Research Article

FORMULATION AND INVITRO EVALUATION OF NEVIRAPINE EXTENDED RELEASE MATRIX TABLETS

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ABSTRACT

Objective: Nevirapine (NVP) is a non nucleoside reverse transcriptase inhibitor widely used in combination with other antiretroviral agents for the treatment of human immunodeficiency virus disease. The present study was aimed to develop generic formulation of extended release (ER) tablets of Nevirapine anhydrous (NVP) using hydrophilic polymer.

Method: Nevirapine NVP(ER) tablets were prepared by different manufacturing technology i.e. direct compression, roller compaction, and wet granulation method by employing hydrophilic polymer (HPMC K15M).The matrix granules were prepared by mixing drug along with polymer and diluents in different polymer ratio from wet granulation technology used water as a granulating fluid.

Results: The prepared granules were evaluated for various physicochemical parameters by official procedure and compressed in tablets. The In-vitro release profile of various batches was prepared by different technologies and has been compared with the innovator product. In-vitro release profiles of NVP from ER tablets were determined using USP apparatus type II (Paddle), 50 rpm and bath temperature 37°C. Dissolution of tablets was carried out in 900 ml of media (phosphate buffer pH 6.8). Samples were withdrawn at predetermined time intervals up to 24 hrs and analyzed using UV detector at a wavelength of 247 nm.

Conclusion: Stress stability studies indicated that the formulation is stable and In-vitro release profile study showed that formulation using wet granulation technology.

Keywords: Nevirapine, Extended release and matrix tablet, human immunodeficiency virus, non nucleoside reverse transcriptase inhibitor, tolerability.

INTRODUCTION

Nevirapine (NVP), is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immune- deficiency virus type 1 (HIV-1), block polymerase activity after binding directly to the HIV-1 reverse transcriptase leading to disruption of the enzyme's catalytic site. Anti-retroviral therapy with NVP has demonstrated significant activity in HIV infected patients in combination drug with highly active antiretroviral therapy[1]. NNRTIs work by binding to and blocking HIV reverse transcriptase, an HIV enzyme. This prevents HIV from replicating and lowers the amount of HIV in the blood. Nevirapine does not cure HIV/AIDS. It is known to reduce the risk of passing HIV to other people. Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The activity of Nevirapine does not compete with template or nucleoside

triphosphates. The most common adverse effect of nevirapine is the development of mild or moderate rash (13%) 2-4.

Ethyl cellulose (EC) is a tasteless, free flowing; white or light tan colored powder which is widely used in oral and topical formulations. The main use of EC in oral formulations is as a hydrophobic coating agent for tablets and granules5. Ethyl cellulose coatings are used to modify the release of a drug, to mask unpleasant taste, or to improve stability of a formulation. Modified release tablet formulations may also be produced using ethyl cellulose as a matrix former. HPMC is an odorless and tasteless, white or creamy white colored fibrous or granular powder. It is pharmaceutically applied as a Coating agent, film-former, stabilizing agent, suspending agent, tablet binder, viscosity increasing agent. Microcrystalline cellulose which is used as a binder or diluents is purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles6. It is commercially available in different particle sizes and moisture grades that have different properties and applications. Magnesium stearate which is incompatible with strong acids, alkalis and iron salts is primarily used as a lubricant in capsule and tablet manufacture at concentrations between 0.25% and 5.0% w/w. It is also used in barrier creams7.

The term controlled or extended release implies a system that provides continuous delivery of the drug for a predetermined period with predictable and reproducible kinetics and a known mechanism of release. This means that the release of drug substance(s) from a controlled release drug delivery system proceeds at a rate that is not only predictable kinetically but also reproducible from one unit to another8. In other words, the system attempts to control drug concentration in the target tissue. The oral route of administration for extended release systems has received greater attention because of more flexibility in dosage form design. The design of oral extended release delivery systems is subjected to several interrelated variables of considerable importance such as type of delivery system, the disease being treated, the patient, the length of therapy and the properties of the drug. Extended release denotes that the system is able to provide some actual therapeutic control whether be it of temporal or spatial nature or both. In other words, the system attempts to provide a constant drug concentration in the target tissue. It is this nature of this system that makes it different from sustained release systems9. The present study was aimed at developing the formulation of extended release tablets of Nevirapine (NVP) using extended release polymer.

MATERIALS AND METHODS:

Materials Used: Nevirapine is obtained from Reddy's labs, India, Lactose used is procured from S.D. Fine chem. LTD, Mumbai, Microcrystalline Cellulose from FMC Biopolymers, Hyderabad. The polymers like HPMC K100 and HPMC K15 are obtained from Colorcon Asia Pvt. Ltd. Carbopol is procured from Dow Chemicals, Hyderabad, Chitosan from Drugs India, Hyderabad and Magnesium stearate from S.D. Fine chem. LTD, Mumbai.

Preformulation Study: The drug sample was evaluated for its colour and odour. The results are shown in Table 9. Melting point of the drug sample was determined by capillary method by using melting point apparatus. The reported and observed melting point is shown in Table 10. The solubility of the Nevirapine was determined by adding excess amount of drug in the solvent and equilibrium solubility was determined by taking supernatant and analyzing it on Perkin Elmer Lambda35, double beam spectrophotometer10. Fourier-transform infrared (FTIR) spectra of the Drug and polymer were obtained on Alpha Brooker FTIR (Tokyo, Japan). The spectra were scanned over the wave number range of 4200 to 500 cm-1.

Construction of Calibration Curve:

Standard Stock solution:

Accurately weighed 100 mg of Nevirapine was dissolved in 100 ml of 6.8 PH phosphate buffer. The resultant solutions were having concentration of 1000 µg/ml (1 mg/ml). 10 ml of this solution was further diluted up to 100.0 ml with 6.8 PH phosphate buffer and to give a solution of Concentrations 100 µg/ml. This resultant solution is used as working stock solution for further study. Further dilutions were prepared from the same solution.

Preparation of calibration curve for Nevirapine:

Appropriate aliquots were pipetted out from the standard stock solution in to a series of 10 ml volumetric flasks. The volume was made up to the mark with to 6.8 PH phosphate buffer get a set of solutions having the concentration range of 50, 75,100, 125, 150 µg/ml for Nevirapine. Absorbance

of the above solutions was measured at 247 nm and a calibration curve of absorbance against concentration was plotted and the drug follows the Beer's & Lambert's law in the concentration range of 50-100 µg/ml. The regression equation and correlation coefficient was determined.

