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Formulation, development and evaluation of econazolenitratenanoemulsion

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ABSTRACT:Theaimofpresentresearchistoformulate and develop nanoemulsion of econazolenitrate as effective treatment for superficial fungalinfection.Econazolenitrateisanimidazoleantifungaldrug.Whichistopicallyusedtotreatinfectioncausedby fungus or yeast such as tinea corporis, tinea pedis,tinea crusis; jock itch, tinea versicolor, candidiasis,Candida albicans. Econazole nitrate is Classified asbiopharmaceuticalclassificationsystemIV(Lowpermeability, Low solubility) drug. The drug efficacyof topicalformulationcanbe limitedby itspoorsolubilityandlowpermeability.Therefore,toovercome these problem nanoemulsions have beendesigned.Topicalnanoemulsionscontaining1%econazolenitratewithoil(oleicacid),Surfactant(Tween 80),Co-surfactant(PropyleneGlycol)arepreparedbyusingultrasonicationmethod.Theformulationsthatpassed thermodynamicstabilitytests were evaluatedforphysicalappearance,pH,viscosity, FTIR, drug content, drug release, pdi andstabilitystudy.

KEYWORDS: Econazolenitrate,Nanoemulsion,Ultrasonication , Thermodynamic stability, viscosityetc.

I. INTRODUCTION

Nanoemulsionisadvancedmodeofdrugdeliverysysteminwhichtwoimmisciblеliquidphases(oilandwater)aremixedtoformsinglephasebymeansofemulsifyingagent(surfactant,co-surfactant). One of these phases is called dispersedphase and other is continuous. Nanoemulsions arenano sized formulation containing nano sized dropletrangingfrom20-500nmandappeartobecleartransparentortranslucent. Thesearethermodynamicallystableandisotropicinnature,comprises of oil, surfactant, and co-surfactant. Due toitssubmicronsizenanoemulsionsaregreatlyinfluenced as drug carrier for systemic, controlled,andtargetdrugdeliverysystem.Theyhavetheabilitytoincorporatevarioushydrophobicandhydrophilic drugs to improve the efficacy of drug at the site ofaction.Brownianmotionofnanoemulsionavoidcreaming,sedimentationandcoalescingwhichiscommonly associate with conventional dosage form,also small droplet size protect it from flocculation.Most of drug shows low solubility and permeabilityissue, less absorption and dose variations, to avoidsuch problem nanoemulsion is the best choice whichprovideincreasedsurfaceareaduetoitssmallerparticlesizeandimprovingthedrugabsorption.Nanoemulsionislipid-basedsystemwhichcandeliver both hydrophilic and lipophilic drug throughtheskinfortherapeuticeffect.

In this formulation antifungal drug econazole nitrateused to formulate nanoemulsion. Most of the topicaldermatologicalformulationssuchascream,and ointments, emulsion have the disadvantages of lessspreadingcoefficient,stickynature,creaming,cracking, sedimentation, coalescence. To overcometheseproblemeconazolenitratenanoemulsionisprepared.Econazolenitrateisanimidazoleringcontainingbroad-spectrumantifungalagent,theyinhabitC-14α-demethylase(acytochromeP450[CYP450]enzymewhichblockmethylationoflanosterol to ergosterol, Depletion of sterol lead tocelldeath.

Objectives

- The main objective of study is to formulateandevaluateEconazolenitrateNanoemulsion.
- Toreducedose frequencyandsideeffect.
- Toincreasethesolubilityofeconazolenitrate.
- Toincreasetherapeuticeffect.

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II. MATERIALANDMETHOD

EconazolenitrateSampleisreceivedfrom,AartipharmaceuticalPvt,Ltd.Mumbai,Tween80,Propyleneglycolarepurchasedfrom,ModernIndustries Malegaon-sinnar, Dist-Nashik, and oleicacidpurchasedfrom,Research-labfinechemIndustries,Mumbai400002.

Methods

Determinationofmeltingpoints

Meltingpointsofdrugisdeterminedbyusingcapillarymethod.Drugisfilledintocapillarytube upto the height of 3 mm by sealing its one end. Thecapillary is introduced into the digital melting pointsapparatusand thepoints atwhichthedrugstartsmeltingnotethatpointuntilentire samplegetmelted.

