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Research Article

APPLICATION OF DOE FOR OPTIMISATION AND EVALUATION OF IMMEDIATE RELEASE TABLET OF LIPID LOWERING AGENT

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ABSTRACT

Aim of the study is to stabilize oral formulation of Atorvastatin calcium tablet. amorphous form of atorvastatin calcium is used for this study because crystalline form is used by innovator which is patent protected upto year 2017. Stabilization is required because Atorvastatin calcium is susceptible to degradation in presence of acidic environment, moisture, heat and light and to evaluate different process parameters. As it has long half life (14 hours), it is not suitable candidate for controlled release formulation. Tablet dosage form is preferable because other than tablet dosage form not having good shelf life in case of Atorvastatin due to its degradation, which imparts the impurity in formulation. Preformulation study and drug excipient compatibility study was done initially and the results obtained directed the way to method of formulation. Atorvastatin calcium (Amorphous) is highly susceptible to heat and it has very poor flow property so dry granulation and direct compression method was avoided. Factorial design was used for the understanding of possible interaction amongst the excipients as sodium carbonate, polysorbate 80 and croscarmellose sodium. Results shown that there is no significant interaction between these factors. In vitro dissolutions were performed and F1 and F2 values were calculated. Dissolution profile of final trial batch was matched perfectly with innovator and F2 value was found to be excellent. Also the impurity profile and stability result of final trial batch was found to be excellent.

Keywords: Atorvastatin calcium, DOE.

INTRODUCTION

Design of experiments (DOE) may be a structured and arranged technique to work out the relationship among factors that influence outputs of a method. once DOE is applied to pharmaceutical method, factors square measure the material attributes (e.g., particle size) and method parameters (e.g., speed and time), whereas outputs square measure the vital quality attributes like mix uniformity, pill hardness, thickness, and breakableness. As every unit operation has several input and output variables also as method parameters, it's not possible to through an experiment investigate all of them. Scientists got to use previous data and risk management to spot key input and output variables and method parameters to be investigated by DOE. DOE results will facilitate determine optimum conditions, the vital factors that almost all influence CQAs and people that don't, also as details like such as On the appropriate vary of CQAs, the planning area of CPPs is determined. When considering scale-up, however, further experimental work is also needed to verify that the model generated at the little scale is prophetical at the massive scale. This can be as a result of some important method parameters ar scale dependent whereas others don't. The operational vary of scale dependent important method parameters can got to modification attributable to scale-up. Previous

information will play a really important role during this consider most pharmaceutical firms use constant technologies and excipients on an everyday basis. Pharmaceutical scientists will usually make the most of past expertise to outline important material properties, process parameters and their operational ranges [1-4].
The best new therapeutic entity in the world is of little value without an appropriate delivery system. Tableted drug delivery systems can range from relatively simple immediate-release formulations to complex extended or modified-release dosage forms. The most important role of a drug delivery system is to get the drug "delivered" to the site of action in sufficient amount and at the appropriate rate; however, it must also meet a number of other essential criteria. These include physical and chemical stability, ability to be economically mass produced in a manner that assures the proper amount of drug in each and every dosage unit and in each batch produced, and, as far as possible, patient acceptability (for example, reasonable size and shape, taste, color, etc., to encourage patients to take the drug and thus comply with the prescribed dosing regimen).[5]

MATERIALS AND METHODS[6-10]

MATERIALS

Atorvastain Calcium were obtained from Alkem Research centre, India as gift samples. All the other excipients, solvents, reagents and chemicals used were of either Pharamcopoeial or analytical grade.
Preformulation testing is the first step in the development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients.
The overall objective of Preformulation studies is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass-produce.
Preformulation study can divided into two sub-class.

Compatibility study

The compatibility of drug and formulation components is important prerequisite before formulation. It is therefore necessary to confirm that the drug does not react with the polymers and excipients under experimental conditions and affect the shelf life of product or any other unwanted effects on the formulation.

Active pharmaceutical ingredient (API) characterization

Organoleptic evaluation

These are preliminary characteristics of any substance, which is useful in identification of specific material. Physical properties of API like Color, Taste, odour.

Loss on drying

0.5g of sample of Atorvastatin calcium was accurately weighed and the powder was kept in a Mettler Toledo apparatus for 5 min. at 105ºC and the moisture content was calculated.

Solubility Analysis

A semi quantitative determination of solubility can be made by adding a solute in small incremental amounts to fixed volume of solvents whose pH ranging from 1.2 to 7.4 including distilled water. After each addition, the system is vigorously shaken and examined usually for any undissolved particles. When some solute remains undissolved the total amount added up to that point servers as a good and rapid estimate of solid.

Table 1: Formula of Atorvastatin calcium Tablets of trials 1 to 5

Sr. No.	Ingredients	Trial No.				
		1	2	3	4	5
Intragranular						
1	Atorvastatin Calcium	80	80	80	80	80
2	Sodium hydroxide	6	--	--	--	--
3	Magnesium hydroxide	--	25	--	--	--
4	Calcium hydroxide	--	--	35	--	--
5	Sodium carbonate	--	--	--	30	30
6	Microcrystalline Cellulose	250	250	250	250	250
7	Lactose	309	300	285	290	290
8	Croscarmellose sodium	50	50	50	50	--
9	Sodium starch glycolate	--	--	--	--	50
10	Crospovidone XL-10	--	--	--	--	--
11	Hydroxypropyl Cellulose	40	30	30	30	30
12	Polysorbate 80	--	--	5	5	5
13	SLS	--	5	--	--	--
14	Purified Water	q.s.	q.s.	q.s.	q.s.	q.s.
Extragranular						
15	MCC	250	250	250	250	250
17	Aerosil	5	5	5	5	5
18	Magnesium Stearate	--	5	10	10	10
19	Sodium stearyl fumarate	5	--	--	--	--
Tablet Weight (mg)		1000	1000	1000	1000	1000
20	Opadry YS-1-7040	30	30	30	30	30
21	Purified water	q.s.	q.s.	q.s.	q.s.	q.s.
Tablet Weight (mg)		1030	1030	1030	1030	1030