Table 1: Calibration curve data for Nevirapine at 247 nm

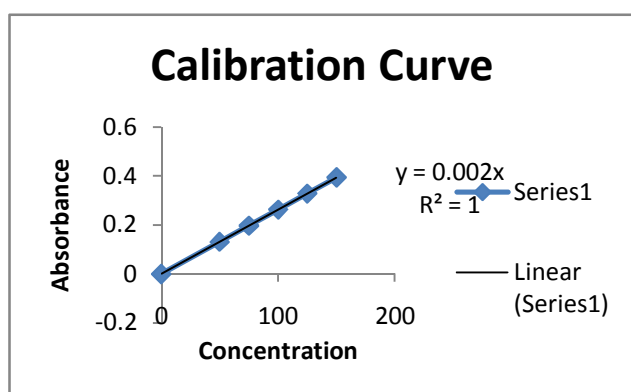| S. No.	Concentration (µg/ml)	Absorbance (247 nm) 6.8 pH phosphate buffer
1	0	0
2	50	0.1314
3	75	0.1971
4	100	0.2628
5	125	0.3285
6	150	0.3942

Fig no 1: Calibration Curve of Nevirapine in phosphate buffer 6.8 pH at 247 nm

Preparation of matrix tablets: Different Nevirapine formulations were prepared by Wet granulation technique (F-1 to F-8). Firstly Nevirapine pure drug, HPMC K15, MCC & Mg-Stearate was weighed accurately and shifted through 40# mesh. Wet granulation was done in Rapid Mixing Granulator (RMG). In RMG, Nevirapine pure drug, HPMC K15 & MCC were mixed and then the RMG was operated at a high speed. Then again sufficient Isopropyl Alcohol was added if necessary. Then lastly for two minutes the RMG operated on slow speed to form granules. The prepared granules were dried at 300C for 20min in presence of dehumidifier, and then it was sifted through sieve #20. In extra granulation step the polymer Magnesium stearate

(Diluent) was accurately weighed and shifted through 40# mesh and mix for 10min. In final Mixing step, in Double Cone Blender firstly dried granules of above mixture was added then lubricating agent magnesium stearate was mixed for 2 minutes11. Finally these granules are ready for compression. Then these granules are compressed by using 8mm punch and maintaining humidity below 50%RH. Different Nevirapine formulations were prepared by Wet Granulations technique (F-1 to F-8).

Evaluation of Matrix Tablets:

The formulated matrix tablets were evaluated for parameters like hardness, weight variation, thickness and friability12.

Content Uniformity Test:

Drug content:

For determination of drug content three tablets from each formulation were weighed individually, crushed and diluted to 100ml with sufficient amount of 6.8 PH Phosphate buffer. Then aliquot of the filtrate was diluted suitably and analyzed spectro photometrically at 247nm against blank.

In-vitro dissolution studies:

The in-vitro release of Nevirapine from formulated tablets was carried out for 24 hours in 6.8 PH Phosphate buffer. The studies were performed in USP dissolution apparatus II (Labindia DS 14000) at $37 \pm 0.5°$ C and 50 rpm speed. Samples were taken at 1, 4, 8, 16, 20 & 24 hours and diluted to suitable concentration and analyzed for Nevirapine content at 247.0 nm by using UV–visible spectrophotometer.

Drug release kinetics: Dissolution data of above two methods was fitted in Zero order, First order and Higuchi equations. The mechanism of drug release was determined by using Higuchi equation[13].

RESULTS AND DISCUSSION:

The selection of release retarding excipients is necessary to achieve a constant in- vivo input rate of the drug. The matrix tablets composed of drug and the release retarding materials offer simplest approach in designing an extended release system.   In this research, extended release tablet of Nevirapine were prepared using pharmaceutically acceptable and easily available inert excipients by wet granulation (Non aqueous granulation) technique14. HPMC (hypromellose) is a pH independent polymer, widely

Table no 2: Formulation series

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8
Nevirapine	100mg	100mg	100mg	100mg	100mg	100mg	100mg	100mg
Lactose	100mg	100mg	100mg	100mg	-	-	-	-
HPMC	65mg	80mg	40mg	-	-	-	-	-
HPMC K15	-	-	-	-	100mg	150mg	-	-
Ethyl cellulose	-	-	40mg	50mg	-	-	-	-
MCC	32mg	17mg	17mg	47mg	97mg	47mg	97mg	97mg
Mg.stearate	3mg	3mg	3mg	3mg	3mg	3mg	3mg	3mg
Carbopal	-	-	-	-	-	-	100mg	-
Chitosan	-	-	-	-	-	-	-	100mg

used for extended release dosage form and also Microcrystalline Cellulose, Ethyl Cellulose. Therefore HPMC was mainly used in this study. The procured drug sample of Nevirapine was tested for its identification by means of organoleptic properties, melting point, UV spectra and FTIR spectrum. The solubility of the Nevirapine was determined and found to be practically insoluble in water, sparingly to slightly soluble in dichloromethane R, slightly soluble in methanol. Infra-red spectroscopy analysis was performed by Fourier Transformation Infrared Spectrophotometer[15]. Alpha Brooker FTIR (Tokyo, Japan).The instrument was calibrated by using polystyrene film. Drug sample showed wavelength of maximum absorption (λ max) 247 nm.

Table no 3: Results of identification tests of drug

S.No	Parameter	Drug
1	Color	White or colorless
2	Odour	Odourless
3	Taste	Unpleasant
4	Appearence	Crystalline powder

Table no 4: Results of Melting point determination test of drug

Reported Melting Point	Observed melting point
196.06	196.00

All the formulations were evaluated for bulk density, tapped density, % compressibility, hausner's ratio and angle of repose[16-18]. The results of % compressibility, hausner's ratio and angle of repose were found to be <9, <1.1 and <30 respectively. These results show that the formulations have very good flow properties. The tablets were evaluated for weight variation, thickness, hardness, friability, swelling index, floating lag time, floating duration, drug content and in- vitro drug release study. All the formulations passed the evaluation tests and showed comparable satisfactory results. The thickness of all tablets was found to be in the range of 3.11-3.19 mm and hardness was found to be in the range of 3.3-3.9 kg/cm2 in all the formulations, the MCC and HPMC together showed good binding properties. In all the formulations, the %friability was (0.22-0.75) below 1% as per USP. The average weight was found to be 300-309 mg which will be within the given limits. Hence all the tablets were found to show less weight variation. The drug content of all formulations ranged from 99% to 100%, which is within the specified IP limits.

