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Formulation and In-vitro Evaluation of Rizatriptan Benzoate Solid Lipid Nano Particles Treatment of Epilepsy

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ABSTRACT:

Rizatriptan, a hydrophilic drug, was successfully encapsulated into Solid Lipid Nano-particles (SLNs) using a modified solvent injection method. The formulated Rizatriptan-loaded SLNs exhibited a nano-metric size range and spherical structure. The solvent injection technique proved to be simple, readily available, and effective for this formulation. The in-vitro drug release profile of the F5 formulation showed controlled release, with 44.50% of the drug released over 10 hours. This study represents a successful endeav or in formulating Rizatriptan using SLN technology, primarily aimed at enhancing the drug's absorption into systemic circulation. Overall, these findings indicate that Rizatriptan Benzoate SLNs show potential as a viable therapeutic approach for epilepsy, offering improved drug delivery characteristics that could potentially improve patient compliance and treatment outcomes. Further research is warranted to elucidate the precise mechanisms underlying these findings

Keywords: Rizatriptan Benzoate SLNs, improve patient compliance, Epilepsy

INTRODUCTION

Epilepsy is one of the oldest known illnesses in Humanity and still the most common neurological condition that affects people of all ages definitely. Estimated 50 million people worldwide have been diagnosed with epilepsy. Higher in developing countries than in developed countries Countries with low economic status and limited Access to Health Care [1, 2, 3, 4].

Our understanding Pathophysiology of epilepsy is advancing dramatically in the last 30 years, especially. Drug treatment Epilepsy has made remarkable progress with it. Since 1978, many new antiepileptic drugs have been introduced. However, the improvement in clinical outcomes. It was below expectations. Up to 1/3 Patients with persistent or unacceptable seizures. Epilepsy is an ancient illness that has been historically described from the time of the ancient Babylonians to the present day. Epilepsy is characterized by an unpredictable frequency of seizures, but epilepsy is a common neuropathy that affects people of all ages. In fact, epilepsy presents with bimodal onset, most commonly in childhood and adulthood (Institute of Medicine 2012). However, it is also a series of illnesses with varying severity, very different types and causes of seizures, and different effects on individuals and their relatives. In addition to living with epilepsy and its seizures and coexisting health conditions, the challenges faced by millions of people with epilepsy include access to quality health care and learning about health care. And as a response to stigma and general public misunderstandings, including coordination, dosing, professional independent living, and other community services. In this way, epilepsy puts a great deal of strain on individuals, families and society as a whole [5-12].

MATERIALS AND METHODS

Materials

Rizatriptan Benzoate was obtained from Sembramky Environmental Management Pvt. Ltd. Delhi (India) as a gift sample. Gleceryl monostearate, Stearic acid, Polysorbate 80 (Tween 80) and Span 80 were purchased from Aditya Overseas Pitampura, New Delhi. For formulation double distilled water was used. All the reagents and solvents were used of analytical grade.

Preformulation Studies

Determination of Melting Point

Open capillary tube method was used for determining the melting point of the drug. This procedure was performed in triplicate and mean of three observations is considered as a melting point.

Solubility Study

The solubility of the drug was performed by placing of a drug in the conical flask by use of different solvents for 24 hours. This practice was performed thrice and a solvent for analysis of drug was finalized.

Calibration curve of Rizatriptan Benzoate

10mg of drug was dissolved in 25ml of methanol and then volume made upto 100 ml with distilled water to get concentration100µg/ml. Stock solution was diluted further to get concentration range between 2µg/ml to 12µg/ml and absorbance was measured at 226nm [13].

FT-IR Spectroscopic Determination

The drug was characterized by using of Infrared absorption spectroscopy. The Required quantity of drug was taken and mixed with potassium bromide and packed into the compact disk and spectrum was recorded [14].

Preparation of Solid Lipid Nano-particles

Rizatriptan Benzoate loaded Solid- Lipid nano-particles were prepared by High speed Homogenization technique. First, lipid was heated 5-10°C above the melting point of the lipid (GMS/ Stearic acid) and OZP was made to dissolve in it. An aqueous phase was prepared by dissolving surfactant (Tween 80/ Span 80) in water and heated to same temperature of oil phase. Hot oil phase was further added to the hot aqueous phase and subjected to high-speed homogenization (10000 rpm- 15000rpm) for specified time (15minutes- 45 minutes) as per DOE (Table no. 1). Thereby produces hot oil in water (O/W) emulsion. The hot nano-emulsion was then cooled down to room temperature, and then lyophilized the sample [15].

