ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Formulation And Evaluation of Rilpivirine Loaded Solid Lipid Nanoparticles.

Yogish D Garhwani[1]*, and Smita S Pimple[2].

[1]Student, Department of Pharmaceutics, PES's Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India.
[2]Professor, Department of Pharmaceutics, PES's Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India.

ABSTRACT

Rilpivirine is an Anti-HIV drug which has very low aqueous solubility. In this study we aimed to develop Rilpivirine loaded solid lipid nanoparticles (SLN) to enhance the bioavailability of the drug. The formulations were optimised and checked for various evaluation parameters like mean particle size, zeta potential, PI, FTIR, in-vitro drug release, SEM, PXRD & TGA. F4 was the optimised batch which showed very good particle size and was a very stable formulation. Solid lipid Nanoparticles protect the encapsulated drug and increases its stability, moreover it is good method to increase solubility of drugs that are lipid soluble.

Keywords: Solid Lipid Nanoparticles, Rilpivirine, Solubility, Optimization.

https://doi.org/10.33887/rjpbcs/2024.15.1.9

Corresponding author

ISSN: 0975-8585

INTRODUCTION

HIV stands for Human immuno deficiency virus which weakens body immune system. Hiv is a deadly disease which has no cure however some drugs can prolong the life of the patient ex:- Rilpivirine hydrochloride. Drug has broad activity against both wild type HIV and has a good pharmacokinetic profile, it's absorption depends on GIT pH, dose of drug is 25 mg/day [1-3].

Solid Lipid Nanoparticles act as colloidal vehicles and have emerged as nanocarriers having excellent protective attributes. SLN play important role in encapsulating the drug and protect it from degradation. They consist of lipid core made from solid lipids. SLN widely focuses on delivery of poorly water-soluble drugs [4-5].

In recent times applications of SLN have been expanded and are used in stabilizing emulsions [1].

Many pharmaceutical compounds are facing the problem of insufficient bioavailability [2]. Nowadays challenging task is also to prepare formulation which have controlled drug release over a specific period of time. SLN which are colloidal carriers made up of high melting point lipids, they have coating of aqueous surfactant layer [2].

Colloidal systems act as very good alternative drug delivery systems for enhancement of bioavailability of drug. They have several advantages over other drug delivery systems. Various methods are employed in preparation of solid lipid nanoparticles such as solvent evaporation & solvent emulsification method, etc [3].

For oral route, SLN specifically focuses on uptake of drug using lymphatic system which prevents the route of first pass effect. SLN production uses various excipients like fatty acids, triglycerides, phospholipids. Solid Lipid nanoparticles have benefits like protection of drug from degradation, good tolerability, no toxic metabolite produced in the body, biocompatibility, improving solubility of lipid soluble drugs and drugs having low aqueous solubility [5].

SLN have a site-specific targeting and have are having excellent physical stability. Also SLN as compared to emulsion formulations have better sustained release action due to drug immobility within lipids [6]. Solid lipid nanoparticles which include benefits of both polymeric nanoparticles and liposomes. Also they have long shelf stability. SLN are made up of biodegradable lipid base.

They are comprised of small particles ranging in size from 100-200 nm size range. They are cleared from blood by RES system in liver and spleen. SLN can be used for variety of routes of administration which includes parenteral, dermal, ocular, etc [7].

A great advantage of SLN over polymeric nanoparticles is that they reduce the risk of acute and chronic toxicity as they are composed of tolerated lipid components [8].

SLN are colloidal particles typically composed of the following constituents:

Lipids: These are the primary building blocks of SLNs and are solid at room temperature. Lipids can be sourced from natural fats or synthetic sources. Common lipids used in SLNs include stearic acid, glyceryl behenate and cetyl palmitate.

Emulsifiers or **Surfactants:** Emulsifiers or surfactants are essential for stabilizing SLNs and preventing their aggregation. They lower the interfacial tension between the lipid and the aqueous phase, allowing for the formation of nanosized particles. Common emulsifiers include lecithin, poloxamer.

Water: SLNs are often prepared in an aqueous medium, where water serves as the continuous phase. This water content can vary depending on the formulation and intended application [9].

