3.3.2 - Number of research papers per teachers in the Journals notified on UGC website during the year

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RAIGIRI (V), BHONGIR (M),
YADADRI BHONGIR (DT)

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Section A-Research paper

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ITRACONAZOLE AND TERBINAFINE IN COMBINE DOSAGE FORMS BY RP HPLC METHOD

Mahesh Banda, Nandini Manthapuram, Sahithi Reddy Gennepally, Srinivas Ampati*
Department of Pharmaceutical Analysis, Unity College of Pharmacy, Raigir (V), Bhongir (M), YadadhriBhuvanagiri (Dist), TS, India
***Corresponding Author:**
Dr. Ampati Srinivas
Professor & Principal
Head of the Department, Dept.of Pharma.Analysis
Unity College of Pharmacy
Raigir (V), Bhongir (M), YadhadriBhuvanagiri (Dist)
Telangana, India
mail: drampaty@gmail.com

ABSTRACT:
This study introduces a novel RP-HPLC technique for simultaneous measurement of Itraconazole and Terbinafine. To get the best results, we ran a series of experiments C18 ODS Agilent make column with 4.6*150mm at a temperature of 5, a pumping flow of 1 ml/min, and a MP ratio of (70:30 v/v) to separate Itraconazole and Terbinafine. The detection wavelength was 254 nm and the medium was methanol: (KH2PO4and KH2PO4) pH 3. Both Itraconazole (101.27%) and Terbinafine (99.99%) passed their purity tests with comparable findings. The analysis method was validated in accordance with ICH standards (ICH, Q2 (R1)). Linearity study conducted on the medications Itraconazole and Terbinafine throughout the concentration ranges of 50 mg to 250 mg and 5 mg to 50 mg, respectively, revealed recoveries of 99.56 and 99.48%, respectively. Intermediate precision had an RSD of 0.1, while repeatability was 0.2. Reliability and repeatability of the research were not compromised in any way.

KEYWORDS: ODSC18, Itraconazole and Terbinafine, RP-HPLCmethod

INTRODUCTION:

Itraconazole is an antifungal agent that inhibits cytochrome P-450-dependent enzymes resulting in impairment of ergosterol synthesis. It has been used against histoplasmosis, blastomycosis, cryptococcal meningitis & aspergillosis. Itraconazole interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Itraconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the

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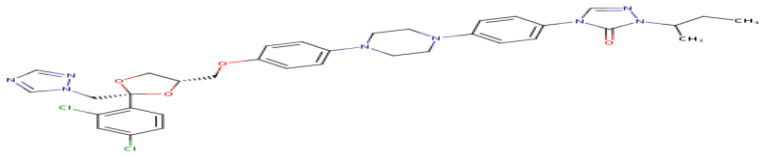
transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis.

Figure 1: Chemical structure of Itraconazole



Terbinafine hydrochloride (Lamisil) is a synthetic allylamine antifungal. It is highly lipophilic in nature and tends to accumulate in skin, nails, and fatty tissues. Like other allylamines, terbinafine inhibits ergosterol synthesis by inhibiting the fungal squalene monooxygenase (squalene 2,3-epoxidase), an enzyme that is part of the fungal cell wall synthesis pathway. Terbinafine is hypothesized to act by inhibiting squalene monooxygenase, thus blocking the biosynthesis of ergosterol, an essential component of fungal cell membranes. This inhibition also results in an accumulation of squalene, which is a substrate catalyzed to 2,3-oxydo squalene by squalene monooxygenase. The resultant high concentration of squalene and decreased amount of ergosterol are both thought to contribute to terbinafine's antifungal activity

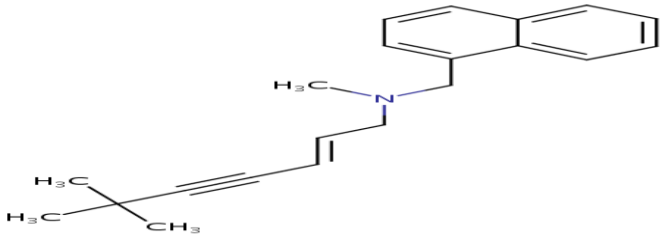


Figure 2: Chemical structure of Terbinafine

MATERIALS AND METHODS:

Equipment: Chromatographic separation was conceded on WATERS HPLC system which is outfitted with the 515 dual head reciprocating pump & a 2489 UV Visible detector. The software used is Empower-2 software and Phenomenex kinetex C_{18} (250mm×4.6mm i.d, 5µm) column is used for the investigation.

Chemicals and reagents: Itraconazole and Terbinafinedrugs were gifted by Aurobindo Pharmaceuticals, Hyderabad, Telangana, India. Acetonitrile, methanol, HPLC grade waterand mono sodium hydrogen orthophosphate and di sodium hydrogen ortho phosphatewere procuredfrom local manufacturers.

Selection of Detection wavelength:

10mg of Itraconazole and Terbinafine was dissolvedin mobilephase. The solution was scanned from200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Itraconazole and Terbinafine. The isobestic point was taken

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as detection wavelength.

Selection of column:

Column is selected basedon solubility, polarity and chemical differences among Analytes [Column: AgilentC18(4.6x250mm,5µm]

Selection of mobile phase:

Methanol:ACN (70:30%v/v) has been selected as mobile phase. If any buffer selected buffer pHshould bebetween 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved.If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response,Area,Tailing factor,Resolution will be achieved.

Preparations and procedures:

Preparation of mobile phase*:*

A mixture of Methanol 700ml (70%),300 mL of ACN (30%) are taken and degassed in ultrasonic waterbath for 5minutes. Then this solution is filtered through 0.45µ filter under vacuum filtration.

DiluentPreparation*:*

Mobile phase is used as Diluent**.**

Preparation of the individual Itraconazole standard preparation:

10mg of Itraconazole working standard was accurately weighed and transferred in to a10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent.(Stock solution). Further 10.0 ml from the above stock solution is pipette into a100ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Terbinafine standard preparation:

10mg of Terbinafine working standard was accurately weighed and transferred in to a10ml clean dry volumetric flask and about 2ml of DMF is added. Then it issonicated to dissolve it completely and made volumeup to the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette in to a100ml volumetric flask and was diluted upto the mark with diluent.

Preparation of Sample Solution:(Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to10mg of Terbinafine and Itraconazole (marketed formulation) sample in to a10mLclean dry volumetric flask and about 7mLof diluents is added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stocksolution)Further 3ml of above stock solution was pipetted in to a10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for Terbinafine and Itraconazole peaks are measured and the %Assayare calculated by using the formulae.

System Suitability:

Tailing factor for the peaks due to Terbinafine and Itraconazole in Standard solution should

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not be more than 2.0.

Theoretical plates for the Terbinafine and Itraconazole p e a k s in Standard solution should not be less than 2000

Preparation of standard stock solution:

Accurately 10mg of Terbinafine and 10mg of Itraconazole working standard were weighed and transferred in to a10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further this Stock was pipette(3mland0.3ml) in to a10ml volumetric flask and dilute upto the mark with diluents.

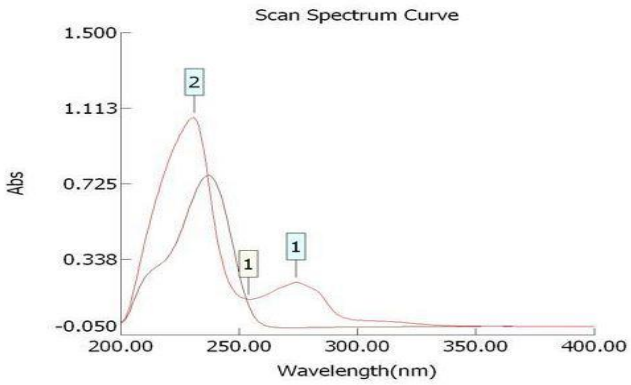
Procedure

The standard solution was injected for five times and the area for all five injections measured in HPLC.The % RSD for the area of five replicate injections was found to be with in the specified limits.

RESULTS AND DISCUSSION

Selection of detection wavelength: The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/m lfor individual and mixed standards. The resulting solution was scannedin U.V range from 200-400nm. The overlay spectrum of Itraconazole and Terbinafine was obtained and the isobestic point of Itraconazole and Terbinafine showed absorbance's maxima at 238nm shown in figure 3.

Figure 3: Overlay spectrum of Itraconazole and Terbinafine



METHOD DEVELOPMENT [4-6]

The chromatographic method development for the simultaneous estimation of Itraconazole and Terbinafine were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for these paration and quantification of Itraconazole and Terbinafine in API and pharmaceutical dosage form by RP-HPLCmethod.

Optimized Chromatographic conditions:

Column: Phenomenex kinetex C_{18} (250mm×4.6mm i.d, 5µm) column

Mobile phase: Methanol: Mono and disodium Hydrogen orthophosphate buffer of pH 6.8: acetonitrile (47:23:30 %V/V)

Flow rate: 1ml/min

Injection volume: 20µl

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Detection wavelength: 287nm
Mode of elution: Isocratic
Column temperature: Ambient

VALIDATION OF THE METHOD [7-10]

System suitability test: Solution for system suitability test was all set by moving 1ml of standard stock arrangement (1000µg/ml) into 10ml volumetric flagon, weakening to check with diluent and sonicated. This preparation was injected six times into the HPLC system for assessing parameters like number of hypothetical plates (N), peak area and tailing factor. The results were shown in table 1 and the overlain chromatogram for system suitability was shown in figure 3.

Table1. System suitability results For Terbinafine (Flowrate)

S.No	FlowRate(ml/min)	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	0.8	3536	1.7
2	1.0	2931	1.7
3	1.2	2713	1.7

*****Resultsforactualflow (1.0ml/min) have been considered from Assay standard.

Table2. System suitability results for Itraconazole (Flowrate)

S.No	FlowRate(ml/min)	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	0.8	2158	1.8
2	1.0	2114	1.7
3	1.2	2069	1.7

*****Results for actual flow(1.0ml/min) have been considered from Assay standard

MobilePhase:

The Organic composition in the Mobile phase was varied from 70% to 60%. Standard solution 300 µg/ml of Terbinafine & 3µg/ml of Itraconazole was prepared and analyzed using the varied Mobilephase composition along with the actual mobile phase compositionin the method.

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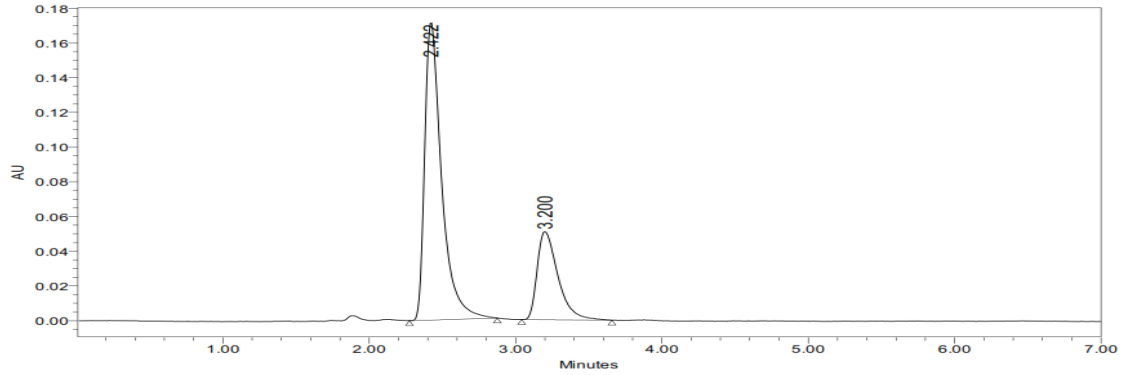

Fig.4.Chromatogram for Robustness more organic

	Name	RT	Area	Height (µv)	Usp plate count	Usptaililng
1	Itraconazole	2.422	1378798	171546	2358.0	1.7
2	Terbinafine	3.200	499679	50843	2616.1	1.6

Table3 Details of Robustness more organic

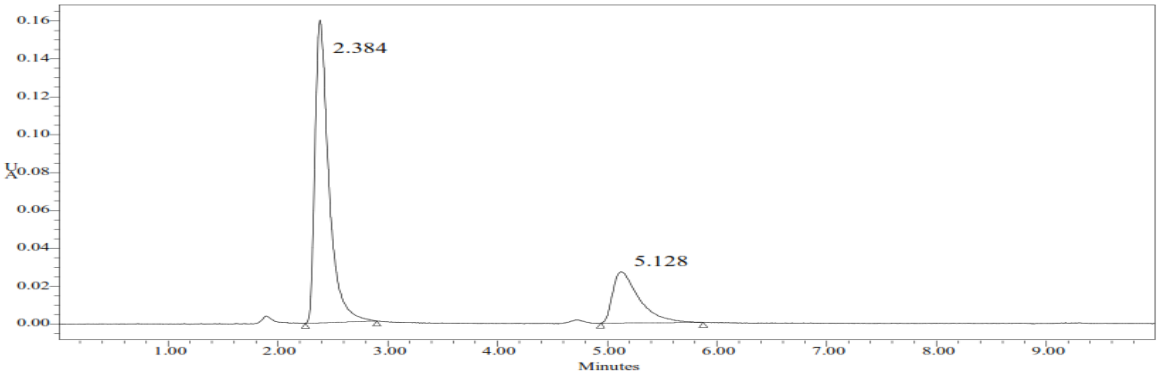

Fig5 Chromatogram for Robustness less organic

	Name	RT	Area	Height (µv)	Usp plate count	Usptaililng
1	Itraconazole	2.384	1404976	159808	2910.4	1.8
2	Terbinafine	5.128	453297	27049	2840.1	1.7

Table4. Details of Robustness les organic

The results are summarized. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobilephase±10

Table5. System suitability results for Terbinafine (Mobilephase)

S.No	Changein Organic Compositionin the MobilePhase	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	10%Less	2910	1.8

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2	Actual	2860	1.7
3	10%More	2358	1.7

*****Results for actual Mobile phase composition (55:45Buffer:Methanol)have been considered from Accuracy standard

Table6.System suitability results for Itraconazole (Mobilephase)

*****Results for actual Mobile phase composition (55:45Buffer: Methanol) have been considered

S.No	**Changein Organic Compositionin the MobilePhase**	**Systemsuitabilityresults**	
		USPPlatecount	**USPTailing**
1	10%Less	2540	1.7
2	Actual	2458	1.7
3	10%More	2616	1.7

from Accuracy standard

Linearity: Working standard solution was prepared according to the procedure and after filtering and sonicating the solution for 5mins further dilutions were madeto getdifferent concentration levels ranging from 20 to 300µg/ml. Every solution was injected into HPLC system as well as linearity was appraised. The calibration curve was designed taking concentration on X-axis along withpeak area on Y-axis. The linearity plots were shown in figure6 and 7

Table 7. Linearity Results

	Name	**RT**	**Area**	**Height (µv)**
1	Itraconazole	2.297	869216	109198
2	Itraconazole	2.264	1148093	145069
3	Itraconazole	2.308	1398858	164962
4	Itraconazole	2.370	1676584	193291
5	Itraconazole	2.322	1936686	238262
6	Terbinafine	3.458	296156	30269
7	Terbinafine	3.351	371946	39434
8	Terbinafine	3.488	452984	45638
9	Terbinafine	3.712	537383	50538
10	Terbinafine	3.535	617463	65483

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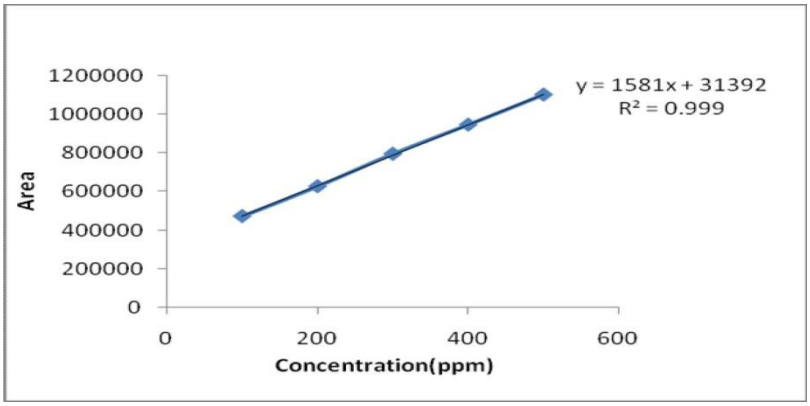

Fig.6 Calibration curve of Terbinafine

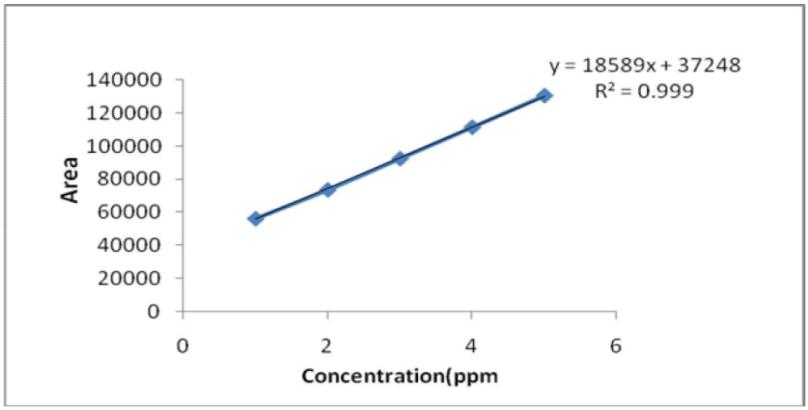

Fig.7 Calibration curve of Itraconazole

Robustness

Aspartof the Robustness, deliberate change in the Flowrate, MobilePhase composition, Temperature Variation was made to evaluate the impacton the method.

Fig8 Chromatogram for Robustness more flow

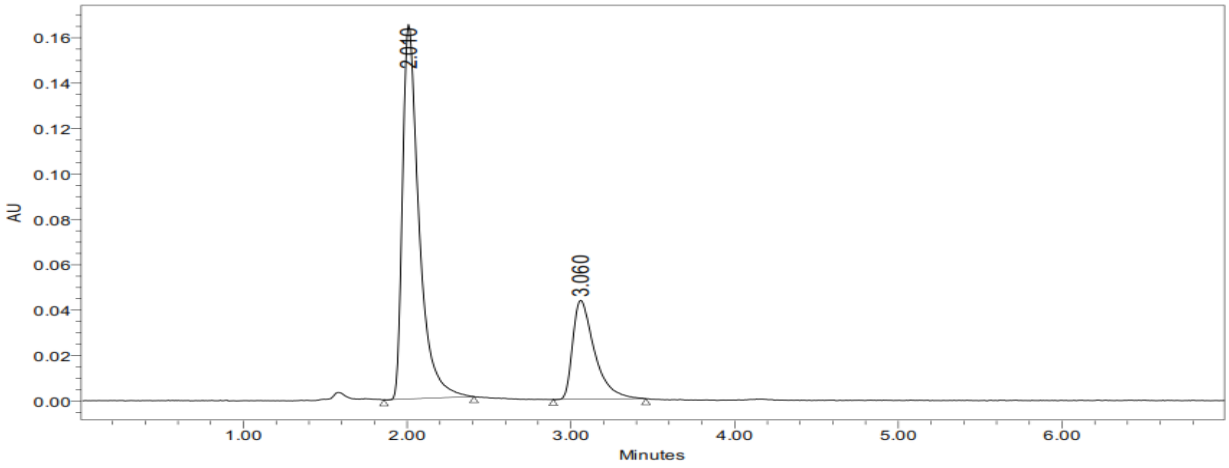

Table8 Details of Robustness more flow

	Name	RT	Area	Height (µv)	Usp plate count	Usptaililng
1	Itraconazole	2.010	1150303	165118	2069.9	1.7
2	Terbinafine	3.060	402322	43574	2713.8	1.7

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Fig.9 Chromatogram for Robustness less flow

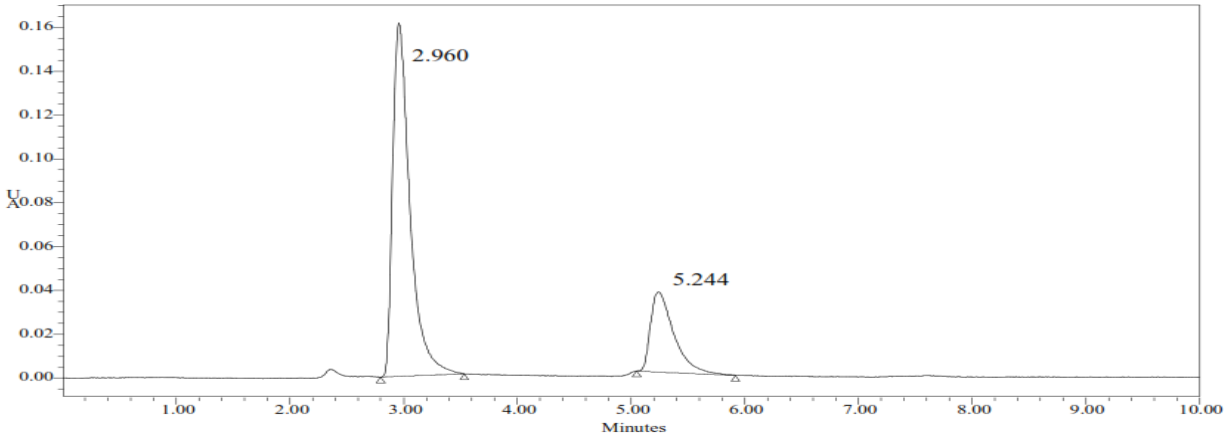

Table9 Details of Robutness less flow

	Name	RT	Area	Height (µv)	Usp plate count	Usptaililng
1	Itraconazole	2.960	1690740	161204	2158.1	1.8
2	Terbinafine	5.244	519208	36602	3536.2	1.7

CONCLUSION: In order to determine Itraconazole and Terbinafine simultaneously, a unique approach based on RP-HPLC was devised. To separate Itraconazole, an Agilent C18 column (4.6 x 150 mm, 5), and a detection wavelength of 254 nm were determined to be optimal. The calculated retention times were 2.34 and 3.28 hours. Terbinafine and Itraconazole were both found to be 99.97% pure, whereas Itraconazole was found to be 101.27 percent pure. In a linearity investigation spanning 50–250 mg of Itraconazole and 5–50 mg of Terbinafine, respectively, we found recoveries of 99.56 and 99.48%, respectively. Intermediate precision had an RSD of 0.1, while repeatability was 0.2. Reliability and repeatability of the research were not compromised in any way. The LOQ values varied from 0.0172 to 0.2125, whereas the LOD values were 3.17 and 5.68.

