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Design, development and evaluation of celecoxib loaded ethosomal gel

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ABSTRACT

The main objective of the present study was to enhance a transdermal penetration of a water-insoluble anti-fungal drug, Celecoxib, by encapsulating it into a Ethozomal gle. The Ethozomal gle of Celecoxib was prepared firstly by preparing transfersomes by modified hand shaking method using surfactants (Tween 80 and Span 80), Lecithin in various concentrations and chloroform and methanol added in 3:1 ratio in each formulation and evaluated for their vesicle shape and size, entrapment efficiency, % drug content and %entrapment efficiency and in vitro permeation studies. Later carbopol gels such as carbopol 940 along with distilled water was used in the aqueous dispersion of Ethozomes to prepare topical Ethozomal gle. The characterization was carried out and in vitro permeation studies carried out. The results were obtained which showed that Ethozomal gle was a promising candidate for transdermal delivery with targeted and prolonged release of a drug. It also enhances skin permeation of many drugs.

INTRODUCTION

Transdermal delivery

Transdermal delivery of drugs is a convenient route of administration for a variety of clinical indications. Transdermal delivery has many advantages over the conventional drug delivery [1, 2, 3]. Transdermal delivery faces significant barrier across the skin, associated mainly with outermost stratum corneum layer of epidermis which limits it to fewer drugs [4, 5]. The skin structure consists of stratum corneum cells are embedded within intercellular lipid lamellae [6]. These lamellae are also responsible for imparting barrier properties to the stratum corneum [7, 8]. As a result, very less quantity (mg) of drug can be delivered by this route. This limits its application to only potent drugs. Work is going on to overcome this limitation. These include augmenting skin permeability using penetration enhancers, using iontophoresis, electroporation, phonophoresis, microneedles, jet injectors, etc., (forces which are independent of concentration gradient) and many more. Transfersomes or vesicles belong to the latter category.

Advantages of TDDS [9]

Transdermal drug delivery offers several advantages over conventional dosage forms, which includes

- The steady permeation of drug increases consistent serum drug levels across the skin.
- Transdermal drug delivery is less invasive than intravenous infusion which also results in consistent plasma level.
- It reduces risk of side effects due to lack of peaks in plasma concentrations. Thus, for transdermal

drug delivery, drugs that require relatively consistent plasma levels are preferable.

- Toxicity is limited to a certain area and can be prevented by removing the patch.
- Transdermal drug delivery is very convenient, especially with patches that require application once in a week. Such simple dosing regimen can aid in patient adherence to drug therapy.
- It is very useful for patients who cannot tolerate oral dosage form.
- It does not result in nausea or unconscious.
- Transdermal delivery avoids direct effects on the stomach and intestine and thus can be a route for drugs causing gastrointestinal upset.
- It can target the gastrointestinal system drug that degrades by the acid and enzymes present in it.
- Transdermal administration avoids first pass metabolism.
- It allows continuation of drug administration with short biological half-life.

Disadvantages of TDDS [9]

- One of the greatest disadvantages to transdermal drug delivery is the possibility of local irritation that may develop at the site of application.
- In patch formulation, the drug, the adhesive, or other excipients, can cause erythema, itching and local edema. For most patients, changing site can minimize irritation.
- The skin's low permeability limits its use for fewer drugs.
- Many drugs with a hydrophilic structure permeate the skin too slowly resulting in no therapeutic benefit.