Solvent evaporation technique [10]

Here's a basic overview of the process:

- **Selection of Lipid:** Choose a biocompatible lipid, often a solid at room temperature, as the core material for your SLNs.
- **Selection of Surfactant:** Select a surfactant or combination of surfactants to stabilize the SLNs and prevent aggregation.
- **Dissolution:** Dissolve the lipid and surfactant(s) in a volatile organic solvent, typically something like chloroform or dichloromethane. This forms a clear solution.
- **Emulsification:** Inject this solution into an aqueous phase (usually distilled water or an aqueous buffer) while stirring vigorously. This forms an oil-in-water emulsion.
- **Solvent Evaporation:** Allow the organic solvent to evaporate over time. This can be done under reduced pressure, with gentle stirring, or by other means, depending on the desired SLN characteristics.
- **Nanoparticle Formation:** The molecules of surfactant and lipid will come together to form SLNs as the solvent evaporates. A number of variables, including temperature, surfactant concentration, and emulsification technique, affect the size of the SLNs. [10].

MATERIALS AND METHODS

All the materials used were of analytical grade & are given in table 1.

Experimental Work

Method of Preparation

Solvent Evaporation Method

- It basically consists of two phases: Lipid phase and aqueous phase.
- In lipid phase: Compritol 1gm and Precirol 2gm (lipids) are dissolved in 10-20ml of chloroform and and drug 10mg is dissolved and kept for magnetic stirring.
- Aqueous phase represents poloxamer F-127 (5mg) dissolved in distilled water (25ml). Aqueous phase is added slowly using syringe to the lipid phase. Assembly was kept for magnetic stirring for 1 hour.
- **Evaporation:** Allow the solvent to evaporate slowly, which will cause the nanoparticles to form as the polymer or active compound precipitates out of the solution.
- **Nanoparticle Collection:** Once the solvent has completely evaporated, you'll be left with nanoparticles suspended in the aqueous phase.
- **Purification:** Often, additional steps such as centrifugation or filtration are required to separate and purify the nanoparticles [10-11]

Formulation and Optimization of Solid Lipid Nanoparticles

Optimization was done using DOE software tool using 2^3 factorial design (Given in Table 2).

Total 8 batches were prepared under this optimization process. From this F4 batch was found to be optimized batch

Evaluation of Optimized Batch

Particle Size Analysis

It was carried out by particle size analyser for all prepared batches. Particle size analysis plays important role in formulations. SZ-100 HORIBA Particle size analyser was used to obtain result regarding particle size and to measure particle size & PDI of solid lipid nanoparticles. All samples were diluted with distilled water and made upto till they reached a suitable concentration. The Z average value and PDI were determined. The SLN were diluted with distilled water (1:100) & measurement was done [12-15].

Polydispersity Index

A Polydispersity Index (PDI) value of > 0.7 indicates a broad particle size distribution in the formulation, while a value of 0.01 (mono dispersed particles) to 0.5-0.7 indicates a narrow distribution. One of the most important aspects of nanoparticle performance is the size and distribution of the particles. A crucial part of formulation stability is played by PI. The SZ-100 Horiba was the analytical instrument used. Using distilled water, a 1 ml sample was diluted to make 10 ml, then placed in a cuvette for analysis [16–20].

Zeta Potential

Measuring the zeta potential of solid lipid nanoparticles (SLNs) is important for assessing their stability and potential for aggregation. Same instrument (SZ-100 Horiba) used for analysis. The samples diluted with water in a ratio of 1:100 before measurement [21-23].

FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) gives idea about the vibration in chemical bonds so by yielding an IR (infra-red) spectrum. FTIR analysis was done by using KBr. Take KBr and sample in ratio of 3:1 in mortar and pestle and mix it thoroughly by the help of spatula; take this mixture in cuvette & run FTIR instrument. JASCO software was used for analysis [24-27].