Dissolution Data of Matrix tablets formulations of Nevirapine by paddle method (USP II) are reported in Table no 8. The in- vitro drug release of tablets has shown a profound effect on the choosing of the type of polymers and their concentrations. Initially, the drug release using HPMC K15M and MCC were compared to see how effectively they could retard the drug release[19, 20]. From the %CDR data of all the formulations, the polymer HPMC K15M showed desirable drug release. Hence HPMC was chosen to make the final

Table no 5: Compatibility studies of Nevirapine with different excipients for one month

| Ingredients	Ratio	Initial Color	After one week 40 °C / 75%RH	After two weeks 40 °C / 75%RH	After three weeks 40 °C / 75%RH	After four weeks 40 °C / 75%RH
Nevirapine	-	White powder	White powder	White powder	White powder	White powder
Nevirapine + HPMC 100	1:1	White powder	White powder	White powder	White powder	White powder
Nevirapine + HPMC K15	1:1	White powder	White powder	White powder	White powder	White powder
Nevirapine + Ethyl Cellulose	1:1	White - yellow powder	White - yellow powder	White - yellow powder	White - yellow powder	White - yellow powder
Nevirapine + Lactose	1:1	White powder	White powder	White powder	White powder	White powder
Nevirapine + Micro Crystalline Cellulose	1:1	White powder	White powder	White powder	White powder	White powder
Nevirapine + Mg. Stearate	1:1	White powder	White powder	White powder	White powder	White powder
Nevirapine + Carbopol	1:1	White powder	White powder	White powder	White powder	White powder
Nevirapine + chitosan	1:1	White powder	White powder	White powder	White powder	White powder

Fig no 2: FTIR of Nevirapine
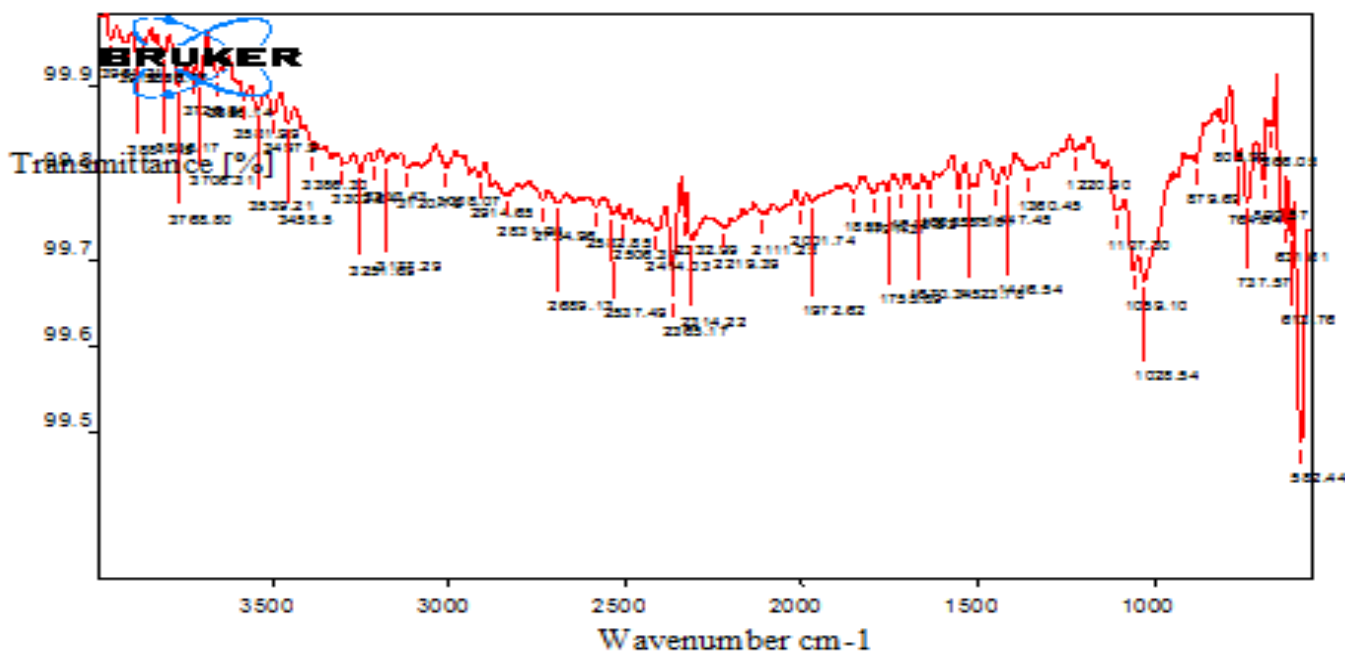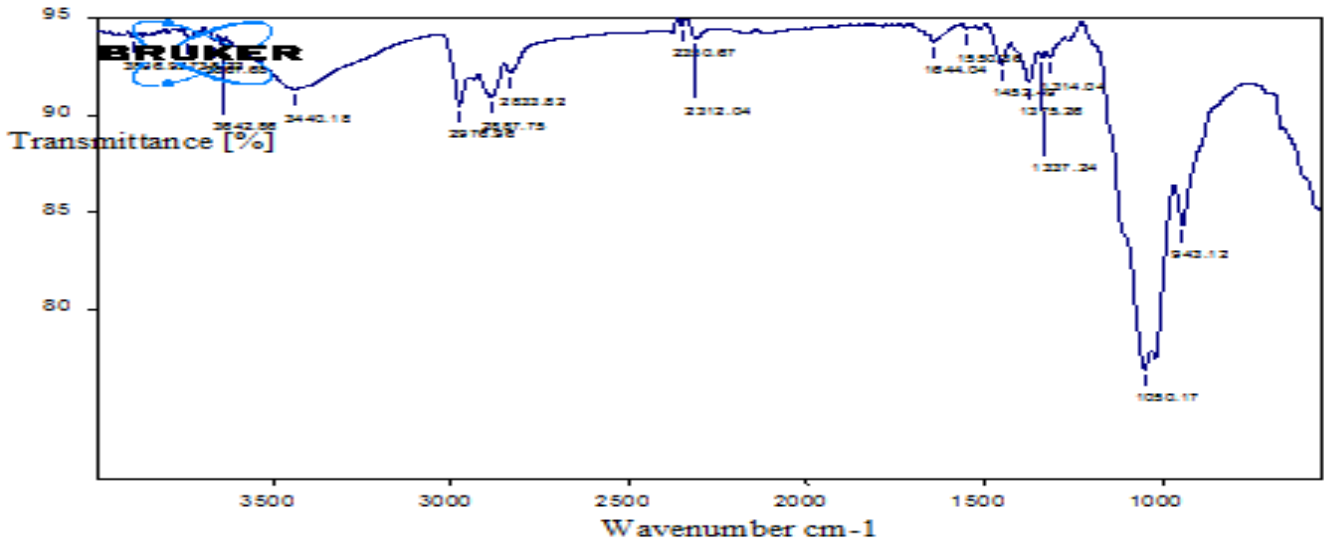
Fig no 3: FTIR of HPMC K15
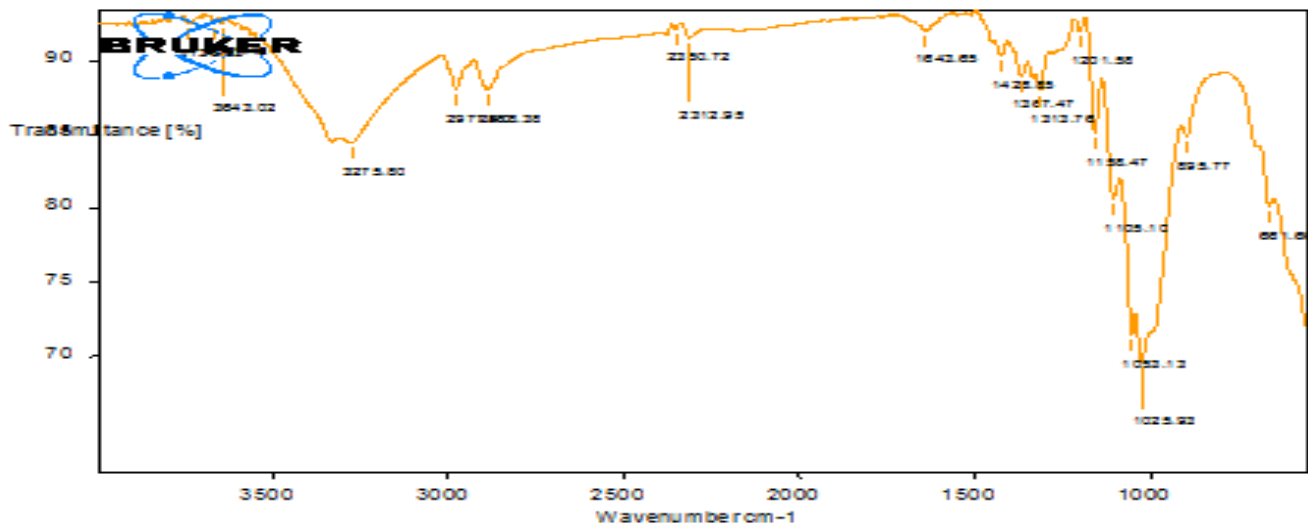