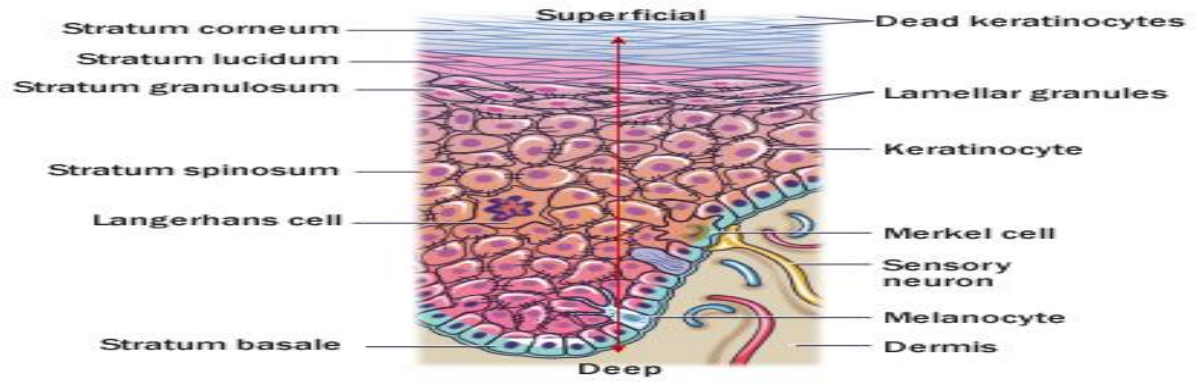


Figure 1: Epidermis

Ethosomes

The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers have been understanding the properties of vesicle structures for use in better drug delivery within their cavities, that would allow to tag the vesicle for cell specificity. Vesicles would also allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and would be able to release just the right amount of drug and keep that concentration constant for longer periods of time. One of the major advances in vesicle research was the finding a vesicle derivative, known as an ethosomes [4, 6].

Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipid (Phosphotidyl choline; PC), ethanol at relatively high concentration and water. It was found that ethosomes penetrate the skin and allow enhanced delivery of various compounds to the deep strata of the skin or to the systemic circulation.

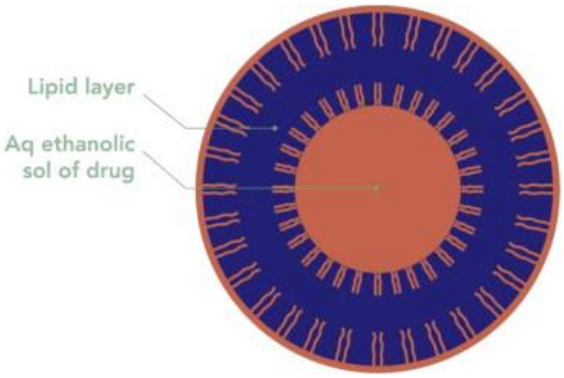


Figure 2: Structure of ethosomes

EXPERIMENTAL WORK

Preformulation studies

Preformulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of the new drug substance, to check for its stability, safety and effective dosage form. Ideally, the Preformulation phase begins early in the discovery process such as the appropriate physical and chemical data is available to aid the selection of new chemical entities that enters the development process during this evaluation possible interaction with various inert ingredients intended for use in final dosage form are also considered in the present study.

Organoleptic properties

The color, odor and taste of the drug were recorded using descriptive terminology.

Solubility

The solubility of the drug sample was carried out in different solvents (aqueous and organic) according to I.P. The results are then compared with those mentioned in the official books and Indian Pharmacopoeia.

Melting point

The melting point of Celecoxib was determined by capillary method using digital melting point apparatus.

ANALYTICAL METHODS

Standard curve

Preparation of standard solution

Stock solution-I

100mg of CELECOXIB was accurately weighed into 100ml volumetric flask and dissolved in small quantity of buffer. The volume was made with 6.8 pH Phosphate buffer to get a concentration of 1000µg/ml (SS-I).

UV Absorption Maxima (λ_{max}) of Celecoxib sample in ph 6.8 Phosphate buffer

Stock II

10ml of above solution was then further diluted to 100ml with 6.8 pH Phosphate buffer to get a stock solution of 100µg/ml. UV scanning was done for 100 µg/ml drug solution from 200-400 nm using pH 6.8 Phosphate buffer as a blank in Shimadzu, UV 2450 spectrophotometer. The wavelength maximum was found to be at 262 nm.

Preparation of working standard solutions

Further, from SS-II aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml were pipetted out into 10ml volumetric flasks. The volume was made up with 6.8 pH Phosphate buffer to get the final concentrations of 2, 4, 6, 8, and 10µg/ml respectively. The absorbance of each concentration was measured at 262nm.

Calibration curve for the estimation of Celecoxib

Calibration curve of Celecoxib was estimated in 6.8 pH buffer.

COMPATIBILITY STUDIES

IR spectroscopy can investigate and predict any physicochemical interactions between different components in a formulation and therefore it can be applied in selection of suitable chemically compatible excipients. The aim of the present study was to test whether there are any interactions between the carriers and the drug. One part of the sample and three parts of potassium bromide were taken in a mortar and triturated. A small amount of triturated sample was taken into a pellet maker and compressed at 10kg/cm^2 using hydraulic press. The pellet was kept on the sample holder and scanned from 4000cm^{-1} to 400cm^{-1} in Bruker IR spectrophotometer. Then it was compared with the original spectra.