ACKNOWLEDGEMENTS: The authors acknowledge Aurobindo Pharmaceuticals Limited, Hyderabad, Telangana, India for providing gift sample of Itraconazole and Terbinafine.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interests.

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Formulation and Evaluation Oral Dispersible Tablets of Vidarabine

Akula Priyanka*, Vishwanadhula Pavani, Mohammad Ismail, Ampati Srinivas
Unity College of Pharmacy, Raigir, Bhongir, Yadadhri Bhuvanagiri, Telangana, India, 508116

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***Corresponding Author Email:** drakulapriyanka.90@gmail.com

Abstract
In the present work, taste masking of Vidarabine was carried out by using HP-β-CD inclusion complex. These taste-masked complexes were further formulated into the Oro dispersible tablet by the direct compression method using Ac-Di-Sol and Avicel as a super disintegrant. Vidarabine is used in the treatment of AIDS. This research has described the production of a taste masked dosage form from initial determination of threshold bitterness concentration of the pure drug through to the development of a final taste masked prototype formulation. It was found that the taste masked 1:2 ratio of LMV: HP-β-CD inclusion complex increases the bulk of final ODT blend (above 1000 mg) which is not feasible for formulation of ODTs. So, in this study the ODTs of LMV: HP-β-CD inclusion complex (1:1 ratio) showing acceptable bitterness in human taste panel studies was used in formulation of ODTs. In all formulations, the dispersion produced was soft (without grittiness) with a good mouth feel, and the bitter taste was fully masked. *In vitro* drug release profile of all optimized ODT formulations showed around 90% of drugs release within 10 to 15 minutes in acidic buffer (pH 1.2), implying that the drug will be absorbed fast, increasing the chances of bioavailability. A three-month stability analysis was carried out. For the optimized formulations, there was no noticeable difference in disintegration time, hardness, friability, or drug content.

Keywords
Vidarabine, super disintegrant, Orodispersible.

*****

INTRODUCTION:
Oral is the most preferred route of drug administration but is not suitable for patients with dysphagia. To overcome this problem or dispersible tablets is one of the famous technological innovations in the contract manufacturing and pharmaceutical field. Taste masking and taste assessment are the two main factors taken into consideration while formulating ODTs as they disintegrate and/ or dissolve in oral cavity. Taste masking in addition is related to patient compliance. Patient compliance is particularly important in pediatrics, geriatric, and long drug therapy patients. An ODT is a drug dosage form available for a small variety of over the counter (OTC) and prescription drugs (4). ODT disintegrates and/ or dissolves rapidly in the mouth without the need for water, which makes it suitable during traveling without immediate access to water. Since swallowing the saliva containing the dissolved or dispersed medication, the drug is consumed normally. Any drugs in ODT are showing fast onset of action and improved bioavailability as compared to same drugs in traditional tablet dosage form. This is due to ODTs pre-gastric absorption. ODT is also the best formulation option for drugs with a first-pass effect.

METHODS

Formulation of ODTs of Vidarabine-HP-β-CD Complex.

After adding superdisintegrants such as croscarmellose sodium (Ac-DI-Sol), sodium starch glycolate (SSG), and a mixture of both in different concentrations, orodispersible tablets of drug: polymer complex was prepared using the direct compression process. As the optimized ratio 1:2 of LMV: HP β-CD complex in the optimization increased the total bulk of ODT, was not selected for further formulation of ODTs of LMV-HP β-CD Complexes. So, the three formulations of LMV: HP β-CD (1:1) complex (which is batch F1 in Table 4-10) were prepared. Mannitol (Perteck M) and microcrystalline cellulose (Avicel PH 102) were mixed thoroughly in a glass mortar using a pestle.

Table 1: Formulation of ODTs of LMV: HP β-CD Complex

Ingredients (Quantity in mg)	F4	F5	F6
LMV-HP β-CD (1:1) equivalent to 100 mg Vidarabine.	676.73	676.73	676.73
Microcrystalline Cellulose PH 102	120	120	120
Sodium Starch Glycolate (SSG)	10	--	--
Croscarmellose Sodium (CCS)	--	10	--
SSG+ CCS	--	--	10
Mannitol	05	05	05
Magnesium Stearate	04	04	04
Talc	04.27	04.27	04.27
Tablet Weight	820	820	820

Results and Discussion

Vidarabine (Azidothymidine)- HP-β-CD Inclusion Complex Formation by Kneading Method

Experimental Design:

The effect of factors X1 and X2 is found to be statistically significant in nature. Response variables i.e., entrapment efficiency and drug content are simultaneously optimized using desirability function using Design Expert software. This process allows the selection of the most suitable level of factors to achieve desired level of drug content and entrapment efficiency. The results of multiple linear regression analysis revealed that for obtaining desirable drug content (91.76%) and entrapment efficiency (more than 67.07%), the formulation should be prepared using 1:2 drug polymer ratio with kneading time of 40 minutes.

Table 2: Independent factors in LMV- HP-Beta CD complexation.

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	LMV: HP Beta CD ratio	2.1754	1	3	0	Actual
B	Kneading time	43.1885	30	50	0	Actual

Table 3: Design Summary of LMV- HP-Beta CD complexation.

Standard	Run	LMV: HP Beta CD ratio	Kneading time	Entrapment efficiency	Drug content
4	1	1	40	76.44	75.9
10	2	2	40	98.66	90.57
2	3	2	30	86.14	67.48
11	4	2	40	97.81	91.11
6	5	3	40	87.85	81.22
5	6	2	40	98.36	91.02
13	7	2	40	96.98	91.21
7	8	1	50	81.02	73.26
8	9	2	50	96.32	79.61
12	10	2	40	98.11	91.76
9	11	3	50	89.96	83.86
1	12	1	30	74.03	67.07
3	13	3	30	88.49	74.38

Effect on Entrapment Efficiency

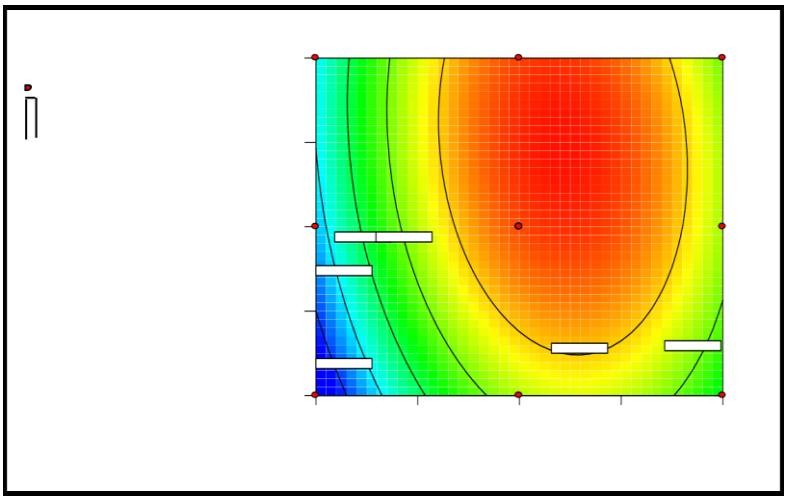


Figure 1: Interaction effect plot

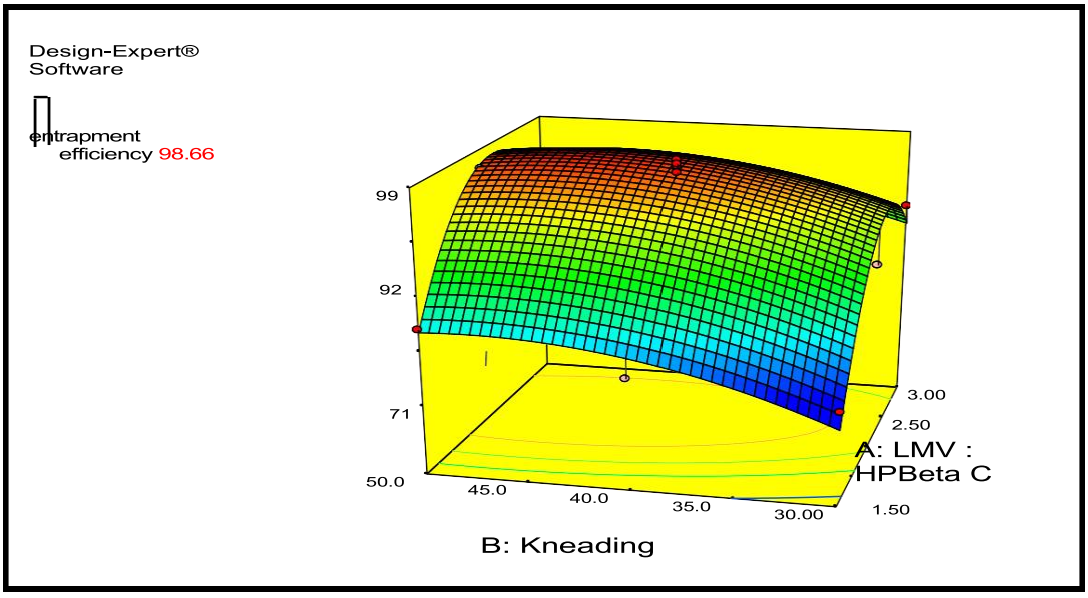


Figure 2: Response surface plot

Sensory Test on Threshold Value of Bitter Taste for Vidarabine/ LMV

Sensory test was performed to determine threshold bitterness concentration of Vidarabine on concentrations 5, 10, 20, 30, 40, 60 and80µg/ml.

Table 1: Sensory Test for Determination of Threshold Bitterness Concentration for LMV

No. of volunteer	Concentration (µg/ml)						
	5	10	20	30	40	60	80
1	0	0	0	0	1	1	3
2	0	0	0	0	0	1	3
3	0	0	0	0	1	2	2
4	0	0	0	0	0	2	3
5	0	0	0	0	0	2	3
6	0	0	0	0	2	1	3

***In vitro* taste masking evaluation of LMV-HP-β-CD (1:1) complex**

Table 2: In vitro taste masking evaluation of LMV-HP-β-CD (1:1) complex in phosphate buffer pH 6.8.

Time (sec.)	Concentration (µg/ml)			
	1	2	3	Average
30	10.56	12.05	11.34	11.31
60	17.74	17.27	19.38	18.13
120	29.84	30.41	29.30	29.85

Threshold Bitterness Concentration for Vidarabine was found to be 40 g/ml. *In vitro* release of Vidarabine from LMV-HP-β-CD (1:1) complex was 29.85 g/ml below threshold bitterness concentration i.e., 40 g/ml up to period of 120 seconds.

***In vitro* taste masking evaluation of LMV: indion 234 (1:1.5) complex**

Table 3: In vitro taste masking evaluation of LMV: indion 234 (1:1.5) complex in phosphate buffer pH6.8.

Time (sec.)	Concentration (µg/ml)			
	1	2	3	Average
30	9.82	10.21	10.41	10.15
60	15.91	15.72	16.21	15.95
120	22.94	22.80	22.99	22.91

Threshold Bitterness Concentration for Vidarabine was found to be 40 g/ml. *In vitro* release of Vidarabine from LMV: indion 234 (1:1.5) complex was 22.91 g/ml below threshold bitterness concentration i.e., 40 g/ml upto time period of 120 seconds.

***In vivo* Taste Evaluation of LMV-HP-β-CD complexes.**

The taste of the drug and complex was checked by time intensity method. The six healthy human volunteers were used for taste masking and informed consent was obtained from all of them. Bitterness was measured by consensus of a trained taste panel, with 20mg of sample held in the mouth for 5 to 10 sec., then spat out: the bitterness level was then recorded.

These volunteers were instructed not to swallow the granules, which were placed on the tongue. They were instructed to thoroughly gargle their mouth with distilled water after the completion of the test.

Table 4: *In-vivo* Taste Evaluation of LMV-HP-β-CD complexes.

Batch	Drug (LMV)						LMV-HP-β-CD complex					
	V1	V2	V3	V4	V5	V6	V1	V2	V3	V4	V5	V6
F1	4	3	4	4	4	4	0	0	1	0	1	1
F2	4	3	4	4	4	4	1	1	0	0	1	1
F3	4	3	4	4	4	4	2	1	1	1	1	1
F4	4	3	4	4	4	4	1	1	2	1	1	1
F5	4	3	4	4	4	4	0	0	0	0	0	0
F6	4	3	4	4	4	4	0	0	0	0	0	0
F7	4	3	4	4	4	4	0	1	0	0	0	1
F8	4	3	4	4	4	4	0	0	0	0	0	0
F9	4	3	4	4	4	4	0	0	0	0	0	0

Table 5: *In-vivo* Taste Evaluation of LMV: Indion 234 (1:1.5) complex.

Volunteers	Bitterness level after taste masking					
	10 sec	1 min	2 min	4 min	6 min	8 min
1	0	0	0	0	0	0
2	0	0	0	0	1	1
3	0	0	0	0	0	2
4	0	0	0	0	1	2
5	0	0	0	0	0	0
6	0	0	0	0	0	0

7	0	0	0	0	1	1
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0

Table 6: Volunteers Opinion Test for Vidarabine before and after Taste Masking (n=10)

Time (seconds)	Before taste masking Mean ± SD	After taste masking Mean ± SD
10	1.9*** ± 0.38	0
60	2.5*** ±0.42	0
120	3.0*** ±0.42	0
240	3.4*** ±0.51	0
360	3.8*** ± 0.43	0.3***± 0.84
480	4.0*** ± 0.48	0.6*** ±0.63

Determination of Drug Content in the Drug-Polymer Complex

Drug content of LMV: indion 234 (1:1.5) complex.

The resinate prepared (containing 10 mg of LMV) was subjected to evaluation of drug content and the data obtained is shown in Table 7. It was observed that the practical concentration obtained was 9.91 ± 0.043 mg, which was almost 99.1 % of theoretical concentration that is 10 mg.

Table 7: Drug content of LMV: indion 234 (1:1.5) complex

Name of Complex	Theoretical Conc. (mg)	Practical Conc. (mg)	% Drug Content
Vidarabine: Indion 234	10	9.91 ± 0.043	99.1 %

Evaluation of Oro dispersibleTablets

Pre-CompressionStudies

The directly compressible tablet blends were evaluated for pre-compression studies to determine their flow and compressibility (86).

Table 8: Micromeritic Properties of tablet blends containing optimized drug: polymer complexes (n= 3)

Property	LMV: HP-β-CD	LMV: Indion 234
Carr's Index (%)	13.6± 0.15	16.90 ± 0.72
Bulk Density (g/ml)	0.532± 0.93	0.473± 1.19
Angle of Repose (0)	25.420± 0.77	16.7 ± 0.691

Post-Compression Studies

Table 9: Evaluation of ODTs of LMV: HP-β-CD Complex

Test	F1	F2	F3
Weight variation test	170.0±1.4	170.4±1.2	170.2±1.6
Hardness (Kg/cm2)	3.5±0.09	3.75±0.08	4.00±0.10
Friability (%)	0.84	0.80	0.72
Drug content (%)	100.8±0.20	100.6±0.56	99.90±0.10
Wetting time (Seconds)	45±1.00	37±1.53	30±2.00
Mouth feel	-	-	-
In vivo disintegration time (Seconds)	57±1.97	48±1.86	30±1.37
In vitro dispersion time (Seconds)	42±1.00	38±2.00	25±1.53

***In vitro* release profile of formulated tablets:**

The dissolution test of tablets was performed using acidic buffer pH 1.2 with USP dissolution type II apparatus at 100 rpm and 37 ± 0.50C temperatures. Test sample (5 ml) was withdrawn at a particular time interval and replaced with fresh dissolution media maintained at 37 ± 0.50C. The test sample was filtered through membrane filter having size 0.45 µm

and analyzed using UV spectrophotometer at λ_{max} values. This test was performed on successive three tablets and mean ± SD calculated.

Table 10: *In vitro* Dissolution Study of Retrovir Vs optimized ODTs Batch F6

Sr. No.	Time (min.)	Retrovir	LMV-HP-β-CD ODTs
1	2	24.23 ± 0.21	71.46 ± 0.68
2	4	55.62 ± 0.73	78.26 ± 0.34
3	6	58.43 ± 0.22	86.21 ± 0.96
4	8	60.62 ± 0.18	89.80 ± 0.32
5	10	62.68 ± 0.27	93.66 ± 0.66

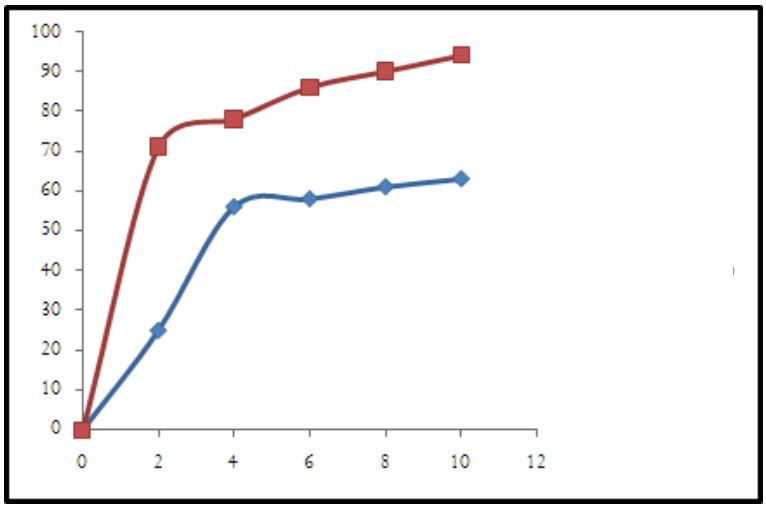


Figure 1: *In-vitro* Dissolution Study of Retrovir Vs optimized ODTs Batch F6

Table 11. Design summary of ODTs containing Vidarabine: indion234 (1:1.5) complex.

Batch No.	Avicel conc.	Ac-Di- Sol conc.	Hardness	Friability	Disintegration time
1	1	7	2.75	0.59	28
2	1	5	2.97	0.33	32
3	2	7	4	0.59	24
4	3	9	3.08	0.59	16
5	2	9	2.84	0.64	14
6	2	7	3.92	0.54	21
7	3	7	3.99	0.4	31
8	2	7	4.16	0.48	20
9	2	5	3.69	0.22	42
10	2	7	3.82	0.57	20
11	1	9	2.5	0.71	15
12	2	7	3.75	0.57	19
13	3	5	3.95	0.39	57

Effect on Hardness (Y1)

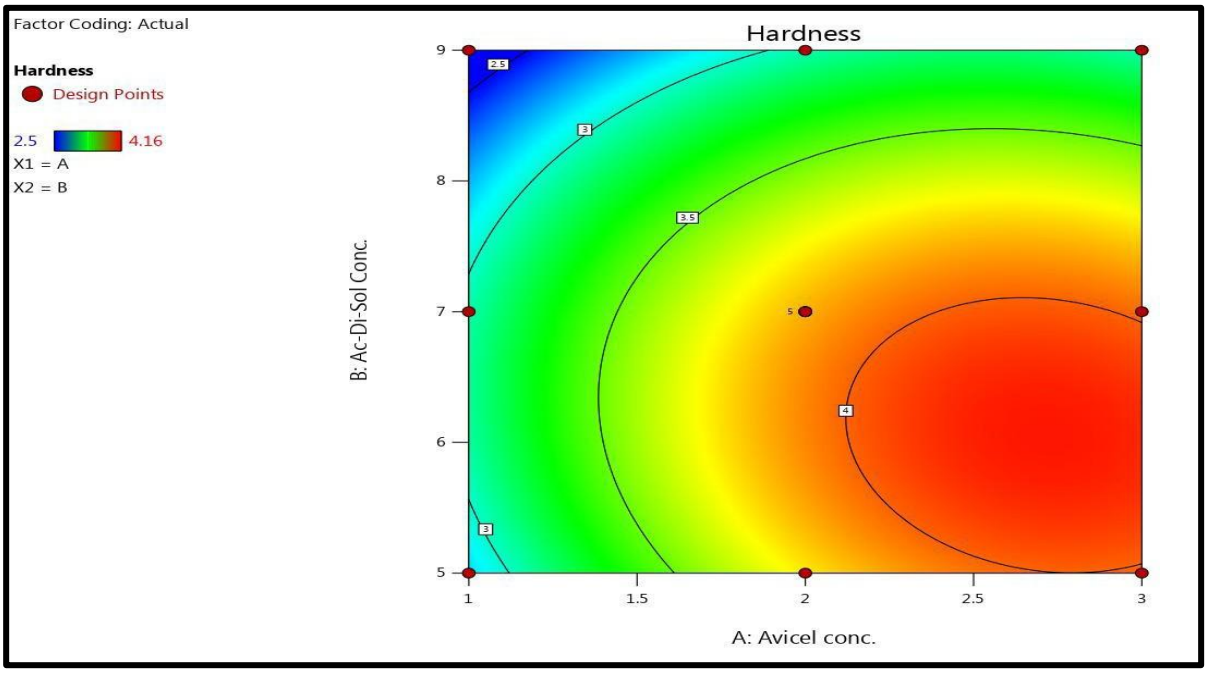


Figure 2: Interaction effect plot for hardness

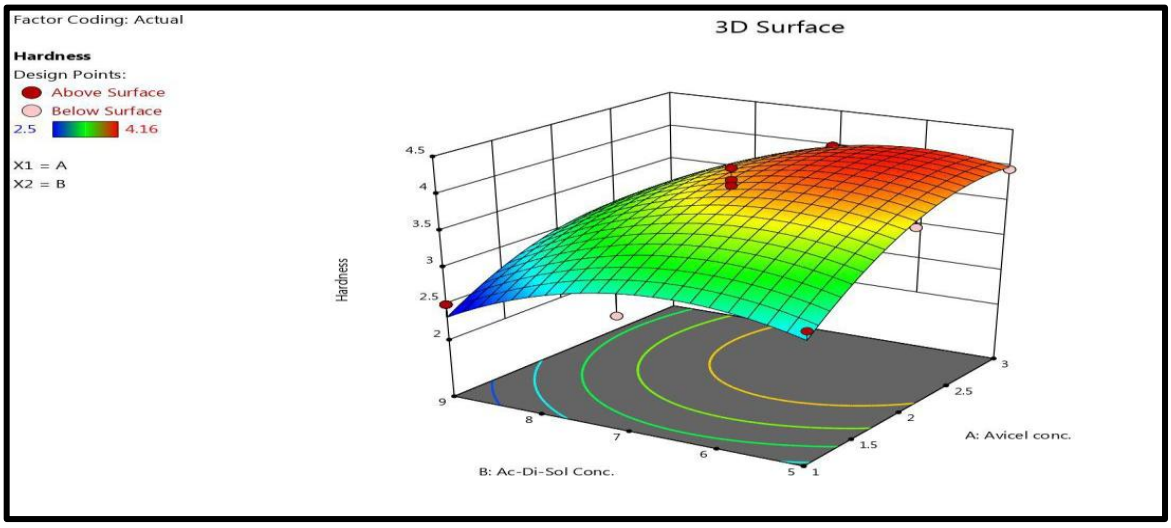
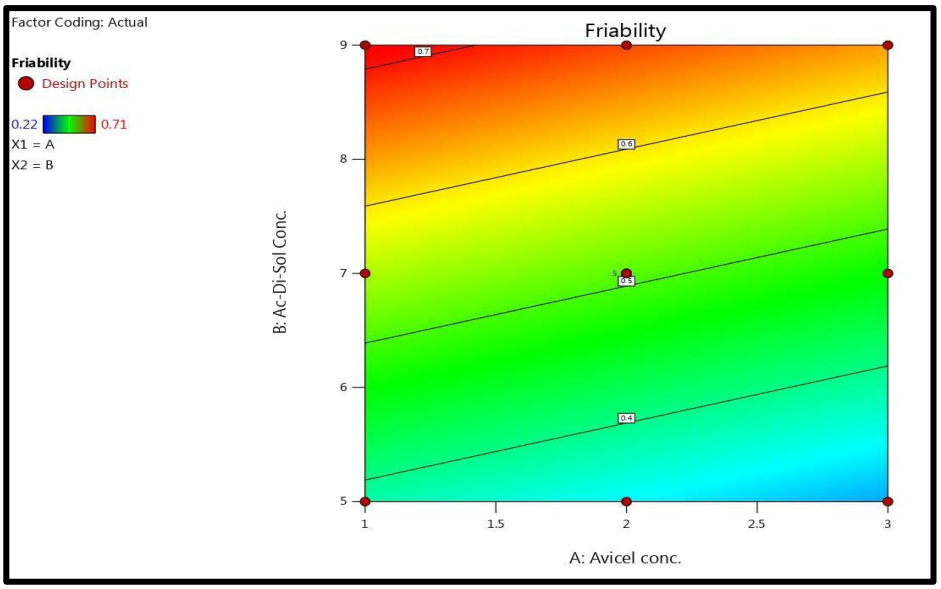


