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Der Pharma Chemica, 2015, 7(8):1-9
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Optimization of an *in vitro* release test for topical formulations containing eberconazole nitrate and mometasone furoate

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ABSTRACT

A simple and reproducible in vitro release test (IVRT) method is developed for the performance evaluation of topical formulations containing Eberconazole nitrate and Mometasone furoate by using Franz Diffusion Cell. The method utilized polysulfone membrane and 1% Sodium lauryl sulfate with ethanol as receptor media that suffice sink condition. The method is able to discriminate release profile of Eberconazole nitrate and Mometasone furoate from market formulations having different viscosities. This method can be utilized in pharmaceutical industries for monitoring of batch to batch reproducibility, discriminate formulations with respect to change in process and formulation composition and for comparative IVRT study of generic formulations to build confidence prior to costly clinical study.

Keywords: Eberconazole nitrate, Mometasone furoate, IVRT, Franz Diffusion Cell

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INTRODUCTION

Percutaneous absorption can be studied using in vitro tests. These are widely used in the preliminary phase of the evaluation of percutaneous absorption mainly due to cost factors, time and reproducibility. In vitro methods allow the use of synthetic membranes and animal or human skin [biological membrane], and contribute toward the reduction, refinement and replacement of in vivo testing. The in vitro experimental conditions should mimic in vivo conditions as closely as possible so that results can then be extrapolated [1, 2]. An in vitro release rate can reflect the combined effect of several physical and chemical parameters, including solubility, particle size of the active ingredient and rheological properties of the dosage forms. Franz diffusion cells are normally used with excised human or animal skin. However, when biological skin is not readily available, synthetic membranes employed in drug diffusion study to check product performance. Synthetic membranes for quality control should have a minimum diffusion resistance to drugs and only act as a support to separate the formulation from the receptor media [3].

The topical formulation is a complex system and the dynamics of the release of the drug has been the subject of investigation from many years. The in vitro [IVRT] of semisolid dosage forms is an official requirement for the pharmaceutical industries to determine the drug availability and to ensure the batch to batch reproducibility . The developments of such methods help to establish the bioequivalence of product after scale up and post approval changes [4, 5]. Such methods applicable for the different types of topical dosage formsand screen experimental

formulations during the product development. Determination of the value of in vitro release helps to cross check the product quality and product comparison.

Currently, the performance testing system employing the Franz diffusion cell is commonly applied to semisolid products, such as creams, ointments and gels, and also to lotions. This procedure quantifies the release of the active component from the formulation, which diffuses through a membrane into a receptor solution [6].

Eberconazole nitrate [EBZ] is an imidazole derivative, used topically in the treatment of superficial fungal infections [7]. Mometasone furoate [MTS] is a glucocorticosteroid used topically in the treatment of inflammatory skin disorders [such as eczema and psoriasis], allergic rhinitis and it has vasoconstrictive properties [8, 9]. Combine therapy of Eberconazole nitrate and Mometasone furoate is approved for the treatment of mild to moderate inflammed cutaneous mycoses. EBZ and MTS are available either alone or in combination in cream and lotion dosage forms. There are no methods available for measurement of release characteristics of EBZ and MTS simultaneously from topical formulations.

The aim of this study was to develop in-vitro release test [IVRT] for Eberconazole nitrate and Mometasone furoate from topical formulations using Vertical Franz diffusion cells and synthetic membranes. Application of the developed method for evaluation of release characteristics of EBZ and MTS from the different marketed formulations.

MATERIALS AND METHODS

Instruments and Reagents

Study was performed on vertical franz diffusion cells (Make: Logan) and samples were analyzed using Waters Alliance HPLC system with UV detection and Empower software for data acquisition. Other instruments such as CAP 2000+ viscometer (Make: Brookfield), XS205 dual range balance (Make: Mettler Toledo), Ultrasonic bath (Make: Bandelin sonorex), Rotary shaker and pH meter (Make: Thermo Orion) were used for the research study. Glasswares of borosil were used for this study.

Reagents such as potassium dihydrogen phosphate, sodium lauryl sulfate, tween 80, ethanol, acetone, isopropyl alcohol and purified water were used for the research study.