Fig no 4: FTIR of MCC

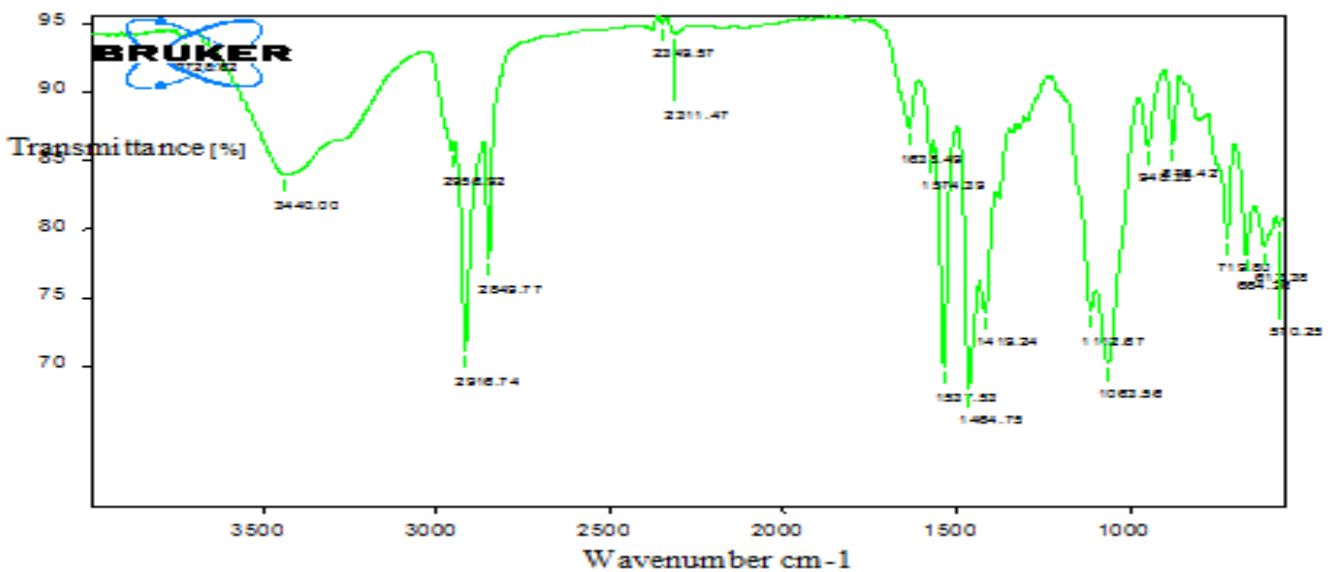**Fig no 5: FTIR of magnesium stearate**

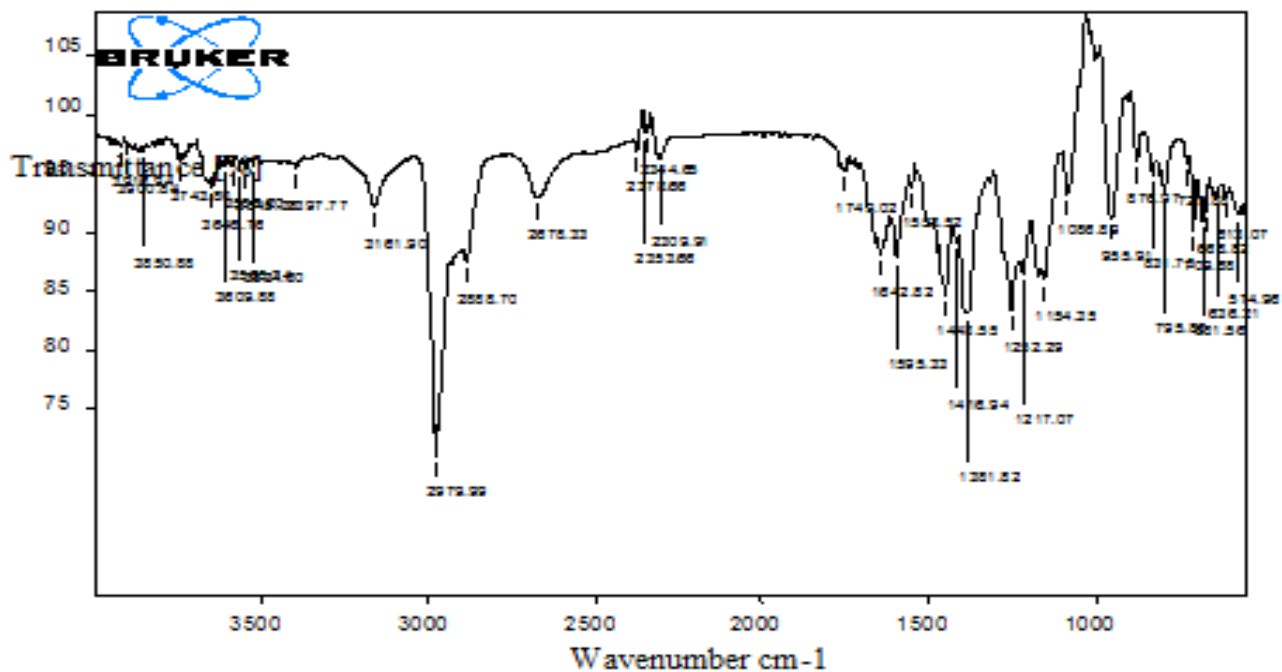**Fig no 6: FTIR OF Nevirapine + HPMC K15 + MG.ST + MCC**

Table no 6: Derived properties and Flow properties of prepared formulation

Formulation code	Derived properties (MEAN±SD)			Flow properties (MEAN±SD)	
	Bulk density	Tapped density	Angle of repose	Carr's index	Hausner's ratio
F1	0.4737±0.01	0.600±0.015	26.45±0.30	21.05±1.97	1.266±0.02
F2	0.45±0.015	0.6428±0.02	27.21±0.39	30.00±1.96	1.4285±0.03
F3	0.45±0.015	0.60±0.01	24.97±0.68	25.00±3.97	1.333±0.05
F4	0.45±0.015	1.00±0.015	23.21±0.96	55.00±1.81	2.222±0.02
F5	0.5294±0.02	0.60±0.03	25.94±0.73	11.76±2.25	1.133±0.03
F6	0.60±0.01	0.642±0.006	24.25±0.36	6.66±3.16	1.074±0.04
F7	0.529±0.025	0.60±0.025	28.21±0.29	11.76±1.19	1.133±0.02
F8	0.50±0.01	0.60±0.017	23.87±0.40	16.66±3.61	1.200±0.05

Table no 7: Physicochemical evaluation of Matrix Tablets of Nevirapine

Formulation code	Thickness (mm)	Weight variation (mg)	Hardness (Kg/cm²)	Friability (%)	Drug content (%)
F1	3.12 ±0.03	309±1.55	3.8±0.15	0.02±0.025	101±0.41
F2	3.19±0.02	302±0.94	3.4±0.25	0.11±0.03	99±0.19
F3	3.11±0.03	300±0.81	3.3±0.31	0.05±0.042	99±0.48
F4	3.15±0.05	301±0.72	3.9±0.21	0.13±0.036	100±0.41
F5	3.18±0.03	300±0.19	3.7±0.2	0.32±0.01	98±0.15
F6	3.19±0.04	303±0.84	3.8±0.26	0.45±0.02	98±0.01
F7	3.13±0.07	300±0.38	3.9±0.31	0.75±0.038	100±0.03
F8	3.16±0.02	300±0.52	3.5±0.25	0.23±0.025	100±0.65

Table no 8: Cumulative % drug released of formulations of nevirapine tablets

TIME	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	66.83	30.83	32.66	36.66	16.66	38.33	53.5	52.66
4	86	66	43.83	74.83	38.33	46.66	63.83	70.83
8	91.66	97.5	48	70.16	54.66	73.16	74.66	71.16
16	95	98	51	74.66	69.16	83.83	79.83	86.33
20	98.66	93	57.5	89.16	84.33	92.33	97	89.33
24	84.33	76.33	79.1	98	99.6	98.33	84.16	99.6