% Entrapment Efficiency

To determine whether a nanocarrier is effective in retaining the drug or active ingredient and delivering a sufficient quantity of the component to the intended site, entrapment efficiency, or EE, is measured. % EE is determined by using the following formula

$$\% \text{ Drug Entrapped} = \frac{\text{Amount of free drug} \quad x100}{\text{Total amount of drug loaded}}$$

% entrapment efficiency of batch was done by taking 5-10ml equivalent to 2mg of drug of sample was subjected for centrifugation for 40 min at 30,000 rpm at 4^0C. The percentage of incorporated Rilpivirine HCL was determined spectrophotometrically. After centrifugation, amount of free drug was detected in supernatant and the amount of incorporated drug was determined by calculating initial drug minus free drug [28-31].

SEM Analysis

Scanning Electron Microscopy (SEM) is a powerful technique for analyzing Solid Lipid Nanoparticles (SLN). SEM allows for high-resolution imaging of the surface morphology and structure of SLN. Also it gives deep insights of size structure and surface morphology of prepared nanocarriers. 10-20 ml sample (liquid) of F4 Batch subjected to centrifugation and sediment was collected. Sediment taken into filter paper for drying and then it is lyophilised to get dried SLN. Dried SLN was sent for SEM analysis. It was done using JEOL SEM analyser. Surface morphology of prepared drug loaded Solid lipid nanoparticles was studied using SEM (Zeis EVO KS10). Samples were fixed on stubs using double side carbon tape. At argon atmosphere, at 20mA for 120 seconds [32-33].

In-Vitro Drug Release

Using a cellophane dialysis membrane bag, the in-vitro drug release study was conducted. Within a cellophane membrane bag, a 5 ml sample of the prepared SLN solution was taken. A 12-hour soak in phosphate buffer (pH 7.4) was done before applying cellophane membrane. A beaker full of buffer (pH 7.4) was used to dip a cellophane membrane after tying it at both ends. At 37 ± 1^0C, a sample of solid lipid nanoparticles was used for the diffusion study. The study used 50 millilitres of phosphate buffer. Each time, a 1 ml sample was taken out, diluted to a 10 ml mark, replaced with a fresh batch of the same buffer, and exposed to UV analysis. To keep the sink conditions, 1 ml of new buffer solution was added each time. Removed 1ml sample diluted upto the mark and is subjected to UV analysis. All the samples were UV Analysed and in-vitro drug release data was compared [34-37].

XRD Analysis

XRD analysis is a valuable tool in characterizing nanoparticles for various applications, such as in materials science and nanotechnology, as it provides essential information about their structural properties. XRD analysis helps to determine crystallinity of sample helps to determine structural properties of prepared nano formulations [38].

RESULTS AND DISCUSSION

Particle Size

Particle size analysis was done for all the batches & particle size of the optimized batch was found to be 271nm and pi=0.5 respectively (Given in Figure 1).

PDI

Polydispersity index (PDI) is a measure of the size distribution of nanoparticles. In the context of nanoparticles, PDI quantifies the variation in particle sizes within a sample. A low PDI value (typically close to 0) indicates a narrow size distribution, meaning most particles in the sample have similar sizes. Conversely, a high PDI value (closer to 1) suggests a broad size distribution, with particles of varying sizes. PI of the formulation was found to be 0.5.

Zeta Potential

It was found to be -29.6mV indicating stable formulation (Given in Figure 2).

FTIR

FTIR analysis was done, drug (Rilpivirine HCl) and lipid Compritol and Precirol were found to be compatible with each other. Formation of SLN were confirmed by FTIR analysis.

% Entrapment Efficiency (EE)

% entrapment efficiency of solid lipid nanoparticles was checked. By altering ratio of Compritol and Precirol, formulations were prepared.

Calculations

$$Y = mx+c$$
$$Y = 0.159x+0.069$$

After all calculations, % EE was found to be 92 %

In-vitro Drug Release Studies

The in-vitro release profile was done for the optimized batch. Release of Rilpivirine from pure drug solution was faster. SLN formulation maintained sustained release of drug. Release kinetics was better. In-vitro drug release was conducted till 180 minutes (3 hour) starting from 15min, 30min, 60min, 120min, 150min, 180min in like wise manner (Given in Table 3 & Figure 3). It was measured as % CDR.

SEM

SEM analysis for the optimized batch was done from SPPU, Pune (Physics department) using instrument JEOL JSM 6360A Analytical SEM. SEM images clearly indicate Rilpivirine is loaded in the solid lipid matrix i.e. SLN formulation (Given in Figure 4 & 5).