Solubilitystudy: Forthe purposeof solubility,beyond saturation additional amount of drug is addedinthe solvent (eitheraqueous ornon-aqueous)atroomtemperatureandkeptfor24hrs.withrareshaking. The supernatant was taken and evaluated byusingShimadzuUV1800doublebeamspectrophotometer.

Identification of drug by FTIR: The pure drugis mixed with IR grade solvent in a proper ratio andapplying pressure on IR plate. The sample of drug isthen scan over the range of 4000-400 cm^{-1} in PerkinElmerFTIRspectrometer.FTIRspectrumofEconazole nitrate shows the presence of the peakswhichcomplieswiththereference spectra.

Identificationofdrugbyuvspectroscopy:A)standard stocksolution

Exactly100mgofeconazolenitrate wasweighedandtransferredto100mlvolumetricflask. 70mlofmethanolwasaddedandstirreduntildissolvedcompletely. Volume was made up to 100 ml withsame solvent to obtain final concentration of 1000mg/ml.

B)workingstocksolution

Standard stock solution was further diluted to getworkingstocksolutionof100mg/mlbypipettingout accurately 1 ml from the standard stock and dilutedup to 10 ml with methanol. A series of dilution weremade from the working stock solution of 100 mg/ml.bypipettingout0.5,1, 1.5, 2,2.5mlrespectivelyintoseparate 10 ml volumetric flask and diluted up to 10ml with methanol to produce concentration rangingfrom 5-25mg/ml. the absorbance was measured withhelpof uv spectrometerat271 nm againstblank(methanol).

Drugexcipientcompatibilitystudy

Todeterminethecompatibilityofdrugwithexcipients,drugexcipientsstudywascarriedout.Drugand excipientwithratioof1:1%w/wwasfilledintheamberc oloredglassvialsandsealedwithaluminumcap.Sealed vialwerekeptat40°C±2°C/75% RH ± 5% for seven days in desiccator anddesiccator filledwithsaturated solutionofsodiumchloride. It was kept in hot air oven for 7 days. Thecontrol and test samples were examined by FT-IRmethodand studiedforany interactions thatmayoccurbetweendrugandexcipient.

Constructionofpseudo-ternaryphasediagrams

Pseudo ternary phase diagram were constructed todetermine the region of nanoemulsion existence andto study the effect of various surfactant/co-surfactantweight ratio on the extent of a stable nanoemulsionregion. Water titration method was used to constructpseudo-ternaryphasediagram.Theoptimizedformulationsare selectedusingcentroidofnanoemulsionregion.variousregionofoiltosurfactant-cosurfactant ratio were used. Three phasepseudo-ternary phase diagram were constructed fordifferentsurfactant-cosurfactantratioof(1:1)and(2:1) by water titration method. For construction ofeach phase diagram oil, surfactant, and cosurfactantmixture were prepared. These mixtures were kept onmagnetic stirrer at room temperature and water wasadded drop by drop. while adding water, a point isachieved where system becomes turbid that readingwas noted. The % quantity of all ingredients werecalculatedforeachdiagramandpseudo-ternaryphasediagramswereconstructed.thephysicalstateisplotted on a pseudo-three-component phase diagramwith each axis represent the aqueous phase, oil phase,andthirdsurfactant-cosurfactantratio.

Preparationof econazole nitratenanoemulsionbyultrasonicprobesonicator

Nanoemulsions(o/w)werepreparedbyultrasonicprobesonicator.Forthiseconazolenitrate1%,oil6%,surfactant-cosurfactantratioof30%(1:1),35%(1:1), 30%(2:1), 35%(2:1), and distilledwaterwithvarious concentrations were used. Accuratelyweighed1%OfEconazolenitrateandoleicacidasoil6%, were dissolved by using sonicator for 30 min attemperature 40°C. tween 80 as surfactant was addedto above mixture and dissolved by sonicator for 10min at 40°C, similarly propylene

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glycol addedas co-surfactant.Water isaddedwithcontinuesstirring,thesolutionwasplacedf orhomogenizationprocessusingprobesonicator.Total timerequiredto obtainedcomplete transparent econazole nitrate nanoemulsionis15-20min.