Standard and product:

Eberconazole nitrate (99.6%) and Mometasone furoate (99.8%) standards of known potency were used. Products such as Ebernet M cream (Eberconazole 1 % and Mometasone furoate 0.1%), Ebernet cream (Eberconazole 1%), Elocon lotion (Mometasone furoate 0.1%) and Momate lotion (Mometasone furoate 0.1%) were purchased from market for the research study.

In vitro release test (IVRT)

The IVRT was performed using six cells (Franz diffusion cell) per formulation. The diffusion area of the cell was 1.76 cm^2 and receptor compartment had a capacity of 11 ml. Polysulfone membranes of 25 mm diameter were saturated with receptor media (1% Sodium lauryl sulfate solution: Ethanol, 70:30, v/v) for 30 minutes prior to experiment run. The cells were filled with degassed receptor media and the membranes were placed on top of the receptor compartment. The donor compartment was placed over the membrane. About 200 mg of formulation containing EBZ and MTS was applied in donor compartment and spread uniformly over the membrane. The receptor media was maintained at 32 ± 1˚C with constant stirring at 400 rpm. Sample aliquot of 200 µL was withdrawn from each cell at 1, 2, 3, 4, 6 and 8 hours for measurement of the drug release rate (Flux) of EBZ and MTS from formulation. The volume collected from the cell was replaced with fresh receptor media. Aliquot of samples were estimated by HPLC method for the measurement of EBZ and MTS releasedas mentioned below.

HPLC method for quantification of IVRT samples

Weighed about 119 mg of EBZ and 10 mg of MTS into 50 ml volumetric flask, added 25 ml of ethanol and sonicated to dissolve it. Made up to volume with ethanol. Diluted 5 ml of above solution to 50 ml with receptor media. Further diluted 5 ml of above solution to 20 ml with receptor media (EBZ: 200 µg/mL and MTS 20 µg/mL). IVRT samples were estimated for release characteristics of EBZ and MTS by HPLC using Waters Xterra C18 (150 × 4.6 mm, 5µm) as stationary phase and mobile phase constituted of water and methanol (35:65, v/v) at a flow rate

of 1.50 mL/min. Injection load was 50µL and column temperature was 30˚C. Both analytes were measured with uv detection at 235 nm and each injection run time was 12 minutes. IVRT samples are analyzed against standard solution containing EBZ (200 µg/mL) and MTS (20 µg/mL). Linearity curves of EBZ and MTS were made in concentration range of 3.5- 270 µg/mL and 0.2- 30 µg/mL respectively. .

Comparative evaluation of market formulations

Different marketed formulations such as Ebernet M cream, Ebernet Cream, Momate lotion and Elocon lotion were run for IVRT and measured the release characteristics of EBZ and MTS from the formulations. Compared flux value of each analyte in different market formulations.

RESULTS AND DISCUSSION

The migration of a drug from a semi-solid matrix into a receptor media is essentially a function of one or a combination of various processes such as drug release from the semi-solid matrix itself, passage of the drug through the membrane and clearance of the drug from below the membrane. Therefore, it is important that the membrane and the receptor media be highly permeable and accessible to the drug in the formulation for efficient drug release.Receptor media, membrane, quantification method and other Franz diffusion cell apparatus parameters are the key components for IVRT method development and were optimized as mentioned below.