ISSN: 0975-8585

XRD

XRD of the Rilpivirine loaded solid lipid nanoparticles was done (Given in Figure 6). The diffraction pattern of sample showed characteristic pointed peaks with 2Ø indicating crystalline nature of sample.

TGA Analysis

TGA analysis was done for pure drug and prepared SLN formulation of the drug (Given in Figure 7 & 8). From the results obtained from graph reveals that there is good effiicient drug loading (80-90%).

Table 1: Formulation Ingredients

Sr No	Ingredients	Source
1	Rilpivirine HCl	Laurus labs
2	Poloxamer F127	Aadhunik industries
3	Compritol 888 ATO	Gattefose
4	Precirol ATO 5	Gattefose
5	Methanol	Merck life sciences Pvt ltd
6	Chloroform	Merck life sciences Pvt ltd
7	Potassium dihydrogen phosphate	Merck specialities ltd

Table 2: Optimization Table

Run	Factor 1 Compritol (gm)	Factor 2 Precirol (gm)	Factor 3 Sonnication time (Minutes)
1	2	2	1
2	0.5	2	3
3	0.5	0.5	3
4	0.5	0.5	1
5	2	2	3
6	2	0.5	3
7	2	0.5	1
8	0.5	2	1

Table 3: In-vitro Dissolution Study

Sr No	Time (minutes)	% Drug Release
1	15	8.42
2	30	12.30
3	60	18.69
4	120	36.82
5	150	64.53
6	180	88.9