Figure 3: Response surface plot for hardness

Effect on Friability (Y2)

Figure 4: Interaction effect plot for friability



Effect on disintegration time (Y3)

Figure 5: Interaction effect plot for disintegration time

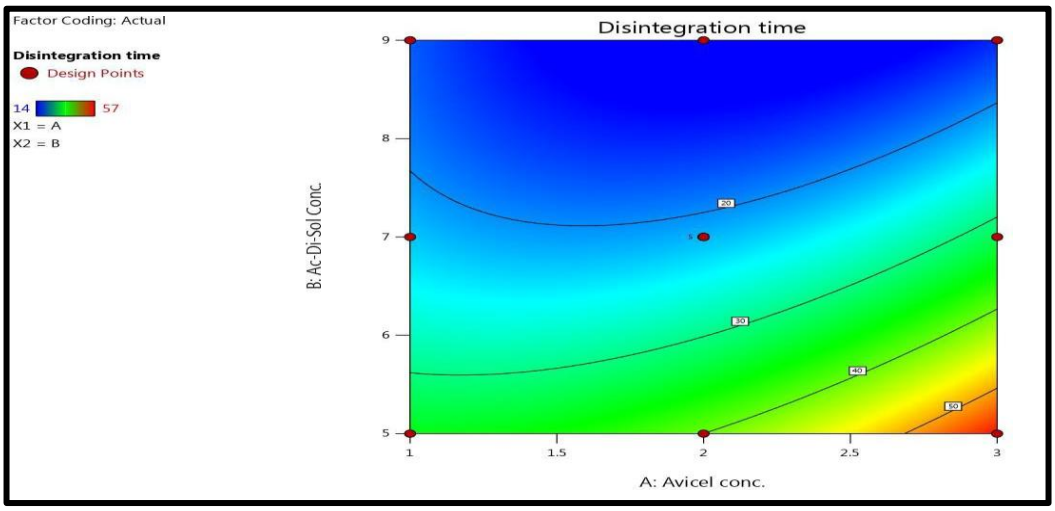


Table 12: Response coefficient table for ODTs of LMV: Indion 234 complex.

	Intercept	A	B	AB	A^2	B^2
Hardness	3.87207	**0.466667**	**-0.365**	-0.1	**-0.357241**	**-0.462241**
p-values		**0.0009**	**0.0037**	0.3702	**0.0250**	**0.0079**
Friability	0.509231	-0.0416667	**0.166667**			
p-values		0.1835	**0.0002**			
Disintegration time	21.7241	**4.83333**	**-14.3333**	**-6**	**5.46552**	3.96552
p-values		**0.0098**	**< 0.0001**	**0.0092**	**0.0308**	0.0913

Figure 6: Dissolution profile for formulation F7, F8, F9

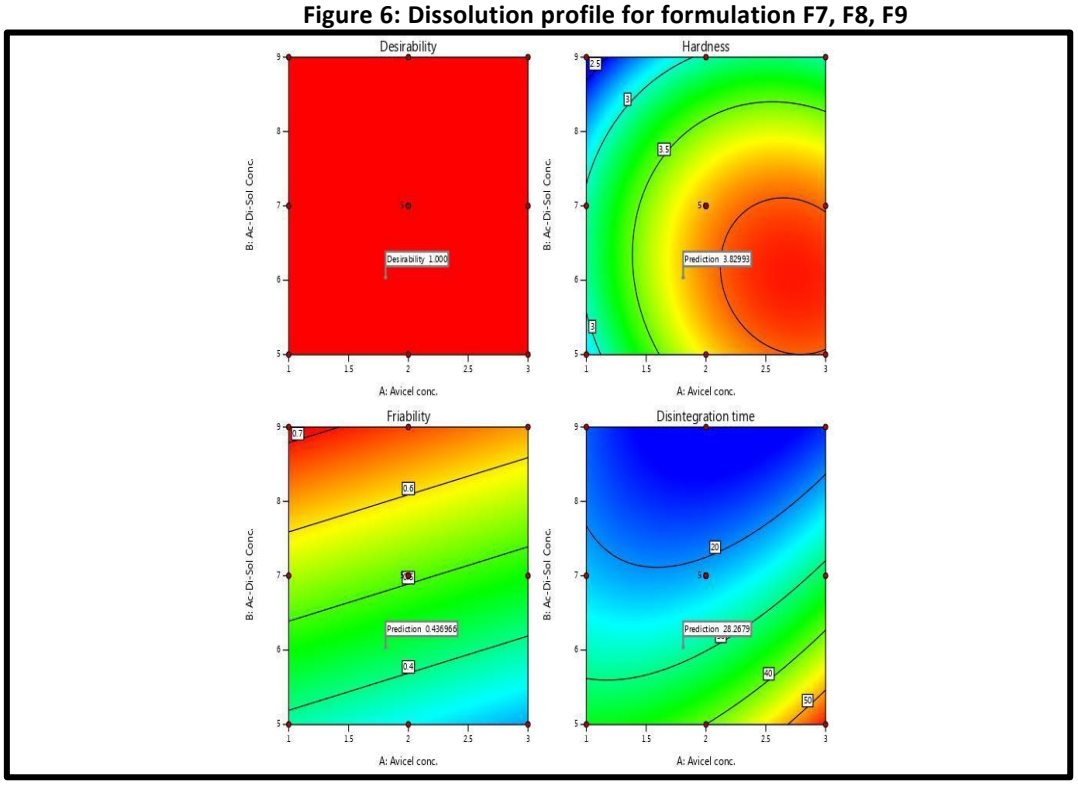


Table 13*:* Evaluation of Orodispersible Tablets of Vidarabine: Indion 234 Resin Complex

Test	F7	F8	F9
Weight variation test	400.0±0.89	409.4±1.11	403.2±0.94
Hardness (Kg/cm^2)	3.5±0.09	3.75±0.08	4.00±0.10
Friability (%)	0.57	0.47	0.49
Drug content (%)	95.69±0.20	98.60±0.56	99.90±0.10
Wetting time (Seconds)	63±1.54	44±1.10	42±2.00
Mouth feel	-	-	-
***In vivo* disintegration time (Seconds)**	47±1.84	41±1.06	50±1.37
***In vitro* dispersion time (Seconds)**	32±1.00	29±2.00	39±1.53

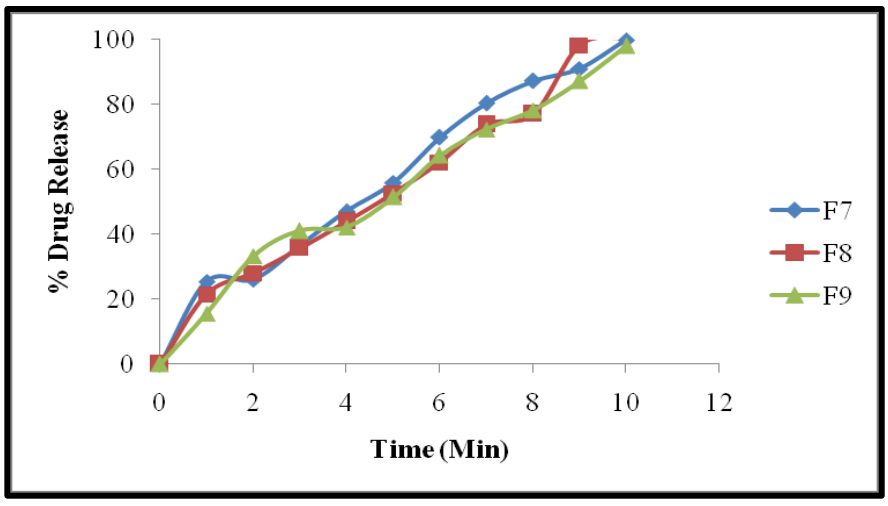


Table 14: Dissolution data for formulation F7 to F9

Time (Min)	F7	F8	F9
0	0	0	0
1	25.50±1.30	21.50±1.32	15.58±0.98
2	26.16±0.70	28.02±1.06	33.36±2.45
3	36.75±0.58	35.77±0.86	41.11±3.51
4	47.02±1.57	43.89±0.66	42.23±3.80
5	55.89±2.21	52.70±0.14	51.58±3.30
6	69.89±3.44	62.16±1.35	64.26±2.76
7	80.43±3.50	73.87±0.68	72.33±3.98
8	87.29±2.49	77.15±2.32	78.29±2.39
9	91.09±1.76	98.34±0.23	87.29±2.49
10	99.88±0.33	100.81±0.32	98.09±1.76

Accelerated Stability Studies of the Optimized ODTs.

Accelerated stability studies were carried out according to an International Conference on Harmonization (ICH) guidelines. The optimized formulations were placed in aluminum capped transparent glass vials for three months under storage conditions of 450C±20C and 75%±5%. At the end of each month, these samples were removed and analyzed for post compression tests.

The stability analysis showed that all the formulations were physically stable when maintained at 450C±20C and 75%±5% RH for three months, with no major differences in the findings. (88).

Table 15: Table 5-49: Effect of Stability Studies on ODTs Prepared by Using LMV-HP-β-CD Inclusion Complex (1:2)

Parameters Evaluated	1stmonth		2ndmonth		3rdmonth	
	Storage Condition		Storage Condition		Storage Condition	
	30±2°C / 60±5%RH	40±2°C / 75±5%RH	30±2°C / 60±5%RH	40±2°C / 75±5%RH	30±22 °C/ 60±5%RH	40±2°C/ 75±5%RH
Hardness (kg/cm2)	4.2 ±0.07	4.7 ±0.18	4.0 ±0.19	4.5 ±0.21	4.5 ±0.13	5.5 ±0.19
Friability (%)	0.89	0.69	0.75	0.78	0.88	0.51
***In Vitro* Dispersion Time (sec)**	39 ±0.25	42 ±0.09	40 ±0.18	45 ±0.11	39 ±0.18	44 ±0.19
Drug Content (%)	99.70 ±0.10	100.42 ±0.17	99.89 ±0.35	98.12 ±0.67	99.87 ±0.25	99.39 ±0.38

Table 16: Effect of Stability Studies on ODTs Prepared by Using LMV-Indion 234 Complex (1:1.5)

Parameters Evaluated	1st month		2nd month		3rd month	
	Storage Condition		Storage Condition		Storage Condition	
	30±2°C / 60±5%RH	40±2°C / 75±5%RH	30±2°C / 60±5%RH	40±2°C / 75±5%RH	30±22 °C/ 60±5%RH	40±2°C/ 75±5%RH
Hardness (kg/cm2)	3.9 ±0.07	4.6 ±0.18	3.57 ±0.19	4.2 ±0.21	4.1 ±0.13	5.2 ±0.19
Friability (%)	0.49	0.37	0.51	0.42	0.73	0.44
***In Vitro* Dispersion Time (sec)**	39 ±0.18	41 ±0.21	36 ±0.11	39 ±0.16	41 ±0.18	47 ±0.07

Drug Content (%)	95.70 ±0.15	98.42 ±0.09	98.98 ±0.21	99.12 ±0.94	96.87 ±0.17	99.53 ±0.07

SUMMARY AND CONCLUSION

Oral is the most preferred route of drug administration but is not suitable for patients with dysphagia. To overcome this problem orodispersible tablets is one of the famous technological innovations in the contract manufacturing and pharmaceutical field. Taste masking and taste assessment are the two main factors taken into consideration while formulating ODTs as they disintegrate and/ or dissolve in oral cavity. Taste masking in addition is related to patient compliance. Patient compliance is particularly important in pediatrics, geriatric and long drug therapy patients.

It was found that the taste masked 1:2 ratio of LMV: HP-β-CD inclusion complex increases the bulk of final ODT blend (above 1000 mg) which is not feasible for formulation of ODTs. So, in this study the ODTs of LMV: HP-β-CD inclusion complex (1:1 ratio) showing acceptable bitterness in human taste panel studies was used in formulation of ODTs.

In vitro drug release profile of all optimized ODT formulations showed around 90% of drugs release within 10 to 15 minutes in acidic buffer (pH 1.2), implying that the drug will be absorbed fast, increasing the chances of bioavailability. A three-month stability analysis was carried out. For the optimized formulations, there was no noticeable difference in disintegration time, hardness, friability, or drug content.

Overall, this study concludes that taste masked ODTs of the drug Vidarabine not only improve patient compliance but also overcome neglected dysphagia associated with these two drug therapies. A greater understanding of patient compliance in any of the drug treatments will allow proper formulations to be developed which in turn will improve treatment outcomes.

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FORMULATION AND EVALUATION TRANSDERMAL DELIVERY OFCELECOXIB MICROEMULSION GEL

Vishwanadhula Pavani, Akula Priyanka, Mohammad Ismail, Ampati Srinivas**
Unity College of Pharmacy, Raigir, Bhongir, Yadadhri Bhuvanagir, TS, 508 116

Abstract:
Celecoxib is an NSAID used to treat inflammatory joint conditions including osteoarthritis. In this study, we set out to create a novel microemulsion formulation for transdermal administration of Celecoxib. To choose excipients with excellent drug loading capacity, solubility of drug in different oils, surfactant, and co-surfactant was assessed in pre formulation tests. Capmul MCM C8 was chosen as the oil in the microemulsion's formulation, while labrasol and ethanol were chosen as the surfactant and co-surfactant, respectively. The concentrations of the oil phase, Smix, and distilled water used in the preparation of the Celecoxib microemulsion were calculated using pseudo ternary phase diagrams.
Three tests-a centrifugation tests, a heating and cooling cycle test, and a freeze thaw cycle testwere used to determine the optimal formulation. The % transmittance, droplet size, and cloud point of the optimized formulations were also measured.Because of its high % transmittance, the MF2 formulation stands out as the best of the bunch. A minimum globule size of 35.2 nm was observed for MF-2. The zeta potential of MF2 was calculated to be +19.0mV. Compared to previous batches, the MF2 formulation demonstrated faster drug release during the in vitro diffusion test, with peak drug concentrations occurring between 4 and 8 hours after the test began.
Microemulgel was created by adding a gelling agent to the microemulsion at a 1:1.2 ratio (microemulsion: gelling agent). When tested under a wide range of stability circumstances, the microemulgel (PN3) in question performed well. The microemulgel formulation (PN3) loaded with Celecoxib was shown to cause no skin irritation in an in vitro testing. Based on these findings, it seems that a microemulsion formulation of Celecoxib might be an effective formulation for transdermal distribution.
Key Words: Microemulsion, Microemulgel, Celecoxib, Arthritis

Corresponding author:
Vishwanadhula Pavani,
Assitant Professor,
Unity College of Pharmacy,
Raigir, Bhongir, Yadadhri, TS, India,.
mail: pavan.chary07@gmail.com

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INTRODUCTION:

New advancements in technology have made transdermal medicine delivery systems a viable choice for dosing patients. The clearance of the digestive system and the first pass of hepatic metabolism are two of the many advantages of transdermal medication delivery. Most failed attempts at oral medication delivery (74%) may be attributed to a number of factors. The skin serves as a barrier against the outside world and can heal itself if it is ever compromised.There has been a rise in the use of transdermal medication administration in recent years. Medication administration through the transdermal route has gained popularity as a result of the ease it provides and the drug's generally positive reputation for safety. Transdermal drug delivery is a practical choice since it is a tried-and-true, easy-to-implement, secure, and low-cost drug delivery method. A larger drug content in the dose form may be necessary if the medication is to be taken orally due to the many possible loss mechanisms. Medication has the potential to cause damage if administered incorrectly. Possible for concentrations to be lower than the MEC when using conventional drug delivery techniques (MEC). Maintaining an adequate supply of MECs is critical in any sickness situation. The needed low concentration is beyond the capabilities of conventional technologies. However, long-term MEC preservation may be possible with the advent of novel medication delivery strategies (transdermal, regulated, etc.). As their names imply, transdermal drug delivery systems strive to localize pharmaceutical administration and control the rate of distribution. Advantages of transdermal drug administration include the potential for regulated absorption of medications applied to healthy skin into the circulation.

MATERIALS AND METHODS:

Celecoxib drug was gifted by Gtosh Enterprises, Pune, India. Capmul MCM C8 from Abitec Corporation, USA, Castor oil, Oleic acid, Labrasol, Ethanol, Polyethylene Glycol 400, Tween 80, Carbopol 940 all are from Research lab, Mumbai.

Solubility Study

Studying medication solubility is the primary goal of solubility testing, since it helps determine which solvent is best for developing dosage forms. Celecoxib solubility was studied by dissolving 10 mg of Celecoxib in water, ethanol, methanol, and acetonitrile, among other solvents.

Solubility of Drug in Various Excipients: -

Celecoxib wads analyzed for their saturated solubility in a series of oils (Capmul MCM C8, Isopropyl myristate, Oleic acid, Castor oil, Labrafil M 2125 CS, Coconut Oil, Lemon Oil, Arachis Oil), surfactants (Span 20, Span 60, Span 80, Labrasol, Tween 20,Tween 60& Tween 80), and co-solvents (PEG200, Overnight at room temperature, 10 ml stopper glass vials were filled to capacity with 5 g of oils, surfactants, and co-solvents, as well as excess quantities of Celecoxib and Celecoxib. After diluting with methanol, the content of Celecoxib in the supernatant was measured at a max of 331 nm using a UV spectrophotometer (Schimadzu), and the concentration of Celecoxib was measured at 252 nm. An oil, surfactant, and co-solvent solution dissolved in methanol served as a control.

Formulations and Development:

Selection of Surfactant and Co-Surfactants Ratio

Surfactant to co-surfactant ratios of 1:1, 2:1, or 3:1 was chosen as a starting point, with further refinement based on visual inspection and practical constraints in formulation. The combination of surfactant and co-surfactant was known as Smix. Swirl 250 ml of distilled water and add 2 ml of Smix at a 1:1, 2:1, and 3:1 ratio. The most transparent solution was chosen after taking into account a number of factors to determine the best formulation dilution. This tactic relies on trial-and-error methods and visual analysis.

Preparation of microemulsion of Celecoxib:

Our previous work established the Microemulsion's composition, which includes Capmul MCM C8, Labrasol, ethanol, oil, surfactant, and co-surfactant, each of which contains Celecoxib as part of the surfactant (1 gm). To create the microemulsion formulations, the researchers used a previously disclosed approach. Celecoxib (1 gm) was dissolved into the combination of oil, surfactant, and co-surfactant, and the mixture was stirred. A high-pressure homogenizer was used to break down the mixture until a clear solution was achieved.

Table 1: Optimized formulation of microemulsion for 100 ml.

Batch No.	Celecoxib	Capmul MCM C8	Labrasol	Ethanol	Water
MF1	1	5	36	24	34
MF2	1	10	32	21	36
MF3	1	20	28	18	33
MF4	1	25	24	15	35
MF5	1	30	20	12	37
MF6	1	35	16	9	39
MF7	1	40	12	6	41
MF8	1	45	8	3	43
MF9	1	5	45	24	25
MF10	1	10	41	21	27
MF11	1	20	37	18	24
MF12	1	25	23	15	36
MF13	1	30	29	12	28
MF14	1	35	25	9	30
MF15	1	40	21	6	32
MF16	1	45	17	3	34
MF17	1	5	39	24.5	30.5
MF18	1	10	35	21.5	32.5
MF19	1	20	31	18.5	29.5
MF20	1	25	27	15.5	31.5
MF21	1	30	23	12.5	33.5
MF22	1	35	19	9.5	35.5
MF23	1	40	15	6.5	37.5
MF24	1	45	11	3.5	39.5

Characterization of Microemulsion: -

Percentage transmittance: -

Microemulsions of Celecoxib were diluted one hundred times with distilled water and examined visually for turbidity. The UV-VIS spectrophotometer was then used to determine its percent transmittance at 331nm using distilled water as a blank.

Cloud point measurement: -

Microemulsions that had been optimised were diluted with distilled water at a 1:250 ratio, then heated in a water bath. The point at which clouds suddenly become visible was identified as the cloud point using a UV-vis spectrophotometer to measure transmittance and ocular observation.

Droplet size determination

In a beaker, 10 milligrams of MF1-MF24 and CF1-CF24 microemulgel formulation was diluted with 50 milliliters of deionized water while being stirred with a glass rod. Analyses of particle size were performed on the resulting emulsion. Dynamic light scattering (DLS) using a zetasizer is used to quantify the size of the resulting droplets (Nano ZS, Malvern Instruments, UK). 25 degrees Celsius; red He-Ne laser; 4.0 milliwatts; 633 nanometers.