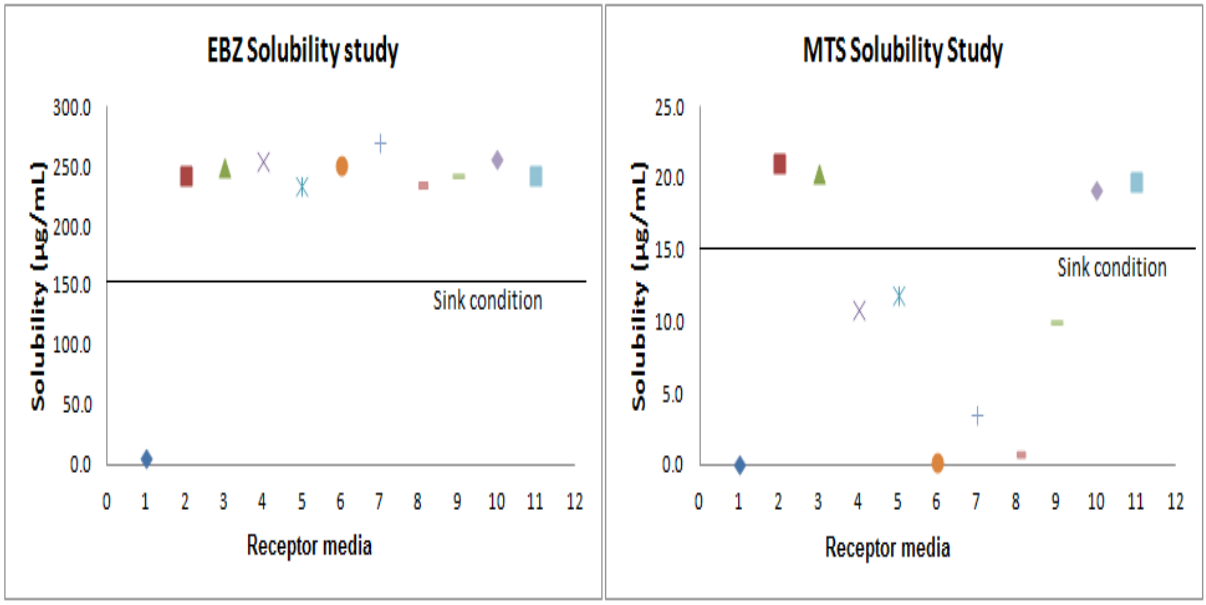
Receptor media selection

While selecting receptor media for in vitro release test, it is necessary that API has to have adequate solubility in media throughout the course of an experiment without impacting on the sink condition of the system. Appropriate receptor media such as aqueous buffer or hydro-alcoholic media for sparingly water soluble drugs or another media with proper justification can be used. In order to facilitate and monitor drug release from such topical formulations, it is necessary to add surfactant and complexing agents or use non-aqueous media in which the drug is more soluble and efficiently released from the matrix during in vitro studies(10-12). Different medias such as pH 7.40 phosphate buffer saline (PBS), 1 % Tween solution, 1% Sodium lauryl sulfate (SLS) solution, pH 7.40 PBS with 1% SLS, pH 7.40 PBS with 1% SLS and mixture of buffers with acetone and ethanol were tried to achieve sink conditions for EBZ and MTS (EBZ: 150 µg/mL and MTS: 15 µg/mL). . Results are depicted in figure 1. Results enabled that desired sink condition is achieved with 1% SLS solution, pH 7.40 PBS with 1% SLS and mixture of 1% SLS and Ethanol as receptor medias (Table 1).

Table 1: Results of solubility study

Receptor media	EBZ (µg/mL)	MTS (µg/mL)	Observation
1 % SLS solution	241.5	21.1	Foam/ bubble formation
pH 7.40 PBS with 1% SLS	249.7	20.3	Foam/bubble formation
1% SLS: Ethanol (80: 20, % v/v)	256.5	19.2	Clear solution
1% SLS: Ethanol (70: 30, % v/v)	242.3	19.8	Clear solution

IVRT experiments were run for topical cream (Ebernet M) for actual release of EBZ and MTS with 1% SLS solution, pH 7.40 PBS with 1% SLS and 1% SLS: Ethanol (70: 30, % v/v) as receptor medias. Study was performed for 8 hours with 0.2µ polysulfone membrane, 32˚C receptor compartment temperature and 400 rpm stirring speed. IVRT samples were analyzed for quantification of EBZ and MTS by HPLC. Results of cumulative release (µg/cm^2) of EBZ and MTS with different receptor medias are depicted in fig 2. Sufficient and precise release of EBZ and MTS from cream formulations was achieved with 1% SLS: Ethanol (70: 30, % v/v) as receptor media. Hence 1% SLS: Ethanol (70: 30, % v/v) was finalized as receptor media for IVRT study.