Table.1: The ingredient quantities of each prepared RZB, SLN formulation

Ingredients (mg/ml)	**Formulation Code**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Rizatriptan Benzoate	10	10	10	10	10	10
Gleceryl monostearate	2	4	6	2	4	6
Stearic acid,	0.5	1	1.5	2	2.5	`3
Polysorbate 80	2	2.5	3	2	2.5	3
Span 80	1%	2%	3%	1%	2%	3%
H2O	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s

Evaluation of Solid-lipid Nano-particles

Particle size determination

The particle size distribution was analysed by using of digital electronic optical microscope (Labomed LX-200). One drop of sample was taken from each batch and diluted with 10 ml of dispersion medium (distilled water). Then particle size was determined with the help of digital electronic microscope under the 90X at room temperature. Then average size of fifteen particles of each formulated batch was taken for analysis [16].

Drug entrapment efficiency

The entrapment efficacy (EE) of Solid lipid nano-particles dispersion was determined by centrifugation method. The SLN dispersion was centrifuged at 2000 rpm for one hour and then collected the supernatant liquid of that dispersion. Then collected liquid was filtered to measure the free drug concentration after making the dilutions with freshly prepared phosphate buffer pH 6.4. The absorbance was measured at 226 nm. Following formula was used for the calculation of EE:

EE (%) = Amount of drug in NP (mg)/ Amount of drug added (mg) X 100

Total drug content

From the prepared SLN formulation 1 ml of suspension is dissolved in the 10 ml of pH7.4 phosphate buffer solution (PBS) and ethanol mixture. The amount of Carbamazepine was determined using UV spectrophotometer at 285nm. The placebo formulation prepared similarly to drug loaded SLN is used as blank. The total drug content was calculated [17].

Scanning electron microscopy (SEM)

The SEM analysis of prepared SLN was performed for morphological studies. The formulations are poured in to circular aluminium stubs using double adhesive tape, and coated with gold in HUS -5 GB vacuum evaporator , and observed in Hitachi S-3000 N SEM at an acceleration voltage of 10 Kv and a magnification of 5000X [18].

Differential Scanning Calorimetry (DSC)

DSC analysis was performed in order to investigate the melting and recrystallization behaviour of crystalline materials like SLNs. The samples were sealed in aluminium pans and

measurements were recorded using DSC instrument. The samples were heated from 25 to 200℃ at a heating rate of 100℃ /min under nitrogen atmosphere [19].

In vitro Drug Release

In vitro drug release of Rizatriptan Benzoate SLN was determined using Franz-diffusion cell. The cellophane membrane was mounted between the donar and receptor compartments. The receptor compartment was filled with phosphate buffer (pH 7.4) at 37℃. The solution was stirred at 100 rpm. The Rizatriptan Benzoate SLN was placed on cellophane membrane and the compartments were clamped together. One ml of sample was withdrawn at predetermined time for 24 hours, from receptor compartments and immediately replaced using phosphate buffer after filtering through 0.45μm filter and appropriate dilutions, the sample were analyzed for drug content at 226nm [20].

Drug Release Kinetics

Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time) & korsmeyer-peppas model were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values (r2) for correlation coefficients for formulations, the best-fit model was decided.

Stability Study as per ICH Guidelines

Stability of a drug has been defined as the ability of a dosage form, in a specific container, to remain within its physical, chemical, and therapeutic specifications.

A drug formulation is said to be stable if it fulfills the following criteria:

i) It should contain at least 90% of its active component.
ii) It should not show discoloration or precipitation.
iii) It should not develop irritation or toxicity.

The stability study of microsphere was carried out at various temperatures and relative humidity levels as per ICH Q1 A guidelines. The duration of the study and the storage conditions were carried out as per the International Conference on Harmonization guidelines (ICH): Long-term testing: 25oC ± 2oC / 60% RH ± 5% for 12 months. Accelerated testing: 40oC ± 2oC / 75% RH ± 5% for 6 months [21].

RESULTS AND DISCUSSION

Preformulation Studies

Melting Point Determination

The melting point of Rizatriptan Benzoate, API was found to be 178-181℃.

Solubility Study

RZB was practically insoluble in water, slightly soluble in Chloroform and freely soluble in Methanol and Ethanol.