Zeta Potential Determination

Laser diffraction examination using a particle size analyser was used to ascertain the Zeta potential of the winning formulation (Malvern Zetasizer Nano Series ZS 90). The samples were diluted with distilled water at a ratio of 1:100 (v/v) and stirred for 1 minute. There were three sets of each experiment.

Preparation of Microemulgel of formulation

Selection of microemulsion and polymer Ratio:

Microemulsion and polymer ratios, including 1:0.5, 1:1, 1:1.2, and 1:1.5, were tested for free-flowing microemulgel before being narrowed down using the table below. Utilize a high-pressure homogenizer to completely dissolve a mixture of microemulsion and polymer at varying ratios of 1:0.5, 1:1, 1:1.2, and 1:1.5. This strategy relies on experimentation and close visual inspection.

Characterization of microemulgel:

Physical appearance:

Color, homogeneity, consistency, and pH were checked visually in the microemulgel formulations after they were created.

pH

Digital pH metre readings were taken from the microemulgels to establish their pH levels (Labindia Instruments, GMPH). After continuously monitoring the microemulgel composition, the electrode was dipped into it. Triplicate pH readings were taken for each batch.

Appearance of microemulgel

The formulas' aesthetic appeal was evaluated by holding them up to the light and taking a look at how they reflected it.

Where + average, ++ good, +++ excellent

Spreadability

The spread ability instrument was used to quantify this quality. The equipment consists of two slides: one is securely fastened in a wooden frame, while the other glides effortlessly over its surface. We stuffed two grammes of microemulgel (2 gm) in between the apparatus's slides. After letting a 1 kilogramme weight sit on the slide for 5 minutes, the air was forced out from between the slides and a homogenous sheet of microemulgel formed. Carefully, we wiped the slides' borders to get rid of the extra gel. An 80-gm weight was pulled on the upper slide while the lower slide was securely fastened. Observe how long it takes centimetres (in seconds). Higher Spreadability is associated with shorter intervals.

Spreadability was then calculated using the following formula:

$$S = M \times L / T$$

Where, S = is the spreadability,

M = is the weight in the pan (tied to the upper slide),

L = is the length moved by the glass slide and

T = represents the time in seconds taken to separate the slide completely.

Extrudability

After the microemulgels were created, they were poured into the compressible tubes. The formula's extrudability has been tested.

Where + average, ++ good, +++ excellent

Rheological study:

Spindle speeds of 0.5, 1.0, 2.0, 2.5, 4.0, 5.0, 10.0, 50.0, and 100.0 revolutions per minute were used on a Brookfield Viscometer (Model RVT, Brookfield Engineering Laboratories, Inc., USA) to examine the flow behaviour of the gel compositions. At 251 °C, the flow behaviour of the various formulations was evaluated by analysing the location.

Drug content determination: -

The 10 mg of Celecoxib and Celecoxib microemulgel was dissolved in 10 ml of dimethyl acetate in a separate 10 ml volumetric flask; the 0.1 ml of stock solution was then properly measured, transferred to a second 10 ml volumetric flask, and filtered using Whatman filter paper. Celecoxib and Celecoxib concentrations in the aforementioned solutions were measured using a UV Spectrophotometer (Shimadzu UV 1800) set to max 331nm and 252nm, respectively. Standard calibration curves of Celecoxib and Celecoxib were used to calculate the exact concentrations of each drug in the formulation.

***In vitro* drug release study: -**

The experiment employed a Franz diffusion cell that had an effective diffusion area of 7.1 cm^2. Franz diffusion cell having donor compartment on the outside and receptor compartment on the inside, with the egg membrane between them. The release patterns of Celecoxib and Celecoxib were measured after being applied to the stratum corneum in the forms of ME (1%, w/w D), MBG (0.5%, w/w D), and 0.1 gm and 0.05 gm, respectively. In order to stimulate receptor activity, 25 ml of physiological saline solution was injected into the receptor chamber (pH 6.8 phosphate buffer). The receptor medium was magnetically agitated at 50 rpm and kept at 37 1 C. Taken at regular intervals, the samples were filtered through a cellulose membrane filter with a pore size of 0.45 m before being subjected to ultraviolet (UV) analysis. After each sample was taken, the buffer solution in the receptor chamber was immediately changed with new. Both the ME and MBG formulations' cumulative drug accumulation in the receptor chamber was shown vs time (t, h).

Stability of microemulgel

Clarity and phase separation observation, as well as UV assays of Celecoxib and Celecoxib, were used to determine the stability of a microemulgel containing

the two drugs at 45 degrees Celsius for three months. For the same purpose of gauging physical stability, centrifuge tests were also performed. 15 minutes of centrifugation at 10,000 rpm were applied to the microemulgel samples.

Table 2: Stability protocol

Stability study (conditions)		
45°C ± 2°C / 75 % RH ± 5% RH		
1 Month	2 Months	3 months

analyzed for drug content by HPLC.

1.8.6. Skin Irritation study on rabbits:

Proctocol Approval

Institutional Animal Ethics Committee (IAEC), Browns College of Pharamcy, Khammam No. IAEC/SGRS/2018/6 approved the in vivo study, which was India).

Animal Study

The irritancy potential of Microemulgel (CF2, PC3) loaded microemulgels was evaluated in rabbits by applying the chosen gels to their freshly shaved backs. Here we detail how we choose the rabbits to use in this experiment.

We used a random number generator to split the rabbits into three groups of three. They had separate cages for each rabbit. Each test animal had the hair on the dorsal surface of its trunk neatly clipped off on both sides about 24 hours before the testing. Each hairless region on the test animals was divided into two portions (A and B) measuring around 6 square centimetres. The SLS solution (sodium lauryl sulphate) at 20% was chosen as the reference point.

The animals were treated as follows:

Group I- 20% w/v SLS solution (area A), Untreated (area B);

Group II- Microemulgel (CF2) (area A), untreated (area B);

Group III- Microemulgel (PC3) (area A), untreated (area B)

Each group had 500mg of the experimental formulation placed on the designated region A. Afterwards, a gauze patch secured with non-irritating tape was placed over the affected region (Transpore 3M surgical tape, 3M India Ltd, India). The B section served as the normative control for each set. The first round of tests was conducted with a single animal. Once the 24 hours of exposure were up, any remaining formulation on the skin was gently washed off with distilled water so as not to compromise the skin's protective barrier. Dermal responses (erythema and edoema) were observed and given a score between 0 and 4 at 1, 24, 48, and 72 hr(s)

1) For erythema and eschar formation:

I. rating zero, no erythema;

II. rating 1, very moderate erythema (barely perceptible);

III. score 2, properly-described erythema;

IV. rating three, slight to extreme erythema;

V. rating four.

(2) For edema formation:

rating 0: no edema;

rating 1: very slight edema (barely perceptible);

score 2: slight edema (edges of place well defined by means of specific elevating);

rating 3: slight edema (raised approximately 1 mm); and

score 4: severe edema (raised greater than 1 mm and extending past region of publicity).

At 1, 24, 48, and 72 hours, all of the rabbits' erythema and edoema scores were added together (s). The following formula was used to get the principal irritation index (PII) from the letter grades.

$$PII = \frac{\text{Sum of erythema grade at 1/24/48/72 hr(s)} + \text{Sum of edema grade at 1/24/48/72 hr(s)}}{\text{Number of animals}}$$

The irritation degree was categorized based on the PII values as negligible (PII = 0-0.4), slight (PII = 0.5-1.9), moderate (PII = 2-4.9) or severe (PII = 5-8) irritation.

RESULTs AND DISCUSSION:

Compatibility Study:

The DSC curve of pure Celecoxib with all excipients are given below in figure. As per DSC graph.

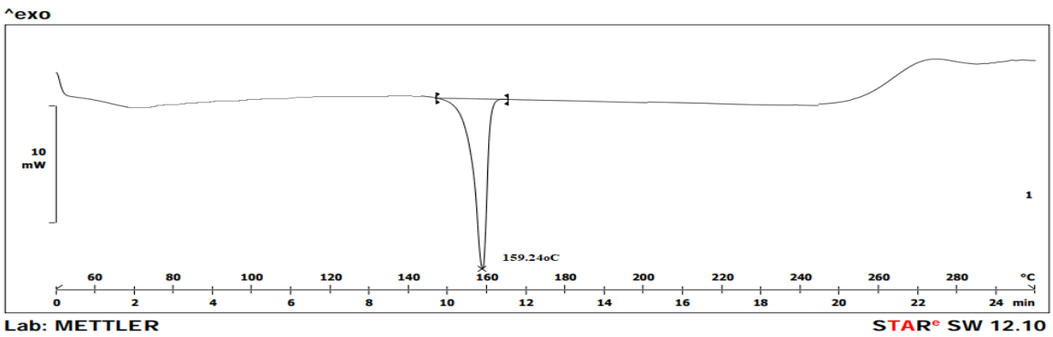


Figure 1: DSC thermogram of Celecoxib +Excipients

FTIR of drug

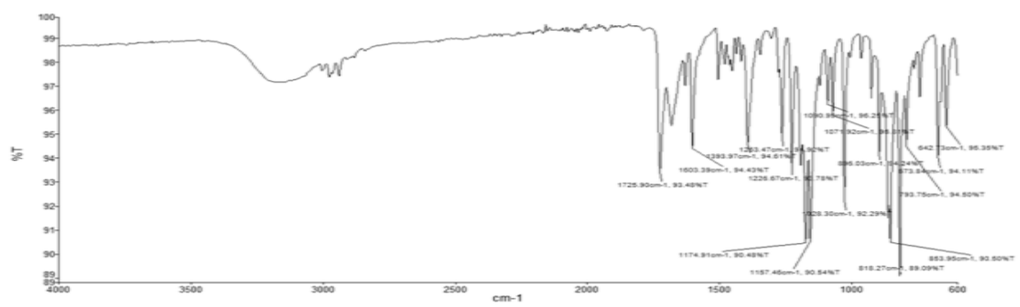


Figure 2: IR spectra of Celecoxib

The FT-IR spectrometer was used to record the IR spectra of pure Celecoxib, and the results were compared to the known frequencies of the drug's functional groups. In Table, we list the most prominent peaks and the functional classes to which they belong.

The solubility of Celecoxib in a variety of oils, surfactants, and co-surfactants.

Table 3: Data for Solubility study of Celecoxib in Various Oils

Sr. No	Oil	*Solubility of Celecoxib (mg/ml) at 25°C
1.	Capmul MCM C8	102.24 ±2.23
2.	Isopropyl Myristate	70.80 ±1.40
3.	Oleic acid	82.00 ±2.68
4.	Castor Oil	60.2 ±1.33
5.	Labrafil M 2125 CS	54.16 ±2.24
6.	Coconut Oil	45.12 ±1.01
7.	Lemon Oil	35.62 ±1.54
8.	Arachis Oil	30.85 ±0.96

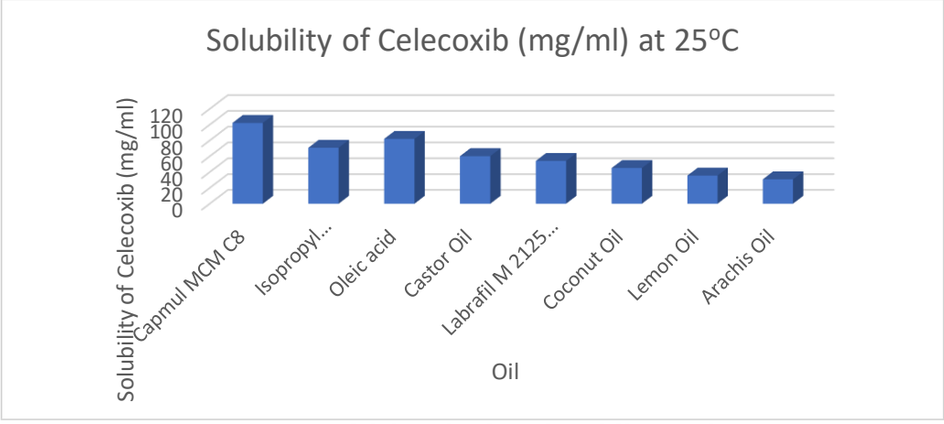


Figure 3: Solubility of Celecoxib in different oils

Compatibility study: -

Pre formulation compatibility studies of Celecoxib and Celecoxib with all excipients were carried out prior to preparation microemulsion. The daily observations of compatibility study for 14 days were taken for colour changes, cake formation, liquefaction, and gas formation.

Table 4: Excipients + Celecoxib Compatibility Study

Sr.no	Physical Mixture	Observations			
		Colour Change	Cake Formation	Liquefaction	Gas formation
1	Celecoxib + Moisture	No	No	No	No
2	Celecoxib + Capmul MCM C8	No	No	No	No
3	Celecoxib + Labrasol	No	No	No	No
4	Celecoxib + Ethanol	No	No	No	No
5	Celecoxib + Carbopol 940	No	No	No	No

Characterization of microemulsion: -

Percentage transmittance: -

100 µl of microemulsion dissolved in 250 ml of distilled water stir the solution up to 2 min and take the absorbance of solution with the help of UV spectrophotometer.

Table 5. Percentage transmittance of formulation of optimized formulation

Sr no.	Formula no.	% transmittance
1	MF2	95.41±0.95
2	MF3	98.14±0.33
3	MF4	65.14±0.45
4	MF12	95.89±0.15
5	MF13	72.10±0.23
6	MF21	70.11±0.63

formulation shows the percent transmittance above 96% except formula number MF4, MF13 and MF21 formulation that indicated that droplet size was nanometer range and transparent microemulsion was formed.

. In vitro drug release study of Microemulsion

Out of the six microemulsion formulations tested for drug release in vitro, the best result came from Formulation MF2 (96.12%).

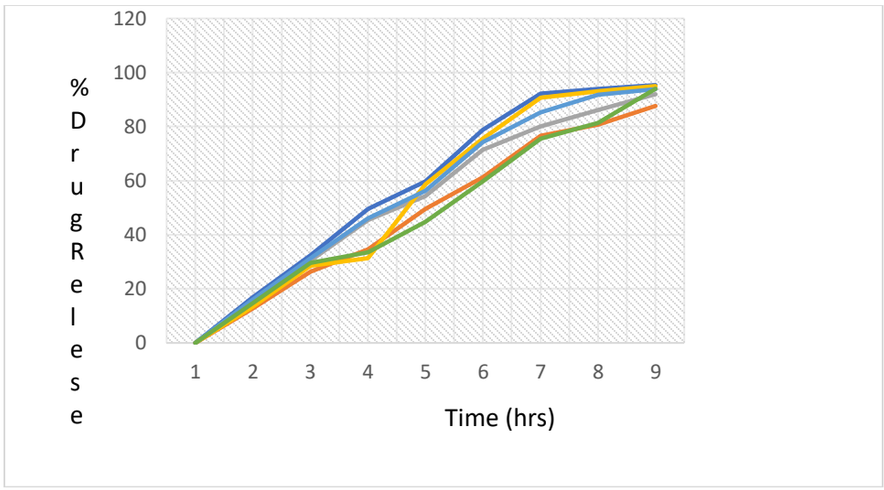


Figure 4: Drug Release Profile of Optimized microemulsion formulation

Figure 4 above compares the medication release of six different lots of preparations. In comparison to batches MF3, MF4, MF12, MF13, and MF21, MF2 had a better in-process drug release. At 8 hours, medication release is greatest with the MF2 formulation.

Based on these results, the MF2 formulation batch was chosen as the final formulation batch for further investigation due to its superior thermodynamic stability, percentage transmittance, drug content, and cloud point, and potential for more drug release in an in vitro diffusion test.

In vitro drug release study of Microemulgel

In vitro analysis of medication release from six distinct microemulgel formulations showed that Formulation P3 had the highest drug release (97.15 percent).

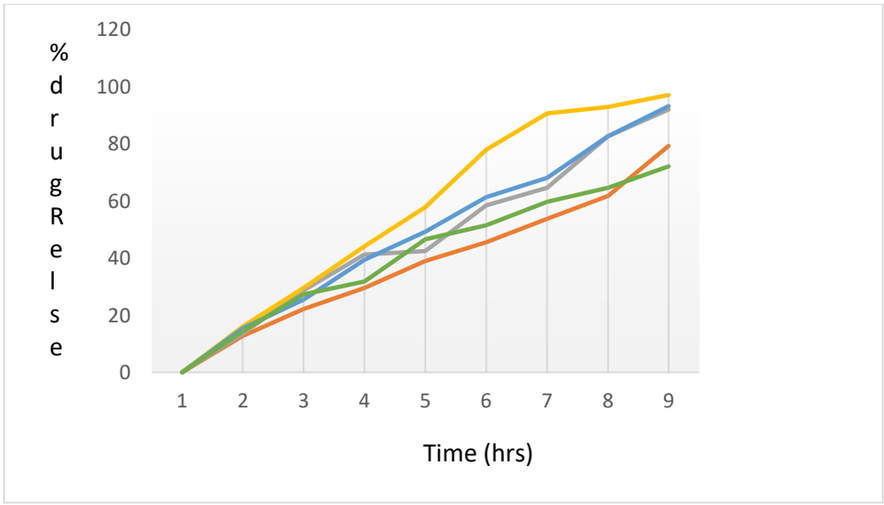


Fig 5. Drug Release Profile of Optimized microemulgel formulation

Above figure depicts a study comparing the rates of drug release from five different lots of preparation. Preparation batch P3 had the highest drug release compared to P1, P2, P4, and P5 formulations. At 8 hours, the B3 formulation exhibits more drug release. Batch P3 was chosen as the final formulation batch due to its superior in vitro diffusion test drug release compared to the other batches studied.

Characterization of microemulgel: -

Physical appearance:

pH

After immersing the glass electrode into the microemulgel, the pH was measured digitally. A table containing the measured values is provided. The pH level shows whether or not the microemulgel may be used topically.

Viscosity

We used a Brookfield viscometer set to spindle no. 5 and 50 rpm to measure the viscosity of each microemulgel formulation at 25 °C. The table displays the microemulgel's viscosity from the first to the fifth performance category.

Appearance

All five batches (PN1 through PN5) seemed to be the same yellowish viscous translucent preparation that was uniform and shiny.

Spreadability

Table 44 displays the results of a measurement of spreadability from PC1 to PC5. It can be seen that the spreadability of a solution decrease as the concentration of carbopol 940 rises.

Extrudability

After the gels were made, they were placed in dismantlable tubes. This formulation's extrudability has been tested, and the findings are listed in Table 44.

Table 6: Physical appearance of microemulgel

Batch code	pH	Viscosity (cps)	Appearance	Spreadability	Extrudability
PN1	7.2±0.86	10245±1.56	+	34.12±0.65	+
PN2	7.10±0.26	10845±0.36	+	35.21±0.44	+
PN3	7.3±1.02	11321±0.44	++	35.10±0.14	++
PN4	6.9±0.23	11254±0.54	+	34.22±0.26	+
PN5	7.4±0.89	12547±0.26	+	33.21±0.91	+

Drug content material dedication: -

The drug content material of Celecoxib in all the components become located to be inside the range 98-ninety-nine% in microemulgel which suggest entire solubilization of drug in formula.

Table 7: Drug content Determination

Formulation	Drug content (%)
PN1	95.21±0.23
PN2	94.74±1.05
PN3	98.15±0.66
PN4	96.12±0.89
PN5	95.41±0.14

From the above study it can be concluded that PN3 formulation shows the higher drug content it means that is degradation of drug and complete solubilization of drug

Stability Study

Batch No.: PN3 was put on stability as below mentioned condition.

Condition: Batch PN3 at 45°C ± 2°C / 75% RH ± 5 % RH

Packaging: Aluminum collapsible tube

Description: Transparent light yellow colored microemulgel.

Table 8:First, Second- & Third-month Stability Data of Tablet at 45°C ± 2°C / 75% RH ± 5 % RH.

Parameters		Initial	1 Months	3 Months
Drug content (%)		PN3	PN3	PN3
		98.78±0.23	98.11±045	97.30±0.22
Diffusion (%) Medium: 25ml of pH 6.8 phosphate buffer, egg's membrane, 50 rpm.	0Hr	0	0	0
	1hr	13.45±0.26	14.10±1.05	13.54±0.23
	2 hr	29.13±0.47	27.14±0.23	28.47±0.29
	3 hr	41.12±0.59	40.22±0.65	42.12±0.44
	4 hr	53.21±0.62	54.64±0.24	53.10±0.15
	5 hr	71.11±0.14	70.14±1.02	70.45±0.26
	6 hr	85.41±0.66	86.52±0.95	84.17±014
Clarity		Clear	Clear	Clear
Phase separation		No phase separation	No phase separation	No phase separation
Centrifugation test		Stable	Stable	Stable

Microemulgel were evaluated for physical appearance, diffusion study, clarity, Phase separation, centrifugation test. There is no change in description of microemulgel after 3-month stability study. There was no variation observed in Clarity, phase separation and centrifugation test.

Skin Irritation study on rabbits:

The protocol explained in methodology part employed for skin irritation study. Saline solution producing skin irritation responses compared with the irritation occurred after the application of given below. Each group containing 3 rabbits in it. Skin irritation was calculated on the bases of criteria 0 to 7 given in methodology part and obtained results represent in Table.