ISSN: 0975-8585

Figure 1: Particle Size of Optimized F4 batch

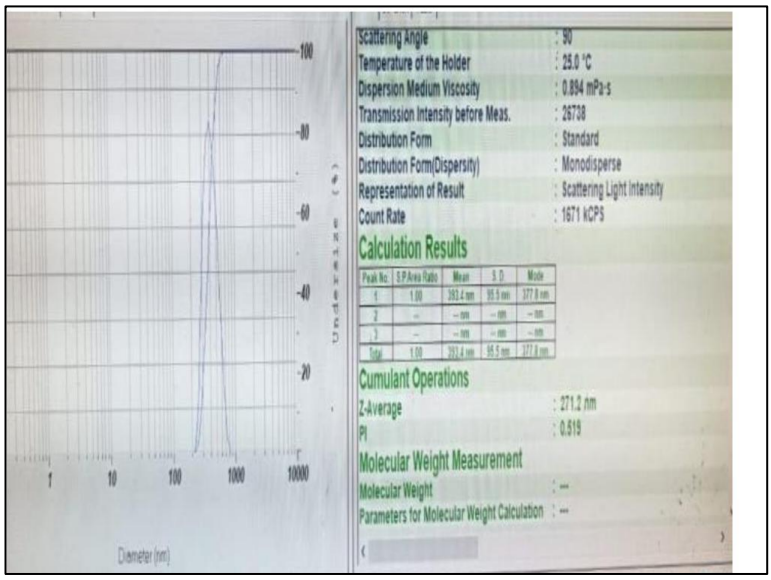


Figure 2: Zeta Potential of Optimized F4 batch

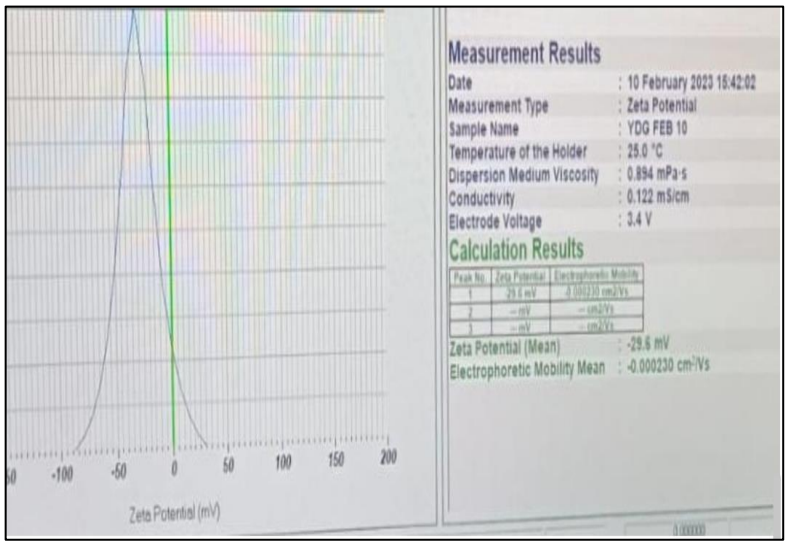
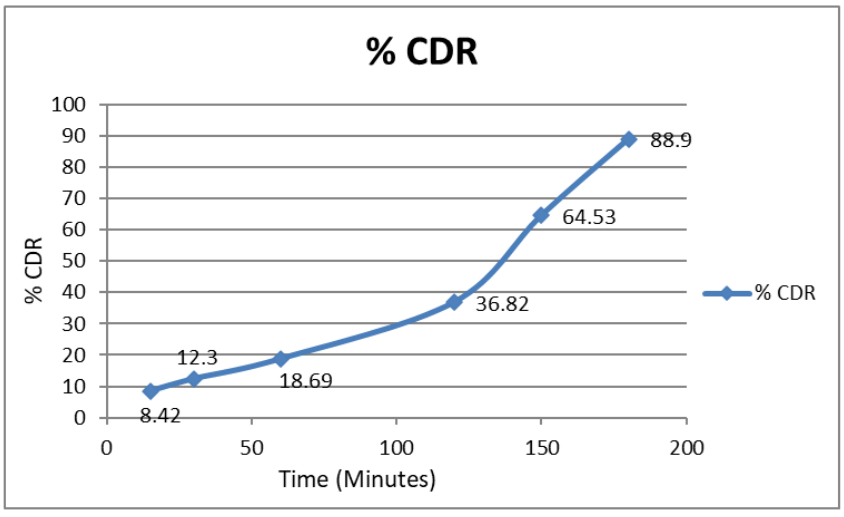


Figure 3: % CDR of SLN loaded drug



ISSN: 0975-8585

Figure 4: SEM image (I)

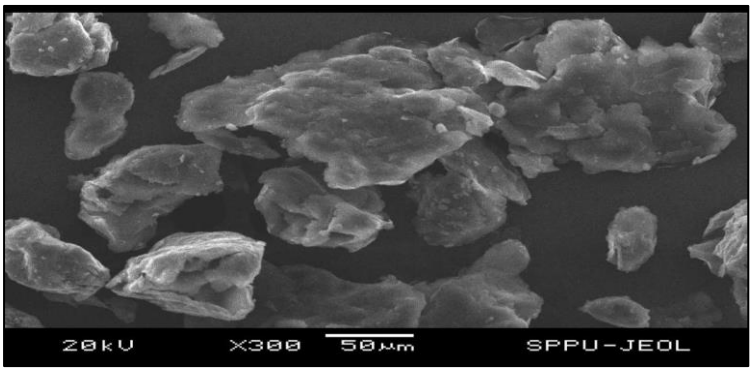


Figure 5: SEM photograph (II)