Table 2: Formula of Atorvastatin calcium Tablets of trials 5 to 10

Sr. No.	Ingredients	Trial No.				
		6	7	8	9	10
Intragranular						
1	Atorvastatin Calcium	80	80	80	80	80
2	Sodium hydroxide	--	--	--	--	--
3	Magnesium hydroxide	--	--	--	--	--
4	Calcium hydroxide	--	--	--	--	--
5	Sodium carbonate	30	30	20	30	30
6	Microcrystalline Cellulose	250	250	250	250	250
7	Lactose	290	285	290	280	320
8	Croscarmellose sodium	--	50	50	50	20
9	Sodium starch glycolate	--	--	--	--	--
10	Crospovidone XL-10	50	--	--	--	--
11	Hydroxypropyl Cellulose	30	30	30	40	30
12	Polysorbate 80	5	10	5	5	5
13	SLS	--	--	--	--	--
14	Purified Water	q.s.	q.s.	q.s.	q.s.	q.s.
Extragranular						
15	MCC	250	250	250	250	250
16	Aerosil	5	5	5	5	5
17	Magnesium Stearate	10	10	10	10	10
18	Sodium stearyl fumarate	--	--	--	--	--
Tablet Weight (mg)		1000	1000	1000	1000	1000
20	Opadry YS-1-7040	30	30	30	30	30
21	Purified water	q.s.	q.s.	q.s.	q.s.	q.s.
Tablet Weight (mg)		1030	1030	1030	1030	1030

Optimization using 2^3 factorial design

Table 3: Factorial design summary

Independent variables	Levels	
	-1	+1
X1: Amount of sodium carbonate(mg)	20	30
X2: Amount of Tween 80(mg)	5	10
X3: Amount of croscarmellose sodium (mg)	20	50

Table 4: Run order and response

Run no.	Sodium Carbonate (mg)	Tween 80 (mg)	Croscarmellose sodium (mg)	% Release In 30 mins.
1	20	5	50	95.1
2	20	5	20	93.6
3	30	5	20	96.8
4	20	10	50	96.5
5	30	10	50	98.9
6	20	10	20	94.6
7	30	10	20	97.5
8	30	5	50	98.1

Table 5: Composition of Factorial batches F1 to F 10

Sr. no.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Intragranular									
1	Atorvastatin calcium	80	80	80	80	80	80	80	80
2	Sodium carbonate	*20*	*20*	*30*	*20*	*30*	*20*	*30*	*30*
3	Polysorbate 80	*5*	*5*	*5*	*10*	*10*	*10*	*10*	*5*
4	Croscarmellose sodium	*50*	*20*	*20*	*50*	*50*	*20*	*20*	*50*
5	Microcrystalline cellulose	250	250	250	250	250	250	250	250
6	Lactose Monohydrate	300	330	320	295	285	325	315	290
7	Hydroxy propyl cellulose	30	30	30	30	30	30	30	30
8	Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Extragranular									
9	Microcrystalline cellulose	250	250	250	250	250	250	250	250
10	Colloidal silicon dioxide	5	5	5	5	5	5	5	5
11	Magnesium stearate	10	10	10	10	10	10	10	10
Total		1000	1000	1000	1000	1000	1000	1000	1000
Coating									
12	Opadry YS-1-7040	30	30	30	30	30	30	30	30
13	Purified water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Total		1030	1030	1030	1030	1030	1030	1030	1030

In-vitro dissolution test

Dissolution study of tablet performed in USP II (paddle) dissolution test apparatus (Electrolab TDT O8L) using 900ml of water as a dissolution media. The tablet was loaded into an each basket of dissolution apparatus; the temperature of dissolution media was maintained at 37°±0.5C with stirring speed of 75 rpm through out the study. Aliquots of dissolution media containing 5 ml of samples were withdrawn at time interval of 5, 10, 15, 30 minutes.

Exposure Study

Exposure study was done for finding the degradation pathways of drug formulation by exposing formulation to stress conditions like 80°C temperature for 2 days & in Autoclave for 15 min. at 121°C after these tests formulation was compared with Innovator formulation which was also kept in same conditions. If any measurable difference seen then that formulation was rejected otherwise selected.

Stability Study

Stability study was done by exposing the formulation to different conditions including stress conditions of temperature & pressure. Generally stability study was done at 40°C/75%RH (for 15 days, 1, 2 and 3 months), After that study was over formulation was checked for its physical & chemical parameters, if all parameters were present within the specification limit then that formulation was selected.

EXPERIMENTAL OBSERVATIONS

Table 6: pH Dependant Solubility Study of API (Atorvastatin Calcium)

Medium	Solubility (mg/ml)	Solubility (mg/ 250 ml)
pH 1.2 Buffer	0.016	4
pH 2.1 Buffer	1.05	262.5
pH 4.5 Buffer	0.048	12
pH 5.5 Buffer	0.367	91.75
pH 6.8 Buffer	1.245	311.25
pH 7.5 Buffer	0.996	249
0.1 N HCl	0.004	1
Water	0.368	92

Table 7: Sieve Analysis of API (Atorvastatin Calcium)

Sieve No. Used	Pore Size in µm	% Retained	Cumulative % Retained
20	850	1	1
30	600	4.94	5.94
60	250	29	34.94
80	180	36.56	71.5
100	150	0.5	72
BASE	NA	28	100

Table 8: Powder Flow Characterization of API (Atorvastatin Calcium)

Parameters	Observations
Angle of Repose	46.960°
Bulk Density	0.279 gm/ml
Tapped Density	0.383 gm/ml
Hauser's ratio	1.37
Compressibility Index	27.15%
LOD	-4.258%

Table 9: Pre Compression Parameters of preliminary Trials 1 to 10

Trial No.	Loss on drying (%w/w)		Bulk density (gm/ml)	Tap density (gm/ml)	Carr's index (%)	Hauser's ratio
	Dried Granules	Final blend				
1	2.22	2.53	0.41	0.55	25	1.34
2	2.13	2.69	0.42	0.57	26	1.35
3	2.42	2.99	0.44	0.59	25	1.34
4	2.50	2.87	0.445	0.549	20	1.23
5	2.62	2.95	0.441	0.573	22	1.29
6	2.42	2.70	0.41	0.52	21	1.26
7	2.36	2.93	0.462	0.562	17	1.21
8	2.48	2.78	0.45	0.58	22	1.28
9	2.26	2.56	0.45	0.55	18	1.22
10	2.19	2.41	0.43	0.54	20	1.26