Table1 :Formulationofnanoemulsion

Srno	Formulations	Oil (%)	(Smix) (Tween80:PropyleneGlycol)	Water(%)	Drug(%)
1	ECZNE1	6	30%(1:1)	63%	1
2	ECZNE2	6	35%(1:1)	58%	1
3	ECZNE3	6	40%(1:1)	53%	1
4	ECZNE4	6	30%(2:1)	63%	1
5	ECZNE5	6	35%(2:1)	58%	1
6	ECZNE6	6	40%(2:1)	53%	1

III. EVALUATION OF NANOEMULSION

Thermodynamicstability

Manufacturing batches of nanoemulsion issubjectedtodifferentstabilitytests.

Centrifugation

Prepared formulations were centrifuged for 30min at 5000 rpm using centrifuge And phaseseparationwasanalyzedvisually.Theformulatio n that did not undergo phase separationwereselectedtofurthertests.

Thermal-stabilityofnanoemulsion

Stability of optimized formulations were detected byplacing the solution in amber color borosil vials atthree different temperatures i.e., 4, 25, and 45°C intemperature-controlled oven for the duration of 48hours.Sampleswereremovedperiodicallyforassess menttodetectanyphysicalchangeslikecoalescence,cl arity,andturbidityetc.

Clarity/dispersibilitytest

2 ml of each formulation was added to 500 ml ofdistilled water and 0.1 N HCL (separately) in USPdissolutionapparatus(type2).Temperaturewasm aintained at 37 ± 0.5°C . and the speed of paddlewas adjusted to 50 rpm for gentle agitation. the resultoftheinvitroperformanceofformulationwasche cked visually for any precipitation/turbidity. Theformulations were examined by comparing it usinggradesystem.

Drugcontent

10 mg of equivalent of drug loaded nanoemulsionwas diluted in 100 ml of solvent (methanol). Fromthisstocksolution1mliswithdrawnanddilutedwit h10 ml of solvent (methanol). The absorbance wasmeasured at 271 nm by uv spectroscopy and drugcontent is calculated by using the equation obtainsfromlinear regressionanalysisofcalibrationcurve.

In-vitrodrugrelease

Theformulationswereplacedonthesurfaceof cellophanemembranewhichwasplacedbetweendono randreceptorcompartmentoftheFranzdiffusioncell.di ffusionstudiesarecarriedoutat37±1°Cusingphosphat ebuffer(pH7.4)asthedissolutionmedium.Thewholeas semblywaskeptonamagneticstirrerandthesolutionwa sstirredcontinuouslyusingmagneticbed.5mlofeachsa mplewaswithdrawnperiodicallyat0.5,1,1.5,2,2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6 hrs. and each sampleare replaced with equal volume of fresh dissolutionmedium in order to maintain sink condition. Samplesare analyzed by UV-spectrophotometer at 271 nm fordrugrelease.

MeasurementofpH

1 gram of nanoemulsion is dissolved in 100 ml ofdistilledwaterandpHwasmeasured.Themeasureme ntofpHwas takenintriplicate.

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Measurementofviscosity
Viscosityofformulationwasdeterminedby Brookfield viscometer.

IV. RESULTANDDISCUSSION

Organolepticproperties
Organoleptic properties of Econazole nitrate arefoundtobesimilartostandard

Identificationtest	Observedresult	Reportedstandard
Appearance	Amorphous	Amorphous
Colour	White	White
Odour	Odourless	Odourless

Table2:Organolepticpropertiesofeconazole nitrate

Meltingpointdetermination
Themeltingpointofeconazolenitratewasfoundto be 162°C which complies with melting range ofstandard160-162°C.

Solubility
Solubility ofeconazole nitratewasfoundto beindifferentsolventaregivenbelow.