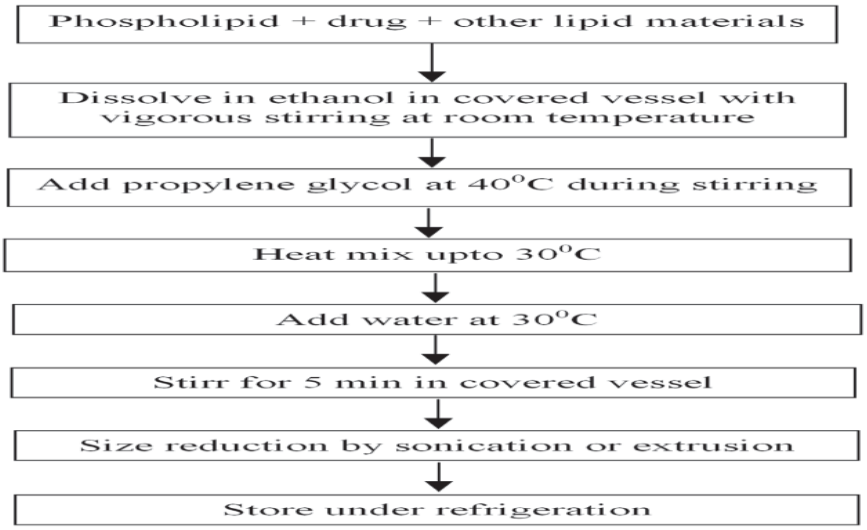
IR spectrum was compared and checked for any shifting in functional peaks and non-involvement of functional groups. From the spectra it is clear that there is no interaction between the selected carriers, drug and mixtures. Hence the selected carrier was found to be compatible in entrapping the Celecoxib with carriers without any mutual interactions.

FORMULATION OF TRANSFERSOME GEL

Preparation of celecoxib ethosomes (by cold method)

Preparation of Celecoxib ethosomes was followed by method suggested by Touitou et al., with little modification [7].

The ethosomal system of Celecoxib comprised of 1-2 % phospholipids, 30-40 % IPA, 10 % of propylene glycol,0.005g of cholesterol and aqueous phase to 100 % w./w. Celecoxib 0.25 g was dissolved in *IPA* in a covered vessel at room temperature by vigorous stirring. Propylene glycol was added during stirring. This mixture was heated to 30^0 in a separate vessel and was added to the mixture drop wise in the center of the vessel, which was stirred for 5min at 700rpm in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication [29] or extrusion [30] method. Finally, the formulation is stored under refrigeration [31].Ethosomes were formed spontaneously by the process.



Composition of different ethosomal formulations

Table no: 1 Composition of different ethosomal formulations

Ethosomal formulation	Lecithin (Soya lecithin %)	IPA (%)	Propylene glycol (%)	Drug (g)	Cholesterol(g)	Water
EF_1	1	40	10	0.025	0.005	q.s
EF_2	1.5	40	10	0.025	0.005	q.s
EF_3	2	40	10	0.025	0.005	q.s
EF_4	1	30	10	0.025	0.005	q.s
EF_5	1.5	30	10	0.025	0.005	q.s
EF_6	2	30	10	0.025	0.005	q.s

Preparation of Celecoxib ethosomal gel

The best achieved ethosomal vesicles suspension, formula EF-6 was incorporated into carbopol gel (1%, 1.5%, 2% w/w).the specified amount of carbopol 934 powder was slowly added to ultrapure water and kept at 100^0c for 20min. tri ethanolamine was added to it dropwise. Appropriate amount of formula EF-6 containing Celecoxib (1% w/w) was then incorporated into gel-base. Water q.s was added with other formulation ingredients with continuous stirring until homogenous formulation were achieved (G-1,G-2 and G-3).

Composition of different ethosomal gel formulation

Table no2: **Composition of different ethosomal gel formulation**

Gel formulation	Celecoxibethosomal suspension(ml)	Carbopol (%)	Triethanolamine (ml)	Phosphate buffer (pH 6.8)
G-1	20	1	0.5	q.s
G-2	**20**	**1.5**	**0.5**	**q.s**
G-3	20	2	0.5	q.s

PREFORMULATION STUDIES

Standard calibration curve

In the pre-formulation studies, the λ max of Celecoxib by spectrophotometric method in phosphate buffer pH 6.8 was found to be 262nm.

