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Formulation, Optimization and Evaluation of Extended Release tablets of Levetiracetam

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Abstract: Levetiracetam is an antiepileptic drug with good bioavailability, rapid achievement of steady state concentration after oral administration. Besides it also has relatively short elimination half-life (6 hours). A drug with a short half-life requires frequent dosing and this makes Levetiracetam an ideal candidate for a extended-release formulation. Hydrophilic and swellable polymer matrix such as HPMC K-15M and PEO are widely used in extended-release delivery because of their flexibility to obtain a desirable drug release profile. To provide a extended-release composition which releases Levetiracetam over a time period of at least about 12 hours when exposed to gastrointestinal milieu thus facilitating a reduction in frequency of drug administration through extended-drug delivery system. Optimization was done using 3^2 full factorial design at 3 levels and 2 factors. From the polynomial equation, both the two independent factors showed significant effect on dependent variables.
Key Words: Levetiracetam, PEO, 3^2 Factorial Design, 0.05m Monobasic Potassium Phosphate.

INTRODUCTION:

The scenario of pharmaceutical drug delivery is rapidly changing conventional pharmaceutical dosage forms are being replaced by new drug delivery systems. These new drug delivery systems are having edge over conventional ones in terms of many biopharmaceutical parameters. One such drug delivery system is sustained-release drug delivery system[1]. The primary objective of sustained release drug delivery system is to ensure safety, improve the efficacy, reduce the dose frequency and ultimately result in improved patient compliance. The aim of present work is to formulate extended-drug drug delivery system of Levetiracetam suitable for twice-a-day dosing [4]. In general, extended-drug -release drug delivery is attempted to maintain constant, effective drug level in the body with concomitant minimization of undesired side-effects. Levetiracetam rapidly and almost completely absorbed after oral administration (99%). Peak plasma concentrations occurring in about an hour following oral administration in fasted subjects. The major metabolic pathway of levetiracetam (24% of dose) is an enzymatic hydrolysis of the acetamide group. No CYP450 metabolism detected. The short biological half life of drug also favours development of a extended-drug release formulation [2,3].

MATERIALS & METHODS

Levetiracetam was obtain as a gift sample from Swiss pharma. pvt. ltd, Ahmadabad, HPMC K15M from Shandong head.ltd, PEO from Colorcon India Ltd, Goa., MCC from Accelmicrocell.pvt.ltd, Baroda, PVP K-30 from Jiaozuo-Yuanhai-China, Magnesium stearate from Lucent pharm, Ahmedabad, Isopropyl Alcohol from Sulab lab, Baroda.

Preparation Of Standard Calibration Curve

Standard calibration curve was prepare with the help of HPLC.

Preparation Of 0.05m Monobasic Potassium Phosphate:

6.8 g/L of monobasic potassium phosphate, adjusted with dilute potassium hydroxide to a pH of 5.6.

Mobile phase: Acetonitrile and Buffer (15:85).

Preparation Of Standard Stock Solution.

Accurately weighed 50 mg levetiracetam was transferred to 100ml volumetric flask, dissolved in 50 ml of mobile phase and diluted up to the mark with mobile phase.

Preparation Of Sample Working Solution.

From the stock solution 0.2, 0.4, 0.6, 0.8 and 1 ml of solution was taken and transfer to 10 ml volumetric flask. The volume was made up to the mark with mobile phase. The dilutions resulted in 10, 20, 30, 40 and 50 mcg/ml concentration respectively [6].

Preformulation Study of Drug

Preformulation can be defined as investigation of physical and chemical properties of drug substance alone and when combined with excipients. Preformulation studies are the first step in the rational development of dosage form of a drug substance. The objectives of preformulation studies are to develop a portfolio of information about the drug substance, so that this information is useful to develop formulation.

Followings studies performed for in the preformulation study.