The animals were treated as follows:

Group I- 20% w/v SLS solution (area A), Untreated (area B);

Group II- Microemulgel (CF2) (area A), untreated (area B);

Group III- Microemulgel (PC3) (area A), untreated (area B);

Table 9: Skin Irritation Study of Group (GroupwithSLS solution)

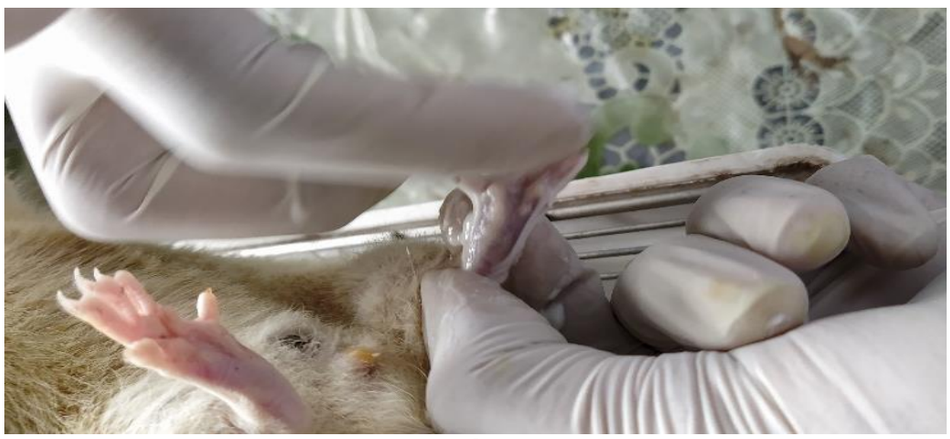
Sr.No.	SkinIrritation Symptom	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	**0**	-	-	-	-	-	-	-	-	p	p	p	p	p	p
2	**1**	-	-	-	-	-	-	-	-	-	p	-	p	p	p
3	**2**	-	-	-	-	-	-	-	-	-	-	-	p	p	p
4	**3**	-	-	-	-	-	-	-	-	-	-	-	-	p	p
5	**4**	-	-	-	-	-	-	-	-	-	-	-	-	-	p
6	**5**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	**6**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	**7**	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 10: Skin Irritation Study of Group (**Microemulgel (CF2)**)

Sr.No.	SkinIrritation Symptom	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	0	-	-	-	-	-	-	-	-	-	-	p	p	p	p
2	1	-	-	-	-	-	-	-	-	-	-	-	p	p	p
3	2	-	-	-	-	-	-	-	-	-	-	-	p	p	p
4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	p
5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

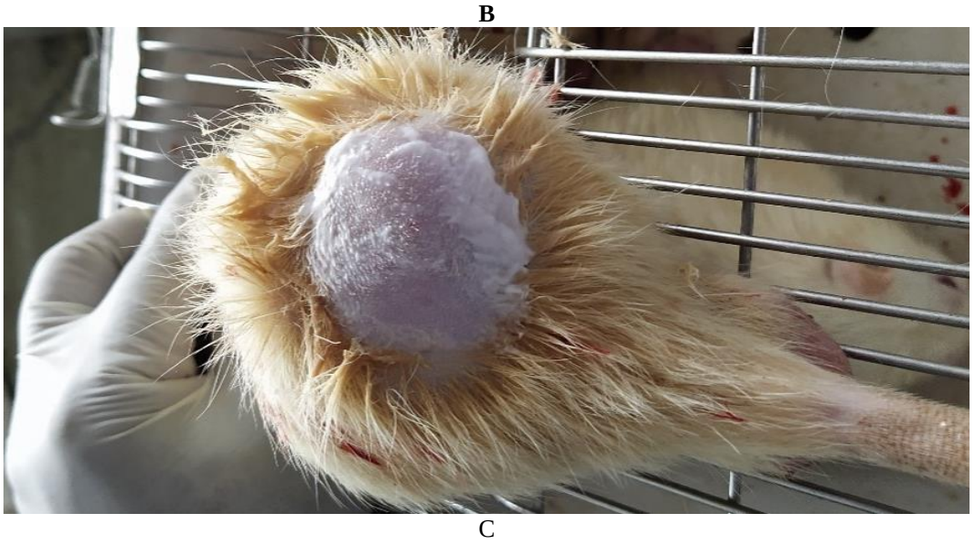
Table 11: SkinIrritationStudyofGroup(Microemulgel (PC3))

Sr.No.	SkinIrritation Symptom	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	0	-	-	-	-	-	-	-	-	-	-	p	p	p	p
2	1	-	-	-	-	-	-	-	-	-	-	-	p	p	p
3	2	-	-	-	-	-	-	-	-	-	-	-	p	p	p
4	3	-	-	-	-	-	-	-	-	-	-	-	-	p	p
5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-



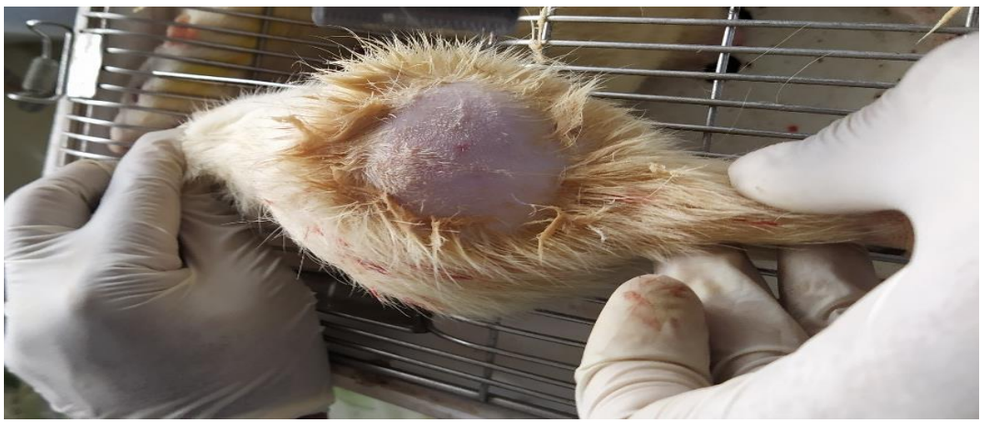
A

B



C

D



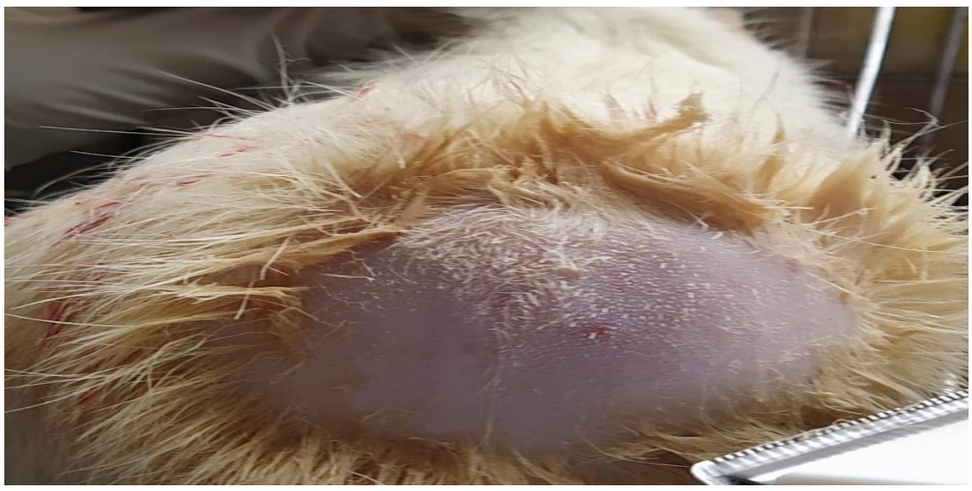


Figure 6: (A) Handling of rabbit for application of microemulgel
(B)SkinIrritationStudy photos after application of formulation 14 days. (Formulation CF2);
(C) SkinIrritationStudy photos after spreading of formulation over skin14 days. (Formulation CF2);
(D) SkinIrritationStudy photos after application of
a) Group I- 20% w/v SLS solution; b) Group II- Microemulgel (CF2); c) Group III- Microemulgel (PC3) (area A) After 14 days.

Results revealed that as saline solution is a skin irritant it was produce irritation with minimal erythema after 10 days and definite erythema, readily visible edema was produced after 12 days. Compared with this both the placebo and optimized batch was not show any type of irritation up to 10 days after that there was little erythema found with light redness at the site of application. These results of in-vivo skin irritation study suggested that both microemulgel does not show any type of major irritation on rat skin up to 14 days.

SUMMARY AND CONCLUSION:

Non-steroidal anti-inflammatory drugs (NSAIDs) taken orally are very efficient, their clinical use is typically restricted due to side effects include gastrointestinal mucosal irritation and ulceration. Microemulsion and microemulsion gel were created for transdermal administration in the current investigation to reduce these adverse effects and improve clinical efficacy. Microemulsions' low viscosity might be a hindrance in certain situations, notably those involving the pharmaceutical business. Efforts were undertaken to improve the microemulsion's viscosity to counteract this shortcoming. The transdermal application of microemulsion gel was shown to be superior to that of microemulsion as a medium for drug delivery. A microemulsion for transdermal administration of Celecoxib

In order to be absorbed through the skin, a medication must first be dissolved in a vehicle, making solubility one of the primary goals of a new pharmaceutical formulation. Therefore, in order to identify appropriate and optimal components of microemulsions, the solubility of the selected medicine was tested in a number of oils, surfactants, and co-surfactants. Microemulsion formulations comprising Celecoxib and Celecoxib were developed using labrasol as the surfactant, ethanol as the co-surfactant, and Capmul MCM C8 as the oil phase, all based on the findings of solubility experiments.

Microemulsion formulation components were chosen for their documented biocompatibility, biodegradability, safety, and efficacy in topical/transdermal applications.Studies of drug-excipient compatibility are conducted to determine the nature and extent of any potential physical or chemical interactions between the two, and to forecast how these interactions will affect drug (Celecoxib and Celecoxib) and excipients were compatible with one another. Analysis by FTIR and DSC indicates that the drug and excipient mixture is stable.Several physicochemical characteristics of the chosen formulations were investigated. All microemulsions tested had a transparent, clear appearance with no visible particles. All of the microemulsion formulations had high percent transmittance when tested using a UV-Vis spectrophotometer. A study of cloud point measurements shows that all formulations create a stable microemulsion even at physiological temperature.Among the six formulations tested for in-vitro skin penetration, formulation MF2 exhibited

the greatest drug release (96.12%) while formulation CF2 showed the highest drug release (97.85%). As a result of the aforementioned analysis, a batch of MF2&CF2 formulation was chosen as an optimized formulation due to its superior for increased drug release in an in vitro diffusion assay. Droplet size was measured to be 35.2 nm for the MF2Celecoxib-loaded microemulsion and 103.2 nm for the CF2 Celecoxib-loaded microemulsion. Microemulsions with a small droplet size tend to be more stable than those with a larger droplet size because bigger droplets are more likely to aggregate or coalesce. The MF2 Celecoxib-loaded microemulsion was found to have a zeta potential of +19.0 mV.Several physicochemical properties of the microemulsion gel were determined after it had been made. According to the results of a skin irritation investigation, the microemulsion gel formulations of Celecoxib did not irritate the skin or result in erythema. Microemulsion and microemulsion gel formulations containing Celecoxib was shown to be stable under all ICH-recommended stability parameters.The produced formulations' spreadability was sufficient, pointing to their convenience in use. An analysis of spreadability revealed that as formulation viscosity increased, spreadability decreased. In a nutshell, it can be concluded that the developed microemulsion gel might be a potential drug delivery vehicle for the transdermal delivery of Celecoxib Taken together these outcomes reveal that the microemulsion is probably a promising approach for the transdermal shipping of Celecoxib. though, giant work still wishes to be performed to clarify the mechanisms of drug delivery into the skin.

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Synthesis and Cardioprotective Activity of a Novel Series of Benzoxazole Derivatives

Singadi Amarnath, Pulugam Nagaraju, Immadi Rajiv and Ampati Srinivas*

Department of Pharmaceutical Chemistry, Unity College of Pharmacy, Raigir (V), Bhongir (M), Yadadhri Bhuvanagiri, Telangana, India

**Corresponding Author*

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Abstract: Novel series of benzoxazole derivatives were prepared by the condensation of methyl-2-(2-aminothiazol-5-ylamino) benzo[d]oxazole-5-carboxylate with various aromatic aldehydes. The structures of the synthesized compounds were VI_1-VI_{15} assigned on the basis of elemental analysis, IR, ^1H NMR and mass spectroscopy. These compounds were also screened for cardioprotective activity against Doxorubicin induced cardiotoxicity in rats. Selected two compounds produced a dose dependent cardioprotective activity by decreasing the doxorubicin elevated parameters like plasma Aspartate aminotransferase (AST), Creatinine kinase (CK-MB), Lactic acid dehydrogenase (LDH) and Triglyceride (TG) levels.

Keywords: Benzoxazole, Cardioprotective activity, Doxorubicin, Aspartate aminotransferase, Creatinine kinase, Lactic acid dehydrogenase, Triglyceride

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Introduction

It has been reported that substituted benzoxazoles and related heterocycles possess potential activity with lower toxicities in the chemotherapeutic approach in man (Haugwitz, *et al*., 1982; Hisano *et al*., 1982). Cautious literature survey exposed that targets containing benzoxazole moiety, either isolated from plants or accessed by total synthesis, have remarkable biological activities (Tipparaju et al., 2008) e.g. antimicrobial (Ilkay *et al*., 1998) Antihistaminic (Sener *et al*., 2018), antiparasitics (Albert *et al*., 2009), herbicidal (Diego *et al*., 2019), antiviral (Medebielle *et al*., 2008), anti-inflammatory (Aruna Devi *et al*., 2014), and antihelmintic (Satyendra *et al*., 2011) activities. Derivatives of thiazole have antibacterial (Khalil *et al*., 2009), antitubercular (Mahendra *et al*., 2009), anticonvulsant activity (Bachir *et al*., 1990) and anticancer (Elif *et al*., 2007) properties. In the present study, the thiazole moiety was connected to the benzoxazole moiety 2-position, (VI_1-VI_{15})

(Table. I), to combine different pharmacophores on one scaffold. Due to broad spectrum of activities reported in the literature so far, we herein report the synthesis of a novel series of methyl-2-(2-(benzylideneamino) thiazole-4-ylamino) benzoxazole-5-carboxylate derivatives (VI_1-VI_{15}) as the target compounds in order to examine their Cardio protective activity.

Myocardial infarction (MI), colloquially known as "heart attack," is caused by decreased or complete cessation of blood flow to a portion of the myocardium. Myocardial infarction may be "silent" and go undetected, or it could be a catastrophic event leading to hemodynamic deterioration and sudden death (Thygesen *et al*., 2007). Most myocardial infarctions are due to underlying coronary artery disease, the leading cause of death in the United States. With coronary artery occlusion, the myocardium is deprived of oxygen. Prolonged deprivation of oxygen supply to the myocardium can lead to myocardial cell death and necrosis (Reimer *et al*., 1983). Patients can present with chest discomfort or pressure that can radiate to the neck, jaw, shoulder, or arm. In addition to the history and physical exam, myocardial ischemia may be associated with ECG changes and elevated biochemical markers such as cardiac troponins (Apple *et al*., 2017). So ischemia, if left untreated for a sufficient period of time, can cause damage or death (infarction) of heart muscle tissue. Consequences of Myocardial infarction include hyperlipidemia, peroxidation of membrane lipids and loss of plasma membrane integrity. Currently numbers of drugs are available to limit the extent of myocardial damage or to prevent myocardium from necrosis. But still there is need of synthetic compounds for management of Myocardial infarction. The present study was aimed to synthesize new benzaxozole derivative for the evaluation of cardioprotective activity against doxorubicin induced cardiotoxicity in rats.

Materials and Methods

The melting points were determined by a digital melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin–Elmer 377 spectrophotometer, ^{1}H NMR spectra were measured on Bruker AV 400 MHz using DMSO as a solvent and TMS as an internal standard.

Drug and chemicals:

Doxorubicin (Cadila pharamceuticals, Hyd, India), Creatine kinase kit, Lactate dehydrogenase kit, Triglyceride kit (Erba Mannheim, Daman, India), Glutamic Oxaloacetic Transaminase Kit (Coral clinical systems, Verna, Goa, India).

Synthesis of Methyl-3-nitro-4-hydroxybenzoate (*I*):

To a solution of aluminium nitrate (40 g) in acetic acid- acetic anhydride (1:1) mixture (160 ml), was added an appropriate phenol (40 g) in small portions, while cooling and shaking occasionally. The reaction mixture was left at room temperature for 1.5 h while shaking the contents intermittently to complete the nitration. The resulting brown solution was diluted to complete the nitration. The resulting brown solution was diluted with ice-cold water and acidified with concentrated nitric acid to get a bulky, yellow precipitate. It was filtered, washed with small quantity of methanol and purified by recrystallization from alcohol to get a yellow crystalline solid (44 g, 85%), m.p. is73℃.

Synthesis of Methyl-3-amino-4-hydroxybenzoate (*II*):

4-carbomethoxy-2-nitrophenol (I, 10 g) was dissolved in boiling alcohol (50%, 100 ml) and sodium dithionite was added to this boiling alcohol solution until it becomes almost colorless. Then the alcohol was reduced to one-third of its volume by distillation and the residual liquid was triturated with crushed ice. The resulting colorless, shiny product was filtered, and dried in the air. Its purification was effected by recrystallization from benzene to get colorless, shiny scales (5.1 g; 60%) m.p. is 143℃.

Synthesis of methyl-2-(*2-chloroacetamido*) *benzoxazole-5-carboxylate* (*III*):

4-carbomethoxy-2-aminophenol (II, 1.3 mol) was dissolved in 1l methyl alcohol and cooled the solution to 5^{0}C by adding chopped ice. A cold

suspension of cyanogenbromide (1.5 mol) in 1l of water was added over a period of 5 min with rapid stirring. The reaction mixture was stirred for 45 min at room temperature, solid sodium bicarbonate (1.3 mol) in small portions over a period of 1.5 h was added to bring the pH 6.5 -7.0. Stirring was continued for another 1h. The solid was separated by filtration, washed with cold water and on recrystallization from ethyl alcohol has resulted white solid, yield 70% and m.p. is 238°C.

Synthesis of methyl 2-(2-chloroacetamido) benzo [d]oxazole-5-carboxylate (IV):

A mixture of methyl-2-aminobenzoxazole-5-carboxylate (III, 0.01mol) and chloroacetyl chloride (0.01mol) was taken in 20 ml of dry benzene and the reaction mixture was refluxed for 5 h on a water bath. The solvent was evaporated and the residue was washed first with benzene and then with Petroleum ether. The compound was recrystallized from suitable solvent(s). The compound was found to be containing yield 72% and m.p. is 177°C.

Microwave synthesis of methyl-2-(2-aminothiazol-5-ylamino)benzo[d]oxazole-5-carboxylate (V):

Methyl-2-(2-chloroacetamido) benzo[d]oxazole-5-carboxylate (IV, 0.01 mol) and thiourea (0.01 mol) were dissolved in 10 ml of absolute alcohol in conical flask. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 5min in LG-Microwave oven. The reaction was monitored by TLC. After the completion of the reaction the contents were cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture found to be containing yield 97% and m.p. is 199°C.

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3450 (NH_2), 3146 (NH), 1672 (C=O), 1626(C=C), 1528 (C=N), 1342 (C-O-C), 1142(C=S).

PMR spectrum (DMSO-d_6) of the compound has been found to exhibit proton signals (δ ppm) at: 8.3(s, 1H, Ar-H), 7.8 (d, 1H, Ar-H), 7.6 (d, 1H, Ar-H), 7.0 (s, 1H, CH, thiazole ring), 6.3 (s, 2H, NH_2), 5.5 (s, 1H, NH), 3.9 (s, 3H, CH_3).

Microwave synthesis of Methyl-2-(2-(aryylideneamino)thiazol-5-ylaminobenzo[d] oxazole-5-carboxylates (VI1-15):

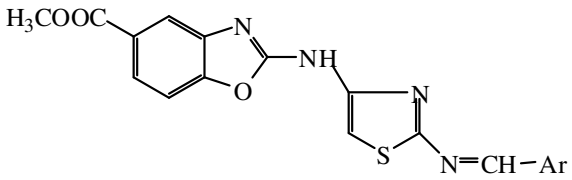
Methyl-2-(2-aminothiazol-4-ylamino)benzoxazole -5-carboxylate (V, 0.01 mol) and appropriate aromatic aldehydes viz. 4-dimethylaminophenyl, *4-t*-butylphenyl, Anisyl, phenyl, 4-hydroxy-phenyl,4-nitrophenyl, Veratryl, Cinnamyl, 3,4,5-trimethylphenyl, 4-tolyl, 2-hydroxyphenyl, 4-bromophenyl, 4-chlorophenyl, 2-naphthyl, 1-naphthyl (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7min in LG-Microwave oven. The reaction was monitored by TLC. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture. The compounds were characterized by spectral data (Table 1).

Compound VI 1: Methyl-2-(2-(4-(dimethylamino) benzylideneaminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-dimethylaminophenyl (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.8 (s, 1H, ArH), 8.2(s, 1H, CH), 8.1 (d, 1H,ArH), 8.0 (d, 1H, ArH),7.5 (d, 2H, ArH), 6.8(d, 2H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.2 (s, 1H, NH), 3.9 (s, 3H, CH_3), 3.0 (s, 6H, $(CH_2)_3$).

Table1: Physical data of methyl 2-(2-(benzylideneamino) thiazol-4-ylamino) benzoxazole-5-carboxylates (VI)



Compd	Ar	Molecular Formula (VI)	Melting Point (°C)	Yield (%)
AS1	4-dimethylamino phenyl	$C_{21}H_{19}N_5O_3S$	208	90
AS2	*4-t*-butylphenyl	$C_{23}H_{22}N_4O_3S$	301	93
AS3	4-methoxyphenyl	$C_{20}H_{16}N_4O_4S$	280	98
AS4	Phenyl	$C_{19}H_{14}N_4O_3S$	201	92
AS5	**4-hydroxy phenyl**	$C_{19}H_{14}N_4O_4S$	228	95
AS6	4-nitrophenyl	$C_{19}H_{13}N_5O_5S$	299	97
AS7	3,4 dimethoxyphenyl	$C_{19}H_{18}N_4O_5S$	226	96
AS8	Cinnamyl	$C_{21}H_{15}N_4O_3S$	240	94
AS9	3,4,5-trimethylphenyl	$C_{22}H_{20}N_4O_3S$	238	95
AS10	4-methylphenyl	$C_{20}H_{16}N_4O_3S$	303	91
AS11	2-hydroxyphenyl	$C_{19}H_{14}N_4O_4S$	234	91
AS12	4-bromophenyl	$C_{19}H_{13}BrN_4O_3S$	305	90
AS13	**4-chlorophenyl**	$C_{19}H_{13}ClN_4O_3S$	222	99
AS14	2-naphthyl	$C_{23}H_{16}N_4O_3S$	341	90
AS15	1-naphthyl	$C_{23}H_{16}N_4O_3S$	322	91

IR (KBr) CM^{-1}: 3096 (NH), 1683 (C=O), 1640(C=C), 1576 (C=N), 1442(C-O-C), 1383(C=S), MS (*m/z):* M^+: 422.1, *Anal.* Calcd for $C_{21}H_{19}N_5O_3S$: C, 59.84; H, 4.54; N, 16.62; O, 11.39; S, 7.61.

Compound VI 2: methyl-2-(2-(4-tert-butylbenzyl ideneamino)thiazol-5-ylamino) benzo[d]oxazole-5-carboxylate:

Methyl-2-(2-aminothiazol-5-ylamino)benzo[d]oxazole-5-carboxylate (V, 0.01 mol) and 4-*t*-butylbenzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.7 (s, 1H, ArH), 8.2(s, 1H, CH), 8.1 (d, 1H,ArH), 8.0 (d, 1H, ArH),7.5 (d, 2H, ArH), 7.1(d, 2H, ArH), 6.0 (s, 1H, ArH, thiazole ring), 5.4 (s, 1H, NH), 3.9 (s, 3H, CH_3), 1.3(s, 9H, $(CH_3)_3$).