Receptor media 1. pH 7.40 PBS **2.** 1% SLS **3.** pH 7.40 PBS with 1% SLS **4.** 1% Tween **5.** pH 7.40 PBS with 1% Tween **6.** pH 7.40 PBS: Ethanol (90:10, %v/v) **7.** pH 7.40 PBS : Ethanol (75: 25, % v/v) **8.** pH 7.40 PBS: Acetone (90: 10, % v/v) **9.** pH7.40 PBS: Acetone (75: 25, % v/v) **10.** 1% SLS: Ethanol (80:20, % v/v) **11.** 1% SLS: Ethanol (70: 30, % v/v)

Fig. 1. Typical plot of solubility study

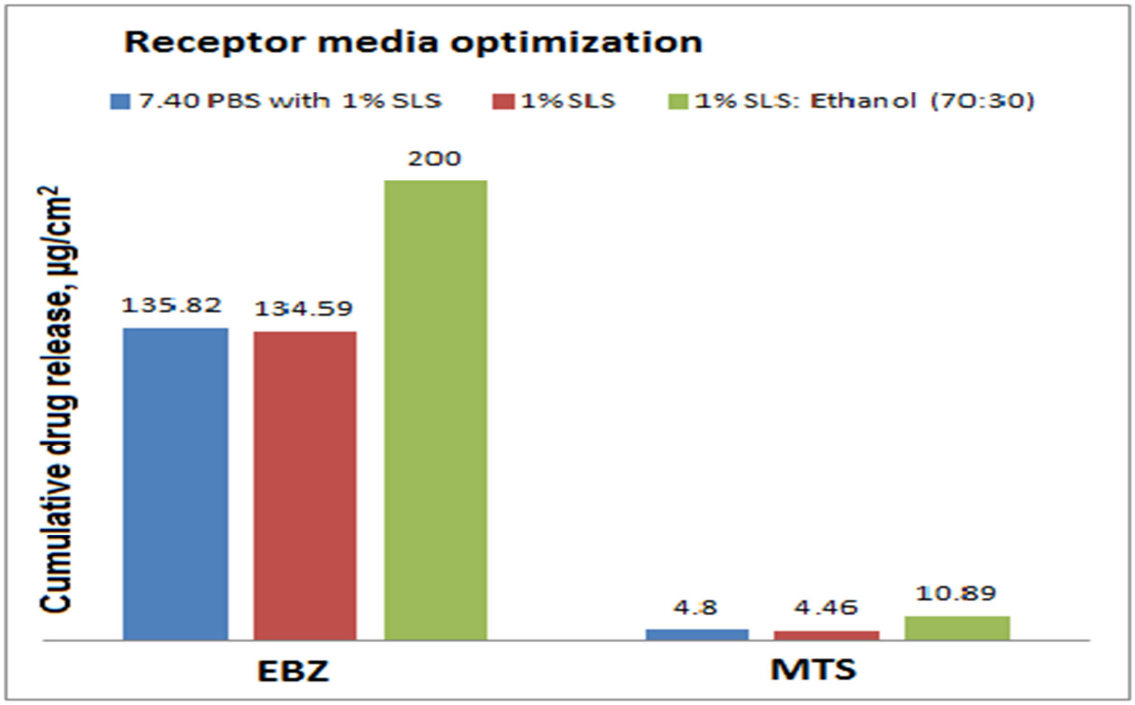


Fig. 2. Typical plot of Receptor media optimization study

Membrane selection:
An important consideration for selecting synthetic membrane for *in vitro* drug release experiments should have low reactivity with formulation components, be compatible with the receptor media and offer the least possible diffusional resistance to the component of interest(13-15). The membrane of choice should be inert and provide a holding surface without barrier properties for the active ingredient and test formulation. Different synthetic membranes such as 0.22µ Polysulfone, 0.45µ Nylon, 0.45µ Cellulose acetate and 0.22µ Teflon were evaluated for drug-membrane binding study and drug release characteristics of EBZ and MTS from topical formulations.

Drug –membrane binding study was performed by preparation of standard containing EBZ (about 200 µg/mL) and MTS (about 20 µg/mL) and filtered through above each of the membranes in triplicates. Checked the recovery of filtered standard solutions against unfiltered standard solution. Results enabled that there are no binding (adsorption) of drug into membrane and recovery of both analytes was found more than 90% in all membranes. The results are summarized in table 2.