Calibration curve of Rizatriptan Benzoate

Table.2: Standard Calibration Curve of RZT Benzoate

S. No.	Concentration (μg/ml)	Absorbance (226nm)
I.	02	0.194
II.	04	0.340
III.	06	0.582
IV.	08	0.691
V.	10	0.844

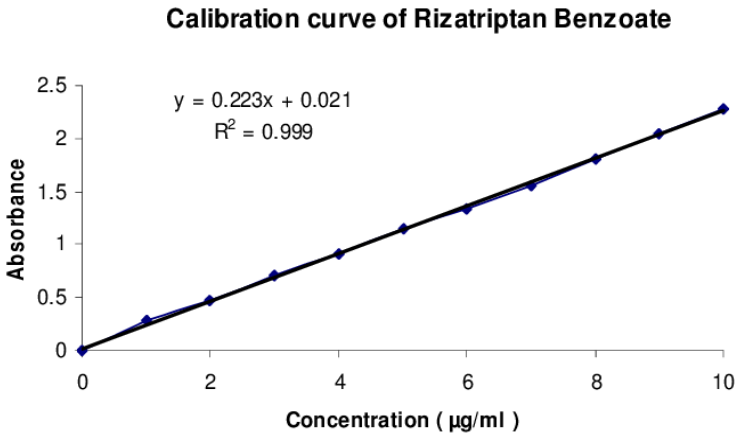

Fig.1: Standard Calibration Curve of RZT Benzoate

Physical drug Excipients Compatibility Studies

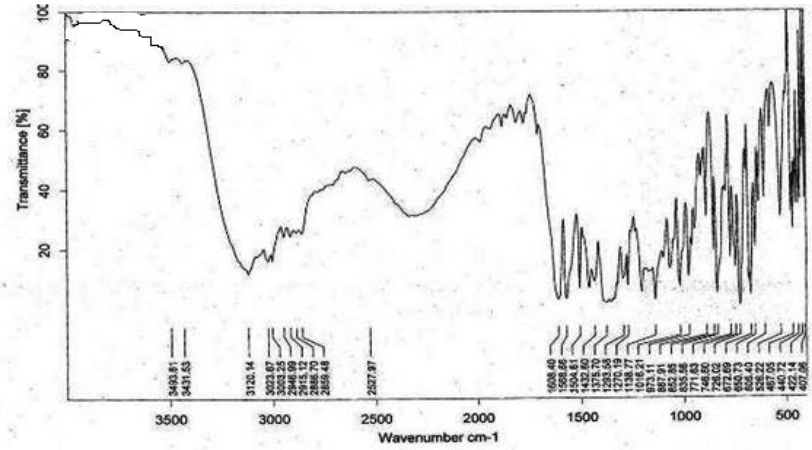

Fig.2: FTIR spectra of RZT Benzoate

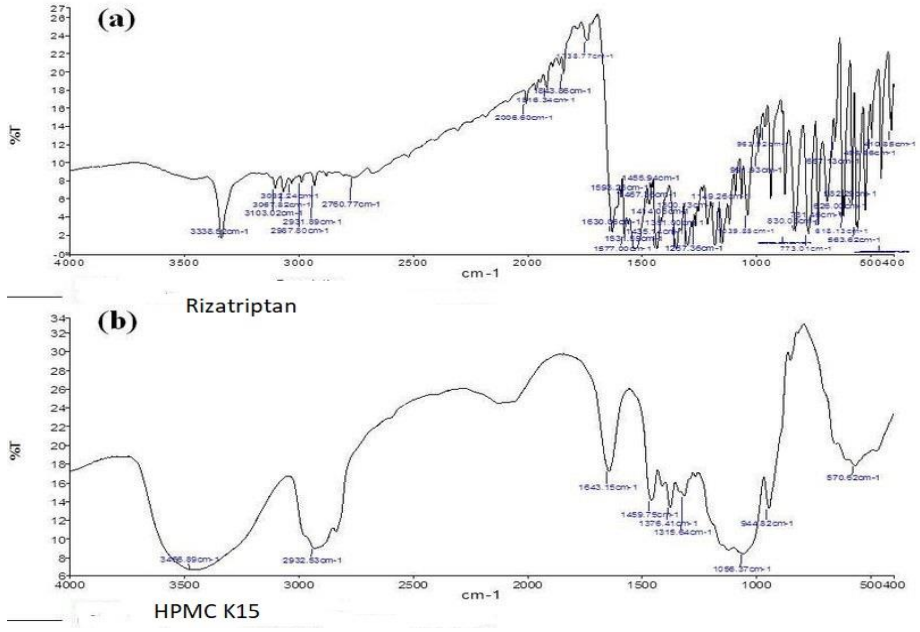

Fig.3: FTIR Spectra of RZT Benzoate+ Gleceryl Monostearate

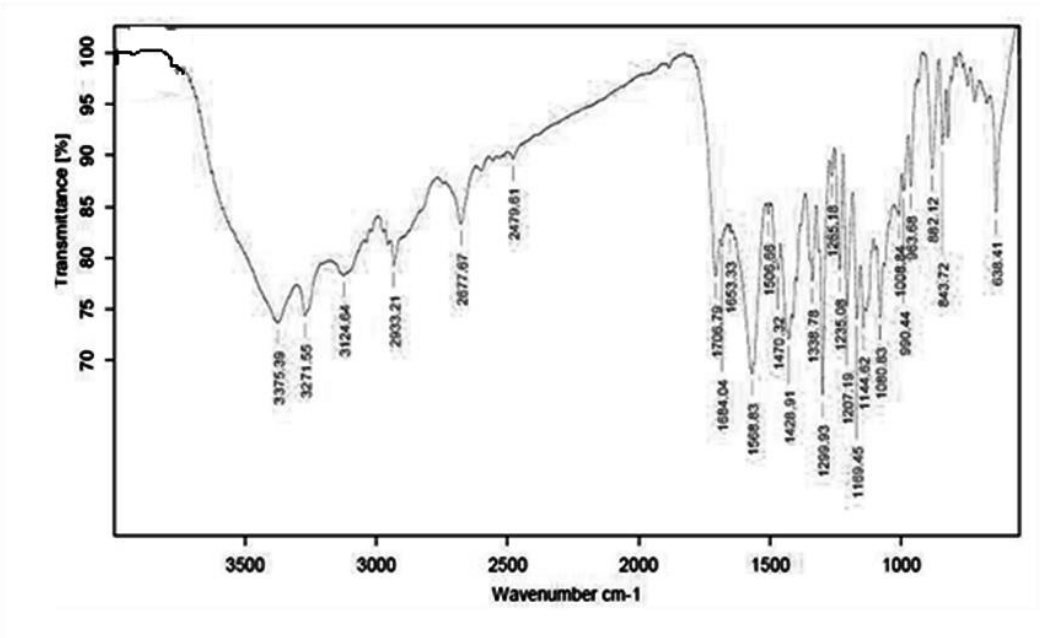