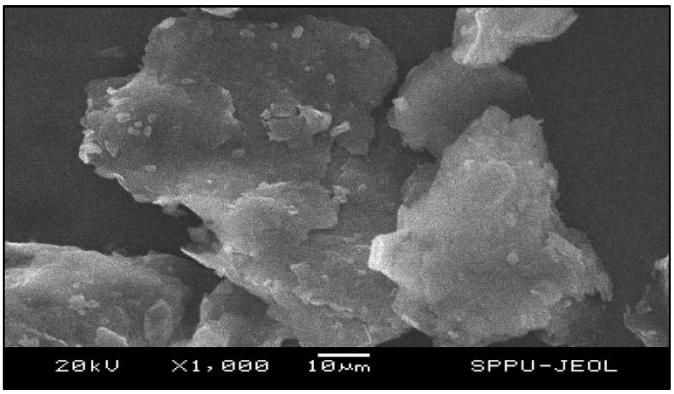
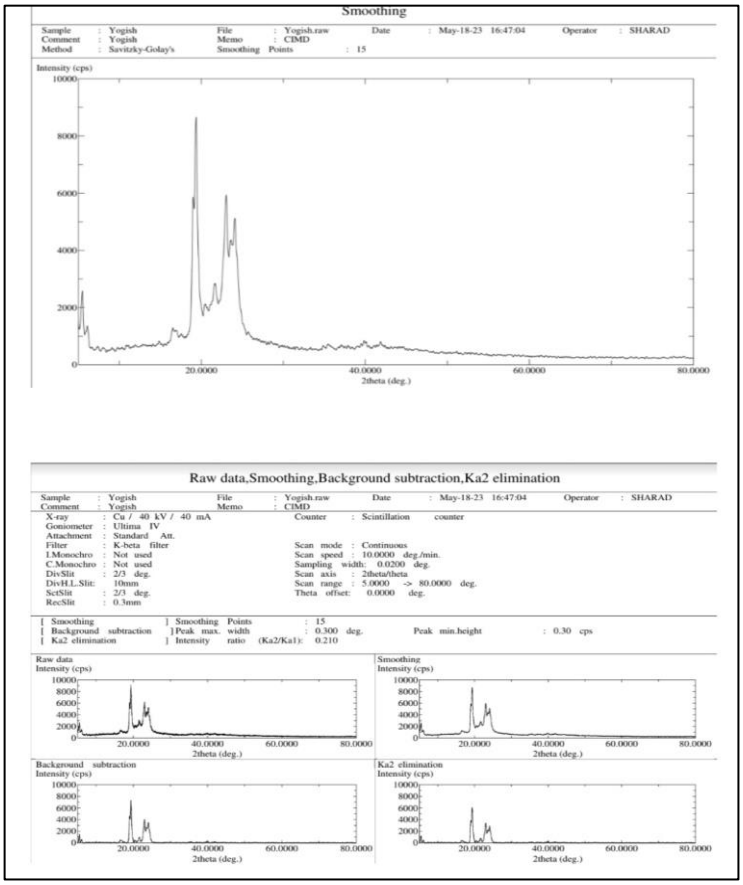


Figure 6: XRD pattern of F4 batch