Table 10: Post Compression Parameters of Trials 1 to 10 (Core Tablets)

Trial No.	Average wt.(mg)	Thickness (mm)	Hardness (N)	Disintegration time(min.)	Friability (% w/w)
1	990- 1010	6.10- 6.20	210-220	3-4	0.12
2	990- 1010	6.10- 6.20	212-225	2-3	0.134
3	990- 1010	6.10- 6.20	213-227	2-3	0.149
4	990- 1010	6.10- 6.20	200-219	2-3	0.138
5	990- 1010	6.10- 6.20	205-220	3-4	0.09
6	990- 1010	6.10- 6.20	202-224	3-4	0.18
7	990- 1010	6.10- 6.20	202-226	2-3	0.11
8	990- 1010	6.10- 6.20	206-227	3-4	0.15
9	990- 1010	6.10- 6.20	205-229	4-5	0.12
10	990- 1010	6.10- 6.20	200-225	9-10	0.19

Table 11: Post Compression Parameters of Trials 1 to 10 (Coated Tablets)

Trial No.	Average wt.(mg)	Thickness (mm)	Hardness (N)	Disintegration time(min.)
1	1028-1036	6.30- 6.50	230-245	4-5
2	1029-1035	6.29- 6.47	225-236	3-4
3	1029-1034	6.26- 6.45	241-250	3-4
4	1026-1033	6.30- 6.49	241-254	3-4
5	1028-1035	6.29- 6.48	235-245	4-5
6	1028-1036	6.28- 6.46	231-239	4-5
7	1030-1035	6.33- 6.47	240-254	2-3
8	1027-1033	6.36- 6.45	241-256	5-6
9	1026-1035	6.25- 6.42	240-246	6-7
10	1031-1034	6.31- 6.46	235-241	10-11

Table 12: Dissolution Profile of Different Trial 1 to 5

Time Point	Formulation					
	Innovator	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0	0	0	0	0	0	0
5	85.6	45.1	54.2	56.6	75.8	69.6
10	94.1	65.6	60.9	67.4	83.6	80.9
15	96.7	70.4	64.7	73.1	86.2	84.6
30	99.8	74.1	67.7	78.8	89.6	87.1
F2	**NA**	**26**	**25**	**30**	**49**	**43**

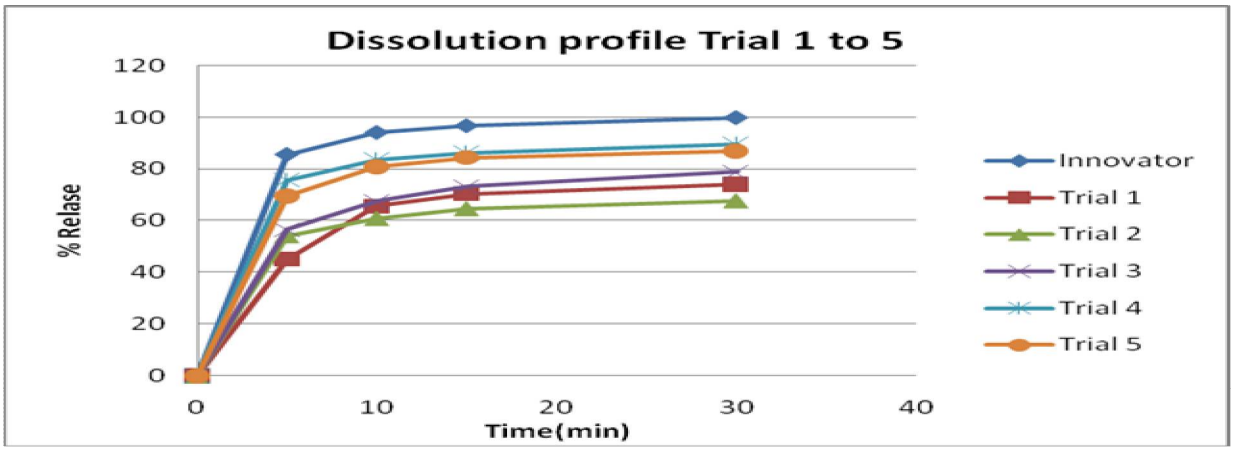

Fig. 1: Dissolution Profile of Different Trial 1 to 5 with innovator

Table 13: Dissolution Profile of Different Trial 5 to 10

Time Point	Formulation					
	Innovator	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10
0	0	0	0	0	0	0
5	85.6	65.7	84.1	69.5	76.8	75.2
10	94.1	78.1	92.9	79.8	85.6	84.2
15	96.7	83.6	94.8	85.9	89.2	88.1
30	99.8	85.8	98.9	87.9	93.1	91.1
F2	**NA**	**40**	**88**	**44**	**55**	**51**

