Archives of Pharmaceutical Sciences and Research
Vol 13 Issue 1 250 - 256 June 2023
Research Article

Development and *In-vitro* Evaluation of Solid dispersion of Resveratrol

Gulnar Tabassum*[1], Mr. Subhash PG[1], Dr. A Geethalakshmi[1], Pavan Kumar[1]

Department of Pharmaceutics, R.R college of Pharmacy, Bangalore-560090, Karnataka

ABSTRACT

The present study was aimed to enhance and evaluate the dissolution rate of Resveratrol (RES) by solid dispersion technique using HP β-Cyclodextrin as a carrier. In the study SD'scontaining RES was prepared applying four different method namely (Physical mixture, Co-precipitation method, Co-evaporation method, kneading method) including HP β-cyclodextrin as a carrier with varied ratios of drug and carrier: 1:2, 1:5, 1:8. Preformulationstudies like determination of calibration curve, lambda maximum, melting point, solubilitystudies in various solvents, ionization study and drug-polymer compatibility study were carried out using FTIR technique. Post formulation studies were carried out like weight variation test, drug content, lock length, moisture permeation test, a *in-vitro* dissolution studies, stability studies.

The results of all preformulation studies were within the specification references. FTIR studies were revealed that there was no interaction between drugand carrier. Drug content, weight variation and pKa tests were found to be within the IP limits. Lock length and moisture permeation test were passed. The poor solubility of RES lead to poor dissolution. Based on the *in-vitro* drug dissolution profiles, formulation "K1" having 1:2 ratio (Drug: Carrier), prepaid by Kneading method using HP β-Cyclodextrin as carrier shows better release i.e., 84.06% compared to other methods.

Keywords: Resveratrol, Solid dispersions, *in-vitro* drug dissolution, solubility.

INTRODUCTION

Absorption of drug and its therapeutic effectiveness will affected by solubility which is a significant physicochemical factor. Poor aqueous solubility can leads to failure in formulation development process. The main reason behind inadequate bioavailability of drug is its low dissolution rate and low solubility in aqueous medium.[1]

The release of drugs is a critical and limiting step in drug bioavailability, especially for drugswith low gastrointestinal solubility and high permeability. It is possible to improve these drugs' bioavailability and side effects by improving their drug release profile.[2]

Solid dispersions are one of the most successful strategies to improve drug release of poorly water-soluble drugs. The term SD has been utilized to describe a family of dosage forms, whereby the drug is dispersed in a biologically inert matrix, usually to enhance the oral bioavailability.

Resveratrol (trans-3,5,4′-tri-hydroxic-stilbene) is a stillbenic structure polyphenol, isolated from the natural plants. Resveratrol became popular in 1992 when it was suggested that it is having cardio-protective effects, and its popularity increased in 1997 when it was proven that resveratrol was able to prevent colorectal cancer in mice. Resveratrol based compounds present anti-oxidant, anti-inflammatory, anti-viral, cardio-protective, neuro-protective, anti-cancer and anti-angiogenetic activities.[3]

In the present study we investigated that the solid dispersion of resveratrol on HP β-CD increases its solubility and bioavailability indicating that enhance biological properties of resveratrol. The purpose of this research work was to develop and in-vitro evaluation of soid dispersion of Resveratrol using HPβ-CD by different method (Microwave oven method, Kneading method, Co-precipitation method, Co-evaporation method) to improve the solubility, dissolution rate as well as bioavailabilty of the drug Resveratrol.

MATERIALS AND METHODS

Material

Resveratrol was collected as gift sample from Sami Labs Limited,Peenya Industrial Area, Bengaluru, HP β-Cyclodextrin collected from Yarrow Chem Pvt ltd, Mumbai, All chemicals and solvents used were of analytical grade.

Methods

Preformulation Studies

Solubility: The solubility study was carried out by using three different solvents alone and in combination i.e., Water, Ethanol and Phosphate buffers using UV spectrometry

method. [4]

Melting Point

Fine resveratrol powder was placed in a glass capillary tube (pre-sealed at one end), attached to a thermometer with a rubber band, and immersed in a Thiel tube containing liquid paraffin. Heating has started. Melting temperature was determined.