Fig.4: FTIR spectrum of Rizatriptan Benzoate+ Stearic acid

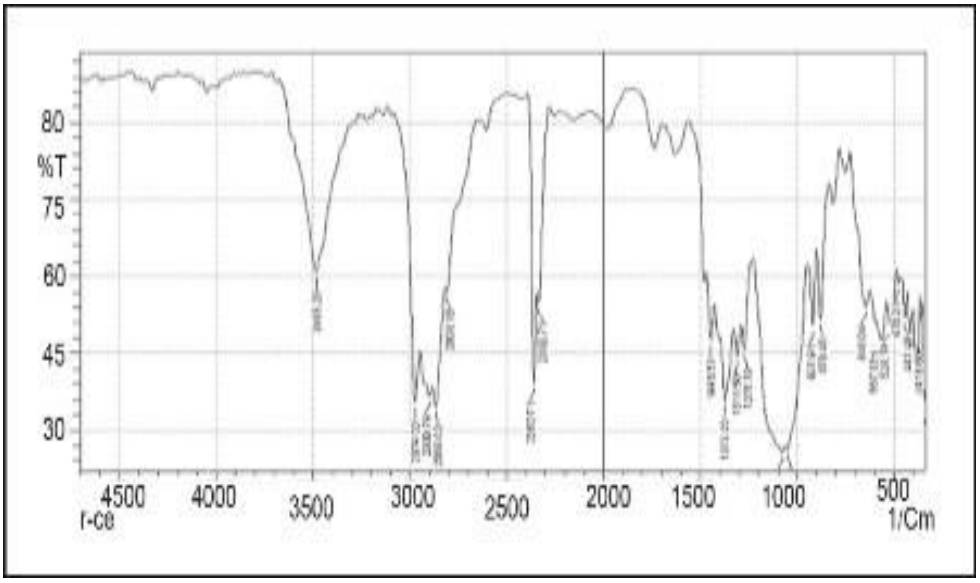

Fig.5: FTIR spectrum of Rizatriptan Benzoate +Polysorbate 80 & Span 80

Discussion FTIR Spectroscopy

The FTIR spectra of pure drug Rizatriptan Benzoate show design due to Nitrogen-Hydrogen extending at 3141.42/cm, Carbon=Oxygen extending at 1631.03/cm and C=Carbon stretching at 1451.0/cm. These assay were meeting the arrive values. The FTIR scope of optimized formulation F5 (Rizatriptan Benzoate +Material) reveal peak N-H stretching at 3214.70/cm, Carbon=Oxygen extending at 1634.05/cm, C=C stretching at 1546.09/cm.