Micromeritic properties evaluation

Bulk Density (BD)

Loose Bulk Density (LBD): Weigh accurately 25 g of drug (W), which was previously passed through 20 # sieve and transferred in 100 ml graduated cylinder. Carefully level the powder without compacting, and read the unsettled apparent volume (V_0). Calculate the apparent bulk density in gm/ml by the following formula

Bulk density = Weight of powder / Bulk volume

Tapped bulk density (TD): Weigh accurately 25 g of drug, which was previously passed through 20 # sieve and transfer in 100 ml graduated cylinder. Then mechanically tap the cylinder containing the sample by raising the cylinder and allowing it to drop under its own weight using mechanically tapped density tester that provides a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute. Tap the cylinder for 250 times initially and measure the tapped volume (V_1) to the nearest graduated units, repeat the tapping an additional 750 times and measure the tapped volume (V_2) to the nearest graduated units. If the difference between the two volumes is less than 2% then final the volume (V_2). Calculate the tapped bulk density in gm/ml by the following formula:

Tapped Density = Weight of powder / Tapped volume

Carr's Index

The Compressibility Index of the powder blend was determined by Carr's compressibility index. It is a simple test to evaluate the BD and TD of a powder and the rate at which it packed down. The formula for Carr's Index is as below

Carr's Index (%) = [(TD-BD) x100]/TD

Hausner's Ratio

It is a number that is correlated to the flow ability of a powder or granular material.

Hausner's Ratio = TD / BD

Angle of repose

Determined by the funnel method. The accurately weight powder blend were taken in the funnel. The height of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation.

$\text{Tan } \theta = h/r$

Where, h and r are the height and radius of the powder cone respectively.

Drug Excipients Compatibility Study

Drug-Excipient Compatibility Studies by FTIR

FTIR is one of the most powerful analytical techniques to identify functional groups of a drug. In the present study, the potassium bromide disc (pellet) method was employed. Chemical stability was confirmed by IR spectrometry [7].

Method of preparation of Extended Release Matrix Tablets By Using 3^2 Full Factorial Designs

A 3^2 randomized full factorial design was utilized in the present study. In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The factors were selected based on preliminary study. The concentration of PEO (X_1)and concentration of HPMC-K15M (X_2) were selected as independent variables. The formulations of the factorial batches (B1 to B9) are shown in Table no 2 [9].

Method: ER tablets were prepared by wet granulation technique. The different stages involved in the process are:

All the raw materials were passed through sieve no. 60 # and weighed accurately as per formulae. Then levetiracetam, polymers, MCC and PVP K-30 were mixed thoroughly by trituration in mortar and pestle to get uniform mix. This thoroughly mixed powder was kneaded with isopropyl alcohol solution till it forms dough mass. This mass was passed through sieve no. 20 # to form granules. The granules were spread on the tray and kept for drying at 50 ^{0}C for 30 min using hot air oven. The dried granules were sifted through sieve no. 40 # to get fines and uniform sized granules and blended with magnesium stearate. The extended release tablets with targeted weight of 1000mg was compressed in 10 station compression machine. The Hardness of all tablets was maintained at 6 to 8 kg/cm^2.

Evaluation of ER tablets

Appearance

Twenty tablets of each formulation were taken to check any discoloration or surface roughness in the tablet formulation.

Weight variation test

To study weight variation, twenty tablets of the formulation were weighed using a Mettler Toledo electronic balance and the test was performed according to the official method.

Hardness

The hardness of five tablets was determined using the Monsanto type hardness tester and the average values were calculated.

Thickness

The Thickness of the tablets was determined by using Digital vernier callipers. Five tablets were used, and average values were calculated.

Friability

The friability of twenty tablets was measured by Roche friabilator for 4min at 25rpm for 100 revolutions. Accurately weighed twenty tablets were placed into Roche friabrilator for 100 revolutions than dedusted and weighed again.

% Friability = W_0 -- W / W_0 × 100

In-Vitro Release study

Drug release studies were carried out using a USP type -II basket type dissolution test apparatus (Apparatus 2, 100 rpm, 37 °C) for 12 hrs in pH-6.8 phosphate buffer (900 ml) and tested for drug release up to complete drug release. At the end of the time period 10 ml of the samples were taken and analyzed for Drug content. A 10 ml Volume of fresh and filtered dissolution medium was added to make the Volume after each sample withdrawal. Samples were filtered through filter (0.45µm) and assayed by HPLC at 210 nm. Cumulative fractions of drug released from the tablets were calculated and plotted as function of time [6].