Estimation of Resveratrol for absorption maxima

A known amount of RES was accurately weighed into 100 ml dissolved in 100 ml ethanol and labeled as SS-I. Pipette 10 mL of solution from SS-I, dilute to 100 mL with ethanol in a100 mL volumetric flask**,** and label SS-II. Standards SS-II were drawn at 0.1, 0.2, 0.3, 0.4,

and 0.5 mL and made up to 10 mL with ethanol to give concentrations of 1, 2, 3, 4, and 5 µg/ml. The absorbance of the prepared solutions was measured using a UV-Visible spectrophotometer at a wavelength of 305.9 nm. Absorbance was recorded.

Drug polymer compatibility study by FTIR

FTIR spectra using KBr, pure drug and the selected polymer HP β-CD **are** coarsely ground and ground with a mortar and pestle. The blank is first run in with KBr, then the milled sample is compressed with dry powder into a disc, placed in a holder and scanned between 4000 and 400cm^{-1}. Each peak is documented and compared to FTIR of the pure drug.

Determination of ionization constant

5mg of drug dilute to 100ml with ethanol, pipette out 10ml from above solution in conical flask and titrate against 0.5N NaoH using methyl red indicator to complete neutralization andburette reading is noted, simillarly 10ml of 50%w/v drug solution is pipetted and titrated against NaoH to 50% neutralization point then the pH is recorded by using pH meter.

Method of preparation of solid dispersion of Resveratrol

In order to optimize drug to carrier ratio Microwave oven method, Kneading method, Co-precipitation and Co-evaporation method of drug with HP β-CD in different ratios were prepared. [5,11,12]

Microwave oven

A predetermined volume of a physical mixture was placed in a glass beaker and microwaved in an oven for five minutes at a power setting of 600W. At any one time, only one beaker was correctly placed into the microwave. To obtain consistent particle size, solid dispersions were then crushed in a glass mortar and put through a 100 mesh sieve. Then, each hard gelatin capsule containing the solid dispersion was filled (size 2). [6]

Kneading method

The kneading mix with HP β-CD was made in the same ratio as the microwave oven. Drugs and excipients were first mixed by grinding with a pestle and mortar. Small amounts of ethanol and water were added to this mixture to form agglomerates. Here, the aggregates were passed through a no. 22 sieve with an opening of 841 µm to obtain granules. These granules were dried in an oven at 60°C for 20 minutes. For further study**,** each ratio of granules was filled into hard gelatin capsules **(**size 2).

Co-Precipitation method

The co-precipitation process with HPβ-CD was performed in the same ratio as microwave oven. First, HPβ-CD was dissolved in 50 mL water and maintained at 75 °C with a magnetic stirrer. RES was dissolved in 10 mL of ethanol. This was added dropwise to an aqueous solution of HPβ-CD. Stirring was continued at 75° C. for up to 1 hour. The solution was gradually cooled to room temperature. The resulting precipitate was filtered,dried, sieved (60#) and stored in a desiccator at 25±2 °C. Each ratio of granules was filled into hard gelatin capsules (size 2).

Co-evaporation method

The co-evaporation process with HPβ-CD was done in the same ratio as microwave oven.A known amount of RES and HPβ-CD was added to 50 mL of 50% ethanol in water to obtain a suspension. The suspension was saved for sonication to obtain a clear solution. The solution was evaporated at 70° C. with a

magnetic stirrer**.** The resulting complexes were dried, sieved (60#) and stored in a desiccator at 25±2 °C. Each ratio of granules wasfilled into hard gelatin capsules (size 2).

EVALUATION OF PREPARED CAPSULES

Weight variation test

To conduct the test, 20 units were individually randomly weighed to determine the average weight. Not more than two individual weights differ from the average weight by more than the percentage deviation specified in the table below, and no more than twice this percentage. [7,8]

Drug content

Approximately 5 mg of drug-equivalent solid dispersion prepared by various methods was accurately weighed and transferred to a volumetric flask (50 mL) containing a few drops of ethanol, the flask was shaken for 15 min, and the final pH was determined using pH. I made the capacity up to the marked line. 7.4 Phosphate buffer. Samples were filtered through whattman filter paper, then diluted to desired levels and analyzed. for drug content by spectrophotometry at a wavelength of 305.9.

Lock Length

It was tested by using Vernier calipers. This is an important check which assuresthe capsules are properly pressed and locking system is intact.