IVRT experiments were run with above synthetic membranes to evaluate drug release characteristics of EBZ and MTS from Ebernent M Cream formulation. Study was performed with 1% SLS: Ethanol (70: 30, % v/v) as receptor media, 32˚C receptor compartment temperature, 400 rpm stirring speed and 8 hour study time.IVRT samples were analyzed for quantitative estimation of EBZ and MTS by HPLC. Calculated cumulative release (µg/cm^2) of each of EBZ and MTS from Ebernet M cream with respect to different synthetic membranes.Results of membrane optimization study are depicted in fig. 3. Results enabled that sufficient and precise release of EBZ and MTS was achieved with 0.22 µ Polysulfone membrane. Hence polysulfone membrane was finalized for IVRT study.

Table 2: Results of membrane binding and optimization study

	EBZ		MTS	
Membranes	**% Recovery ± SD* (Membrane binding)**	**Flux (µg/cm^2/√t)**	**% Recovery ± SD* (Membrane binding)**	**Flux (µg/cm^2/√t)**
Polysulfone	98.44 ± 0.82	70.48	98.32 ± 0.63	3.48
Nylon	96.39 ± 1.12	60.40	96.01 ± 0.51	2.49
Cellulose	96.05 ± 1.47	60.81	96.25 ± 1.90	2.62
Teflon	98.18 ± 0.60	57.83	95.77 ± 1.22	1.38

**Study performed in triplicates for each membrane.*

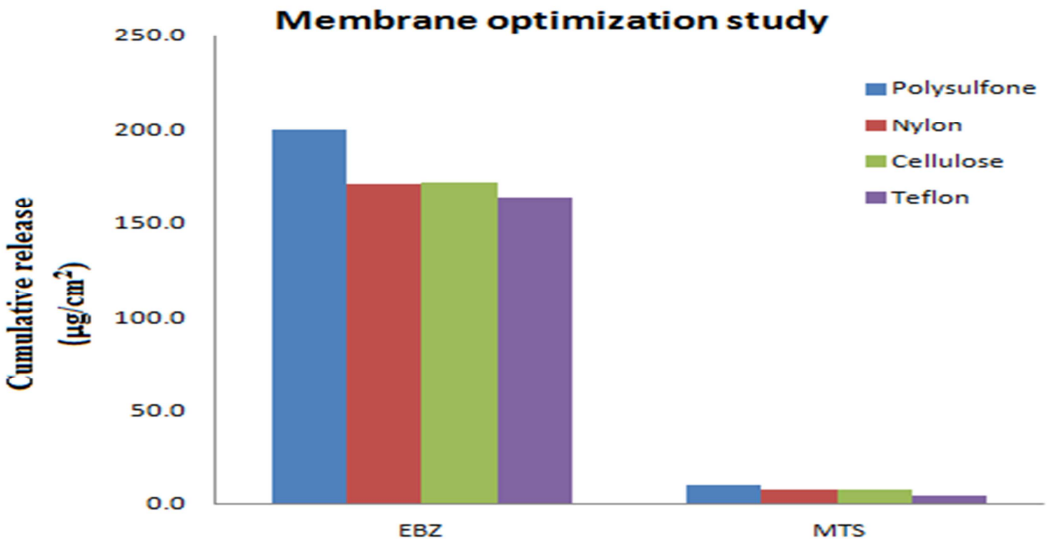


Fig. 3. Typical plot of Membrane optimization study

Franz diffusion cell parameters and Drug release calculation
Quantity of sample application on membrane was selected based on amount required to cover membrane area and spread uniformly. About 200 mg of sample quantity was selected for IVRT study. Basic application of EBZ and

MTS containing topical formulations on skin, hence 32˚C temperature for receptor compartment was used for this study. Generally six diffusion cells are used for a test as in dissolution testing to nullify individual dosage form variability. Sampling intervals of this study were 1, 2, 3, 4, 6 and 8 hours based on sufficient release of EBZ and MTS from topical formulations. Sample aliquot of 200 µL was withdrawn at each time point and replaced with fresh receptor media. Cumulative drug release (µg/cm^2) of analyte is determined by following equation.