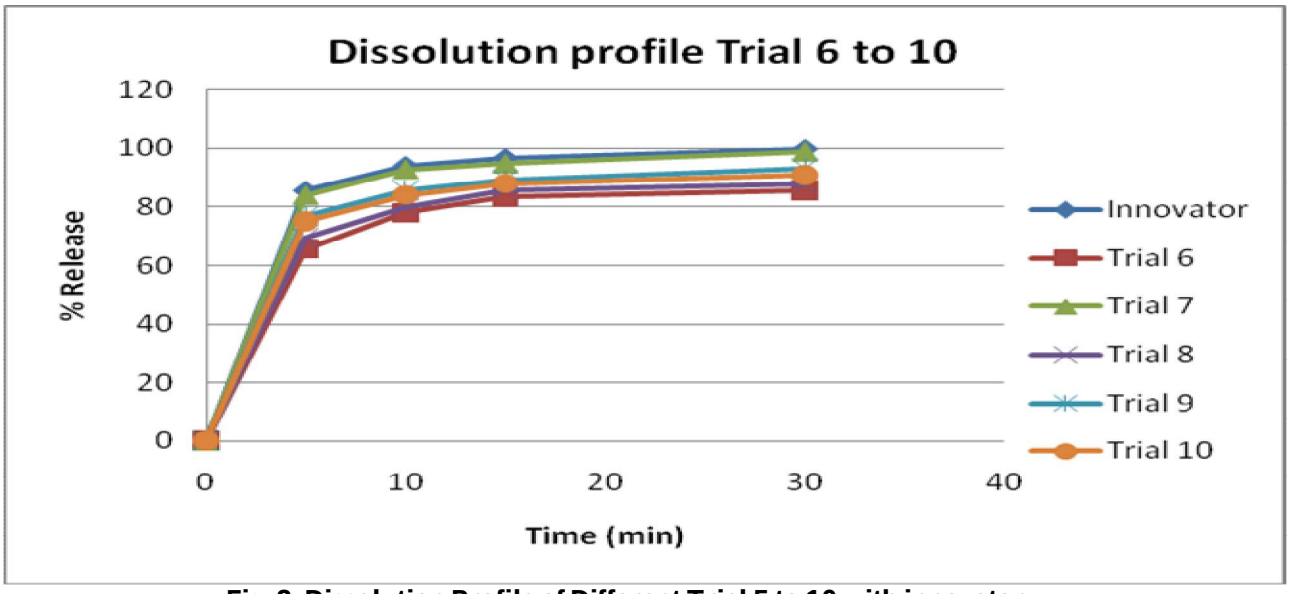

Fig. 2: Dissolution Profile of Different Trial 5 to 10 with innovator

Table 14: Pre Compression Parameters of Factorial Trials F1 to F8

Trial No.	Loss on drying (%w/w)		Bulk density (gm/ml)	Tap density (gm/ml)	Carr's index (%)	Hausner's ratio
	Dried Granules	Final blend				
F1	2.29	2.45	0.45	0.575	23	1.28
F2	2.33	2.50	0.44	0.55	20	1.25
F3	2.45	2.65	0.45	0.58	22	1.29
F4	2.41	2.68	0.435	0.541	20	1.24
F5	2.52	2.59	0.465	0.564	18	1.21
F6	2.26	2.39	0.44	0.57	23	1.30
F7	2.42	2.59	0.46	0.567	21	1.23
F8	2.63	2.78	0.45	0.57	21	1.27

Table 15: Post Compression Parameters of Factorial Trials F1 to F8 of Core Tablets

Trial No.	Average wt.(mg)	Thickness (mm)	Hardness (N)	Disintegration time(min.)	Friability (% w/w)
F2	990- 1010	6.10- 6.20	210-224	5-6	0.104
F3	990- 1010	6.10- 6.20	210-230	5-6	0.159
F4	990- 1010	6.10- 6.20	205-215	2-3	0.168
F5	990- 1010	6.10- 6.20	215-230	2-3	0.08
F6	990- 1010	6.10- 6.20	212-229	2-3	0.154
F7	990- 1010	6.10- 6.20	210-225	2-3	0.103
F8	990- 1010	6.10- 6.20	205-225	5-6	0.128

Table 16: Post Compression Parameters of Factorial Trials F1 to F8 of Coated Tablets

Trial No.	Average wt.(mg)	Thickness (mm)	Hardness (N)	Disintegration time(min.)
F2	1030-1034	6.30- 6.44	223-239	6-7
F3	1028-1033	6.30- 650	235-254	6-7
F4	1027-1031	6.25- 6.47	241-254	3-4
F5	1027-1032	6.30- 6.49	230-246	3-4
F6	1027-1034	6.30- 6.45	232-248	3-4
F7	1029-1034	6.33- 6.47	240-254	3-4
F8	1028-1033	6.35- 6.55	235-252	6-7

Table 17: Dissolution Profile of Factorial Trials F1 to F4

Time Point	Formulation				
	Innovator	F1	F2	F3	F4
0	0	0	0	0	0
5	85.6	79.1	77.5	80.9	81.2
10	94.1	89.6	86.9	91.1	91.1
15	96.7	91.5	88.7	92.3	92.5
30	99.8	95.1	93.6	96.8	96.5
F2	NA	63	56	70	70

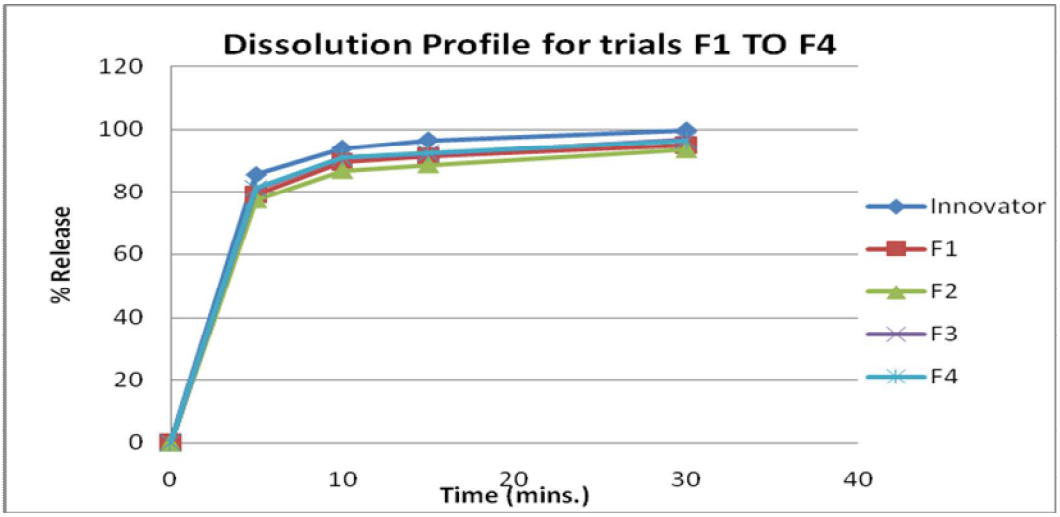

Fig. 3: Dissolution Profile of Different Trial F1 to F5

Table 18: Dissolution Profile of Factorial Trials F5 to F8

Time Point	Formulation				
	Innovator	F5	F6	F7	F8
0	0	0	0	0	0
5	85.6	84.6	78.5	82.1	84.6
10	94.1	94.5	89.5	92.4	91.5
15	96.7	96.5	89.9	95.5	95.8
30	99.8	98.9	94.6	97.5	98.1
F2	**NA**	**96**	**61**	**80**	**85**