Evaluation of Solid-lipid Nano-particles

Table.3: Solid-lipid Nano-particles Evaluation

Formulation	Particle size determination	Drug entrapment efficiency	Total drug content
F1	168.7 ± 1.6 nm	14.1%	78.63
F2	178.7 ± 1.3 nm	24.5%	72.56

F3	187.5 ± 2.1 nm	16.2%	88.34
F4	154.0 ± 1.2 nm	26.2%	80.22
F5	188.0 ± 1.4 nm	28.3%	85.60
F6	173.0 ± 1.6 nm	30.1%	90.87

Scanning Electron Microscopy (SEM)

SLNs with a narrow size distribution were prepared by modified solvent injection method using Gleceryl Monostearate as a solid lipid. The small and uniform sized SLNs can be prepared by this method without using any sophisticated instruments. The SEM study revealed that most of the SLNs were fairly spherical in shape, the surface of the particle showed a characteristic smoothness, and that the particle size was in the nano-metric range, as depicted in Fig.6.

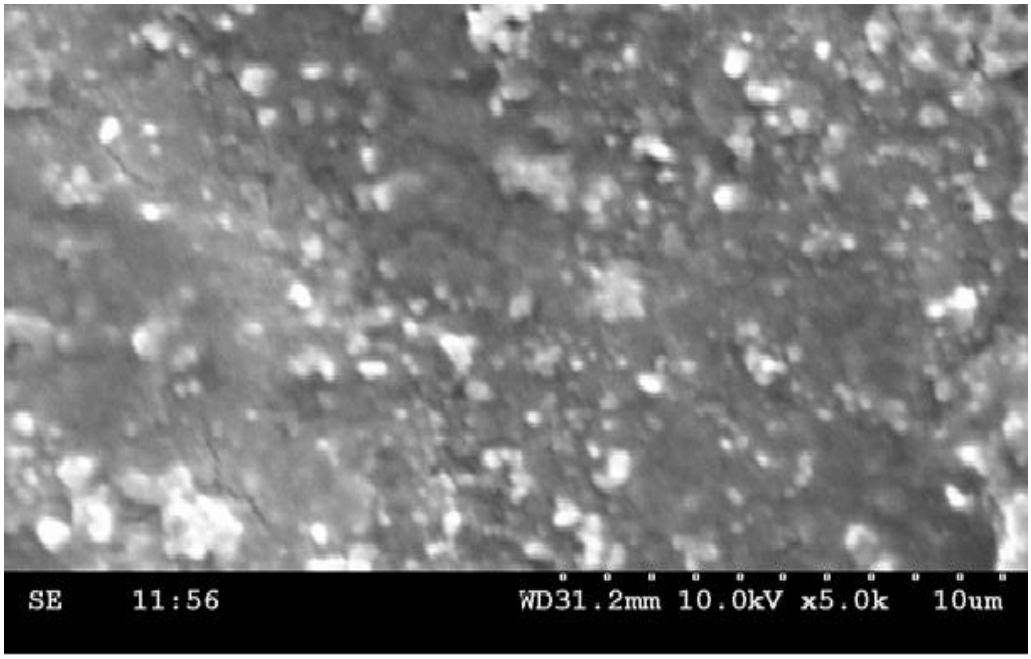

Fig.6: Scanning Electron Microscopy of SLNs

Differential scanning Calorimetry (DSC)

Fig.7 reveals the thermal behaviors of the pure components Rizatriptan, Gleceryl monostearate, Polysorbate-80 and Span-80, together with the thermal behaviour of the final SLNs prepared and the melting point peaks were Gleceryl Monostearate in table 2. The DSC curve of pure drug Rizatriptan benzoate exhibits a sharp endothermic peak at 1840C. The thermo grams of Gleceryl monostearate and Polysorbate-80 and Span-80, displayed broad endothermic peaks at 85 and 74.5℃. On the other hand, the SLNs Thermogram displayed complete disappearance of characteristic peak of Rizatriptan; a fact that the drug was molecularly dispersed within the lipid matrix. That was accompanied by the formation of a new endothermic peak at 126.5℃.

Table.4: DSC data of pure components, physical mixture and SLN formulation

S.No	Sample	Melting Peak
1	Rizatriptan benzoate	184
2	Gleceryl monostearate,	82
3	Polysorbate-80 & Span-80,	76.5
4	Rizatriptan benzoate Physical Mixture	74.5, 178.2
5	Rizatriptan benzoate SLN formulation	180.4