$$Q = [C_nV + \sum_{i=1}^{n-1} CiS] / A$$

Where,
Q= Cumulative amount of EBZ/ MTS released per surface area of membrane (µg/cm2)
C_n= Concentration of EBZ/MTS (µg/mL) determined at nth sampling interval
V= Volume of individual Franz diffusion cell, 11 ml
n-1
$\sum_{i=1}^{n-1} Ci$ = Sum of concentration of EBZ/MTS (µg/ml) determined at sampling intervals 1 through n-1
S = sampling volume, 200 µL
A = Surface area of diffusion, 1.766 cm^2

HPLC method for quantification

IVRT samples were estimated for release characteristics of EBZ and MTS by HPLC using Waters Xterra C18 (150 × 4.6 mm, 5µm) as stationary phase and mobile phase constituted of water and methanol (35:65, v/v) at a flow rate of 1.50 mL/min. Both analytes were measured with UV detection at 235 nm. EBZ and MTS were eluted at about 5.0 minutes and 8.0 minutes respectively. Typical diagram of IVRTand HPLC chromatogram is depicted in fig. 4.

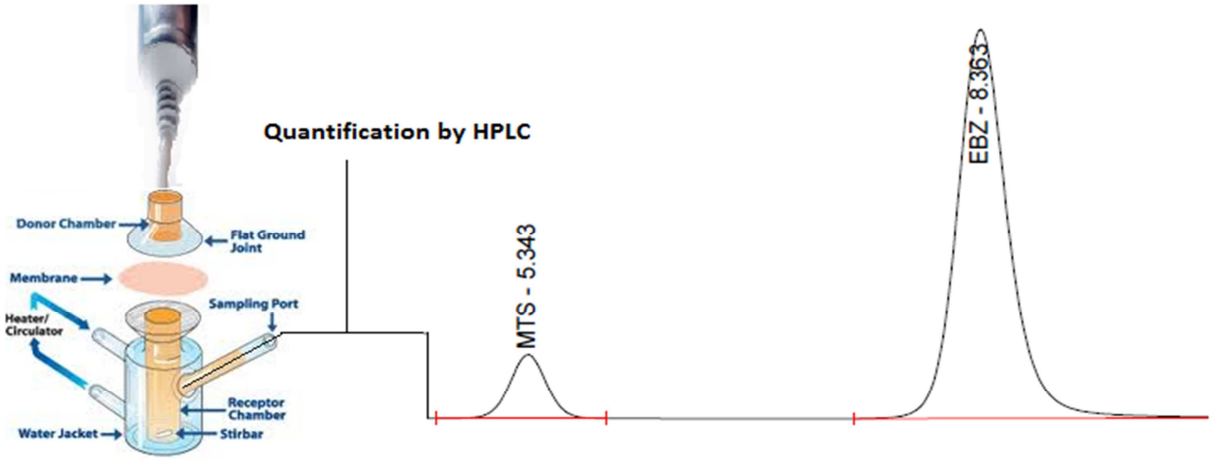


Fig. 4. Typical diagram of IVRT study

Linearity curves of concentration Vs peak area were plotted for EBZ and MTS in the concentration range of 3.5-270 µg/mL and 0.2- 30 µg/mL respectively. Linearityplots of EBZ and MTS are depicted in fig. 5. The correlation co-efficients of both analytes were found > 0.99.

Solution stability of standard and IVRT samples

Standard solution and IVRT sample (8 hours time point) were analyzed at initial and after 24 hours for stability at room temperature. No significant drop was observed in response of EBZ and MTS in standard and sample solution after 24 hours. Hence concluded that standard and sample solutions are stable at room temperature for 24 hours.