Table3:Solubilityofeconazolenitrateindifferentsolvent

Sr.no	Solventsystem	Standard	Observed
1	Methanol	Freelysoluble	Freelysoluble
1	Ethanol	Freelysoluble	Freelysoluble
3	Tween80	Soluble	Soluble
4	Oleicacid	Soluble	Soluble
5	PropyleneGlycol	Soluble	Soluble
6	Water	Slightlysoluble	Slightlysoluble
7	Chloroform	Insoluble	Insoluble

IdentificationofdrugbyUVspectroscopy

Table4:Standard calibrationcurveofEconazole nitrateinmethanol

Concentration	Absorbance
2	0.034
4	0.104
6	0.198
8	0.281
10	0.374

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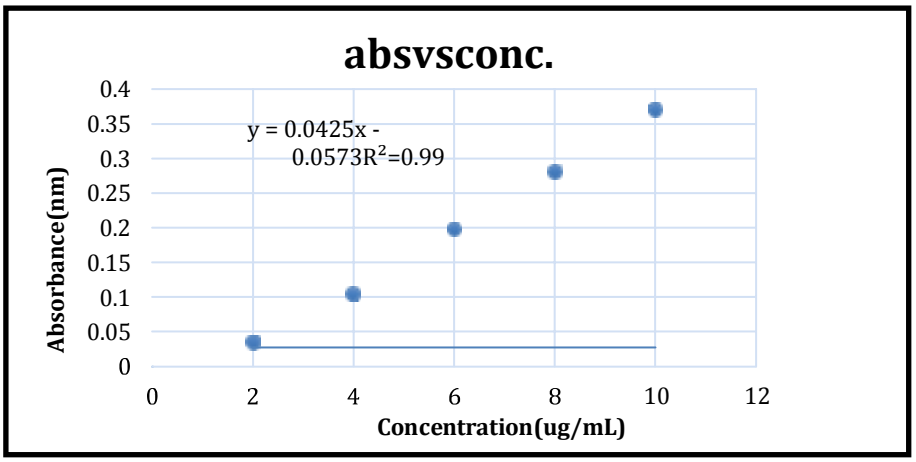