Experimental work done:

Using 3^2 factorial design and nine batches were prepared using Polyox WSR 303 & Eudragit RS at three different levels (low, medium, high). Tablets were characterized by various physic-chemical parameters, in-vitro drug release study. Cumulative percentage drug released at 2 hr, 12 hr are $Q_{2,}$ Q_{12}.The observed and predicted responses were critically compared. Plots between predicted and observed responses were critically compared. The residual graphs plotted were plotted and the percent error was also calculated with respect to the observed responses[9].

Stability Studies

To assess the drug and formulation stability, stability studies was done according to ICH guidelines. Prepared formulation was kept in humidity chamber maintained at 40°C and 75% relative humidity (RH) for one month. The sample was analyzed for the physical appearance, drug content, and drug release characteristics[10].

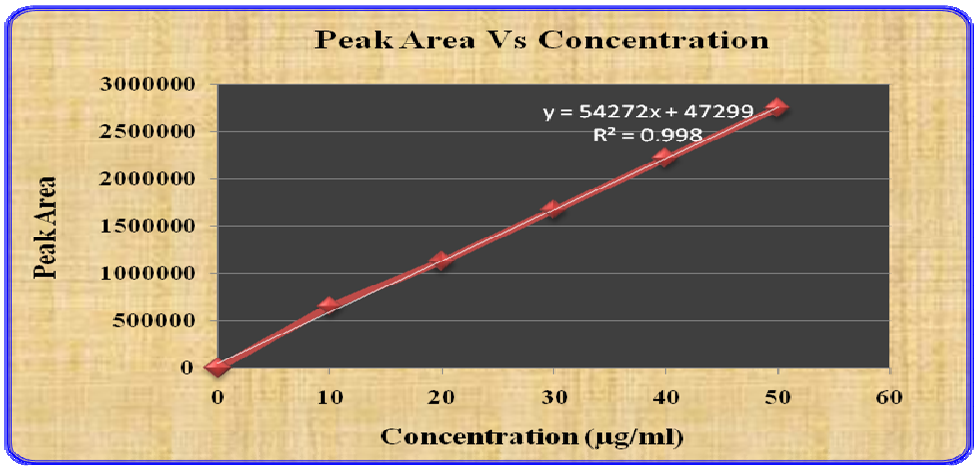

Figure No. 1: Calibration Curve of Levetiracetam

RESULT AND DISCUSSION

Preparation Of Standard Calibration Curve

From the standard calibration curve we find out the value of Y =54272x +47299 and value of R^2 =0.998 as shown in **following fig no 1.**

Preformulation Study of Drug

Micromeritic properties evaluation

The results of bulk density, tapped density, Carr's index, hausner's ratio and angle of repose are given in following **table no 1.**

Table No. 1: Evaluation of powder blend of Factorial batches

Batch code	Angle of Repose (θ)	Bulk Density (g/ml)	Tapped Density (g/ml)	Carr's Index (%)	Hausner's Ratio
B1	21.56	0.404	0.473	15.22	1.17
B2	23.13	0.452	0.512	14.06	1.13
B3	25.1	0.429	0.504	14.88	1.17
B4	24.38	0.464	0.528	12.12	1.14
B5	22.9	0.42	0.489	14.11	1.16
B6	22.15	0.451	0.512	11.39	1.14
B7	19.8	0.437	0.515	15.15	1.18
B8	20.35	0.449	0.521	13.82	1.16
B9	20.82	0.447	0.511	12.52	1.14

Drug Excipients Compatibility Study

From the results of FTIR it is concluded that there is no interaction between drug and polymer. The FTIR spectra of pure levetiracetam, excipient and formulation in **Fig no 2 to 4.** Pure levetiracetam presents characteristic infrared spectra in the region of 1336 cm^{-1} C-N functional group, while 3300 cm^{-1} suggests N-H group. It also exhibits characteristic infrared spectra in the C=O stretching region of functional carbonyl group band at 1641 cm^{-1}, showing its crystalline nature. The characteristic C-H stretching band at 1288 cm^{-1} of pure levetiracetam appeared unchanged in physical mixture and complex.

