate is calculated by using the formula: (392.46/476.59) (15C) (Ru/Rs) = (392.46/476.59 (15x0.2) (Ru/0.881)=2.80Ru;where Ru is peak area ratio of betamethasone valerate to beclomethasone dipropionate in the assay preparation.

Table 3.4 Assay of betamethasone valerate cream

Manufacturer	Peak area of		Ru	mass of BV (mg)	mass of BV (%)
	Betamethasone valerate	Beclomethason dipropionate			
GlaxoSmithKline (England)	2798903.57	3032353.99	0.9230	2.58	103.20
GlaxoSmithKline (England)	2645921.00	2949087.16	0.8972	2.51	100.40
GlaxoSmithKline (England)	2991290.81	3032392.48	0.9864	2.76	110.40
GlaxoSmithKline (England)	2759506.01	2920112.17	0.9450	2.65	106.00
GlaxoSmithKline (England)	2996894.02	3333957.06	0.8989	2.52	100.80
GlaxoSmithKline (Saudi Arabia)	2894305.00	3110817.93	0.9304	2.61	104.40
GlaxoSmithKline (Saudi Arabia)	2991290.81	3032392.48	0.9864	2.76	110.40
GlaxoSmithKline (Saudi Arabia)	2807943.03	3205414.38	0.876	2.45	98.00
GlaxoSmithKline (Saudi Arabia)	2799804.47	2867476.92	0.9764	2.73	109.20
GlaxoSmithKline (Saudi Arabia)	2879654.04	3350772.68	0.8594	2.41	96.40
Zygpharma Pvt.Ltd (India)	2757059.11	3095909.35	0.8905	2.49	99.72
Zygpharma Pvt.Ltd (India)	2730600.78	3046617.07	0.8963	2.51	100.40
Zygpharma Pvt.Ltd (India)	2778463.06	3123900.51	0.8894	2.49	99.60
Hoe Pharmaceuticals (Malaysia)	2887022.63	3255423.80	0.8868	2.48	99.20
Hoe Pharmaceuticals (Malaysia)	2795500.78	3165952.44	0.8830	2.47	98.88
Hoe Pharmaceuticals (Malaysia)	2873931.67	3253379.19	0.8834	2.47	98.92
Pharma Inkl (Jordan)	2906473.17	3316128.79	0.8765	2.45	98.00
Pharma Inkl (Jordan)	2885762.01	3257849.26	0.8858	2.48	99.20
Pharma Inkl (Jordan)	2874430.29	3246655.56	0.8853	2.47	99.16

The above calculated extracted amounts of betamethasone valerate (96.4-110.4%) indicated that all the products comply with the USP specification (90-110%) of betamethasone valerate in the cream.

As can be seen from the above tables: the studied clobetasol propionate cream products (table 3.2) contained 90.80-110.80 % of the active ingredient, CP, the USP 2008 (90.0 -115.0%) and that of the betamethasone valerate cream products (table 3.4) contained 96.40 - 104.40 % of the active ingredient, BV, the USP 2008 (90.0 -110.0%), in which all the surveyed creams of the two products comply with the USP 2008 specifications and are potentially stable. In one way or the other this may be accounted with the following scientific justifications:

The storage conditions in terms of temperature was appropriate (Evoy, 2003), the packaging materials are strong enough to protect the entrance of light (BP, 2004), the dosage forms contain the correct kind and amount of surfactants, which lowers the free energy of the system by reducing the generation of new interfaces (Myers, 1988), existence of stable interfacial film and interfacial rheological properties, such as elasticity and viscosity (Sjöblom, 1996), commercial pharmaceutical creams are complex polydispersed systems containing several surfactants, which complement the properties of each other (Eccleston, 1990). As the number of components in a cream is higher, the internal structure of the cream is more complex and the concentration of the surfactant increased, the excess of the monomolecular interfacial films interacts with other components either at the interfaces or in the bulk phases to produce complex and stable products and the emulsion maintain the same number and size distribution of dispersed droplets per unit of volume or weight of the continuous phase (Garrett, 1965).

4. CONCLUSIONS

The issue of quality evaluation of clobetasol propionate and betamethasone valerate topical corticosteroid creams marketed in Addis Ababa was addressed in terms of organoleptic properties (appearance, color and odor), microbiological, identification and quantification (assay) tests were assessed based on USP pharmacopoeial specifications.

From the findings of this study, it can be concluded that all the studied cream products retained their characteristic original white color. The microbiological (*Staphylococcus aureus* and *Pseudomonas aeruginosa)* tests were also negative as indicated in the result above (figure 3.1). The results are may be because of the air-tight packaging material which did not allow permeation of moisture, humidity and heat which are favorable conditions for bacterial growth. As the USP recommends, the presence of the active ingredients in the dosage forms was checked by comparing the retardation (retention) factors in the reference standards and dosage forms (extracts). The results revealed that the retention factors for the standard and the extracts of clobetasol propionate and betamethasone valerate were 0.56 and 0.33, respectively. The USP requirement specifies that the drug substances in clobetasol propionate (90-115%) and betamethasone valerate (90-110%), what was found in this study is (90.8-114.9%) and (96.4-110.4%), respectively. The quantitative assay also supported that the products comply with the USP specifications.

Generally, this study disclosed that the surveyed cream products (clobetasol propionate and betamethasone valerate) topical corticosteroids fulfilled the USP 2008 quality indicating requirements in terms of organoleptic properties, microbiological limit, absence of the suspected active ingredient (identification) and quantification (the proper amount of the active pharmaceutical ingredient in the dosage forms). Even though these creams do not have quality problems in terms of the mentioned attributes, peoples are being using them as a cosmetic (skin lighteners) without prescriptions so that they become a victim of their

serious systemic and topical side effects. Consequently, health professionals have had to reinforce and disseminate information on the disadvantages of topical corticosteroid abuse.

5. SUGGESTION FOR FUTURE WORK

The following follow-up works are suggested

- Stability of clobetasol propionate and betamethasone valerate topical corticosteroid creams collected from areas with tropical climatic conditions

- Stability tests of clobetasol propionate and betamethasone valerate topical corticosteroid creams at accelerated and intermediate storage condition

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DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university.

Name: Abebayehu Terefe

Signature: _____

This thesis has been submitted for examination with our approval as University Advisor

Name: prof. Tsige Gebre-Mariam

Signature: _____

Place and date of submission: Addis Ababa, Ethiopia