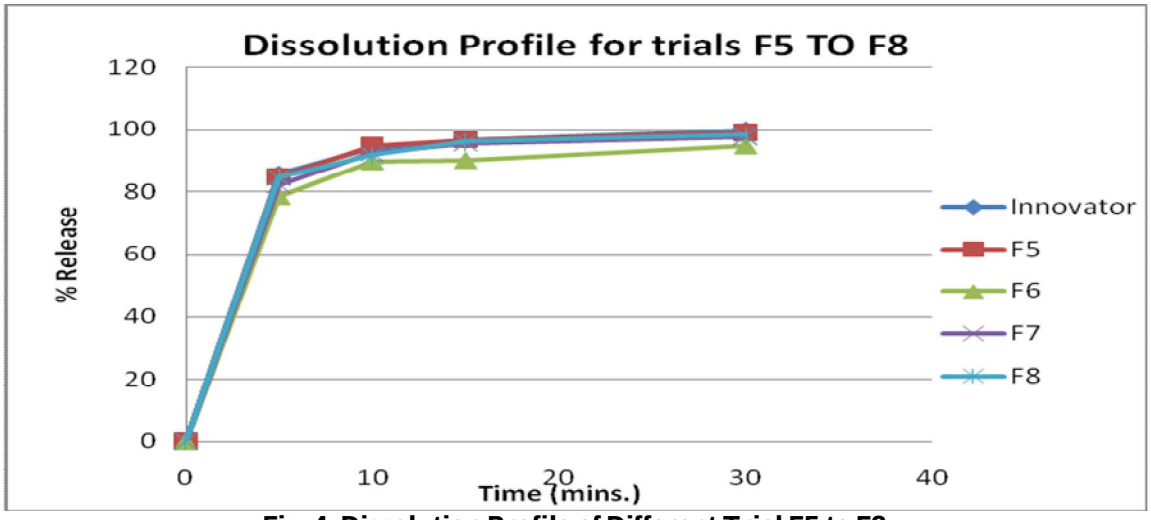

Fig. 4: Dissolution Profile of Different Trial F5 to F8

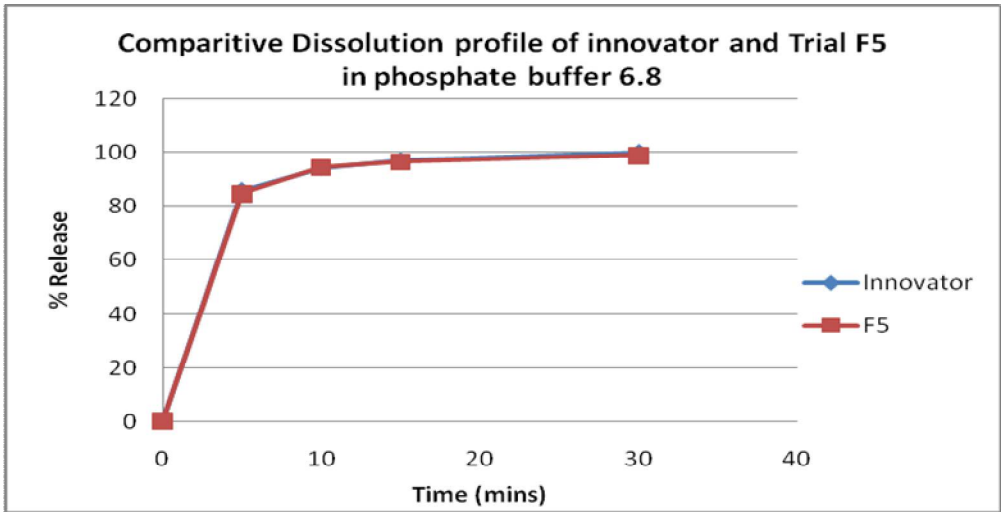

Fig. 5: Comparative dissolution Profile of Innovator and Trial F5 to F8

Statistical Analysis

Analysis of data by design expert software

The 2^3 Fatorial design was applied to study the effects of formulation variables such as amount of sodium carbonate, polysorbate 80 and croscarmellose sodium on the response factor as % Release in 30 mins.

Table 19: Summary of statistical design

Factor	Name	Units	Type	Actual values		Coded values	
				Low	High	Low	High
A	Sodium carbonate	Mg	Numerical	20	30	-1	+1
B	Polysorbate 80	Mg	Numerical	5	10	-1	+1
C	Croscarmellose sodium	Mg	Numerical	20	50	-1	+1

Table 20: Summary for response

Response	description	observations	Min	Max	Mean
Y1	% Release	8	93.6	98.9	96.39

The response data was analysed by using stat ease design expert software. The software gives statistical analysis of data. The interaction effect of these formulation factors on the drug release can be studied using the results of statistical analysis.