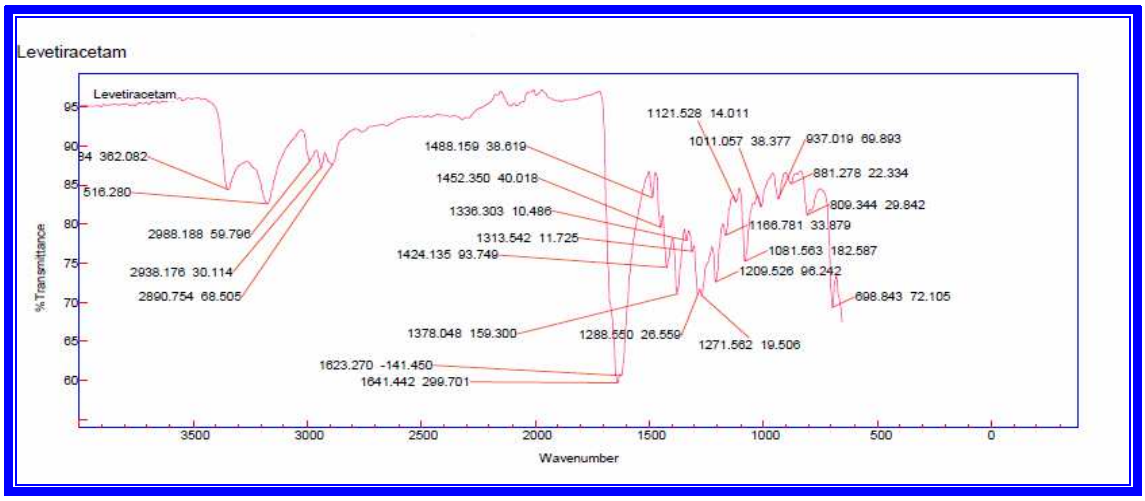

Figure No. 2: FTIR of Levetiracetam

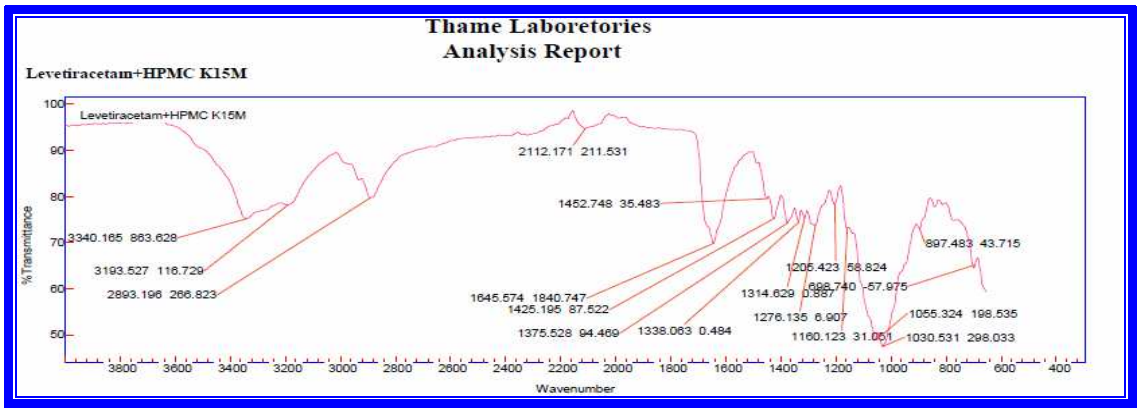

Figure No. 3: FTIR of Levetiracetam + HPMC K-15M

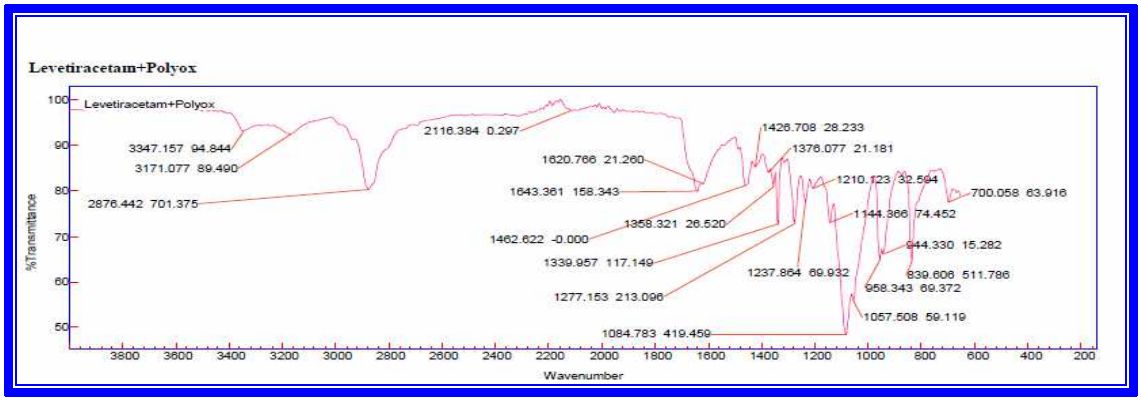

Figure No. 4: FTIR of Levetiracetam + Polyox

Evaluation of Tablets

The physical attributes of the tablet were found to be satisfactory. Typical tablet defects, such as capping, chipping, and picking, were not observed. Results for other physical evaluations were also found to be within an acceptable range. For instance, Wight variation ranges for all formulation 998 mg to 1003 mg, % Drug content ranges for all formulation are from 98.57% to 101.23%. Hardness of the tablet was found to be 7 kg/cm^2 to 7.5 kg/cm^2. Thickness was found to be fixed during the compression cycle; values were 3.8 mm to 4.2 mm. The range of Friability of the batches was calculated 0.23 to 0.76 which was well within the acceptable range of 1% and it indicates that tablet surfaces are strong enough to withstand mechanical shock or attrition during storage and transportation and until they are consumed.

In Vitro Dissolution Studies

Result of in vitro dissolution study of all nine batches are shown in following table no 4. It was found that B1, B2, B3, B4, B5, B6, B7, B8 and B9 have 99.25%, 98.67%, 90.25%, 98.39%, 99.4%, 92.27%, 97.44%, 95.13%, 91.5% of the drug released during the twelve hour.

Statistical Analysis

Total 9 batches were prepared by applying 3^2 factorial design. All the batches were evaluated and different polynomial equations were derived for Cumulative % drug released at 2 hr (Q_2) and 12 hr (Q_{12}). Derived equations were checked for validity by preparing a check point batch. Also contour plots were prepared for dependent variables. The statistical analysis of the factorial design batches was performed by multiple regression analysis using Design-Expert 8.0.7.1 software. A statistical model incorporating interactive and polynomial terms was used to evaluate the response.

$$Y=b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{11}X_{12}+b_{22}X_{22}$$