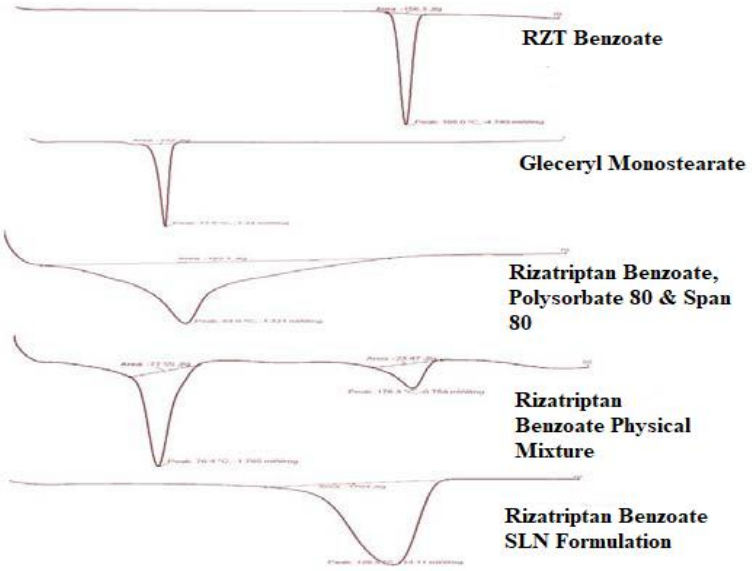

Fig.7: Overlaid DSC thermo-grams of SLN

In Vitro Drug Release Study

Table.5: In Vitro Drug Release Study of Nano-particles

Time	% Drug Release					
Formulation	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
1	19.90	17.21	20.90	19.34	18.24	16.78
2	35.76	32.24	30.52	34.65	32.66	31.78
3	46.72	49.68	47.90	47.12	47.56	46.54
4	53.34	53.42	53.54	50.32	57.94	50.65
5	66.78	68.82	62.75	69.65	68.52	67.74
6	76.23	76.64	79.67	76.89	78.81	75.76
8	87.74	85.92	84.87	84.22	90.50	85.81

In vitro drug release study were performed for all the formulations F1 to F6 which shows the sustained release of drug 87.74%, 85.92%, 84.87%, 84.22%, 90.50% and 85.81% respectively after 8h. The results are shown in Table.5.

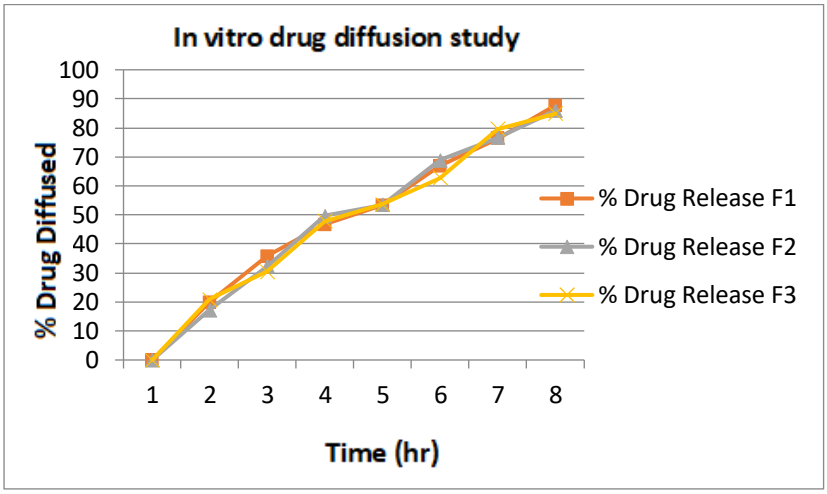

Fig.8: In Vitro Drug Release Study of Formulation F1-F3 Nano-particles

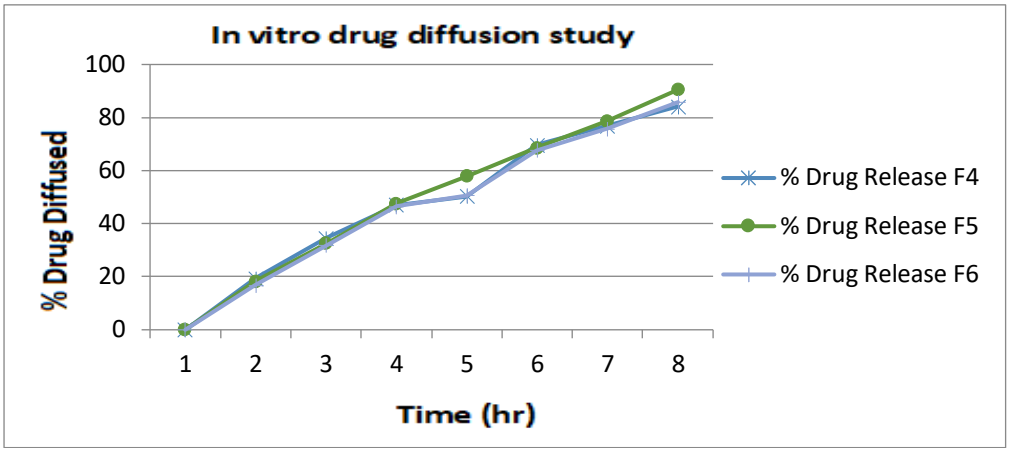

Fig.10: In Vitro Drug Release Study of Formulation F4-F6 Nano-particles

Drug Release Kinetics

The release kinetics was evaluated by fitting the data into first order, zero order, Higuchi, Peppas equations. The drug release kinetic data of different SLN formulations was depicted in table.6.