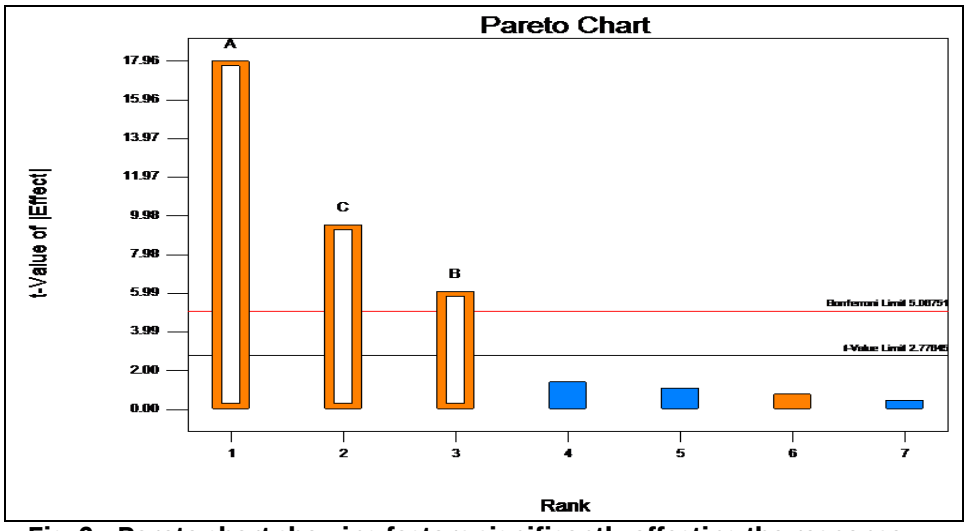

Fig. 6: Pareto chart showing factors significantly affecting the response

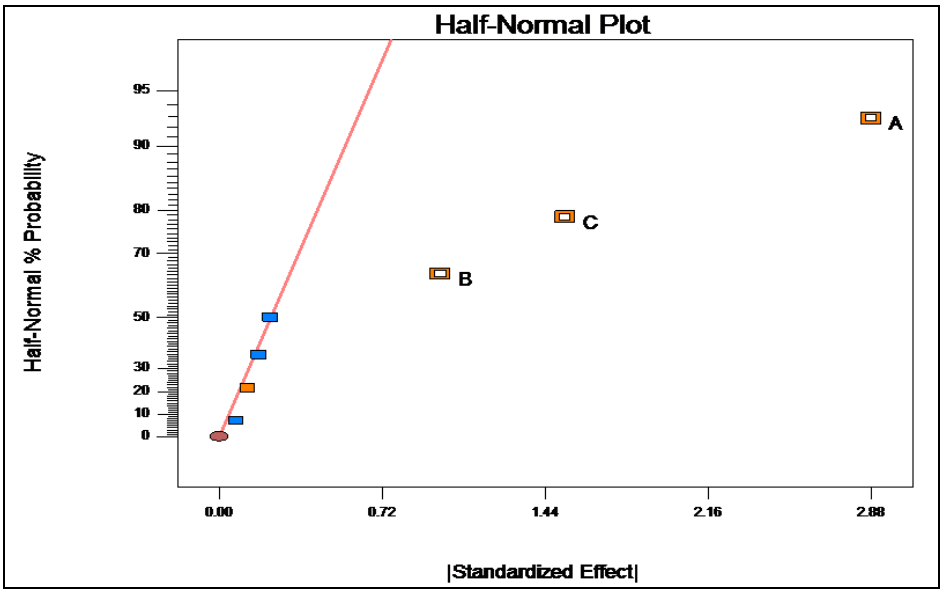

Fig. 7: Half normal plot - factors to the right side of line are significant

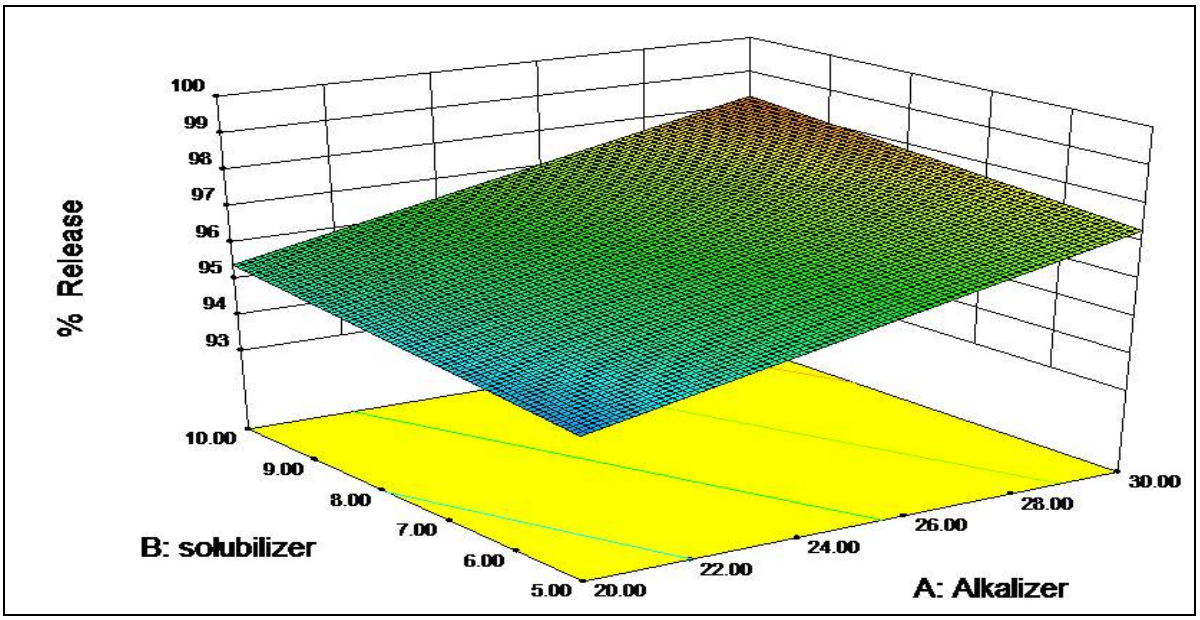

Fig. 8: 3D Graph showing the effect of formulation variables on % Release

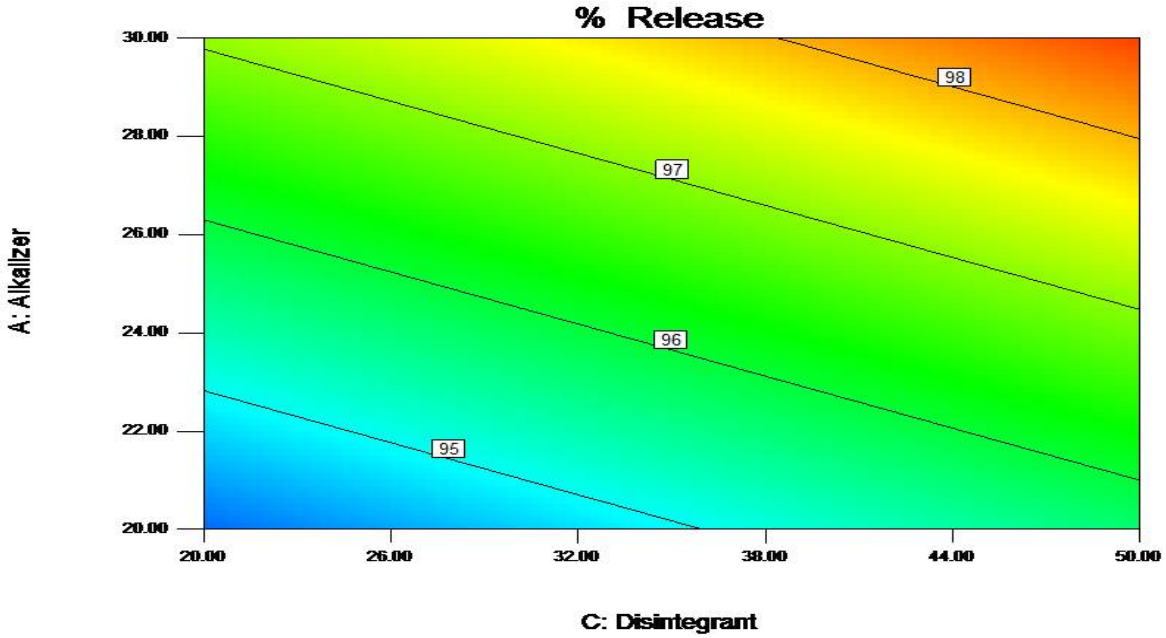

Fig. 9: Contour plot showing the effect of formulation variables on % Release

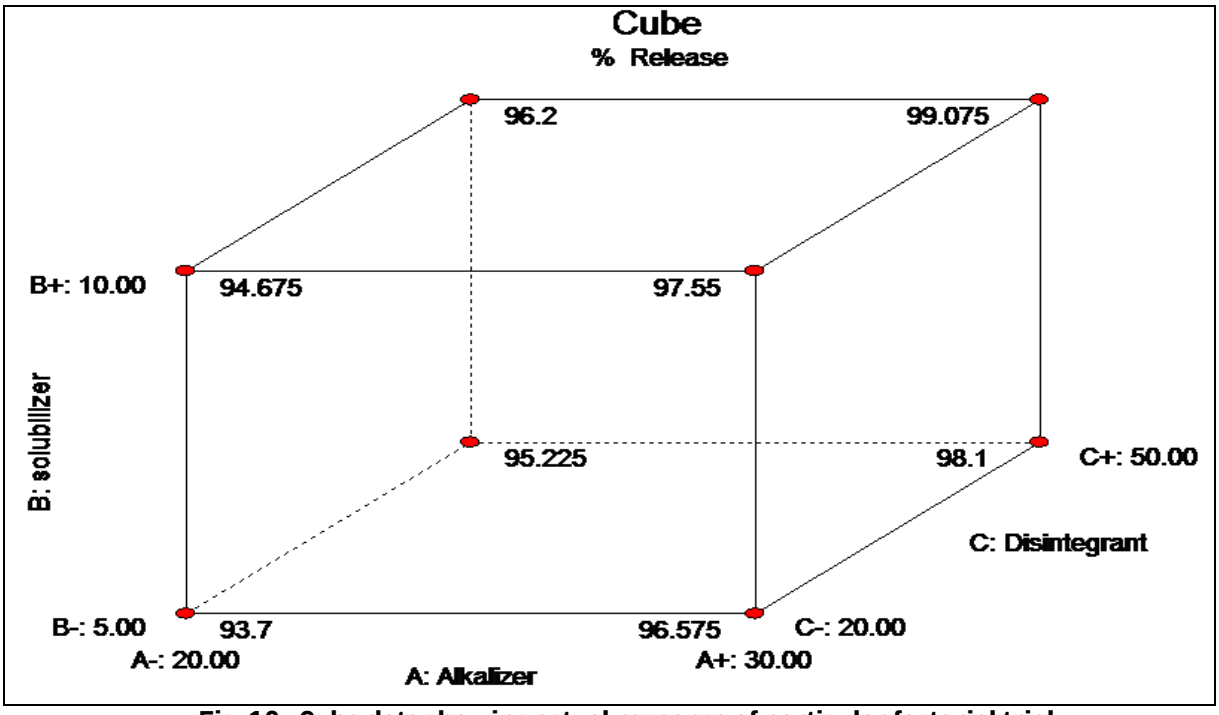

Fig. 10: Cube data showing actual response of particular factorial trial

Table 21: Statistical analysis of % Release

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	P Value	Significance
Model	23.08375	3	7.694583333	150.1382114	0.0001	Significant
A-Sodium carbonate	16.53125	1	16.53125	322.5609756	< 0.0001	Significant
B-Polysorbate 80	1.90125	1	1.90125	37.09756098	0.0037	Significant
C- Croscarmellose sodium	4.65125	1	4.65125	90.75609756	0.0007	Significant
Significant Residual	0.205	4	0.05125	150.1382114		
C or Total	23.28875	7		322.5609756		

The Model F-value of 150.14 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.
Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C are significant model terms.

Final equation in terms of coded factors
% Release = 96.39+ 1.44A + 0.49B + 0.76C

Final equation in terms of actual factors
% Release =
85.958 + 0.2875(Sodium carbonate) + 0.195(Polysorbate 80) + 0.0508(Croscarmellose sodium)