Where Y is dependent variable, b_0 is the arithmetic mean response of nine runs, b_1 is the estimated coefficient for factor X_1.The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how response changes when two factors simultaneously changed.X_{12} and X_{22} are quadratic term to indicate non-linearity.

The polynomial equations were generated by linear multiple regression that quantitatively explain the effect of the different variables on the attributes.

Data Analysis of Y_1 (Q_2)

The observed value for Q_2 for all 9 batches B1-B9 varied from 27% to 39%. The result clearly indicates that Y_1 is strongly affected by the independent variables selected for the study. The response (Y_1) obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation no.1.

Equation 1

$$Y_1 = 33.13-4.02X_1-0.81X_2+1.31X_1X_2-1.14X_{11}+0.39X_{22}$$

F- Value - 103.88

P - Value - <0.0001

R^2 – Value - 0.9905

The above equation clearly reflects the wide range of values of various co-efficient (b). Among the independent variables selected X_1 and X_2 have negative value indicating unfavourable effect on Y_1 (Q_2). But their interaction have positive value of co-efficient (1.31) indicating favourable prominent effect on Y_1. The variable X_1 and X_2 were also found to be significant ($P<0.05$).

Data Analysis Of Y_2 (Q_{12})

The observed value for Q_{12}% for all 9 batches B1-B9 varied 90% to 99%. The result clearly indicates that Y_2 is strongly affected by the independent variables selected for the study. The response (Y_2) obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation no. 2.

Equation 2

$$Y2= 98.64-3.50X_1-0.68X_2+0.76X_1X_2-2.92X_{11}-1.32X_{22}$$

F-value: 19.11

P-value: 0.0028

R^2: 0.9503

The above equation clearly reflects the wide range of values of various co-efficient (b). Among the independent variables selected X_1 and X_2 have negative value indicating unfavourable effect on Y_2 (Q_{12}) But their interaction have positive value of co-efficient (0.76) indicating favourable prominent effect on Y_2. The variable X_1 and X_2 were also found to be significant ($P<0.05$).