Fig no 7: Dissolution profile of formulations F-1 to F-8
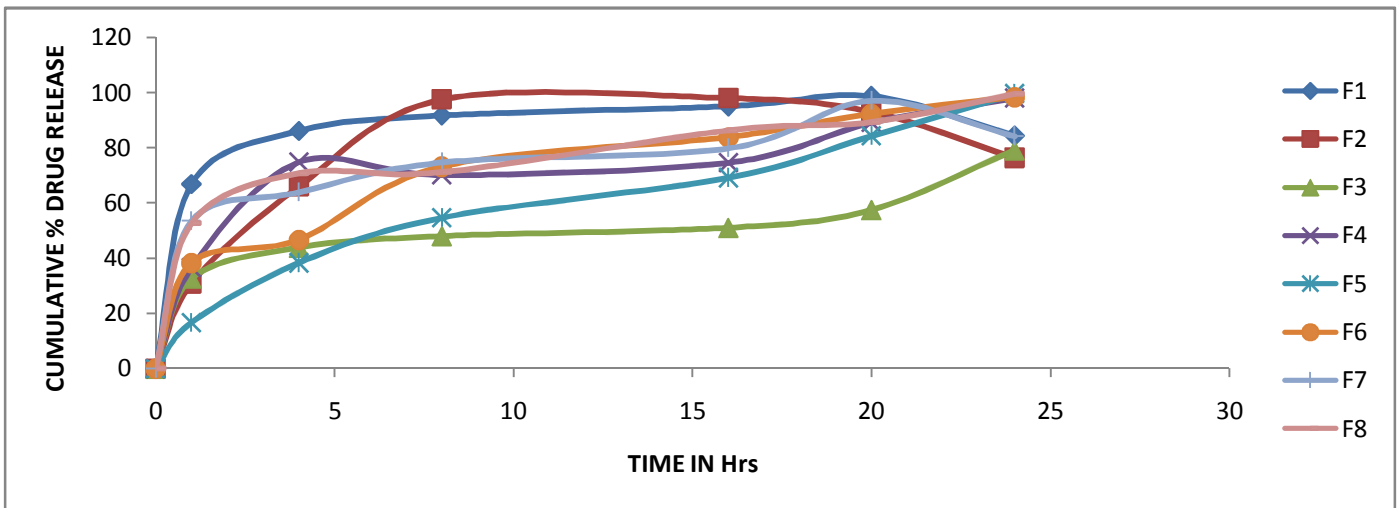
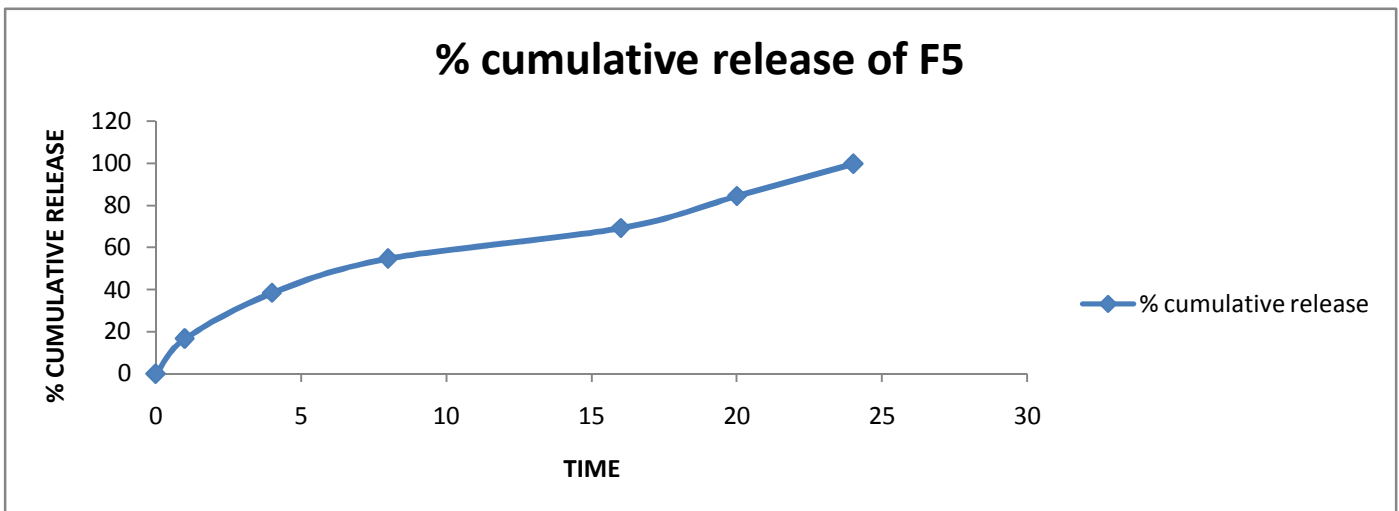**Fig no 8: Cumulative % drug released from Nevirapine Matrix tablets in 6.8 PH Phosphate buffer**

% cumulative release of F5

Fig no 9: Zero order treatment for F-5 Formulation

zero order

$y = 4.410x$
$R^2 = 0.869$
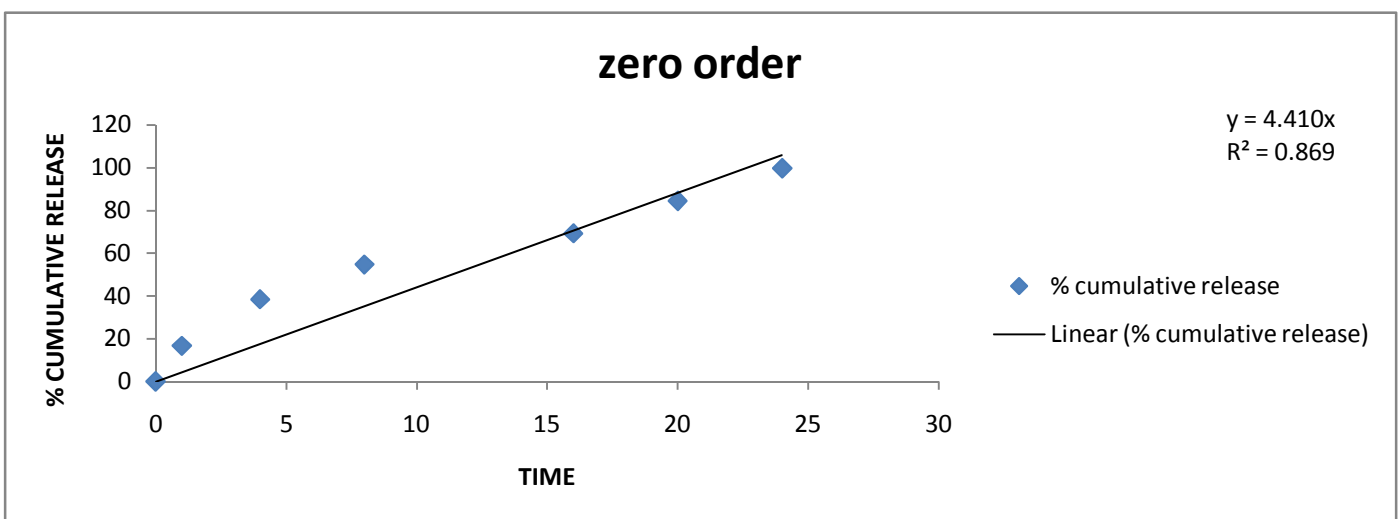
Fig no 10: First order treatment for F-5 Formulation