ISSN: 0975-8585

Figure 7: TGA of Pure Drug

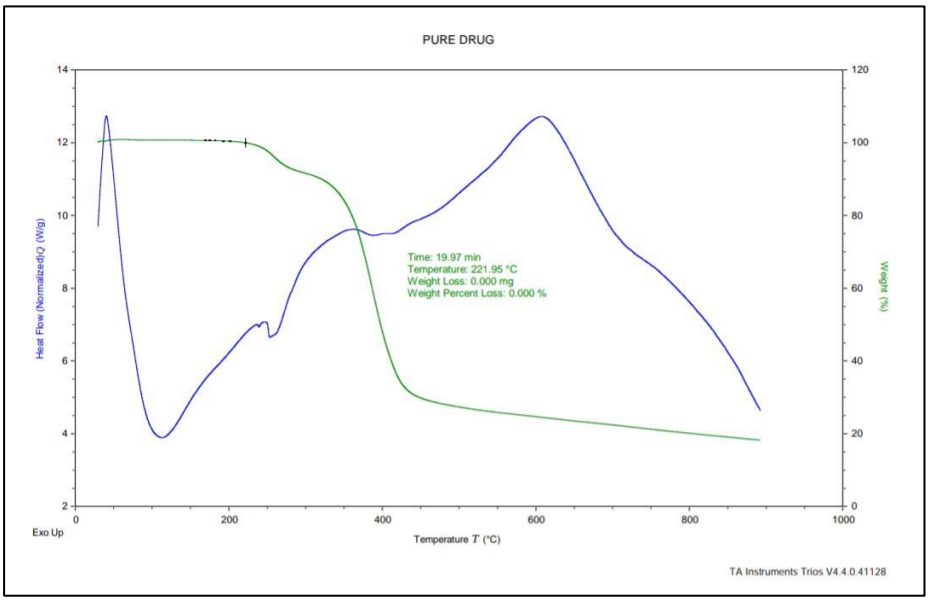
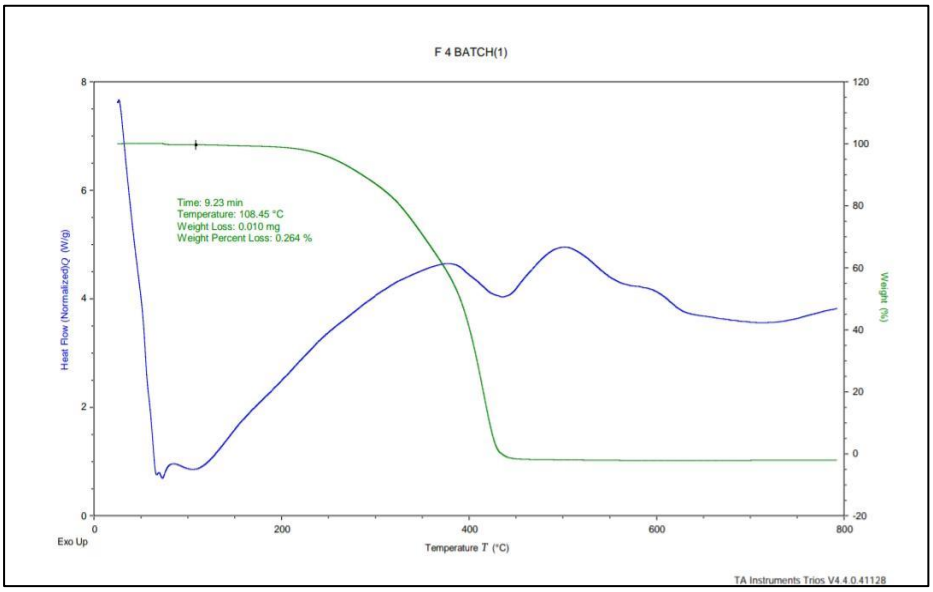