Stability Studies

Result of stability study was given in the following table no 7. It indicates there are not much changes occurring in tablet's properties in one month of storage.

Table No. 2: 3^2 Full Factorial Design Of Formulation Batches Qty (mg/Tab)

Factorial Batch	B1	B2	B3	B4	B5	B6	B7	B8	B9
Levetiracetam	500	500	500	500	500	500	500	500	500
HPMC K-15M	100	100	100	150	150	150	200	200	200
PEO	100	150	200	100	150	200	100	150	200
MCC	250	200	150	200	150	100	150	100	50
PVPK30	40	40	40	40	40	40	40	40	40
Mg. Stearate	10	10	10	10	10	10	10	10	10
IPA	qs	qs	qs	qs	qs	qs	qs	qs	qs
Total weight	1000	1000	1000	1000	1000	1000	1000	1000	1000

Table No. 3: Evaluation of Tablets of Factorial Batch

Batch code	Weight variation (mg)	Hardness (kg/cm^2)	Thickness (mm)	Friability (%)	Drug content (%)
B1	998	7	4.1	0.65	99.90
B2	1000	7	4	0.45	101.23
B3	1000	7	3.9	0.34	99.56
B4	1001	7.5	4.2	0.47	98.57
B5	1003	7	4	0.23	100.44
B6	1001	7.2	4.1	0.57	99.21
B7	999	7.4	3.8	0.76	99.88
B8	999	7.5	4	0.54	100.10
B9	1000	7.5	4	0.38	98.78

Table No. 4: % Cumulative Drug Release of B1 To B9

Sr. No.	Time (hr)	B1	B2	B3	B4	B5	B6	B7	B8	B9
1	**1**	28.6	25.6	20.6	27.5	26.7	21.3	30.2	25.8	22.3
2	**2**	38.37	34.2	28.12	36.11	33.28	27.29	34.3	32.26	29.28
3	**4**	48.3	47.55	45.33	50.3	49.33	48.32	51.30	47.32	46.30
4	**6**	62.5	60.25	64.24	63.5	60.12	59.37	62.25	61.77	59.92
5	**8**	81.34	80.33	78.17	81.25	81.26	79.26	80.92	82.26	78.85
6	**10**	92.56	90.26	84.27	91.26	89.82	86.27	90.32	88.25	85.89
7	**12**	99.25	98.67	90.25	98.34	99.4	92.27	97.44	95.13	91.5

Table No. 5: Summary of Quadratic Polynomial Equation for Dependent Variables Y_1 and Y_2

Quadratic Model	Quadratic polynomial equation
Y_1	$Y1=33.13-4.02X_1-0.81X_2+1.31X_1X_2-1.14X_{11}+0.39X_{22}$
Y_2	$Y2=98.64-3.50X_1-0.68X_2+0.77X_1X_2-2.92X_{11}-1.32X_{22}$

Table No. 6: Formula of Optimized Batch

Sr. no.	Ingredient	Quantity (mg)
1.	Levetiracetam	500
2.	HPMC k-15M	100
3.	PEO	200
4.	Microcrystalline cellulose	150
5.	PVP K-30	40
6.	Magnesium Stearate	10
7.	Isopropyl Alcohol	Qs

Table No. 7: Result of Stability Study of Optimized Batch

Condition	**40˚C/75%RH**			
Batch No.	**B3**			
Time period	**Hardness Kg/cm^2**	**Friability (%)**	**Drug content (%)**	**% Drug release at 12 hrs**
Initial	7	0.34	99.56	90.25
After 1 month	6.5	0.38	99.32	90.01