Moisture permeation test

To perform this test, the capsules are packaged in a unit container along with dehydrated pellets that have the property of changing color in the presence of moisture. The packaged capsules are then placed in an atmosphere of known humidityfor a specified period of time. A color change in the dry pellet indicates water absorption. The weight of this test capsule is then compared to the weight of the tested capsule. The difference in weight indicates the amount of water absorbed.

In-Vitro dissolution studies

The dissolution test was carried out in USP apparatus (rotating paddle method). The samples were placed in a hard gelatin capsules. 900 ml of phosphate buffer pH 7.4 was used as dissolution media at 37 ± 0.5°C and maintaining stirring speed at 50 rpm. The aliquots were withdrawn at 30, 60, 90,120,150and 180 mins interval. Freshvolume of the dissolution medium phosphate buffer pH7.4 was replaced with the withdrawn volume to maintain the sink conditions. Samples withdrawn were analyzed for the percentage of drug released at 305.9nm.

Stability studies

Accelerated stability studies of the prepared solid dispersions were performed at 40°C/75% relative humidity for up to 3 months. Accurately weighed amounts of samples were placed in aluminum-coated capped glass vials and stored in a microprocessor-controlled humidity chamber. Samples were taken at 30, 60, and 90days and analyzed for drug content and in vitro dissolution testing and compared to standard deviations of these tested immediately after preparation. [9]

RESULTS AND DISCUSSION

The melting point of Resveratrol was determined by the Thiels tube method. The melting point was found to be 254.6^{o}C. *FT-IR* of the Resveratrol was determined by FTIR spectra as mentioned. Drug-polymer Compatibility studies were carried out by *FT-IR* Spectroscopy to establish theany possible interaction of excipients with the drug in the formulation. The *FT-IR* Spectrum of drug alone as well as combination of drug with excipient were obtained and analyzed for compatibility. *FT-IR* Spectra of drug Resveratrol and the physical mixture of drug and carrier were given in table. Pure drug showed principal absorption peaks at 3267.21cm^{-1} (OH-Str), 1628.23cm^{-1} (C-O), 955.88cm^{-1} (Olefinic bond) The *FT-IR* Study showed that there is no interaction between drug and excipients because it shows the characteristic peak of drug andexcipients. pKa value of RES was found to be 9.03 and itis meeting the standard as specified in official limits.

All the capsules exhibited uniform weight and

there was no much deviation in the weight of any formulation. Among various batches the uniformity of weightindicates that the drug is well dispersed in the capsules. The results of % weight variation wasfound to be in between (2.128 – 4.377%) were shown in the table 2.

Drug content estimation was done and the absorbance was measured by UV Spectrophotometer. Drug content of the developed formulations from M1 to CE3 lies in the range of 60.86-111.4% were given in the table 2. Drug content of formulation CP1 was foundto be higher than other formulations.

The lock length of Resveratrol capsule was determined by the vernier caliper method. The lock length was found to be in the range 17.605-17.672mm. Lock length of formulation CE2 was found to be higher than other formulation. The difference in the moisture permeation may be due to the increase in concentration of hydrophilic polymers and difference in resistance of matrix network structure to the movementof water molecule through the formulation. The moisture permeation was found to be decreased in formulations with the decrease in content of HP β-CD. The values obtained for all the formulations was found to be in between 1.74-4.19%were given in the above table.

In-vitro dissolution studies were carried out for all the prepared formulations from M1 to CE3 and the result of release profiles for all the formulations were tabulated in table 3. Among all theformulations, formulation K1& CE1 in the ratio 1:2 (drug: HPβ-CD), showed a better drug release over 3hr**.**

Formulation code	Solid dispersion	Drug & carrier ratio	Method employed
M1	RES : HP β-CD	1:2	Microwave oven
M2	RES : HP β-CD	1:5	Microwave oven
M3	RES : HP β-CD	1:8	Microwave oven
K1	RES : HP β-CD	1:2	Kneading method
K2	RES : HP β-CD	1:5	Kneading method
K3	RES : HP β-CD	1:8	Kneading method
CP1	RES : HP β-CD	1:2	Co- Precipitation
CP2	RES : HP β-CD	1:5	Co- Precipitation
CP3	RES : HP β-CD	1:8	Co- Precipitation
CE1	RES : HP β-CD	1:2	Co-evaporation
CE2	RES : HP β-CD	1:5	Co-evaporation
CE3	RES : HP β-CD	1:8	Co-evaporation