Figure 8: TGA of F4 batch



CONCLUSION

In the present study, Rilpivirine loaded solid lipid nanoparticles were prepared by solvent evaporation method and was evaluated for its various characteristic properties. Formulation was evaluated for its particle size, zeta potential, PI, in-vitro drug release, SEM, XRD, TGA, etc. SLN showed sustained release of drug upto 3 hours as compared to conventional.

ACKNOWLEDGEMENTS

The facilities and tools needed to complete this work were provided by PES's Modern College of Pharmacy, Nigdi, Pune, for which the authors are grateful.

REFERENCES

[1] S Mukherjee, S Ray and RS Thakur. Ind J Pharm Sci 2009; 349-358.

[2] MR Mozafari. 41-50 (2006).
[3] Rainer H Muller, Karsten Mader and Sven Gohla. Eur J Pharm. Biopharm 2000;50(1):161-177.
[4] Wolfgang Mehnart and Karsten Mader. Adv Drug Deliv Rev 2001;47: 165-196.
[5] Houli Li, Xiaobin Zhao, Yukun Ma and Guangxi Zhai, Ling Bing Li and Hong Xiang, Lou. J Cont Release 2009;133: 238-244.
[6] Melike Uner, Gulgun Yener. Int J Nanomedicine 2007;2(3): 289-300.
[7] Annette Zur Mehlen, Cora Schwarz and Wolfgang Mehnart. Eur J Pharm Biopharm 1998; 45: 149-155.
[8] Elena Ugazia, Roberta Cavalli and MR Gasco. Int J Pharm 2002; 241: 341-344.
[9] Indu Pal Kaur, Rohit Bhandari, Swati Bhandari and Kakkur. J Cont Rel 2008;127: 97-109.
[10] Ghada Abdelbary and Rania H Fahmy. AAPS Pharm Sci Tech 2009; 10(1).
[11] N Al-Haj and A. Rasedee, Int J Pharmacol 2009; 5(1): 90-93.
[12] Dong Zhi Hou, Chang Sheng Xie, Kaijn Huang and Chang Hong Zhu. Biomaterials 2003;24: 1781-1785.
[13] Alessandro Bargoni, Roberto Cavalla, Otto Caputo and M. R Gasco. Pharm Res 1998;15(5): 745-750.
[14] Milan Stuchlík and Stanislav Žák. Biomed Papers 2001; 145(2): 17-26.
[15] C Olbrich and RH Muller. Int J Pharm 1999; 180: 31-39.
[16] D Schwarz, W Mehnert, JS Lucks and RH Muller. J Cont Release 1994;30: 83-96.
[17] Wei Liu, Meling Hu, Wehsuang Liu and Chengbin Xue, Huibi Xu. Int J Pharm 2008; 364: 141-146.
[18] Qing Zhi Lu, Aihua Yu, Yanwei Xi and Houli Li, Zhimei Song, Jing Cui and Fengliang Cao, Guangxi Zhai. Int J Pharm 2009; 372: 191–198.
[19] Yi Fan Luo, DaWei Chen, Li Xiang Ren and Xiu Li Zhao, Jing Qin. J Cont Release 2006;114: 53–59.
[20] Paliwal, Shivani Rai, Bhuvaneshwar Vaidya, Kapil Khatri, Amit K. Goyal, Neeraj Mishra, Abhinav Mehta and Suresh P. Vyas. Nanomedicine, Nanotechnology, Biology and Medicine 2009;5(2):184-191.
[21] Zhenghong Xu, Lingli Chen, Wangwen Gu and Yu Gao, Liping Lin, Zhiwen Zhang and Yong Xi, Yaping Li. Biomaterials 2009; 30:226.
[22] Rathapon Asasutjarit, Sven – Iver Lorenzen, Sunee Sirivichayakul and Kiat Ruxrungtham, Uracha Ruktanonchi and Garnpimol C. Ritthidej. Pharm Res 2007; 24(6): 1098–1107.
[23] Carsten Rudolph, Ulrike Schillinger, Aurora Ortiz and Kerstin Tabatt, Christian Plank, Rainer H. Müller and Joseph Rosenecker. Pharm Research 2004; 21(9): 1662-1669.
[24] Robhash Kusam Subedia, Keon Wook Kanga and HooKyun Choi. Eur J Pharm Sci 2009; 37(3-4): 508-513.
[25] Suresh Gande, Kopparam Manjunath, Vobalaboina Venkateswarlu and Vemula Satyanarayana, AAPS Pharm Sci Tech 2007; 8(1):Article 24.
[26] Nagi A Alhaj, Rasedee Abdullah, Siddig Ibrahim and Ahmed Bustamenn. Amer J Pharmacology And Toxicology 2008;3(3): 219–224.
[27] Michael D Triplett, E James, F Rathman. J Nanopart Res 2009; 11: 601–614.
[28] Yung-Chih Kuo and Hung-Hao Chen. Int J Pharm 2009;365: 206-213.
[29] K Vivek, Harivardhan Reddy and Ramachandra SR Murthy. AAPS Pharm Sci Tech 2007;8(4).
[30] S Mukherjee, Subhabrata Ray and RS Thakur. Pak J Pharm Sci 2009; 22(2): 131-138.
[31] EQ Hu, H Yuan, HH Zhang and M Fang. Int J Pharm 2002;239: 121-128.
[32] Niladi Chattopadhyay, Jason Zastre, Ho-Lun Wong and Xiao Yu Wu, Reina Bendayan. Pharm. Research 2008;25(10).
[33] Katja Jores, Annekathrin Haberland, Siegfried Wartewig and Karsten Mader, Wolfgang Mehnart. Pharm Res 2005;22(11): 1887-1879.
[34] Bin Lua, Su-Bin Xionga, Hong Yanga and Xiao-Dong Yina, Ruo- Bing Chaoa. Eur J Pharmaceutical Sci 2006;28(1-2): 86-95.
[35] Meyer E Heinzelmann and Wiesendanger R. Springer Verlogg 1992; 99-149.
[36] Pallavi V Pople and Kamalinder K Singh. AAPS Pharm Sci Tech 2006; 7(4): Article 91.
[37] Lang Sc, Lu L. F, Cai Y and Zhu J. B, Liang BW and Yang CZ. J Controlled Release 1999;59: 299-307.
[38] Biswajit Basu, Kevin Garala, Ravi Bhalodia and Bhavik Joshi, Kuldeep Mehta. J Pharm Res 2010; 3(1): 84-92.