Calibration Curve Data of Celecoxib

Table no3: Calibration Curve Data of Celecoxib

CONCENTRATION (µg /ml)	ABSORBANCE
0	0
2	0.097
4	0.178
6	0.250
8	0.345
10	0.438

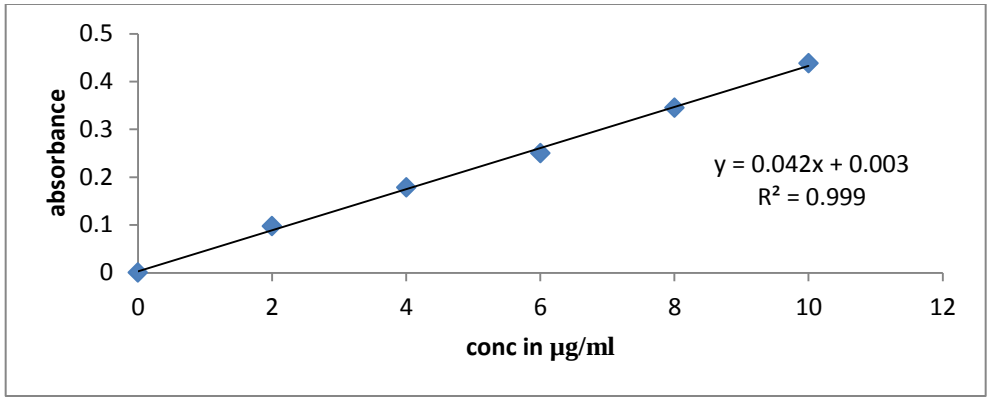


Fig 3: calibration cuve plot of Celecoxib

Drug excipient compatibility study

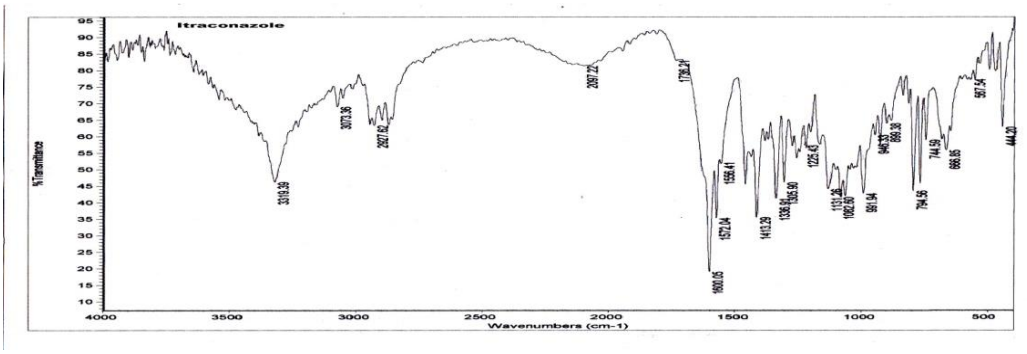


Fig 4: FTIR spectra of Celecoxib

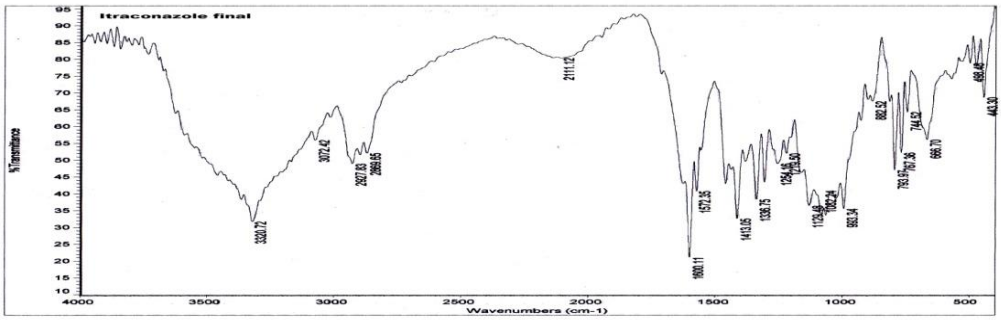


Fig 5: FTIR spectra of Celecoxib final formulation

EVALUATION OF ETHOZOMAL GLE

Size and shape analysis

Microscopic analysis was performed under different magnification to visualize the vesicular structure, lamellarity and to determine the size of ethosomal preparations.