Fig1:StandardcalibrationcurveofEconazolenitrateinmethanol

Determinationofdrugexcipientcompatibilitystudy

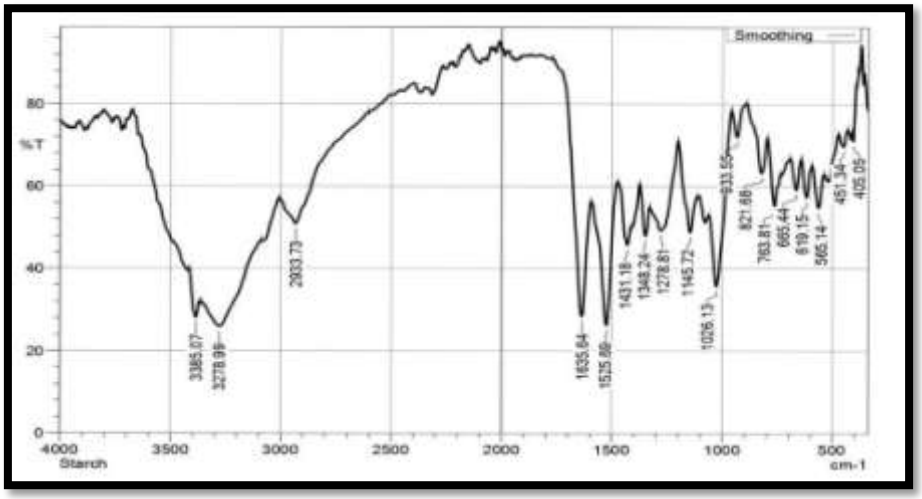

Fig2:FTIRofEconazolenitrate

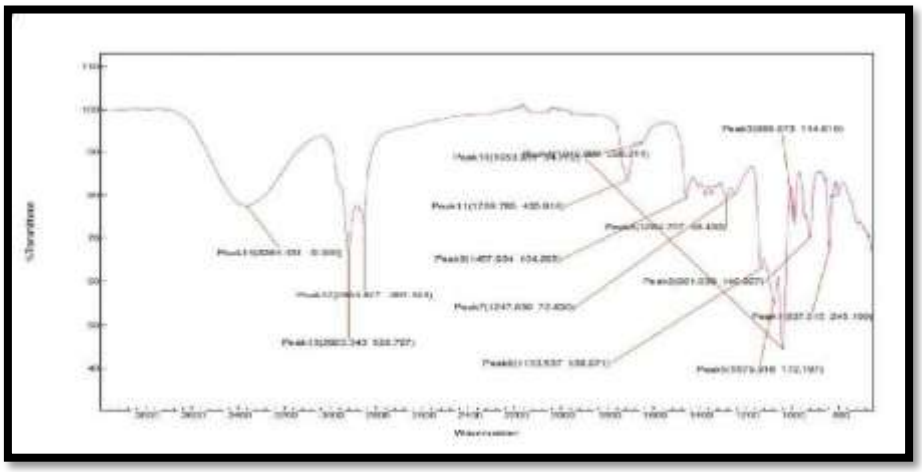

Fig3:FTIRof Econazolenitrate+oleicacid,Tween80,Propyleneglycol

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After FTIR study functional group of drugs shows sharp peak at standard range. There is no change in spectra ofdrugwithallexcipients.Hence,Econazolenitrateiscompatible withallexcipients.

Thermodynamicstability

Table5 :Observationofthermodynamicstability

Formulationcode	**Thermal-stability**	**Centrifugation**
F1	Stable	Nophaseseparation
F2	stable	Nophaseseparation
F3	Not stable	phaseseparation
F4	stable	Nophaseseparation
F5	stable	Nophaseseparation
F6	Not stable	phaseseparation

Afterthermodynamicstabilitytestingit wasobserved that formulationF1,F2,F4andF5arethestableSmixratiosas they didn't show phase separation after thermodynamic stability tests. Hence, formulation F1, F2, F4, and F5wereselected forfurthertestswithformulationcodeECZNEI,ECZNEII,ECZNEIII,ECZNEIV.

Clarity/dispersibilitytest

Table6 :Observationofclaritytest

Formulationcode	**Visualobservation**	**Grade**	**%Transmittance(420 nm)**
ECZNEI	Dispersewithin1min andappeartobeclear	A	93.75
ECZNE II	Dispersewithin1min andappeartobeclear	A	85.31
ECZNEIII	Dispersewithin1min andappeartobeclear	A	85.90
ECZNEIV	Dispersewithin1min andappeartobeclear	A	88.51

pH,Viscosity and%Drug content

Table7:Characterizationofnanoemulsion

Formulationcode	**pH**	**Viscosity(cp)**	**Drug content(%)**
ECZNEI	6.2	1121.14	84.54
ECZNEII	5.9	1050.45	88.07
ECZNEIII	6.4	990.76	89
ECZNEIV	6.1	1020.48	88.16

pHofprepared nanoemulsionformulationswasfoundinrangeof5.9–6.4andtabulatedintable7. Themeanaverageviscositywasfoundtobe1000to1200cp. ECZNEIIIshowssignificant viscosityandhighest %drugcontentvaluetabulatedintable 7.

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In-vitrodrugrelease

Table8:In-vitrodrugreleasein(%)

Time inhours	**ECZNE**I	**ECZNE** II	**ECZNE**III	**ECZNE** IV	**Marketedproduct**
0.5	5.85	5.26	4.9	6.27	3.21
1	9.77	8.59	11.86	10.86	7.08
1.5	16.04	14.19	16.29	14.43	12.88
2	27.75	22.37	28.04	29.35	17.40
2.5	35.13	28.02	34.22	31.16	22.30
3	46.22	40.38	43.22	42.06	28.90
3.5	57.60	52.61	56.12	53.89	36.40
4	66.30	63.26	68.05	64.27	44.70
4.5	71.56	72.11	76.19	74.58	51.12
5	77.23	76.47	80.16	78.66	56.89
5.5	83.74	81.63	84.92	80.52	64.29
6	90.33	89.96	94.70	92.23	67.32