Table :1 List of Formulations from M1 to CE3

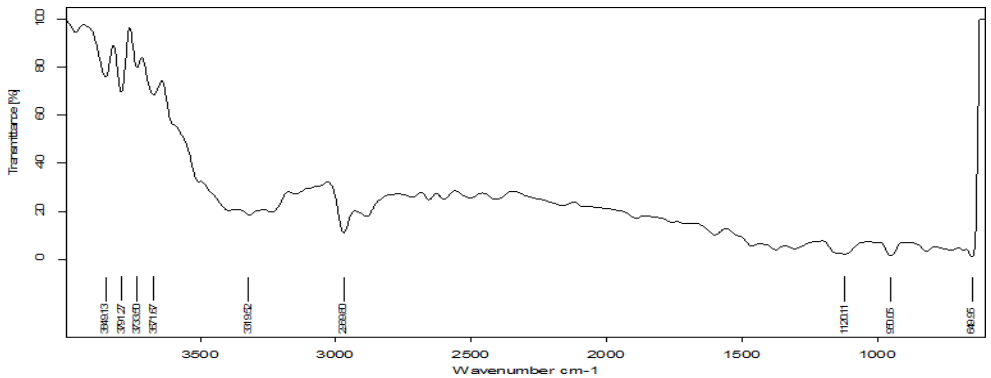


Fig 1: *FT-IR* Spectrum of drug Resveratrol

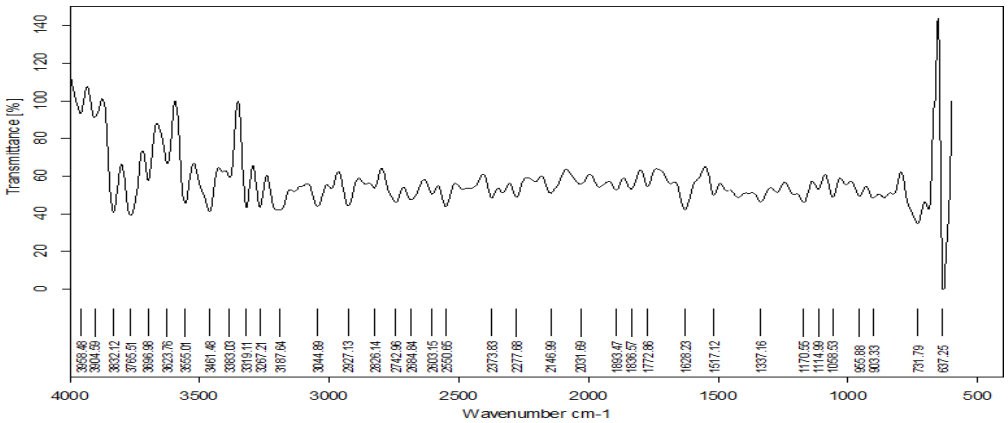


Fig 2: *FT-IR* Spectrum of RES +HP β – CD

Solvent	Absorbance	Concentration (µg/ml)	Description
Water	0.876	39.81	Practically insoluble
Water + Ethanol (Ratios) 5:5	1.291	56.68	Slightly soluble
3:7	0.988	44.90	Slightly soluble
7:3	0.7808	35.49	Slightly soluble
1:9	1.6210	73.68	Soluble
9:1	0.669	30.409	Very slightly soluble
Phosphate Buffer 6.8	1.366	62.090	Slightly soluble
7.2	1.568	71.21	Sparingly soluble
7.4	1.599	72.68	Soluble

Table :2 Solubility Studies

Formulation code	PARAMETERS			
	%Weight Variation	Drug content(mg)	Lock length(mm)	%Moisture permeation
M1	2.754%	65.57	17.653	2.99%
M2	3.030%	60.86	17.605	3.77%
M3	4.191%	73.92	17.622	4.19%
K1	2.790%	86.72	17.611	1.74%
K2	3.636%	88.33	17.627	1.85%
K3	2.808%	84.03	17.632	2.38%
CP1	4.377%	111.4	17.613	3.55%
CP2	2.753%	96.99	17.633	2.29%
CP3	2.128%	97.78	17.664	2.35%
CE1	2.576%	88.22±0.299	17.663	1.84%
CE2	2.926%	84.24±0.732	17.672	1.82%
CE3	3.636%	89.82±0.195	17.624	1.851%