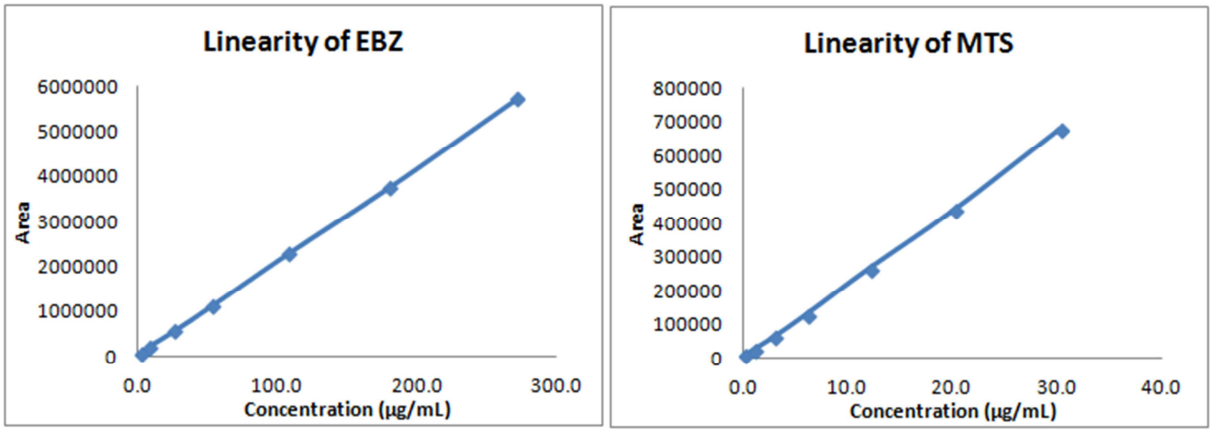


Fig. 5. Linearity plots of EBZ and MTS

Comparative IVRT study of marketed formulations

Different market formulations such as Ebernet M cream, Ebernet cream, Momate lotion and Elocon lotion were studied for viscosity test and IVRT study. Study performed with six cells for each market formulation.Calculated cumulative amount release (µg/cm^2) and flux value (µg/cm^2/√t) of EBZ and MTS from each market formulation. Plotted graph of cumulative amount release of EBZ and MTS in µg/cm^2 Vs √t for each market formulation (fig. 6). Viscosity and flux values of formulations are summarized in table 3. Results enabled that Flux value of MTS in Momate lotion and Elocon lotion are comparable, While Ebernet M cream is showing significantly less flux value of MTS compared to Momate and Elocon lotions. Results enabled that viscosity of formulation is playing critical role on flux value of EBZ and MTS. % RSD of cumulative drug release of EBZ and MTS at 8th hour from six diffusion cells for respective formulation was found less than 10% (table 4).