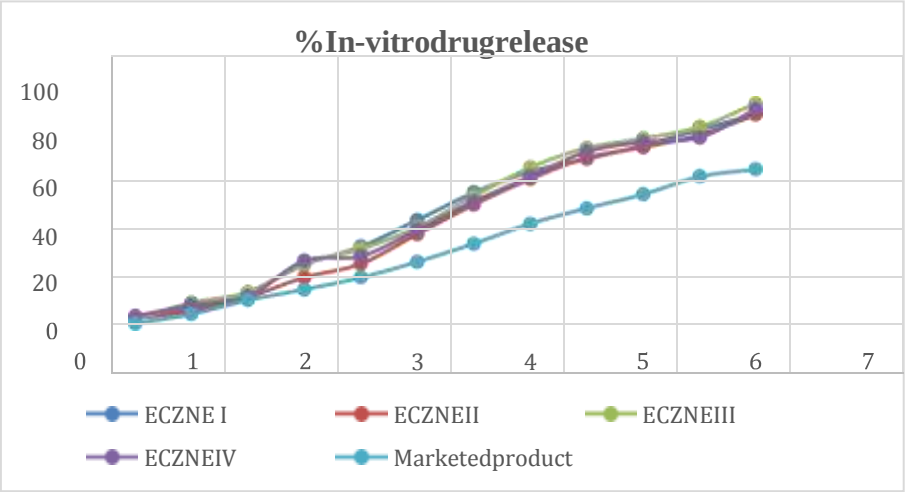

Fig4:%In-vitrodrugrelease

Result of in vitro drug release from different formulations are tabulated in **Table 8** and graphically shows in **fig.4.**All optimized batches show better drug release profile than marketed formulation. The prepared formulation batch**ECZNE III** shows the better drug release profile as compared to other preparation ECZNE I, ECZNE II, andECZNEIV.

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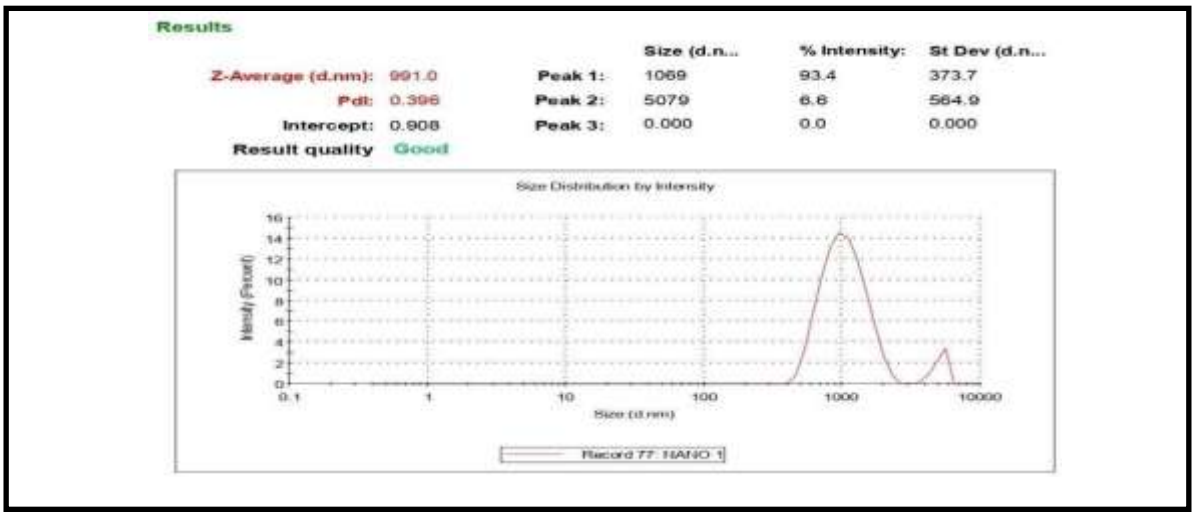

Fig5 :PdiofoptimizedbatchECZNE III

On the basis of nature of nanoemulsion formulation for the optimized batch ECZNE III was showedmidrangeMonodispersibilitystandardwithinrangeofpolydispersityindex(0.08-0.8).Hencetheoptimizedbatchofnanoemulsioniscons ideredasstable.

V. CONCLUSION

Econazole nitrate nanoemulsion was formulated andevaluated for topical treatment of superficial fungalinfection. The optimized formulation **ECZNE III**showed good result as thermodynamic stability wasfound to be in standard, clear nanoemulsion, drugcontent89%,drugrelease94.70%, pH6.4,viscosity 990.76 cp, pdi 0.396. Greater drug release profile incomparisonwith marketed formulationShows nochange in physical characteristic over three-monthperiod. Thus, main objective of this research wasachieved.

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