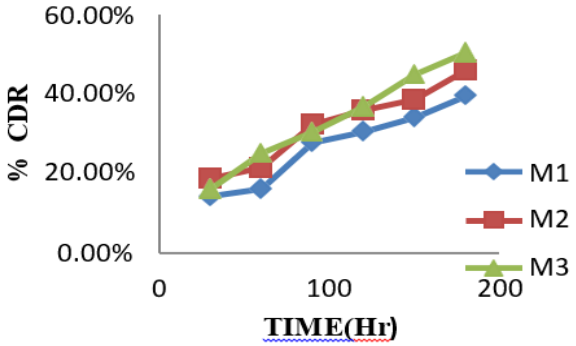
Table :3 Physicochemical Evaluations

Time (Min)	%CDR											
	M1	M2	M3	K1	K2	K3	CP1	CP2	CP3	CE1	CE2	CE3
30	14.41	18.92	16.23	23.26	18.02	15.32	21.62	20.72	19.82	19.82	20.72	18.02
60	16.23	21.64	25.25	31.56	20.74	18.94	27.93	27.05	24.35	28.85	29.75	25.25
90	27.96	32.41	30.68	52.32	33.38	30.67	36.09	33.39	30.68	48.71	46.91	39.69
120	30.69	36.12	37.02	64.09	46.93	39.72	47.85	40.64	37.92	63.18	56.87	45.65
150	34.33	38.86	45.17	70.46	68.61	59.58	53.31	49.69	49.68	74.06	67.75	60.51
180	39.78	46.20	50.63	84.06	78.59	74.06	65.08	61.45	59.65	82.25	76.83	72.29

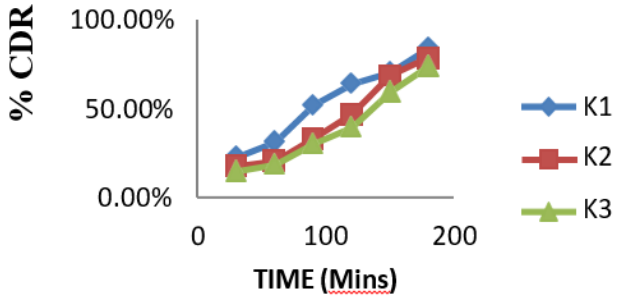
Table No 4: *In-vitro* Drug release studies from M1-CE3

Retest time	K1 formulation		CE1 formulation	
	%Drug content	%CDR	%Drug content	%CDR
0days	86.72	84.06	88.22	82.25
30 days	86.66	83.96	88.06	82.19
60 days	86.05	83.88	87.97	82.11
90days	85.91	83.83	87.83	81.96

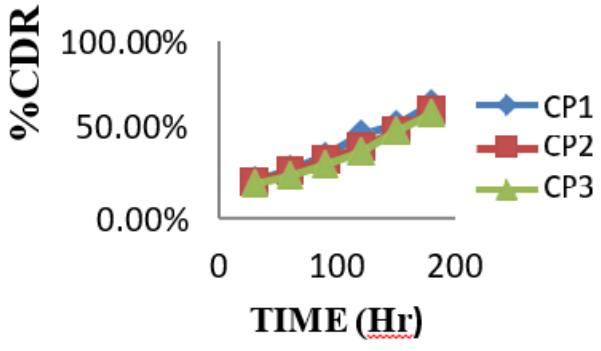
Table No 5: Stability Study Details



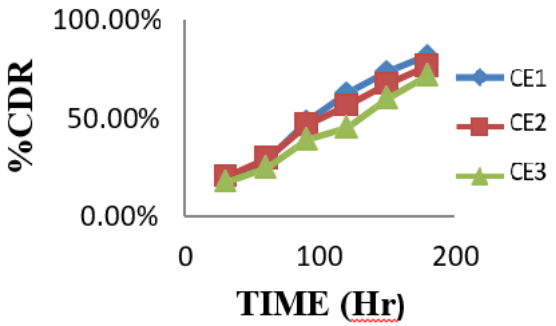
Comparative *in-vitro* dissolution profile of M1, M2 & M3



Comparative *in-vitro* dissolution profile of K1, K2 & K3



Comparative *in-vitro* dissolution profile of CP1, CP2 & CP3



Comparative *in-vitro* dissolution profile of CE1, CE2 & CE3

Stability studies of K1 and CE1 formulations were conducted according to ICH guidelines up to 3 months at 40°C/75% RH. Among all selected formulations, K1 and CE1 showed satisfactory results. There were no significant differences in physicochemical parameters and in vitro drug release profiles. Therefore, K1 and CE1 formulations are stable and retain their original properties with minor differences.