Scanning electron microscope (SEM)

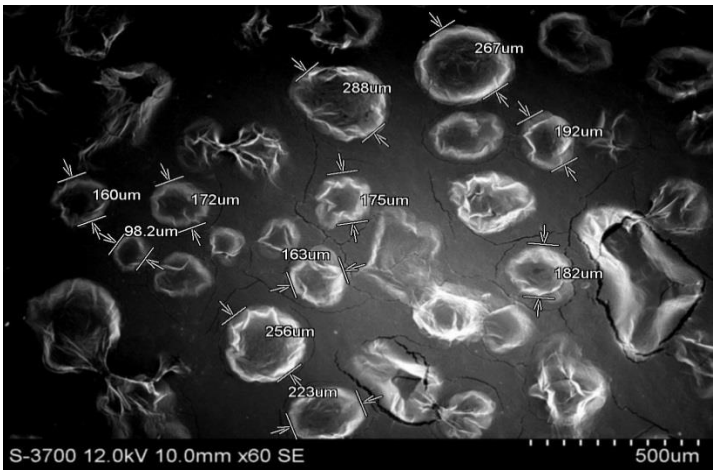


Fig 6: Scanning electron microscope image

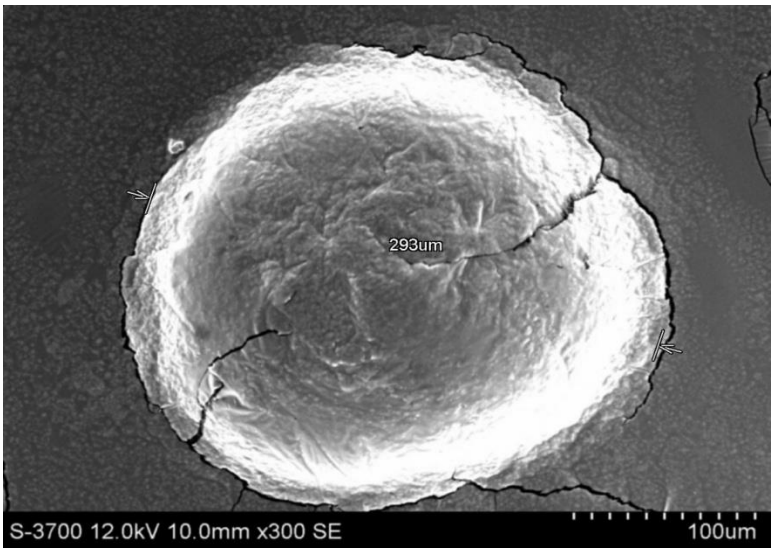


Fig 7: Scanning electron microscope image (Figure -16)

Entrapment efficiency

Once the presence of bilayer vesicles was confirmed in the ethosomal system, the ability of vesicles for entrapment of drug was investigated by ultra-centrifugation. Ultra-centrifugation was the method used to separate the ethosomal vesicles containing drug and un-entrapped or free drug, to find out the entrapment efficiency.

Table no 4: Drug entrapment efficiency of CelecoxibEthosomal Gel (Table-15)

Formulation code	Entrapment efficiency(%)
EF1	81.2
EF2	85.6
EF3	77.5
EF4	75.2
EF5	90.1
EF6	89.5

The maximum entrapment efficiency of ethosomal vesicles as determined by ultracentrifugation was 89.5% for ethosomal formulation containing 30% IPA (EF6). Results of entrapment efficiency also suggest that 2% phospholipid is optimal concentration for entrapment efficiency and hence increased or decreased in concentration of phospholipid reduces the entrapment efficiency of vesicles. These result further supported by observation made by Jain NK et al., [14]

Entrapment efficiency of ethosomal formulations are significantly different and are reported in Table 15. Increase in entrapment efficiency may be due to the possible reduction in vesicle size. The detrimental effect on the vesicle during ultra-centrifugation which are larger in size. Sonication gives the more uniform lamellae, smaller vesicle and uniform size and hence it may be the reason for higher vesicular stability and lesser vesicular disruption during ultra centrifugation.