IR (KBr) CM^{-1}: 3091 (NH), 1681 (C=O), 1642 (C=C), 1577 (C=N), 1443 (C-O-C), 1381 (C=S), MS (*m/z):* M^+: 435.1, *Anal.* Calcd for $C_{23}H_{22}N_4O_3S$: C, 63.58; H, 5.10; N, 12.89; O, 11.05; S, 7.38.

Compound VI 3: methyl-2-(2-(4-methoxybenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-methoxybutylbenzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.9 (s, 1H, CH), 8.7 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.8 (d, 2H, ArH), 7.1 (d, 2H, ArH), 6.2 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3068 (NH), 1687 (C=O), 1645 (C=C), 1512 (C=N), 1432 (C-O-C), 1371 (C=S), MS (*m/z):* M^+: 409.0, *Anal.* Calcd for $C_{20}H_{16}N_4O_4S$: C, 58.81; H, 3.95; N, 13.72; O, 15.67; S, 7.85.

Compound VI 4: methyl-2-(2-(benzylideneamino) thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.6 (s, 1H, ArH), 8.1(s, 1H, CH), 8.0 (d, 1H,ArH), 7.9 (d, 1H, ArH),7.5 (d, 2H, ArH), 7.0(t, 3H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 5.4 (s, 1H, NH), 3.8 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3076 (NH), 1679 (C=O), 1646(C=C), 1580 (C=N), 1448(C-O-C), 1373 (C=S), MS (*m/z):* M^+: 379.0, *Anal.* Calcd for $C_{19}H_{14}N_4O_3S$: C, 60.31; H, 3.73; N, 14.81; O, 12.68; S, 8.47.

Compound VI 5: methyl-2-(2-(4-hydroxybenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-hydroxy benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ9.2 (S,1H,OH), 8.9 (s, 1H, ArH), 8.5 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.7 (d, 2H, ArH), 6.8(d, 2H, ArH), 6.4 (s, 1H, ArH, thiazole ring), 4.6(s, 1H, NH), 3.7 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3091 (NH), 1699 (C=O), 1676 (C=C), 1580 (C=N), 1455 (C-O-C), 1371 (C=S), MS (*m/z):* M^+: 395.0, *Anal.* Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; O, 16.23; S, 8.13.

Compound VI 6: methyl-2-(2-(4-nitrobenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl - 2- (2- aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-nitro benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.6 (s, 1H, CH), 8.4 (s, 1H,ArH), 8.3 (d, 2H, ArH),8.1 (d, 1H, ArH), 7.9 (d, 1H, ArH), 7.8 (d, 2H, ArH), 6.5 (s, 1H, ArH, thiazole ring), 5.5 (s, 1H, NH), 3.8 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3091 (NH), 1692 (C=O), 1684 (C=C), 1582 (C=N), 1449 (C-O-C), 1373 (C=S), MS (*m/z):* M^+: 424.0, *Anal.* Calcd for $C_{19}H_{13}N_5O_5S$: C, 53.90; H, 3.09; N, 16.54; O, 18.89; S, 7.57.

Compound VI 7: methyl-2-(2-(3,4-dimethoxybenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl – 2 -(2 -aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 3, 4-dimethoxy benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-

Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.9 (s, 1H, ArH), 8.5 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (s, 1H, ArH), 7.4 (d, 1H, ArH), 6.9 (d, 1H, ArH), 6.5 (s, 1H, ArH, thiazole ring), 4.8 (s, 1H, NH), 3.8 (s, 9H, $3OCH_3$).

IR (KBr) CM^{-1}: 3071 (NH), 1687 (C=O), 1663 (C=C), 1575 (C=N), 1451 (C-O-C), 1373 (C=S) , S (*m/z):* M^+: 439.0, *Anal.* Calcd for $C_{21}H_{18}N_4O_5S$: C, 57.53; H, 4.14; N, 12.78; O, 18.25; S, 7.31.

Compound VI 8: methyl-2-(2-(3-phenylallyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl – 2 - (2 - aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and cinnamaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.8 (s, 1H, ArH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (t, 2H, ArH), 7.5 (s, 1H, CH), 7.4 (t, 2H, ArH), 7.3(t, 1H, ArH),7.0 (s, 1H, CH), 6.4 (s, 1H, ArH, thiazole ring), 5.6 (s, 1H, CH), 4.2 (s, 1H, NH), 3.7 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3088 (NH), 1681 (C=O), 1667 (C=C), 1573 (C=N), 1455 (C-O-C), 1371 (C=S), MS (*m/z):* M^+: 405.1, *Anal.* Calcd for $C_{21}H_{16}N_4O_3S$: C, 62.36; H, 3.99; N, 13.85; O, 11.87; S, 7.93.

Compound VI 9: methyl-2-(2-(3,4,5-trimethylbenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl – 2 - (2- aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 3,4,5-trimethyl benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.7 (s, 1H, ArH), 8.6 (s, 1H,CH), 8.1 (d, 1H, ArH),8.0 (d, 1H, ArH), 7.3 (d, 2H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 4.8 (s, 1H, NH), 3.8 (s, 3H, OCH_3), 2.3 (s, 6H, $2CH_3$), 2.1 (s, 1H, CH_3).

IR (KBr) CM^{-1}: 3090 (NH), 1674 (C=O), 1660 (C=C), 1569 (C=N), 1449 (C-O-C), 1367 (C=S), MS (*m/z):* M^+: 421.1, *Anal.* Calcd for $C_{22}H_{20}N_4O_3S$: C, 62.84; H, 4.79; N, 13.32; O, 11.41; S, 7.63.

Compound VI 10: methyl-2-(2-(4-methylbenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-methyl benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.8 (s, 1H, CH), 8.6 (s, 1H,ArH), 8.1 (d, 1H, ArH),8.0 (d, 1H, ArH), 7.7 (d, 2H, ArH), 7.2 (d, 2H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.1 (s, 1H, NH), 4.0(s, 3H, OCH_3), 2.3 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3092 (NH), 1695 (C=O), 1689 (C=C), 1589 (C=N), 1458 (C-O-C), 1371 (C=S), MS (*m/z):* M^+:393.1, *Anal.* Calcd for $C_{20}H_{16}N_4O_3S$: C, 61.21; H, 4.11; N, 14.28; O, 12.23; S, 8.17.

Compound VI 11: methyl-2-(2-(2-hydroxybenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 2-hydroxy benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 11.2 (S,1H,OH), 8.9 (s, 1H, ArH), 8.6 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.6 (d, 1H, ArH), 7.5 (t, 1H, ArH), 7.2 (t, 1H, ArH), 7.0 (t, 1H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s,3H,CH_3).

IR (KBr) CM^{-1}: 3088 (NH), 1697 (C=O), 1666 (C=C), 1578 (C=N), 1454 (C-O-C), 1375 (C=S), MS (*m/z):* M^+: 395.0, *Anal.* Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; O, 16.23; S, 8.13.

Compound VI 12: methyl-2-(2-(4-bromobenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-bromo benzaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.7 (s, 1H, CH), 8.5 (s, 1H, CH), 8.0 (d, 1H,ArH), 7.9(d, 1H, ArH),7.6 (d, 1H, ArH), 7.5 (d, 2H, ArH), 7.3 (d, 2H, ArH), 5.9 (s, 1H, ArH, thiazole ring), 5.0 (s, 1H, NH), 3.9 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3093 (NH), 1693 (C=O), 1679 (C=C), 1583 (C=N), 1446 (C-O-C), 1376 (C=S), MS (*m/z):* M^+: 458.1, *Anal.* Calcd for $C_{19}H_{13}BrN_4O_3S$: C, 49.90; H, 2.87; Br, 17.47; N, 12.25; O, 10.50; S, 7.01.

Compound VI 13: methyl-2-(2-(2-chlorobenzyl ideneamino)thiazol-5-ylamino)benzo[d]oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 4-chloro benzaldehyde (0.015mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^1H NMR (DMSO-d_6): δ 8.8 (s, 1H, CH), 8.6 (s, 1H, CH), 8.1 (d, 1H,ArH), 8.0(d, 1H, ArH),7.7 (d, 1H, ArH), 7.5 (d, 1H, ArH), 7.4 (t, 1H, ArH), 7.3 (t, 1H, ArH), 6.4 (s, 1H, ArH, thiazole ring), 5.2 (s, 1H, NH), 3.6 (s, 3H, CH_3).

IR (KBr) CM^{-1}: 3094 (NH), 1691 (C=O), 1680 (C=C), 1581 (C=N), 1447 (C-O-C), 1374 (C=S), MS (*m/z):* M^+: 413.0, , *Anal.* Calcd for $C_{19}H_{13}ClN_4O_3S$: C, 55.28; H, 3.17; Cl, 8.59; N, 13.57; O, 11.63; S, 7.77.

Compound VI 14: methyl-2-(2-(naphthalen-2-ylmethyleneamino)thiazol-5-ylamino)benzo[d] oxazole-5-carboxylate:

Methyl- 2- (2- aminothiazol-5-ylamino) benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 2-naphthaldehyde (0.015 mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$

solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.7 (s, 1H, CH), 8.5 (s, 1H, CH), 8.4 (d, 1H,ArH), 8.3 (t, 1H, ArH),8.2 (d, 1H, ArH), 8.1(d, 1H, ArH), 8.0(d,1H, ArH),7.9(d, 1H, ArH),7.7(t, 1H, ArH), 7.4 (t, 1H, ArH), 6.3 (s, 1H, ArH, thiazole ring), 4.1 (s, 1H, NH), 3.9 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3090 (NH), 1692 (C=O), 1683 (C=C), 1585 (C=N), 1453 (C-O-C), 1370 (C=S), MS (*m/z):* M^+: 428.0, *Anal.* Calcd for $C_{23}H_{16}N_4O_3S$: C, 64.47; H, 3.76; N, 13.08; O, 11.20; S, 7.48.

Compound VI 15: methyl-2-(1-(naphthalen-2-ylmethyleneamino)thiazol-5-ylamino)benzo[d] oxazole-5-carboxylate:

Methyl - 2- (2- aminothiazol-5-ylamino)benzo[d] oxazole-5-carboxylate (V, 0.01 mol) and 1-naphthaldehyde (0.015mol) were taken into a conical flask and were dissolved in 10 ml of absolute alcohol. The conical flask was hanged with a funnel and was subjected to microwave irradiation at 480 Watts for 5-7 min in LG-Microwave oven. The reaction mixture was cooled and triturated with crushed ice; the separated solid was filtered, washed with 1% $NaHCO_3$ solution and purified by recrystallization from ethanol and water mixture.

^{1}H NMR (DMSO-d_6): δ 8.8 (s, 1H, CH), 8.5 (s,1H, CH), 8.6 (d, 1H,ArH), 8.2 (t, 1H, ArH),8.0 (d, 1H, ArH),7.9(d,1H, ArH), 7.8(d,1H, ArH), 7.7 (d, 1H, ArH),7.6 (t, 1H, ArH), 7.5 (t, 1H, ArH), 6.1 (s, 1H, ArH, thiazole ring), 4.8 (s,1H, NH),3.4 (s, 3H, OCH_3).

IR (KBr) CM^{-1}: 3093 (NH), 1689 (C=O), 1687 (C=C), 1581 (C=N), 1458 (C-O-C), 1365 (C=S), MS (*m/z):* M^+: 428.0, *Anal.* Calcd for $C_{23}H_{16}N_4O_3S$: C, 63.74; H, 5.17; N, 12.04; O, 12.20; S, 6.85.

Evaluation of Test compounds for Cardioprotective activity:

Among all the synthesized compounds, the compounds coded as AS1 and AS2 were selected for further screening of cardioprotective activity in rats.

Experimental animals:

36 female Wistar rats (180-200 g) were obtained from Mahaveera Enterprises (Hyderabad, India). The animals were housed in cages under hygienic conditions and placed in a controlled environment (12 h light–dark cycle, 25±2 °C and 45±10% humidity) with free access to water and fed with standard diet *ad libitum*. Care was taken to avoid stressful conditions.

Study design:

Rats were randomly divided into 6 groups (n = 6) and treated as follow:

Group 1: received 0.5% CMC (orally) daily for 7 days and served as control.

Group 2: received 0.5% CMC (orally) daily for 7 days and on 6th day single dose of doxorubicin (15 mg/kg., i.p.) (Nagi and Mansour, 2000) and served as cardiotoxic control.

Group 3: received compound AR1 (50 mg/kg/day, orally) daily for 7 days and on 6th day single dose of doxorubicin (15 mg/kg., i.p.).

Group 4: received compound AR1 (100 mg/kg/day, orally) daily for 7 days and on 6th day single dose of doxorubicin (15 mg/kg., i.p.).

Group 5: received compound AR2 (100 mg/kg/day, orally) daily for 7 days and on 6th day single dose of doxorubicin (15mg/kg., i.p.).

Group 6: received compound AR2 (100 mg/kg/day, orally) daily for 7 days and on 6th day single dose of doxorubicin (15 mg/kg., i.p.) (Reddy *et al*., 2012).

On the 7th day of the experiment, blood samples were collected through retro-orbital puncture method. After centrifugation, plasma was separated and stored at -20°C for biochemical analysis.

Analysis of blood samples:

Plasma samples were analyzed for cardiotoxic biomarkers like Aspartate amino transferase (AST), lactic acid dehydrogenase (LDH) and Creatine kinase (CK-MB) and also for plasma lipid

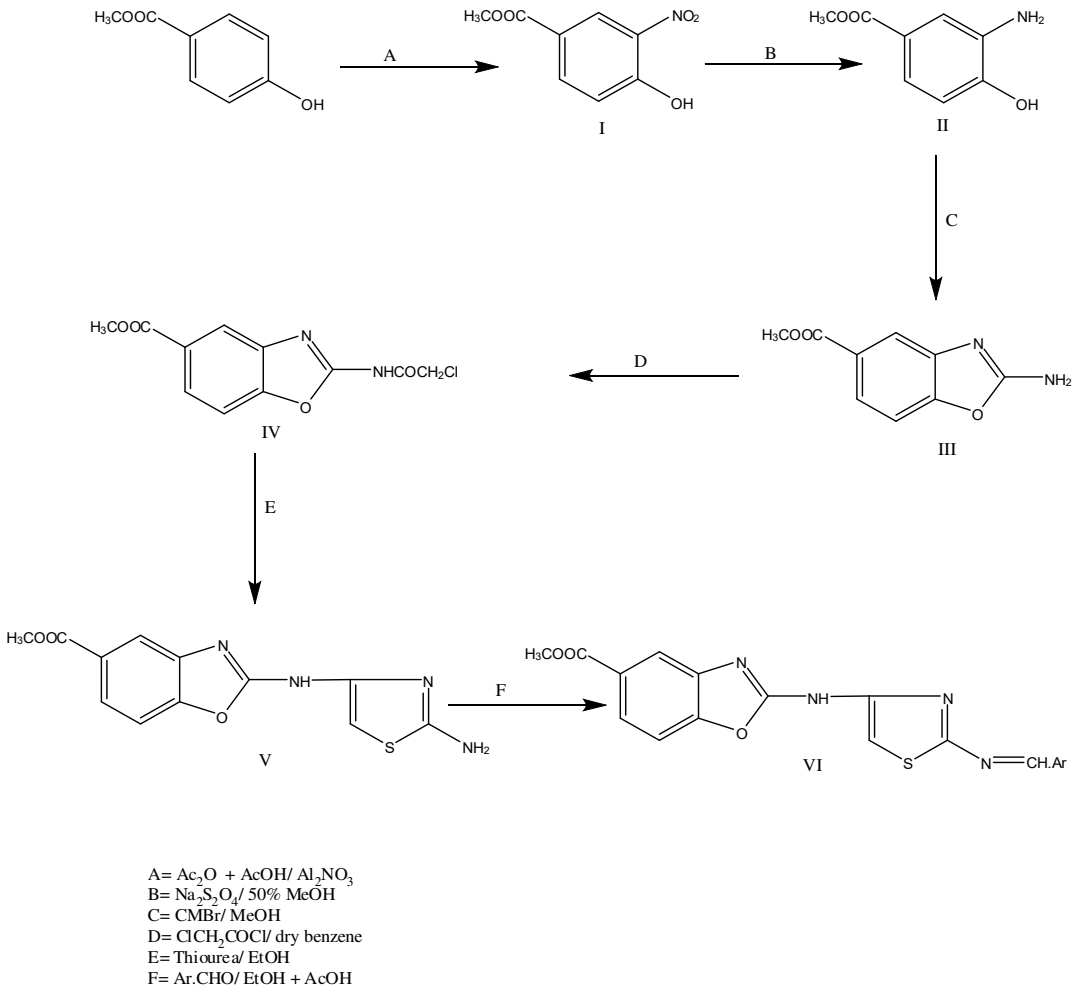


Fig. 1: Synthesis of target compounds.

levels, especially Triglyceride levels using commercially available diagnostic kits (George, 1975).

Statistical analysis:

All the values were expressed as Mean ±Standard deviation (S.D) (n=6). Statistical comparisons between different groups were done by using one way analysis of variance followed by Tuckey post test. $P < 0.05$ was considered as statistically significant.

Results and Discussion

The target compounds were synthesized according to Figure 1. The required starting material, methyl 3-amino-4-hydroxybenzoate (II) was prepared in good yield (85%). The starting material (II) on cyclization with cyanogen bromide in methyl alcohol on rapid stirring at room temperature gave the product, methyl 2-aminobenzo[d]oxazole-5-carboxylate (III). Methyl-2-aminobenzoxazole-5-carboxylate (III) on reaction with chloro acetyl chloride in dry benzene yields the compound, methyl-2-(2-chloroacetamido) benzo[d]oxazole-5-carboxylate (IV). methyl 2-(2-chloroacetamido) benzo[d] oxazole-5-carboxylate (IV) on cyclization with thiourea gave the compound methyl-2-(2-aminothiazol- 5 - ylamino) benzo [d] oxazole-5-carboxylate (V) which on reaction with various aromatic aldehydes viz, 4-dimethylaminophenyl, *4-t*-butylphenyl, Anisyl, phenyl, 4-hydroxy-phenyl,4-nitrophenyl, Veratryl , Cinnamyl, 3,4,5-trimethylphenyl, 4- tolyl, 2- hydroxyphenyl, 4-

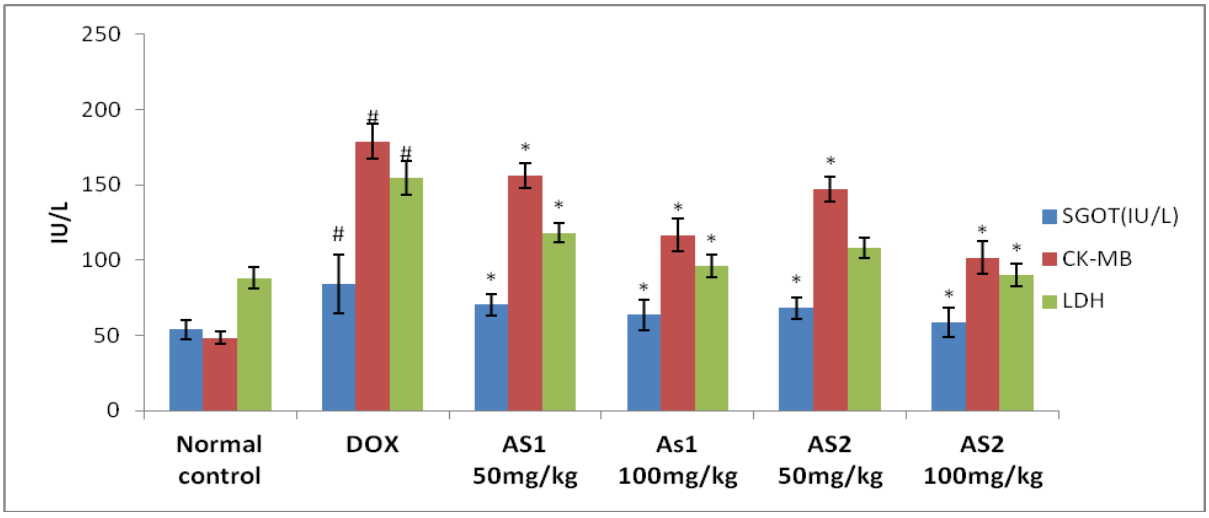


Fig. 2: Effect of test compounds on AST, CK-MB, LDH levels in rats treated with doxorubicin.

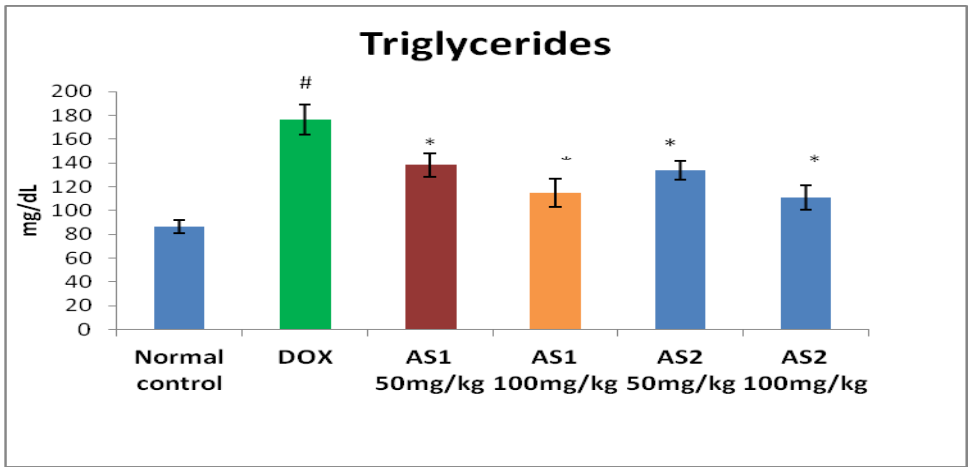


Fig 3: Effect of Test compounds on TG levels in rats treated with doxorubicin.

bromophenyl, 4-chlorophenyl, 2-naphthyl, 1-naphthyl conveniently converted into the targeted compounds Methyl -2 - (2- (aryylideneamino) thiazol-5-ylamino) benzo [d] oxazole – 5 - carboxylates (VI).

The yields, melting points and physical data of newly synthesized compounds are summarized in Table 1. Among synthesized fifteen compounds we have selected two compounds i.e. AS5 where the Ar group is 4-hydroxy phenyl and AS13 where the Ar group is 4-chloro phenyl for further investigations.