Positive Sign Before each coefficient indicates that with increasing the level of each factor increases the response

Table 22: Exposure Study of Final Trial F5

Storage condition→	Room Temperature		80°C		Autoclave	
Period →	Initial		2 Days (open)		At 121°C for 15 min	
Formulations →	Innovator	Trial F5	Innovator	Trial F5	Innovator	Trial F5
Parameters↓	Observations					
Physical Appearance	White	White	White	White	White	White
Hardness (N)	264 N	242	290	258	Not Applicable	
LOD (%)	7.48	7.54	6.10	6.22	12.27	12.97
D.T. (min.)	2-3	2-3	5-6	6-7	Not Applicable	
Assay (%)	99.24	99.56	94.25	93.72	95.85	94.99
Dissolution (at 30 min.)	99.6	98.9	97	96	Not Applicable	

Table 23: Stability Observations of Trials

Storage condition→		Room Temperature		40°C/75%RH						Specifications
Period→		Initial		1 Month		2 Months		3 Months		
Formulations →		Innovator	Trial F5	Innovator	Trial F5	Innovator	Trial F5	Innovator	Trial F5	
Parameters↓		Observations								
Physical Appearance		White	White	White	White	White	White	White	White	No change should observed
Hardness (N)		264	242	268	244	269	246	269	255	NLT 240N
LOD (%)		7.48	7.54	7.52	7.57	7.43	7.57	7.52	7.36	NMT 8.0%
D.T. (min.)		2-3	2-3	2-3	2-3	2-3	2-3	2-3	2-3	NMT 15 min.
Impurities	Highest unknown Impurity (%)	0.03	0.12	0.038	0.13	0.04	0.13	0.04	0.14	NMT 0.2%
	Total Impurity	0.93	1.63	0.94	1.65	1.02	1.70	1.09	1.70	NMT 3%
Assay (%)		99.24	100.7	101.8	100.72	100.12	101.36	99.25	101.32	95-105%
Dissolution (at 30 min)		99.6	98.9	98.9	97.7	98.6	97.25	98.1	96.9	NLT 85% in 30 min.