FIRST ORDER PLOT

$y = -0.061x + 2.085$
$R^2 = 0.780$
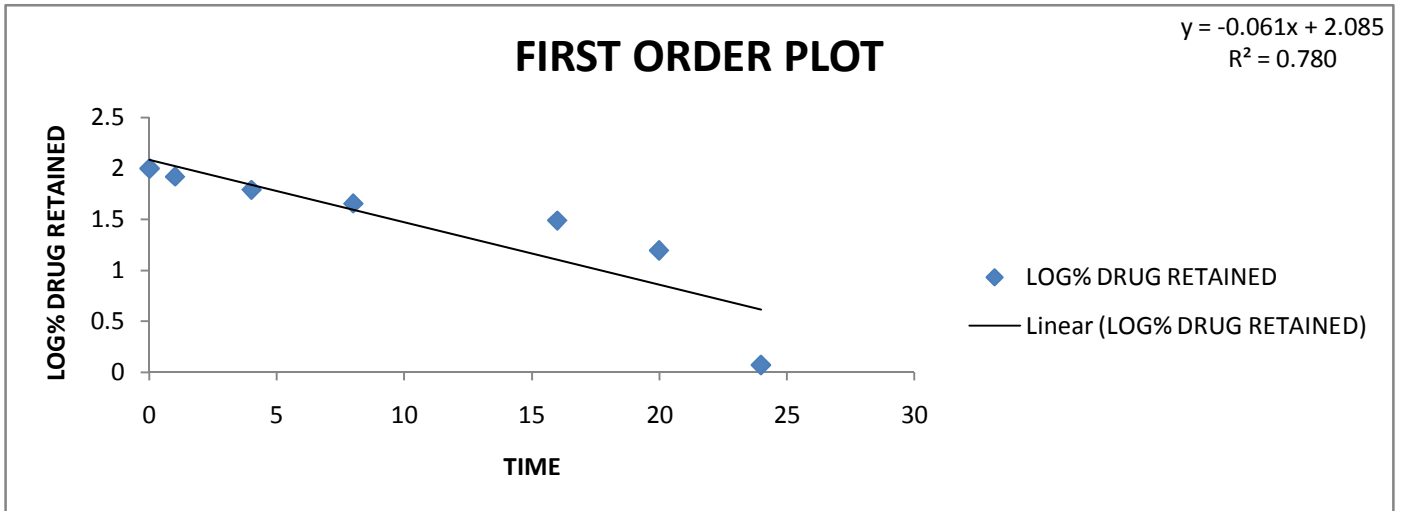
Fig no 11: Higuchi treatment for F-5 Formulation

HIGUCHI PLOT

$y = 19.40x - 1.407$
$R^2 = 0.988$
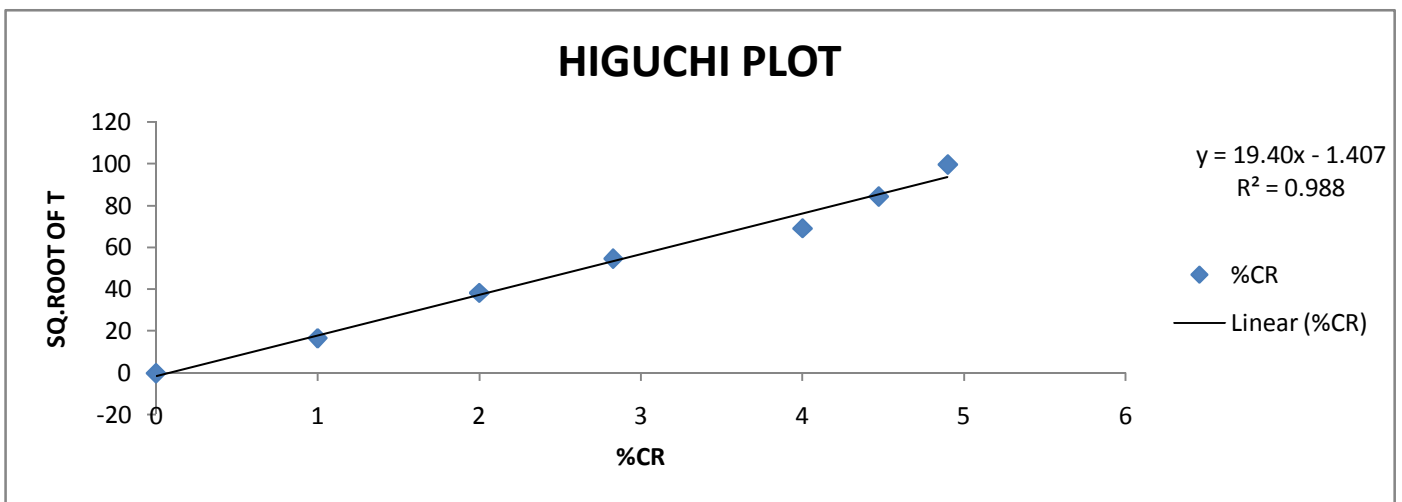
Fig no 12: Kores mayer peppas treatment for F-5 Formulation

KORESMAYER PEPPAS PLOT

$y = 0.262x - 0.279$
$R^2 = 0.928$
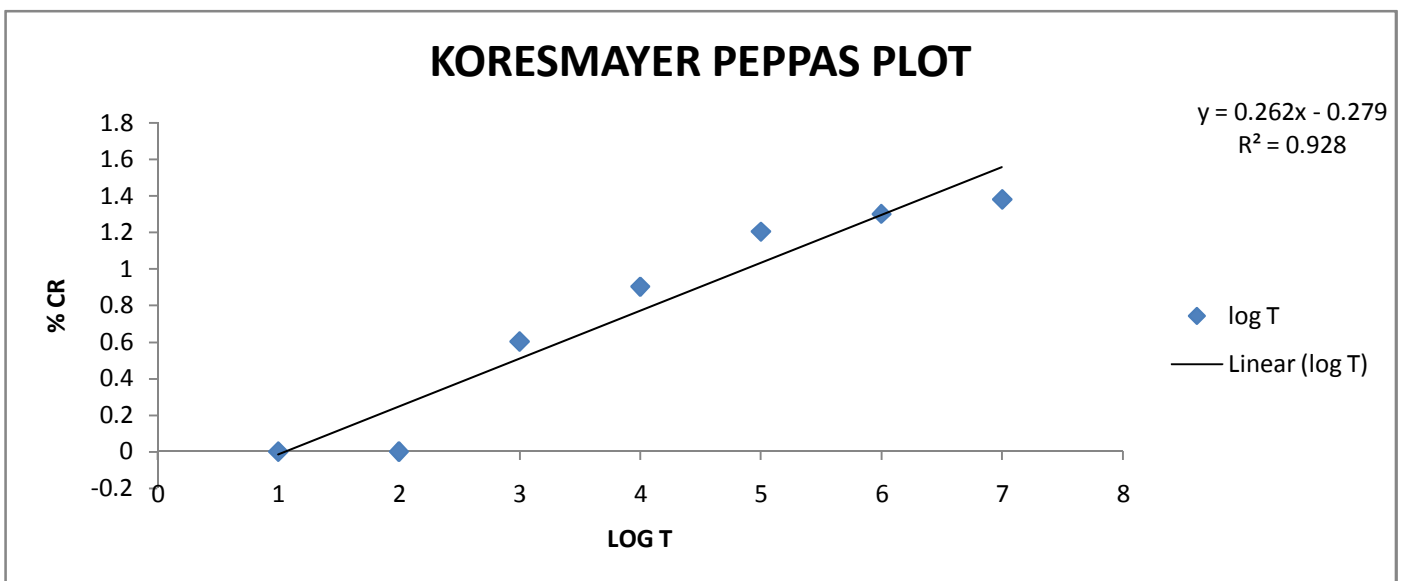
Fig no 13: Hixon crowell tretment for F-5 Formulation