Cardioprotective activity:

The general appearance of all rats was observed every day after the treatment. No mortality was observed in all groups. Rats in the DOX alone treated group showed scruffy fur and developed a light yellow tinge. These animals appeared sicker, weaker and lethargic as compared to AS+DOX treated group. In this study, we evaluated the Cardioprotective effects of test compounds in rats by measuring plasma Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), Creatine kinase (CK-MB), Triglyceride (TG) and the results are shown in Figures 2 and 3.

In this study, plasma markers indicating myocardial injury like plasma AST, LDH, CK-MB and TG were significantly elevated ($P<0.001$) in Doxorubicin treatment group when compared

with normal control group (Figs. 2, 3). Pre-treatment with test compounds in AS+DOX groups at different doses (50 mg/kg/day and 10 mg/kg/day, respectively) almost restored the raised AST, CK-MB,LDH and TG levels when compared with DOX only treated group (P<0.001).

In the present study, Doxorubicin (15 mg/kg, i.p.) was used to induce cardiotoxicity. Evaluation of cardiotoxicity was done by measuring plasma AST, LDH, and CK enzyme activities, which are important measures of both early and late phases of cardiac injury (Saad *et al*., 2001). DOX Administration to rats significantly elevated plasma AST, LDH, CK-MB, TG levels (P<0.001) which are released from damaged myocytes and sensitive markers of cardiac injury (Reddy *et al*., 2012). Treatment with test compounds (AS1 and AS2) results in significant inhibition of DOX elevated plasma AST, LDH, TG and CK enzyme levels (Figs. 2,3). In conclusion, it was found based on the above findings that test compounds has produced a mild to moderate cardioprotective effect as evidenced by decreased cardiac injury markers in rats.

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Research Article

FORMULATION AND IN VITRO, IN VIVO EVALUATION OF NATEGLINIDE-GEMFIBROZIL BI LAYER TABLETS GASTRO RETENTIVE DRUG DELIVERY SYSTEM

Mohammad Ismail*, Akula Priyanka, Vishwanadhula Pavani, Ampati Srinivas
Unity College of Pharmacy, Raigir, Bhongir, Yadadhri Bhuvanagir, TS, 508 116

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ABSTRACT

In this research work nateglinide, gemfibrozil was selected as model drugs. Nateglinide is an amino acid derivative that induces an early insulin response to meals decreasing postprandial blood glucose levels which act by binding to β cells of the pancreas to stimulate insulin release.Gemfibrozil is used with diet changes (restriction of cholesterol and fat intake) to reduce the amount of cholesterol and triglycerides in the blood in certain people with very high triglycerides who are at risk of pancreatic disease.Formulation of sustained release floating bilayer tablets of gemfibrozil-nateglinide with HPMC K4M, Polyox WSR 303, Carbopol 971P.The compatibility of nateglinide and excipients used in study was determined using DSC and this study revealed that no interaction between drug and excipients used in the formulation. The optimized nateglinide-gemfibrozil floating formulations (NDT3, NDT9) showed satisfactory results with respect to in vitro buoyancy and sustained drug release, and was physically stable during 3 months period. The in vivo radiographic studies showed that the BaSO4-loaded floating tablets were retained in the stomach for 4.16 ± 1.57 h (n=3). The relative bioavailability of floating- tablets (NDT3) was found to be 1.7 times to that of IR tablets. This improved relative bioavailability is due to the combined effect of sustained release and increased GRT of tablets.

KEYWORDS: *Nateglinide, Gemfibrozil, floating, Natiz*

INTRODUCTION:

Bilayer tablet is suitable for sequential release of two drugs in combination, separate two incompatible substances and also for sustained release tablet in which one layer is immediate release as initial dose and second layer is maintenance dose. In which the one layer is formulated to obtain immediate release of the drug, with the aim of reaching a high serum concentration in a short period of time. The second layer is a controlled release, which is designed to maintain an effective plasma level for a prolonged period of time. The pharmacokinetic advantage relies on the fact that drug release from fast releasing layer leads to a sudden rise in the blood concentration. However, the blood level is maintained at steady state as the drug is released from the sustaining layer.

***Corresponding Author:**

Mohammad Ismail*
Assitant Professor
Unity College of Pharmacy
Raigir, Bhongir, Yadadhri, TS, India
Email: *mdismail.599@gmail.com*
DOI:

One of the most feasible approaches for achieving a prolonged and predictable drug delivery profiles in gastrointestinal tract is to control the gastric residence time (GRT) using gastroretentive dosage forms that offer a new and better option for drug therapy.Dosage forms that can be retained in the stomach are called gastroretentive drug delivery systems (GRDDS). Gastroretentive floating drug delivery system was first described by Davis in 1968. Over the last two decades, numerous GRDDS have been designed to prolong gastric residence time (Talukder and Fassihi, 2004a). Figure 1.3 describes how the drug absorption takes place in the case of conventional dosage forms and GRDDS. GRDDS can improve the controlled delivery of drugs that have an absorption window. This system releases the drug continuously for a prolonged period of time before it reaches its absorption site, thus ensuring its optimal bioavailability (Brahma and Know, 2000).

MATERIALS AND METHODS

MATERIALS

Nateglinde was collected as a gift sample from splendid pharma, Ltd, Puna, Gemfibrozil collected from Hetero drugs Ltd, Hyderabad. Hydroxy propylmethylcellulose (HPMC K4M) and Polyox WSR303 were received as gift samples from Orchid pharma Ltd., Chennai, India. Sod.CMC and Carbopol 971P were

received from M/s Aurobindo Pharma. Ltd., Hyderabad, India. Sodium bicarbonate, magnesium stearate and talc were purchased from S.D. Fine Chem. Ltd., Mumbai, India. Acetonitrile and methanol HPLC grade were purchased from Sigma Aldrich chemicals Dombivli, India. All other solvents and reagents used were of analytical grade.

METHODS

Solubility study of gemfibrozil gemfibrozil, nateglinide

Excess amount of drug was placed in 0.1 N HCl (pH 1.2), pH 4.5 acetate and pH 6.8 phosphate buffer and water in order to determine its solubility. The samples were shaken for 48 hrs at 37 °C in a horizontal shaker. The supernatant was filtered and the filtrate was diluted with the appropriate buffer and analyzed by using UV/Visible spectrophotometer (Elico, SL210) at λmaxof 276, 216 nm.

Determination of acid stability of gemfibrozil,nateglinide

Stock solution of drug was prepared in 0.1 N HCl in order to determine its acid stability. At predetermined time points like 1, 2, 3, 4, 6, 8, 10, 12 and 24 h, the samples were assayed using UV/Visible spectrophotometer (Elico, SL210) at λmax of 276, 216 nm.

Evaluation of final blend

The powder blend of all formulations was evaluated for Bulk density, Tapped density, Compressibility Index, Hausner ratio and Angle of repose (Carr, R.L., 1965).

Stock solution of drug was prepared in 0.1 N HCl in order to determine its acid stability. At predetermined time points like 1, 2, 3, 4, 6, 8, 10, 12 and 24 h, the samples were assayed using UV/Visible spectrophotometer (Elico, SL210)at λmaxof 276, 216 nm.

Table 1. Formulation of immediate release tablets of gemfibrozilin combination of Cross povidone, Lactose and Sorbitol

(Weights in mg/tablet)

Ingredients	GF1 mg	GF2 mg	GF3 mg	GF4 mg	GF5 mg	GF6 mg	GF7 mg	GF8 mg	GF9 mg	GF10 mg
Gemfibrozil	10	10	10	10	10	10	10	10	10	
Cross povidone	**47**	**57**	**67**	**77**	**87**	**-**	**-**	**-**	**-**	**-**
Lactose	**40**	**30**	**-**	**-**	**-**	**87**	**87**	**87**	**-**	**-**
Sorbitol	**-**	**-**	**20**	**10**	**-**	**-**	**-**	**-**	**87**	**87**
Magnesium stearate	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**

Formulation of floating tablets of nateglinide

Accurately weighed quantities of drug, Polyox WSR303/HPMC K4M/Carbopol 971P/Sod CMC, sodium bicarbonate and Avicel PH102 were passed through a sieve, no. 40, to get uniform sized particles, and then they were triturated for 10 min with the help of mortar and pestle. At that point the blend was moved into a poly sack and further blended for 5 min to warrant a. homogeneous mass. To the blend, magnesium stearate and talc were included and preceded with the blending for another 2 min. At long last, each blend was weighed and fed manually into the die of a 16station punching machine (Cadmach, Ahmedabad, India) to produce the desired tablets using flat- faced round punches. The hardness is adjusted to 5 kg/cm^2

Table 2. Formulation of sustained release floating bilayer tablets of ezetimibe-nateglinide with Polyox WSR 303, Carbopol 934 P, (weights in mg/tablet)

Ingredients	NDT1	NDT 2	NDT 3	NDT 4	NDT5	NDT6	NDT7	NDT 8	NDT 9	NDT 10
Nateglinide	120	120	120	120	120	120	120	120	120	120
Polyox WSR 303	40	60	80	100	120	-	-	-	-	-
HPMC K4M	-	-	-	-	-	40	60	80	100	120
Carbopol 934P	80	60	60	40	40	-	-	-	-	-
SCMC	-	-	-	-	-	80	60	60	40	40
Sodium bicarbonate	60	60	60	60	60	60	60	60	60	60
Avicel PH102	88	88	68	68	48	88	88	68	68	48
Mg. Stearate	6	6	6	6	6	6	6	6	6	6
Talc	6	6	6	6	6	6	6	6	6	6
Total Tablet weight	500	500	500	500	500	500	500	500	500	500

Physical characterization of prepared tablets

The prepared floating tablets were evaluated for hardness, thickness, weight variation, friability, drug content.

In-vitro buoyancy studies

The in vitro buoyancy was characterized by floating lag time (FLT) and total floating time (TFT). The test will be performed using United States Pharmacopeia (USP 24) type-2 apparatus using 900 mL of 0.1N HCl with a paddle rotation of 50 rpm at 37°C ± 0.5°C. Nateglinide sustained release effervescent floating bilayer tablets were placed in dissolution vessels and time required for the tablet to rise to the surface of the dissolution medium and the duration of time the tablet constantly floated on the dissolution medium were noted as FLT and TFT, respectively (Baumgartner et al., 2000).

In-vitro dissolution studies

The in vitro drug release studies will be conducted using USP 24 type-2 apparatus (Electrolab, TDT-06T). The dissolution test is performed using 900 mL of 0.1N HCl (pH 1.2), at 37 ± 0.5°C and 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at pre-determined time intervals, and replaced with fresh dissolution medium. The samples were filtered through a 0.45-µm membrane filter and diluted to a suitable concentration with 0.1N HCl. Absorbance of these samples were measured using UV/Visible spectrophotometer (Elico, SL 210, India) at λmax276, 276 nm.

Analysis of drug release kinetics

There are number of kinetic models, which described the overall release of drug from the dosage forms. Because qualitative and quantitative changes in a formulation may alter drug release and in vivo performance, developing tools that facilitate product development by reducing the necessity of bio-studies is always desirable. In this regard, the use of in vitro drug dissolution data to predict in vivo bio-performance can be considered as the rational development of controlled release formulations (Dressman et al., 1984).

The model dependent methods all rely upon a curve fitting procedure. Different mathematical functions were used to model the observed data (Costa and Lobo, 2001). Both the linear and non-linear models are being used in practice for dissolution modeling. Linear models include Zero order, Higuchi, Hixon – Crowell, Quadratic and Polynomials, whereas the nonlinear models include First order, Weibull, Korsmeyer – Peppas, Logistic etc.

Determination of tablet swelling and erosion levels

The swelling behavior of the tablets will be determined in triplicate The nateglinidesustained release effervescent floating bilayer tablets, nateglinidesustained release effervescent floating bilayer tablets were weighed (W0) and placed in a glass beaker containing 200 mL of 0.1 N HCl, maintained at 37 ± 0.5oC. At regular time intervals, the tablets were removed and the excess surface liquid was carefully removed by a filter paper (Patel et al., 2009). The swollen individual tablet was then reweighed (W1). The wet tablets were then dried in an oven at 40o C for 24-h and finally weighed until constant weight was achieved (final dry weight, W2). The percentage swelling and erosion at different times was estimated from the following equations:

% Swelling= ((W1-W0))/W0×100

% Erosion= ((W0-W2))/W0×100

Physical stability studies

Physical stability studies were conducted according to ICH guidelines. One of the optimized formulations of nateglinidesustained release effervescent floating, floating bilayer tablets (NGT3) were enclosed in polyethylene bottle and placed in a desiccator containing saturated sodium chloride solution (75% RH). The desiccator was stored at 40oC for 3 months (Tadros, 2010). At predetermined time intervals, the tablets were examined for hardness, FLT, TFT, drug content and drug release. Finally, the tablets were tested for any statistical difference using the student paired t-test, the differences were considered to be significant at $p < 0.05$.

Formulation of nateglinide sustained release effervescent floating, bilayer tablets for in-vivo radiographic studies

BaSO4 was used to make the tablet X-ray opaque. For this study, BaSO4 was loaded in optimized formulation of nateglinide sustained release effervescent floating tablets (NGT3) with following composition: 125 mg drug, 75 mg BaSO4, 60 mg Sod. CMC, 60 mg NaHCO3, 130 mg Avicel PH102, 5 mg magnesium stearate and 5 mg talc. The tablets were prepared by direct compression method.

In-vivo evaluation of gastric residence time in healthy volunteers

Three healthy male volunteers will participate after giving an informed written consent. The subjects weighed in between 64-75 kg, in height from 165-173 cm, and in the age group of 22-26 years.The in vivo gastric residence time of GRDDS can be determined by a variety of techniques such as x-ray, endoscopy, gamma-scintigraphy (Jagdale et al., 2009). In this study, x-ray technique will be used to determine the gastric residence time of gastroprotective tablets. To make the tablet X-ray opaque, BaSO4 was used. The study was conducted under the guidance of an

expert radiologist. After overnight fasting, the volunteers were fed with low calorie food (100 g of bread). Half an hour later, BaSO4-loaded optimized formulation of nateglinide floating tablet (NDT3) was given to every volunteer with a glass of water. During the study, the subjects were not allowed to eat but water was made available ad libitum. At different time intervals like, 0.5, 2.5, 4.5 and 5.5 h, the volunteers were exposed to abdominal x-ray imaging in a standing position. The distance between source of x-rays and the subject was kept constant for all images. Thus, the observation of the tablet movements could be easily noticed (El Gamal et al., 2011). The mean gastric residence time was calculated.

Comparative bioavailability study in human volunteers

Subjects: The mean age of volunteers was 22.5 ± 3.2 years, mean height was 167.5 ± 8.5 cm, and mean body weight was 63.5 ± 6.4 kg.

Nine healthy male volunteers for nateglinidefloating tablets were selected for the study. Before starting the study, each candidate signed an informed consent form. They were judged to be healthy based on medical history, physical examination, hematological and biochemical laboratory tests. The bioavailability protocol was approved by an Institutional Human Ethical Committee, Talla Padmavathi College of Pharmacy, Warangal, Telangana, India

Study design:

Nateglinidefloating tablets:

A single dose, randomized, three-way cross-over study was designed with nine subjects in each treatment group. A one-week washout period existed between treatments of the study. After overnight fasting, in three study periods for each subject the assigned formulation (floatingNDT3/Natiz 120 mg) aside policy phoneticly amidst 240 ml of water.One week before and during the study, they were not allowed to take alcohol or any other medication. The subject's fasted overnight and 5 hrs after tablet administration, but water was made available ad libitum. Study medication was administered according to randomization schedule. Subjects received standard meals after 5 hrs of tablet administration. Blood samples were collected at predetermined time intervals such as 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h. Blood samples (5 ml) were obtained from forearm vein using sterile disposable needle and collected into 10 ml sterile test tubes. The samples were centrifuged immediately at 4000 rpm for 15 min. and the separated plasma was transferred into 2.5 ml of Eppendorf tubes and stored at −80o C till the time of analysis.

Sample preparation for analysis

The serum samples were extracted by liquid-liquid extraction method. To 1 ml of serum, 0.4 ml of phosphate buffer PH 7 and 100 µl of internal standard (furosemide, 2 µg/ml) was added and vortexed for 5 min in a test tube. To this mixture 7 ml of chloroform: isopropyl alcohol was added as extracting agent in 95: 5 v/v proportion and vortexed for 3 min then centrifuged at 2500 rpm on cooling centrifuge for 15 min at 4 oC (Manglani et al., 2006). The organic phase was separated into another test tube and evaporated to dryness in a vacuum oven. To the dried residue 0.4 ml of diethyl ether was added and shaken for 10 sec and the ether layer was discarded. The dried residue was reconstituted with100 µl of mobile phase from which 20 µl was injected into the HPLC column.

Pharmacokinetic analysis

The pharmacokinetic parameters of test formulation and reference formulation were estimated for each volunteer by using a computer programme, Kinetica 2000 (Version 3.0, Innaphase Corporation, and Philadelphia, USA). Non-compartmental analysis was used to calculate pharmacokinetic parameters, Cmax, tmax, t1/2, AUC0-∞ and MRT values. Cmax and tmax were read directly from the observed mean plasma drug concentration against time profile. AUC0-t was calculated by the trapezoidal rule and the total AUC0-∞ was calculated according to the equation.

$$AUC0\text{-}\infty = AUC0\text{-}t + Ct/KE$$

Where, Ct is the last measurable concentration and KE is the elimination rate constant obtained from terminal log-linear portion of the plasma concentration-time profile. The mean residence time (MRT) was calculated using following equation (Shargel et al., 2005).

$$MRT = AUMC0\text{-}\infty$$

$$AUC0\text{-}\infty$$

Where, AUMC is the area under the first moment of the concentration time curves.

RESULTS AND DISCUSSION

Equilibrium Solubility Study of Nateglinide

The solubility studies were conducted in different media and values are shown in Table 2. The solubility of the drug was determined in different media like, 0.1 NHCl (pH 1.2), pH 4.5, pH 6.8 and water. The drug showed greater solubility in 0.1 N HCl (1.85 ± 0.32 mg/mL) and lesser solubility in water (1.32 ±0.26mg/mL).

Table3: Solubility of Nateglinide in different media (mean±SD).

Medium	Solubility(mg/mL)
pH1.2	1.85±0.32
pH4.5	1.66±0.22
pH6.8	1.46±0.43
Water	1.32±0.26

Determination of acid stability of Nateglinide:

From the acid stability study results it was observed that there was no change in drug concentration until 24h indicating stability of drug in 0.1 NHCl.

Physical characteristics of prepared tablets of Ezetimibe:

All the prepared formulations were subjected to the hardness, thickness, weight variation, and friability, drug content. The hardness of all tablets ranged from 5.18 ± 0.23 to 5.31 ± 0.45 kg /cm2 and that of thickness from 5.28 ± 0.13 to 5.58 ± 0.16 mm. All the tablet formulations showed acceptable physicochemical properties and complied with the pharmacopoeial specifications for weight variation, drug content and friability (Banker and Anderson, 1987).

Table 4: Physicochemical characters of prepared tablets of Gemfibrozil

Formulation code	Hardness (kg/cm2)	*Thickness (mm) (n=6)*	*Tablet weight(mg) (n=20)*	*Friability (%) (n=10)*	*Drug content (%) (n=3)*	*Disintegration Time (mins)n=3 Mean ±S.D*
GF 1	4.20±0.2	4.17±0.12	101.80±6.1	0.53	99.03±1.9	1.43 ± 0.61
GF 2	4.28±0.2	4.24±0.11	102.20±7.1	0.57	101.30±1.	1.01 ± 0.22
GF 3	4.38±0.2	4.38±0.16	101.90±7.7	0.52	99.40±1.4	0.50 ± 0.06
GF 4	4.30±0.4	4.28±0.13	99.30±6.62	0.56	98.55±1.4	0.56 ± 0.08
GF 5	4.36±0.2	4.28±0.16	99.20±5.90	0.53	99.67±1.4	0.42 ± 0.07
GF 6	4.22±0.2	4.34±0.11	101.20±6.9	0.59	99.88±1.3	0.57 ± 0.07
GF 7	4.34±0.3	4.52±0.17	100.10±6.1	0.58	98.91±1.8	1.12 ± 0.04
GF 8	5.30±0.3	4.52±0.13	102.10±6.1	0.57	99.28±1.3	0.50 ± 0.02
GF 9	5.26±0.4	4.42±0.14	103.80±7.0	0.52	99.38±1.2	0.66 ± 0.05
GF 10	5.30±0.5	4.50±0.15	101.60±7.3	0.58	99.40±1.7	0.66 ± 0.05

Table 5: Physicochemical characters of nateglinide sustained release effervescent floating bilayer tablets

Formulation code	Hardness (kg/cm2)	Thickness (mm) (n=6)	Tablet weight (mg) (n=20)	Friability (%) (n=10)	Drug content (%) (n=3)
NDT 1	5.31±0.28	5.16±0.12	501.80±6.16	0.54	99.03±1.96
NDT 2	5.36±0.23	5.25±0.11	502.20±7.16	0.56	101.30±1.7
NDT 3	5.28±0.29	5.29±0.16	501.90±7.75	0.53	99.40±1.49
NDT 3	5.22±0.42	5.28±0.13	499.30±6.62	0.57	98.55±1.46
NDT 5	5.26±0.26	5.28±0.16	499.20±5.90	0.57	99.67±1.44
NDT 6	5.28±0.29	5.34±0.11	501.20±6.97	0.59	99.88±1.33
NDT 7	5.29±0.36	5.52±0.17	500.10±6.10	0.68	98.91±1.89
NDT 8	5.30±0.36	5.52±0.13	502.10±6.16	0.63	99.28±1.39
NDT 9	5.27±0.47	5.42±0.14	503.80±7.07	0.65	99.38±1.24
NDT 10	5.30±0.51	5.50±0.15	501.60±7.35	0.68	99.40±1.79

The physicochemical characteristics of the tablets are summarized in table 5. The hardness of all tablets ranged from 5.18 ± 0.23 to 5.31 ± 0.45 kg/cm2 and that of thickness from 5.28 ± 0.13 to 5.58 ± 0.16 mm. All the tablet formulations showed acceptable physicochemical properties and complied with the pharmacopoeial specifications for weight variation, drug content and friability (Banker and Anderson, 1987).