P^H measurements

Fig no: 8 P^H measurements

The pH of organogels was measured by using electrode based digital pH meter.

The pH values for all formulations were in the range of 6.6 to 6.8

Drug content

Table no 5: Drug Content

Formulation code	Drug content (%)
EF1	78.6
EF2	80.3
EF3	79.6
EF4	81.3
EF5	82.3
EF6	89.3

In-vitro release studies

Table no: 6 In-vitro cummulative % drug release profile for Celecoxib Ethosomal gel

Time (min)	EF1	EF2	EF3	EF4	EF5	EF6
5	2.31	1.46	4.57	3.6	5.42	6.26
10	9.42	8.4	11.8	10.08	13.5	15.95
15	24.4	22.97	26.04	25.2	27.15	30
30	31.24	30.01	33.02	32.04	35.68	41.68
60	37.02	35.68	39.6	38.7	41.68	53.3
120	49.7	47.1	53.3	52.4	55.5	57.7
240	55.5	53.5	60.44	58.6	62.22	64.4
360	64.4	62.22	67.1	65.7	68.5	70.6
720	73.3	70.6	77.3	75.5	78.2	82.6
1440	78.2	75.5	89.3	87.11	88.4	93.7

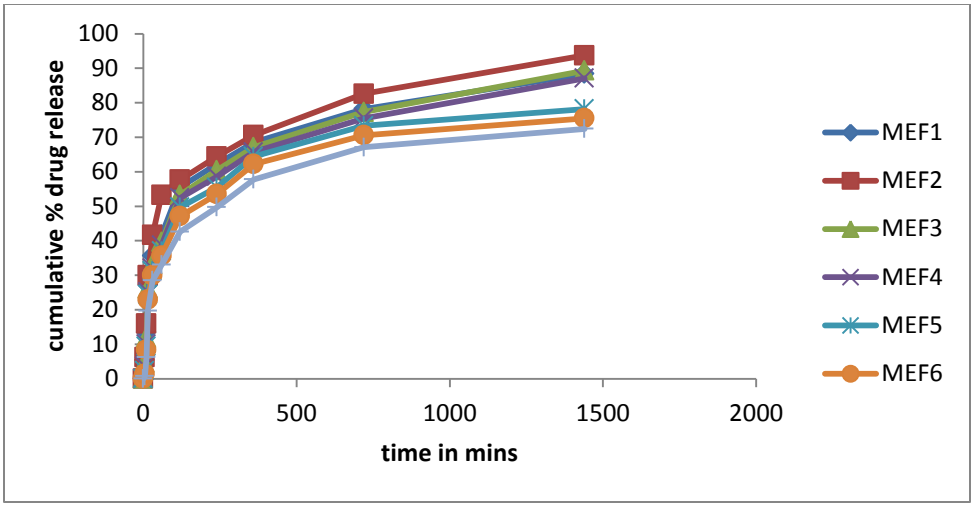


Fig no9: Cumulative drug release of in-vitro studies

Stability study

Ethosomal formulations were observed for any change in appearance or color for a period of 8 weeks. There was no change in appearance in Ethosomal formulations throughout the period of study.

In-vitro stability release study

Since the stability of drug and stability of vesicles are the major determinant for the stability of formulation, studies were carried to evaluate total drug content at room temperature (27±2° C) and refrigeration temperature (4±2° C). Stability study could not be carried out at higher temperature (>room temperature) because phospholipid was used as the component for ethosomes and gets deteriorated at higher temperature.

Loss in percentage of drug was not more than 4 percentages. Highest drug loss was observed at room temperature after 8 weeks as compared to refrigeration temperature. Results also showed that there was no significant change.

Loss in percentage of drug release during stability studies of the optimized batch (4±2 ° C & 27±2 ° C)

Table7: Loss in percentage drug during stability studies

Formulation code (EF6)		Percentage of drug release	Loss in percentage
Initial	4±2 ° C	93.7	0
	27±2 ° C	93.7	0
After 2 weeks	4±2 ° C	93.7	0
	27±2 ° C	92.8	0.96
After 4	4±2 ° C	92.9	0.85
Weeks	27±2 ° C	91.5	2.34
After 6weeks	4±2 ° C	92.2	1.60
	27±2 ° C	90.4	3.52
After 8 weeks	4±2 ° C	91.5	2.34
	27±2 ° C	90.2	3.73

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