HIXON CROWELL PLOT

$y = 4.357x - 7$
$R^2 = 0.963$
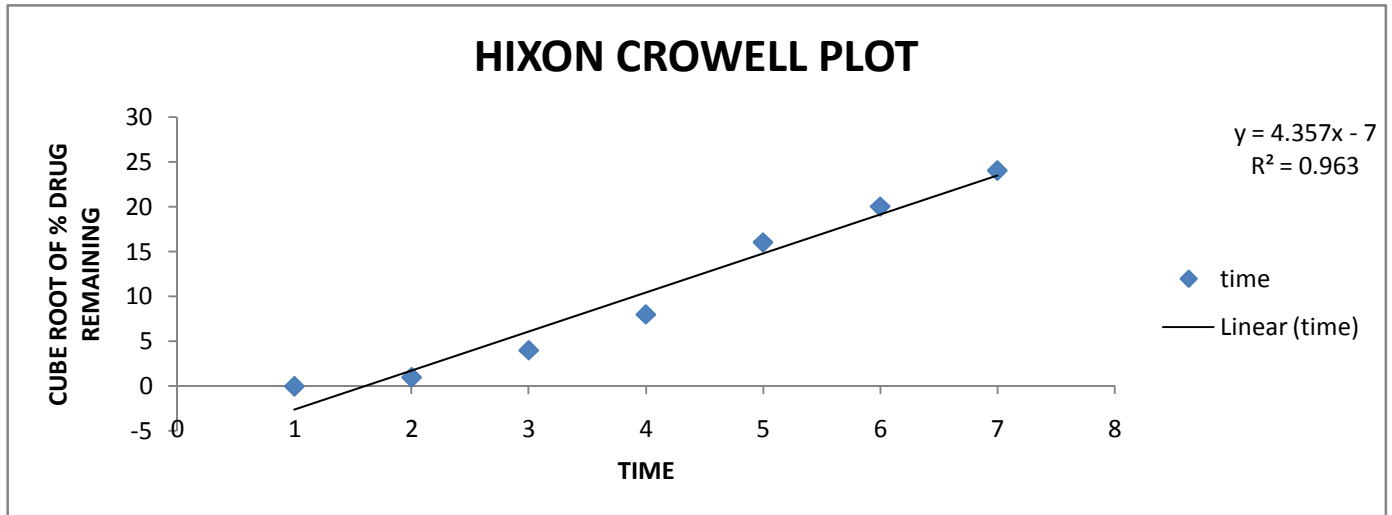
Table no 9: Dissolution Study in 6.8 PH buffer solution of Optimised batch F-5

TIME	F5
0	0
1	16.66
4	38.33
8	54.66
16	69.16
20	84.33
24	99.60

Table no 10: Zero order plot of F-5 Formulation

S. NO	TIME	% CUMULATIVE RELEASE
1	0	0
2	1	16.66
3	4	38.33
4	6	54.66
5	8	69.16
6	20	84.33
7	24	99.6

Table no 11: First order plot of F-5 Formulation

S.NO	TIME	LOG % CUMULATIVE DRUG RETAINED
1	0	2
2	1	1.92
3	4	1.79
4	8	1.656
5	16	1.489
6	20	1.195
7	24	0.068

Table no 12: Higuchi plot of F-5 Formulation

S.NO	Root T	% CR
1	0	0
2	1	16.66
3	2	38.33
4	2.828	54.66
5	4	69.16
6	4.472	84.33
7	4.898	99.6

Table no 13: Korsmeyer peppas plot of F-5 Formulation

S.NO	LOG TIME	LOG %CR
1	0	0
2	0	1.22167
3	0.602	1.58353
4	0.903	1.7376
5	1.204	1.83985
6	1.301	1.92598
7	1.38	1.99825

formulae with modifications using as a retarding polymer. The hydrophilic polymer (HPMC K15M) although was able to retard the drug release in the initial stage, could not show effective retardation of drug release in the later stage. Hence, in the later stages of dissolution study, the tablets started to release more amount of drug. The % Cumulative drug release of all the formulations F1-F8 were within the range of 79.1% to 99.66% for 24 hrs. The optimized formulation F5 showed a %drug release of 99.66% for 24 hrs which showed a constant release in a extended manner compare to all other formulation. According to R2 value obtained, best formulation, i.e, F-5 formulation follows higuchi's model which shows that drug is released from matrix.

CONCLUSION:

In this study, extended release tablet of Nevirapine were prepared using pharmaceutically acceptable and easily available inert excipients by wet granulation (Non aqueous granulation) technique. The procured drug sample of Nevirapine was tested for its identification by means of organoleptic properties, melting point, UV spectra and FTIR spectrum. The drug sample showed similar results as reported in literature. In present study, the high viscosity grade HPMC K15 as specified by USP was used as hydrophilic matrix forming agent. It forms a strong gel in aqueous media which may be useful to control the drug release of both, water soluble as well as water insoluble drugs from formulations.

The research work suggests that Formulation F5 containing HPMC K15 & MCC in different concentration shows the extended drug release for up to24 hrs, among all the formulations, F5 is considered as optimized formulation because it shows similar drug release pattern with that of innovator.

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