In vitro buoyancy studies

Table 6: In vitro buoyancy of nateglinide sustained release effervescent floating bilayer tablets

Formulation code	Floating lag time (s) (n=3)	Floating duration (h) (n=3)
NDT 1	15.3±2.5	>12
NDT 2	17.7±2.6	>12
NGT 3	20.0±2.0	>12
NDT 4	24.7±3.5	>12
NDT 5	29.3±5.5	>12
NDT 6	35.7±3.1	>12
NDT 7	17.3±3.1	>12
NDT 8	19.0±3.0	>12
NDT 9	21.3±3.1	>12
NDT 10	35.7±3.1	>12

All the formulations were prepared by effervescent technique. Sodium bicarbonate was used as a gas generating agent. Formulations NDT1-NDT10, prepared with combination of Polyax WSR 303 and CP 971P floated lag time of 15.3±2.5 (NDT1) to 35.7±3.1 (NDT10) sec. Tablets of all formulations showed good in vitro buoyancy with maximum floating lag time of 35.7±3.1 sec (Table 6& Table 7), regardless of viscosity of polymer used. This was mainly due to evolution of carbon dioxide entrapped inside the hydrated polymeric matrices, resulting from the interaction between gas generating agent (NaHCO3) and dissolution medium (0.1N HCl), and this led to the lowering of density of matrices to float. The floating lag time could not change with different viscosity grades of polymers and the type of filler used. All formulations remained buoyant for more than 12 h in dissolution medium (0.1 N HCl, pH 1.2).

In vitro drug release studies:

Table 8: In vitro immediate drug release of Gemfibrozil

Time (Min)	GF 1	GF 2	GF 3	GF 4	GF 5
0	0	0	0	0	0
1	18.87±1.54	22.61±166	24.26±1.32	26.96±1.34	30.04±1.22
5	32.70±1.76	34.52±1.54	35.09±1.54	36.78±1.55	38.00±1.89
10	43.65±1.92	43.65±1.99	44.39±1.22	46.09±2.22	48.74±2.15
15	65.78±2.12	54.91±1.86	56.57±1.77	59.35±1.98	60.43±1.56
20	74.83±1.88	69.96±2.21	67.78±1.54	69.39±2.23	72.52±1.89
25	87.87±1.56	85.83±1.12	85.48±2.23	89.09±1.87	89.22±1.45
30	91.61±1.97	94.43±1.83	94.09±2.12	96.74±1.77	99.74±1.29

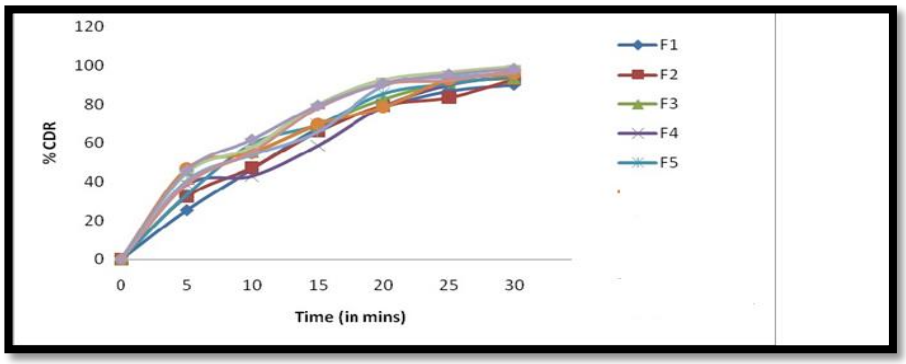

Fig: 1 In vitro immediate drug release of gemfibrozil

In vitro sustained release effervescent floating bilayer tablets of nateglinide

Table 9: Cumulative percentage release of nateglinide from floating tablets prepared with combination of Polyox WSR 303 and CP 934 P and (n=3, Mean±SD)

Time (h)	NDT1	NDT 2	NDT 3	NDT 4	NDT 5
0	0	0	0	0	0
1	24.87 ± 1.54	26.61±1.66	20.26±1.32	18.96±1.34	19.04±1.22
2	37.70±1.76	39.52±1.54	32.09±1.54	30.78±1.55	29.00±1.89
3	48.65±1.92	52.65±1.99	41.39±1.22	40.09±2.22	39.74±2.15
4	60.78±2.12	62.91±1.86	51.57±1.77	50.35±1.98	48.43±1.56
6	74.83±1.88	77.96±2.21	66.78±1.54	62.39±2.23	60.52±1.89
8	88.87±1.56	90.83±1.12	82.48±2.23	78.09±1.87	75.22±1.45
10	101.61±1.97	94.43±1.83	91.09±2.12	90.74±1.77	87.74±1.29
12	101.09±1.54	98.39±1.96	98.04±1.67	98.26±1.86	95.35±1.33

All the formulations were subjected to in vitro drug release studies in 0.1 N HCl. The drug release profiles of formulations NDT1-NDT5 prepared with combination of Polyox WSR 303 and CP 934 P shown in figure 1. Formulation NDT1 and NDT2 released about 88.87±1.56% and 94.43±1.83 % drug in 8 and 10 h respectively and couldn't sustain the drug release for 12 h, indicating less concentration polymer. The formulation NDT 3 released about 98.04±1.67% of drug in 12 h. Similarly, formulation NDT4 sustained the drug release for 12 h and released 98.26±1.86% of drug in 12 h. Formulations NDT 5 released about 95.35±1.33% in 12 h. From the results, it was also observed that as increasing concentration of CP 934 P, the drug release was decreased.

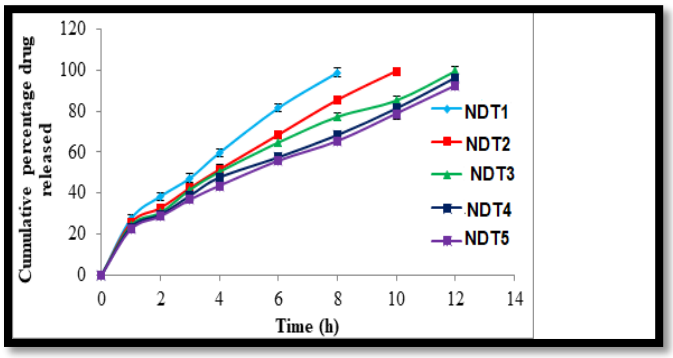

Fig. 2: Cumulative percentage release of nateglinide from floating tablets prepared with combination of Polyox WSR 303 and CP 934 P

All the formulations were subjected to in vitro drug release studies in 0.1 N HCl. The formulations NDT6-NDT10 prepared with combination of HPMC K4M and Sodium CMC were shown in figure 2. Amount of drug release from these formulations ranged from 84.48±1.85% (NDT 6) to 97.35±2.42% (NDT10). All the formulations sustained the drug release for 12 h. The optimized formulation released about 99.91±1.84% of drug in 12 h.

Table 10: Cumulative percentage release of nateglinide from floating bilayer tablets prepared with combination of HPMC K4M and Sodium CMC (n=3, Mean±SD)

Time (h)	NDT6	NDT 7	NDT 8	NDT 9	NDT 10
0	0	0	0	0	0
1	*14.17±1.53*	*15.74±1.51*	*17.61±1.54*	*18.65±1.41*	*19.52±1.89*
2	*22.26±2.87*	*23.09±1.58*	*26.78±1.58*	*29.78±1.48*	*30.87±1.78*
3	*30.13±1.42*	*33.96±1.44*	*35.78±1.37*	*37.91±1.43*	*38.96±2.64*
4	*39.61±2.12*	*41.52±1.07*	*43.30±2.08*	*45.48±2.01*	*48.43±1.65*
6	*51.48±2.10*	*53.30±2.16*	*55.04±2.16*	*56.13±2.16*	*60.22±1.68*
8	*63.52±1.14*	*65.48±2.86*	*67.22±1.72*	*69.30±1.86*	*71.57±1.56*
10	*73.48±1.97*	*77.39±1.45*	*80.17±1.31*	*82.35±1.43*	*83.17±1.87*
12	*84.48±1.85*	*87.74±1.36*	*91.35±1.51*	*94.13±1.12*	*97.35±2.42*

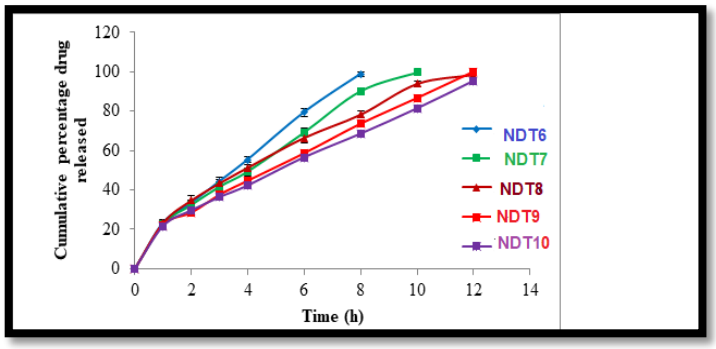

Figure 3: Cumulative percentage release of nateglinide from floating bilayer tablets prepared with combination of HPMC K4M and Sodium CMC (n=3, Mean±SD)

Kinetics of drug release profiles

The drug release profiles of all the formulations of nateglinide floating were fitted to different kinetic equations. The R2 values for Zero-order model ranged from 0.921 (NDT1) to 0.983 (NDT10). Similarly, r2 values for Higuchi model ranged from Higuchi model ranged from 0.949 (NDT9) to 0.973 (NDT10). All the formulations followed the Peppas model and r2 values were ranged from 0.962-0.998 due to high coefficient of determination (r2). The optimized formulation NDT10 followed Peppas model (r2=0.998) followed non-Fickian diffusion drug release mechanism (n=0.636). The value of release exponent for all the formulations ranged from 0.652 (NDT1) to 0.0.679 (NDT7) and that of optimized formulation was 0.635. All the formulations have n values between 0.5 and 1, indicating anomalous transport (non-Fickian). The release rate constants (k) of all the formulations were significantly different. The value of k for formulations NDT1-NDT6 prepared with combination of Polyox WSR303 and CP 971P was ranged from 4.32 (NDT6) to 7.71 (NDT1), and that of formulations NDT7-NDT10, prepared with combination of HPMC K4M and Sod.CMC was ranged from 5.89 (NDT10) to 6.60 (NDT 7). Higher k values meant higher quantities of drug released.

Determination of tablet swelling and erosion levels:

Table 11: Percentage swelling of nateglinide floating tablets in 0.1 N HCl ((Mean ± SD, n=3).

Time (h)	NDT3		NDT9	
	Mean	**SD**	**Mean**	**SD**
1	48.79	2.38	43.12	1.65
2	58.48	2.53	53.59	2.88
4	76.11	3.25	71.94	1.99
6	110.49	2.98	102.19	2.83
8	91.38	3.21	87.69	2.27
10	59.81	2.25	55.70	0.83
12	44.57	2.84	37.87	0.95

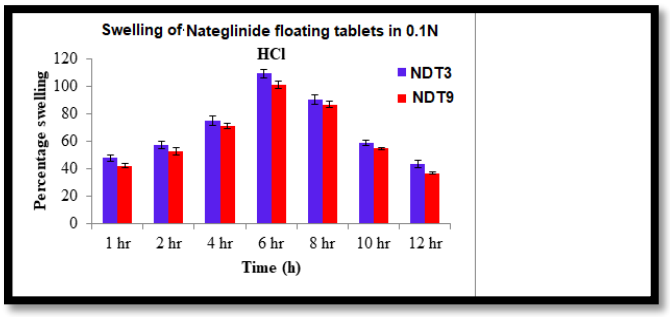

Figure 4: Extent of swelling of different formulations of nateglinide floating tablets in 0.1 N HCl ((Mean ± SD, n=3).

The hydration ability of the formulation is important because it influences: (i) tablet buoyancy, (ii) adhesion ability of swellable polymers in contact with the dissolution medium and (iii) drug release kinetics. The swelling and erosion studies were performed on the optimized formulations NDT3, NDT9. The percentage swelling of optimized nateglinide floating tablets (NDT3, NDT9) were determined at different time intervals. The maximum swelling was observed at 6 h and was found to be 110.49 ± 2.98%, 102.19 ± 2.83 and, respectively. The erosion increased with increase in time for both the formulations. At 12 h, the erosion was found to be 62.54 ± 2.60%, 66.06 ± 2.24% for NDT3, NDT10. The nominal swelling and erosion differences were due to the different polymers used.

Table 12: Percentage erosion of optimized nateglinide floating bilayer tablets in 0.1 N HCl

Time (h)	NDT3		NDT9	
	Mean	SD	Mean	SD
1	20.46	1.48	22.40	1.24
2	23.62	2.14	24.41	2.18
4	29.89	1.99	30.88	1.66
6	39.62	1.81	40.34	2.25
8	55.45	1.41	55.64	3.27
10	62.64	2.01	62.45	1.54
12	66.54	2.60	65.06	2.24

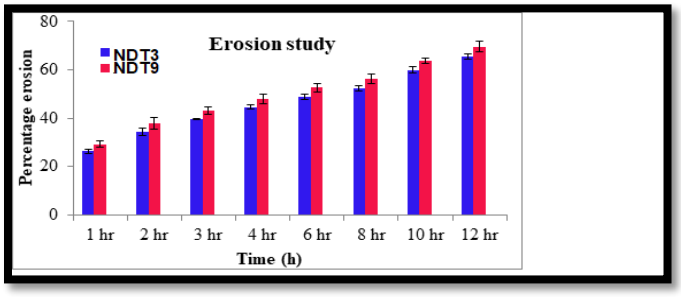

Figure 5: Extent of erosion of optimized nateglinide floating bilayer tablets in 0.1 N HCl

Physical stability studies

The optimized nateglinide floating bilayer tablets (NDT3) was selected for stability study based on physical characters and in vitro drug release. Before and after conducting the stability studies for 3 months, the results were analyzed statistically by using Student's paired t-test. No significant difference ($p > 0.05$) was observed in the tablet hardness, FLT, TFT, drug content or in vitro dissolution. The drug content was slightly decreased from 101.26 ± 1.59% to 100.85 ± 1.69% after storage at 40oC under 75% RH. But the difference was not statistically significant ($p > 0.05$). Thus, the NGT3 nateglinide sustained release floating bilayer tablets were found to be stable.

Table 13: Physical characters during storage - stability study of nateglinide floating-bio adhesive bilayer tablets (NDT3).

Characteristic parameter	*0 day **	*15th day **	*30th day **	*60th day **	*90th day **
Hardness (kg/cm2)	*5.43± 0.50*	*5.43± 0.45*	*5.65± 0.53*	*5.62± 0.44*	*5.40± 0.38*
Floating lag time (s)	*28.30± 3.20*	*28.25±3. 05*	*28.21± 2.51*	*28.22±2. 31*	*28.20± 2.64*
Duration of floating (h)	*>12*	*>12*	*>12*	*>12*	*>12*
Drug content (%)	*102.26±1 .59*	*101.31± 1.63*	*101.32 ±1.74*	*101.26± 1.75*	*101.85± 1.69*
Drug released at 12 h	*99.91±1. 84*	*99.69±1. 44*	*99.45± 1.32*	*99.31±1. 31*	*99.26± 1.43*

* Statistically not vital ($p > 0.05$).

In vivo evaluation of gastric residence time

Table 14: Location of nateglinidefloating bilayer tablet in the GIT of volunteers

Time (h)	Position of tablet in the GIT of volunteer		
	A	B	C
0.5	Stomach	Stomach	Stomach
2.5	Stomach	Stomach	Stomach
4.5	Stomach	Stomach	Stomach
5.5	Stomach	Intestine	Stomach

The floating tablets (NDT3) prepared for radiological studies were characterized for hardness (5.40 ± 0.33 kg/cm2), thickness (5.65 ± 0.11 mm), weight variation (501.45 ± 5.65 mg), friability (0.37%), FLT (68.65 ± 5.42 sec) and TFT aside greater than 12 h. The maximized lag time of BaSO4-loaded nateglinide bilayer tablets, compared to the original formulation NDT3 (28.3 ± 3.1 s) was expected because of high density of BaSO4 (4.5 g/cm3).

Figure shows the radiographic images taken at different periods after administration of BaSO4-loaded nateglinide floatingtablets in one volunteer (A). The first radiographic image was taken at 0.5 h post-administration of tablet and the tablet was observed in the stomach. The next pictures were taken at 2.5, 4.5 and 5.5 h; the tablet had altered its position, yet remained within the stomach till the end of 5.5 h. The mean gastric residence time was found to be 5.13 ± 0.64 h (n=3).

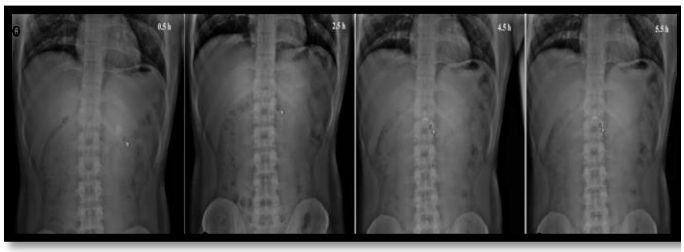

Fig 6: Radiographic images showing the presence of BaSO4-loaded sustained release effervescent floating bilayer tablets of nateglinide in the stomach of volunteer-A at different time points (the location of the tablet is shown with an arrow).

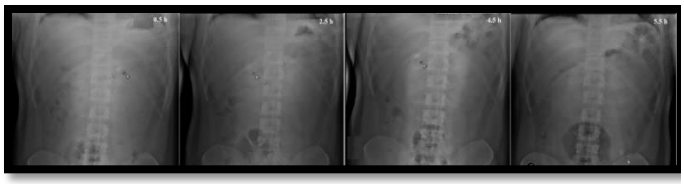

Fig 7: Radiographic images showing the presence of BaSO4-loaded sustained release effervescent floating bilayer tablets of nateglinide in the stomach of volunteer-B at different time points (the location of the tablet is shown with an arrow).

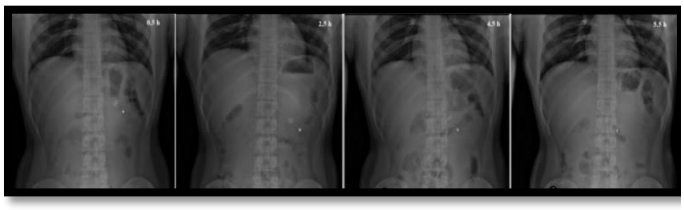

Fig 8: Radiographic images showing the presence of BaSO4-loaded sustained release effervescent floating bilayer tablets of nateglinide in the stomach of volunteer-C at different time points (the location of the tablet is shown with an arrow).

Table 15: Pharmacokinetic parameters of nateglinide test (NDT 3) and reference (Natiz) formulation, n=8.

Pharmacokinetic parameter	Nateglinide reference formulation (Natiz Tab)	Pharmacokinetic parameter
Cmax (µg/ml)	0.295±0.047	0.252±0.011
tmax (h)	2.167±0.500	3.444±0.500
t1/2 (h)	2.029±0.200	4.116±0.407
AUC0-24 (µg.h/ml)	9.461±0.760	16.110±1.580
AUC0-∞ (µg.h/ml)	9.564±0.772	16.289±1.667
MRT (h)	4.731±0.211	8.791±0.409

Aside student paired t-test, $p < 0.05$ is heeded statically vital in all the criterion.

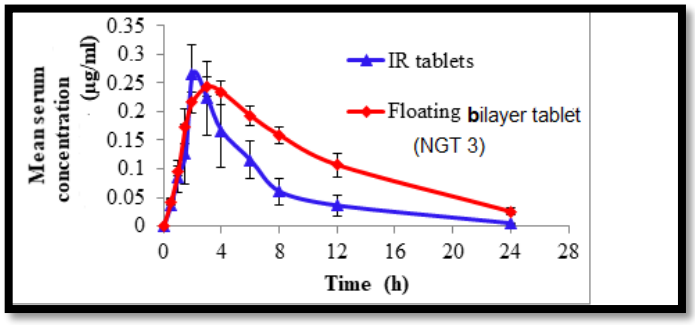

Figure 9: Mean serum concentration (µg/ml) of nateglinide test (NDT 3) and reference (NatizTab) formulation in healthy human volunteers (m=9, Mean±SD)

The bioavailability study was successfully conducted according to the study protocol. The drug was well tolerated with no other symptoms or disturbances during the two study periods. The serum samples were analyzed by RP-HPLC method. The HPLC chromatogram of blank serum is shown in figure and that of sample is shown in figure9. The retention time of drug nateglinide is 4.81 min and the internal standard furosemide is 7.42 min. These retention times were nearly closer to the reported values (Manglani et al., 2006). The pharmacokinetic parameters used to assess the bioavailability of test versus reference were AUC0-∞ for the extent of absorption and Cmax, tmax for the rate of absorption. The mean serum concentration-time curves for test (NDT3) and reference (Natiz Tab 120 mg) immediate release formulations are show in figure 5.41. The Cmax value for Natizformulation (reference) apart met to be 0.295±0.047 µg/ml and that of test formulation (NDT3) aside met to be 0.252±0.011 µg/ml. The tmax values for both reference and test formulation were found to be 2.167±0.500 h and 3.444±0.500 h certainly.

Half-life value for reference was found to be 2.029±0.200, and that of test is 4.116±0.407 h. The AUC0-24 values for reference and test were 9.461±0.760 µg×h/ml and 16.110±1.580 µg×h/ml, certainly. AUC0-∞ value reference formulation was 9.564±0.772 µg×h/ml and that of test formulation was 16.289±1.667 µg×h/ml. Similarly, mean residence time (MRT) value reference formulations were 4.731±0.211 and that of test formulation was 8.791±0.409 h. In the present study student's paired t- test was used to compare pharmacokinetic data of reference and test formulation. The data showed that there was significant difference ($P < 0.05$) between two formulations in their tested pharmacokinetic parameters, AUC0-24, AUC0-∞, Cmax, tmaxand MRT. The increase in relative bioavailability of test formulation was found to be 1.7 times when compared to reference formulation.

Conclusion:

Initially, drug-excipients interaction study was determined using DSC and found that the drug was compatible with all the excipients used in the formulation. Floating tablets of nateglinidewere prepared with combination of Polyox WSR 303 and CP 971P/ HPMC K4M and Sodium CMC. The optimized formulation (NDT 9) floated with a lag time of 28.3±3.2 sec, duration of floating 12 h and released about 99.91±1.84% of drug in 12 h, and then followed non-Fickian diffusion release mechanism with n value of 0. 635. The NDT 3 tablets with BaSO4 remained in stomach for 5.13 ± 0.64 h (n=3) in radiological studies.The bioavailability studies were carried out for the optimized formulation (NDT9) and compared with that of reference IR tablets, Natiz in nine healthy human volunteers. Based on invivo performance significant difference was observed between Cmax, tmax, t1/2, AUC0–∞, and MRT of F10 and IR. The increase in relative bioavailability of NDT 3 was 1.7-fold when compared to reference, IR tablets. The increased relative oral bioavailability may be due to the floating mechanism of dosage form, which is desirable for drugs absorbed from the upper part of gastrointestinal tract.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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