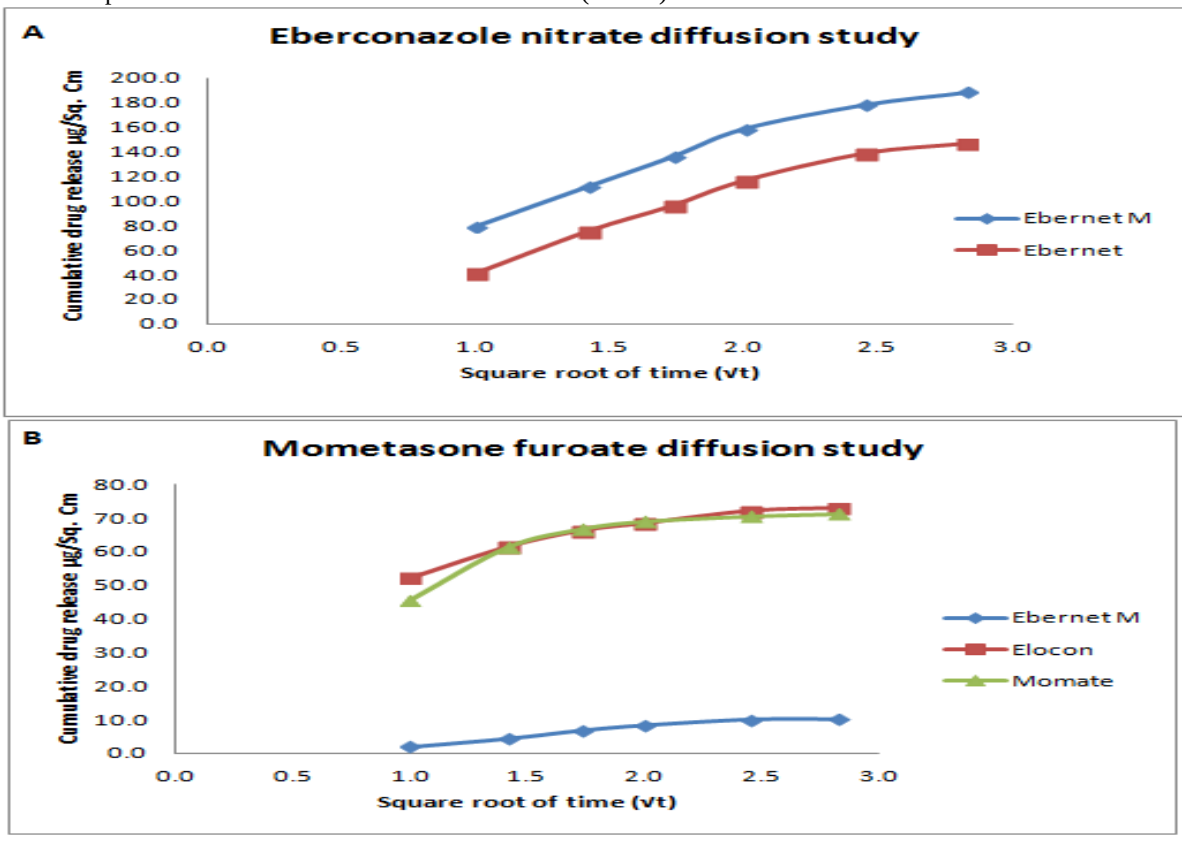


Fig. 6. Comparative plots of drug release of EBZ and MTS Vs √t in different market formulations

Table 3: IVRT study data of EBZ and MTS in different marketed formulations

	EBZ		MTS		
Market formulations	**Flux ± SD* ($\mu g/cm^2/\sqrt{t}$)**	**Correlation coefficient**	**Flux ± SD* ($\mu g/cm^2/\sqrt{t}$)**	**Correlation coefficient**	**Viscosity** (Poise)**
Ebernet M Cream	68.49 ±2.35	0.991	3.97 ± 0.19	0.989	20.33
Ebernet Cream	52.40 ±1.58	0.990	-	-	9.72
Elocon Lotion	-	-	26.21 ± 0.23	0.959	1.28
Momate Lotion	-	-	25.95 ± 0.18	0.921	1.27

**Study performed on six cells (n=6) for each formulation, **Viscosity parameters: Brookfield CAP 2000+ viscometer, spindle no. 1, 25 rpm and temperature 25˚C.*

Table 4: Results of precision

	EBZ		MTS	
Market formulations	**Cumulative release ($\mu g/cm^2$, 8th hour)**	**% RSD# (NMT 10%)**	**Cumulative release ($\mu g/cm^2$, 8th hour)**	**% RSD# (NMT 10%)**
Ebernet M Cream	193.71	3.44	11.24	4.84
Ebernet Cream	148.21	3.01	-	-
Elocon Lotion	-	-	74.13	0.88
Momate Lotion	-	-	73.41	0.69

#% RSD calculated for cumulative release of six cells.

CONCLUSION

The developed IVRT method proposed the use of synthetic membrane (0.2µ polysulfone) at early product development stage and 1% SLS: Ethanol (70: 30, % v/v) as receptor media for in vitro release study of EBZ and MTS from topical formualtions. Quantification of IVRT samples was performed by sensitive HPLC method, which is established for linearity of EBZ and MTS in concentration range of 3.5- 270 µg/mL and 0.2- 30 µg/mL respectively. The in vitro release test provides the good evidence for product evaluation and performance. The use of four different formulations ensures that proposed method helps to differentiate between the formulations of different manufacturers. The Method showed precise release profile within the set of six cells This method was utilized to evaluate release profile of EBZ and MTS from different topical formulations. The Proposed method showed discrimination in release profile with different viscosities formulations. This method can be utilized in pharmaceutical industries for monitoring of batch to batch reproducibility, discriminate formulations with respect to change in process and formulation composition and for comparative IVRT study of generic formulations to build confidence prior to costly clinical study.

Acknowledgement
The authors would like to thankful to Dr. Reddy's laboratories, Hyderabad, India for providing research facilities to carry out this research work.

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