Fig.11: Zero-order release kinetics

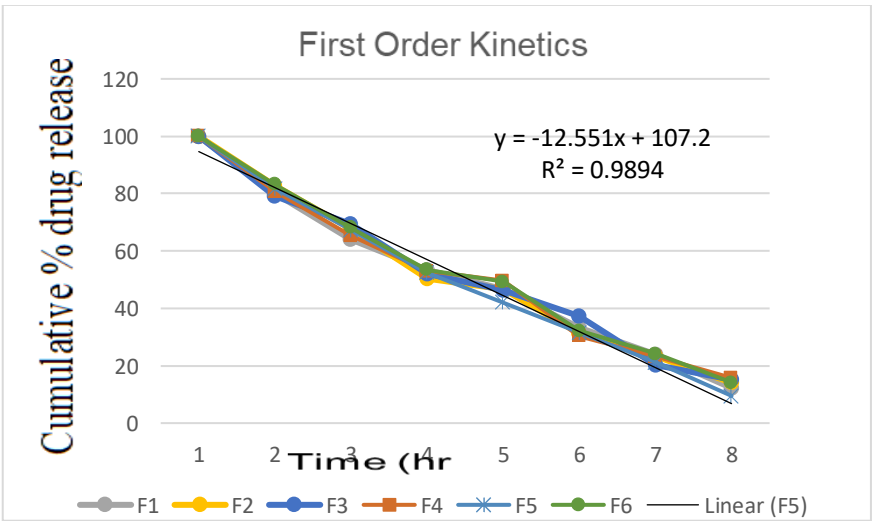

Fig.11: 1st-order release kinetics of Rizatriptan

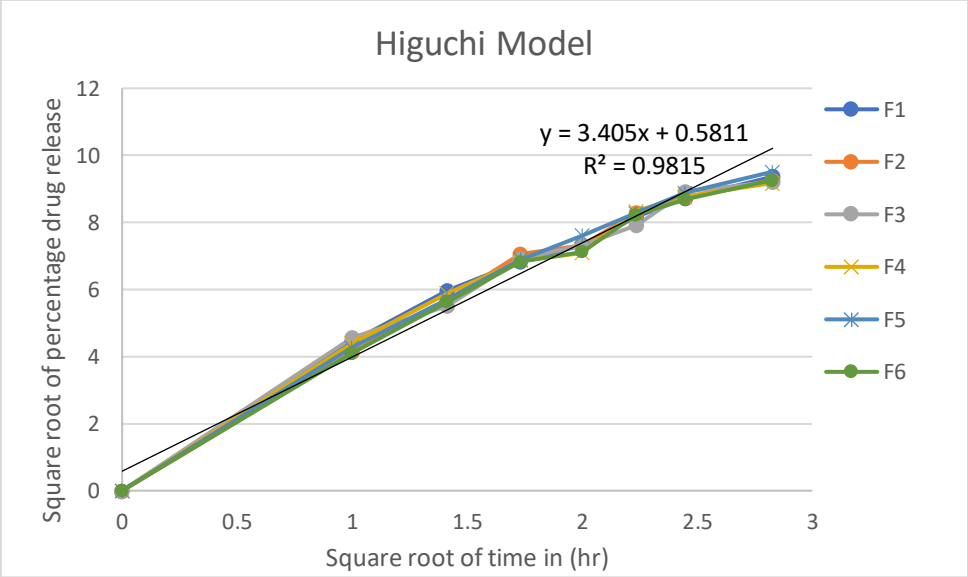

Fig.12: Higuchi model unleash kinetics of Rizatriptan

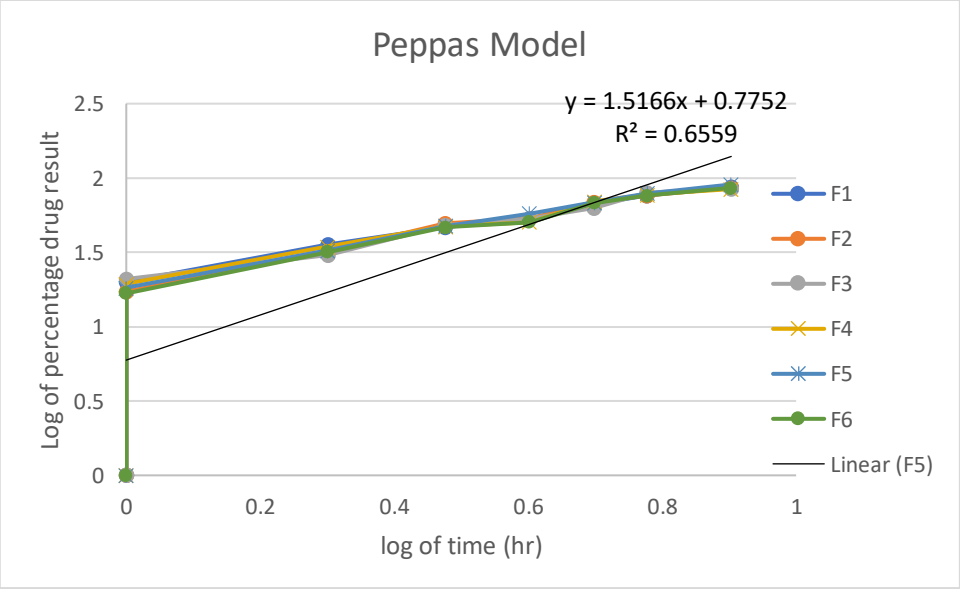

Fig.13: Peppas model **release kinetics of Rizatriptan**

Table.7: Release kinetics for all Nano-particles formulation

Formulation code	**Zero-order**	**1st-order**	**Higuchi**	**Peppas**
	r^2	r^2	r^2	r^2
F1	0.982	0.989	0.973	0.630
F2	0.979	0.980	0.975	0.645
F3	0.980	0.982	0.971	0.626
F4	0.973	0.973	0.968	0.630
F5	0.989	0.989	0.990	0.664
F6	0.985	0.985	0.981	0.665

Based on the results, the release of rizatriptan from SLNs best-fitted Higuchi equation and the possible mechanisms for the drug release might be diffusion of the drug from the matrix. From the results it was observed that, larger particles showed slower release compared to

smaller particles. This may be due to the larger surface area of smaller particles leading to faster drug release. In general the drug release from all formulation followed a steady pattern. The drug release may be mainly controlled by drug diffusion through the lipid matrix.

Stability Study

The stability studies of optimum formulation were carried out and the results state that there is no change in drug release and entrapment efficiency was observed over a period of 60 days. No significant change was observed at 40±2ºC, 75% RH hence formulation F5 prepared with Physical Mixture was found to be stable for 2 months.

Table.8: Stability study of Optimized Formulation F5

Stability (40 ±2ºC)	Percentage yield (%)	Entrapment Efficiency (%)	In vitro drug release (%)
Initial	90.87	30.1	90.50
15 days	90.75	30.45	90.46
30 days	90.64	30.78	90.40
45 days	90.49	30.45	90.35
60 days	90.30	30.60	90.33

CONCLUSION