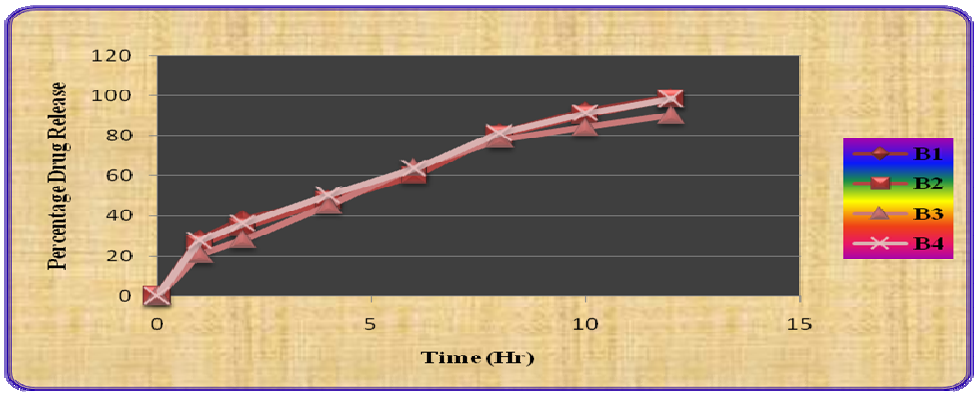

Figure No. 5: In-Vitro Release From Batches B1 To B4

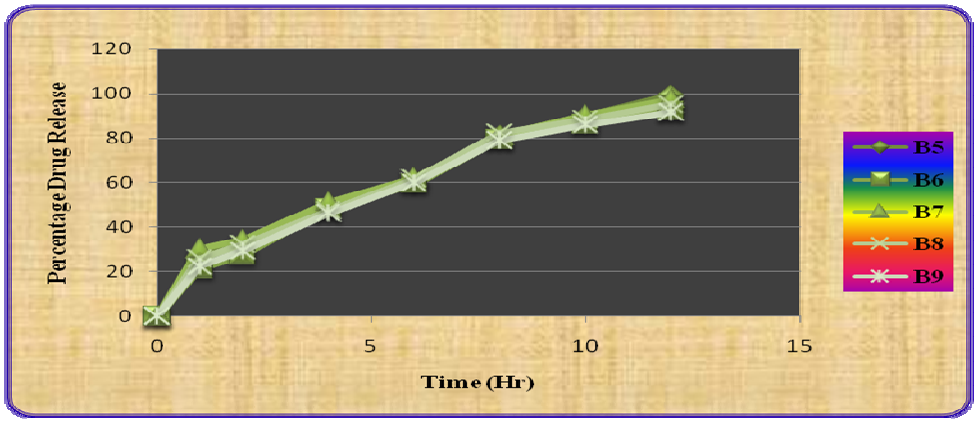

Figure No. 6: In-Vitro Release From Batches B5 To B9

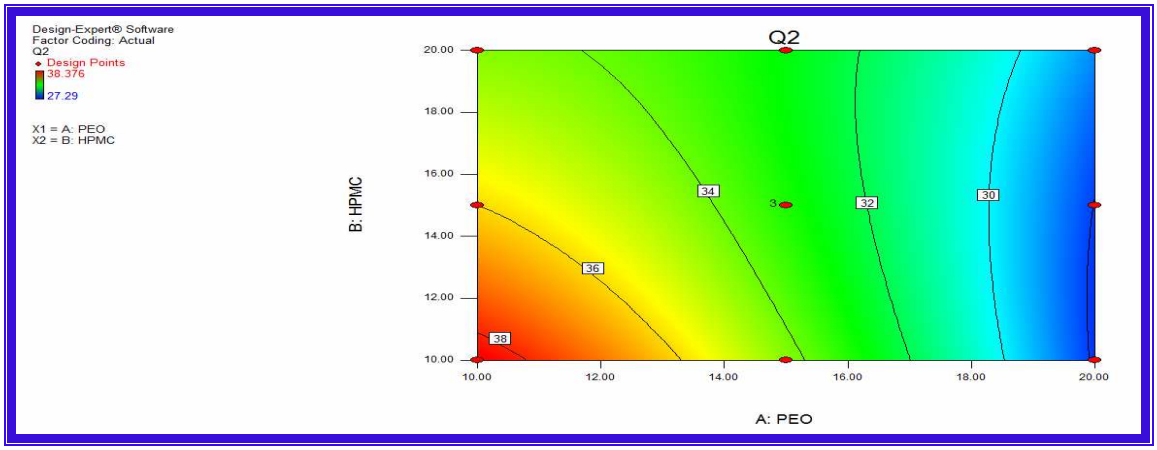

Fig. No. 7: Contour Plot of Q_2 V/S X_1, X_2 for 3^2 Full Factorial Design

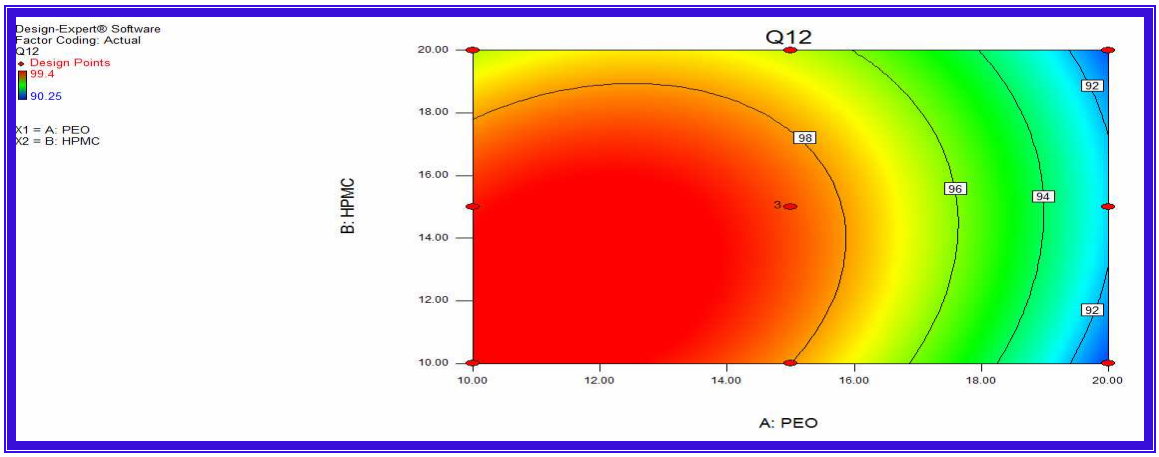

Fig. No. 8: Contour Plot of Q_2 V/S X_1, X_2 for 3^2 Full Factorial Design

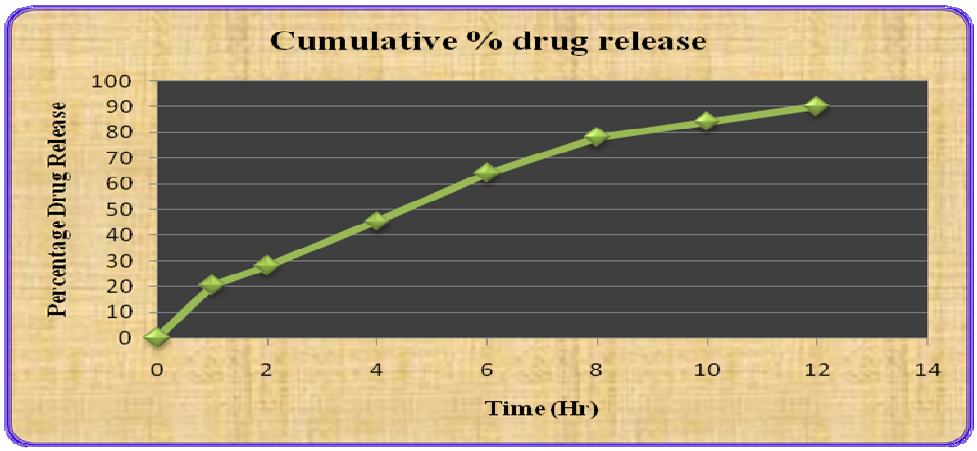

Fig. No. 9: In-vitro drug release profile of optimized batch

SUMMARY AND CONCLUSION

It is evident from the results ER tablet prepared by using 3^2 factorial design is a better system for twice daily extended release tablet. The matrix former Polyox WSR 300 and Eudragit RS can control the release levetiracetam effectively for 12 hours. *In vitro* drug dissolution of different formulations is carried out for 12 hours, Formulation B1, B2, B3, B4, B5, B6, B7, B8 show 99.25%, 98.67%, 90.25%, 98.39%, 99.4%, 92.27%, 97.44%, 95.13%, 91.5% drug release. By using 3^2 factorial design the check point was prepared. It show 20.6% drug release in 1st hour and 90.25% drug. Post compression parameters like weight variation, tapped density, bulk density, Carr's index, hausner's ratio and angle of repose are within the pharmacopeia limit. Stability studies on the optimized extended release tablet formulation on stability storage conditions 40 ± 2 °C and 75 ± 5 % RH for 1 month. Good storage stability as assessed by short term stability studies as per ICH guidelines.

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