CONCLUSION

The purpose of this study was to improve the dissolution rate of the sparingly soluble drug RES by solid dispersion method. Pre-formulation studies in various solvents were determinedand drug-polymer compatibility studies were performed. Post-prescription studies were determined and were within standard limits. The λmax obtained was found to be 305.9 nm. The melting point of the drug was 254.6°C. The solubility profile of the drug was best seen in1:9 ratios of solvent mixtures of water and ethanol. In this study, the solubility and stabilityof RES in different aqueous solutions with different pH values were evaluated. FT-IR studiesshowed no interaction between the drug and polymer, as the main drug peak was unchanged in the spectra obtained for solid dispersion mixtures of drug and carrier. Drug content estimates were made for formulations M1 through CE3, with a maximum drug content of 111.4 mg anda minimum of 60.86 mg. Evaluation and dissolution characteristics were performed on all solid dispersion batches. Among all formulations, the 'K1' and 'CE1' formulations showed superiordrug release over 3 hours. According to ICH guidelines, he conducted a 90-day stability studyof K1 and CE1 formulations and no significant changes in physicochemical parameters were observed, thus confirming their stability. From these results, it was concluded that the K1 & CE1 formulations are stable and retain their original properties with minor differences. HPβ- CD as carrier 1:2 proved to be the most promising dosage form for SD. From the above experimental data, we can conclude that the SD technique is excellent for improving the solubility and resolution of RES. In the future, solid oral dosage forms (tablets, capsules) of RES can be formulated using his prepared solid dispersions of RES and HPβ-CD.

REFERENCE

1. Tekade AR, Yadav JN. A review on solid dispersion and carriers used therein for solubility enhancement of poorly water soluble drugs. Adv Pharma Bull 2020 Jul;10(3):359
2. Ahire BR, Rane BR, Bakliwal SR, Pawar SP. Solubility enhancement of poorly water soluble drug by solid dispersion echniques. Int J Pharm Tech Res 2010;2:2007-2015.
3. Spogli R, Bastianini M, Ragonese F, Iannitti RG, Monarca L, Bastioli F, Nakashidze I, Brecchia
4. G, Menchetti L, Codini M, Arcuri C. Solid dispersion of resveratrol supported on magnesium dihydroxide (Resv@ MDH) microparticles improves oral bioavailability. Nutrients. 2018 Dec 5;10(12):1925.
5. Stieger N, Liebenberg W, Wessels JC. UV spectrophotometric method for the identification and solubility determination of nevirapine. Die Pharmazie. 2009 Oct 1;64(10):690-1.
6. Pinakin P, Narendra KP, Sachin KS et al. Formulation and characterization of ternary complex of poorly soluble duloxetine hydrochloride. J App Pharm Sci 2015;5(06):2231- 3354.
7. Zawar L, Bari S. Microwave induced solid dispersion as a novel technique for enhancing dissolution rate of repaglinide. Adv Pharmacol Pharm 2013;1:95-101.
8. Vaibhav G, Vithaldas A, Shivarkar D. Solubilty enhancement of poorly water soluble drug by using solid dispersion technique. Int J Pharm 2013;2(4):91-96.
9. Tiwari S et al. Pamam dendrimer based luliconazole gel formulation and efficiency evalutions. Eur J Mole Clini Med 2020;7(8):4881-4594.
10. Sharma A, Jain CP. Preparation and characterization of solid dispersions of carvedilol with -PVP K30. Research in pharmaceutical sciences. 2010 Jan;5(1):49.
11. SirishaGubbala, A Geethalakshmi, ReshmaLamsa, L.Sambath.Co-solvent evaporation method for anti-HIV drug nevirapine to enhance solubility and dissolution rate. World journal of pharmacy and pharmaceutical sciences, 3(8), 2014, 972-981.
12. Anusha N Raj, A. Geethalakshmi. Enhancing the Solubility of Poorly Water Soluble Statins by Different Techniques, Formulation and Evaluation. IJPRS. V-4, I-2, 2015. pp 382-389.