Rizatriptan, a hydrophilic drug was successfully incorporated into SLNs by modified solvent injection method. The formulated Rizatriptan loaded SLNs were in nanometric range with spherical structure. The release of drug from SLNs best-fitted Higuchi equation and the possible mechanisms for the drug release might be diffusion of the drug from the matrix and matrix erosion resulting from degradation of lipids. This experiment confirmed the evidence that solvent injection technique was a simple, available and effective method. The in- vitro drug release of F5 formulation was found to be 90.50% over 8 hours in controlled manners, hence the present study was a successful attempt to formulation of Rizatriptan by SLN system. The main purpose of formulating this formulation has been based on the enhancement of absorption of the drug in the systemic circulation. Based on the findings, the release of Rizatriptan from SLNs is best described by the Higuchi equation, and one plausible mechanism for drug release is diffusion from the matrix. The results indicated that bigger particles released more slowly than smaller ones. This might be owing to the increased surface area of smaller particles, resulting in quicker drug release. In general, medication release from all formulations followed a consistent pattern. Drug diffusion across the lipid matrix may be the primary mechanism controlling drug release. Overall, the findings suggest that Rizatriptan Benzoate SLNs hold promise as a viable therapeutic option for epilepsy, offering improved drug delivery characteristics and potentially enhancing patient compliance and treatment outcomes. Further study is necessary to investigate the exact mechanism related to findings of the study. The stability studies of optimum formulation were carried out and the results state that there is no change in drug release and entrapment efficiency was observed over a period of 60 days. No significant change was observed at 40±2ºC, 75% RH hence formulation F5 prepared with Physical Mixture was found to be stable for 2 months.

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