Table 24: Worst Case Study of Final Formulation

Critical Steps	Justification	Challenge	Parameters
Dry Mixing	Dry mixing impacts Content Uniformity	Samples from 10 different location	Content Uniformity
Granulation	Granulation impacts Drug Release & Physical Parameters of tablet	High, Optimum, Low	Micromeritics of Granules, Drug Release
Compression	Hardness Impacts Physical Parameters & Drug Release	200-250N, 250-300N, 300-350N	Friability, D.T, Drug Release, Thickness

Table 25: Dry Mixing Challenge

Stage	Parameter	High	Optimum	Low
Dry Mixing	Trial no.	A	B	C
	Batch Size (Tablets)	5000	5000	5000
	RMG (capacity)	25.0 L	25.0 L	25.0 L
Mixing in RMG	Impeller Time	15 min	10 min	5 min
	Impeller Speed	150rpm	150rpm	150rpm
	Chopper Time	NA	NA	NA
Environmental Conditions		25ºC/55%RH	25ºC/55%RH	25ºC/55%RH
Content Uniformity at Different Location (%)				
Upper Left		97.5	98.2	90.4
Upper Right		100.6	99.3	92.8
Upper Middle		99.6	101.3	95.6
Middle Left		98.6	98.7	104.6
Middle Right		93.5	97.9	108.8
Middle		103.8	99.9	99.9
Lower Left		100.2	100.1	95.7
Lower Right		96.8	101.5	91.7
Lower Middle		99.9	98.2	104.1
Composite Sample		99.8	100.3	102.4

Table 26: Granulation Challenge

Parameters	Parameter		High	Optimum	Low
Granulation	Trial no.		A	B	C
	Batch Size (tabs)		5000	5000	5000
	RMG (capacity)		25.0 L	25.0 L	25.0 L
Granulation In RMG	Impeller Time		12 min	7min	3 min
	Impeller Speed		150rpm	150rpm	150rpm
	Chopper Time		10 min	5 min	3 min
	Chopper Speed		2500rpm	2500rpm	2500rpm
Environmental Conditions			25ºC/55%RH	25ºC/55%RH	25ºC/55%RH
Micrometrics of lubricated granule	TD gm/ml		0.769	0.6	0.8
	BD gm/ml		0.625	0.483	0.615
	CI		18.73%	19.50%	23.13%
	HR		1.23	1.24	1.3
	LOD		3.24%	2.86%	2.99%
Sieve Analysis% Retained	20#		1.2	1.1	0.8
	30#		1.1	8.5	6.21
	40 #		19.25	3.5	7.5
	60 #		30.3	12	8.5
	80 #		6	3.5	13.06
	100 #		6.75	26	3.37
	BASE		34.7	45	60.56
Compression Parameters	Weight Variation		-0.60%	3.70%	0.90%
	Thickness		6.30-6.50mm	6.30-6.50mm	6.30-6.50mm
	Hardness (N)		240-260N	240-255N	220-240N
	Friability (100rtn's)		0.14%	0.16%	0.10%
	D.T (min.)		3-4min	2-3min	2-3min
DR Profile Innovator Vs Trial F5(pH 6.8 phosphate buffer)	*Minutes*	*Innovator*			
	5	85.6	72.9	83	95.9
	10	94.1	80.01	91	97.01
	15	96.7	87.25	95	98.8
	30	99.8	94.3	98	99
F2 Value			**47.92**	**79.47**	**62.76**

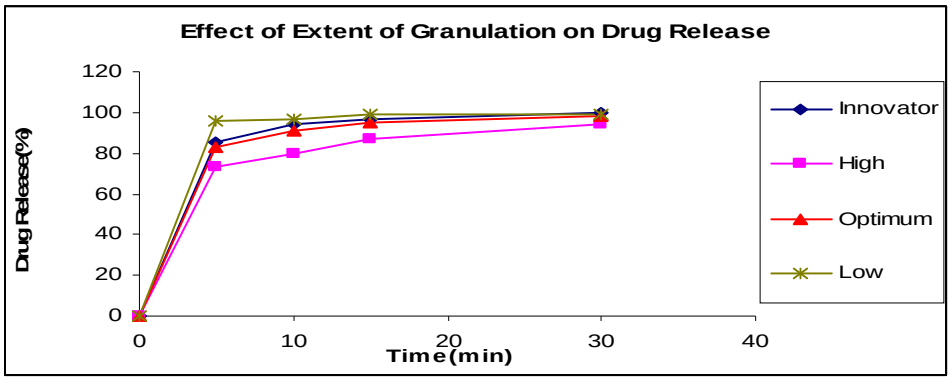

Fig. 11: Effect or Extent Granulation on Drug Release.

Table 27: Compression Challenge

Parameter	High	Optimum	Low
Trial no.	A	B	C
Machine Speed	18 rpm	18 rpm	18 rpm
Environmental Conditions	25ºC/55%RH	25ºC/55%RH	25ºC/55%RH
Hardness (N)	300-350N	250-300N	200-300N
Appearance	OK	OK	OK
Weight Variation	-1.125% to +4.5%	-1.625% to +4.0%	-1.89 to +1.50%
Thickness (mm)	6.30-6.41mm	6.40-6.54mm	6.55-6.70mm
Friability (100rtn's)	0.12%	0.10%	1.52%
DT (min)	5-6min	2-3min	1-2min
Dissolution Profile of All 3 Conditions			
Time Point (Min)	***Cumulative % Drug Release***		
	Innovator	*High*	*Optimum*	*Low*
5	85.6	60	83	85
10	94.1	74	88	92
15	96.7	87	94	98
30	99.8	95	99	100
F2 Value		**38.25**	**81.02**	**89.52**

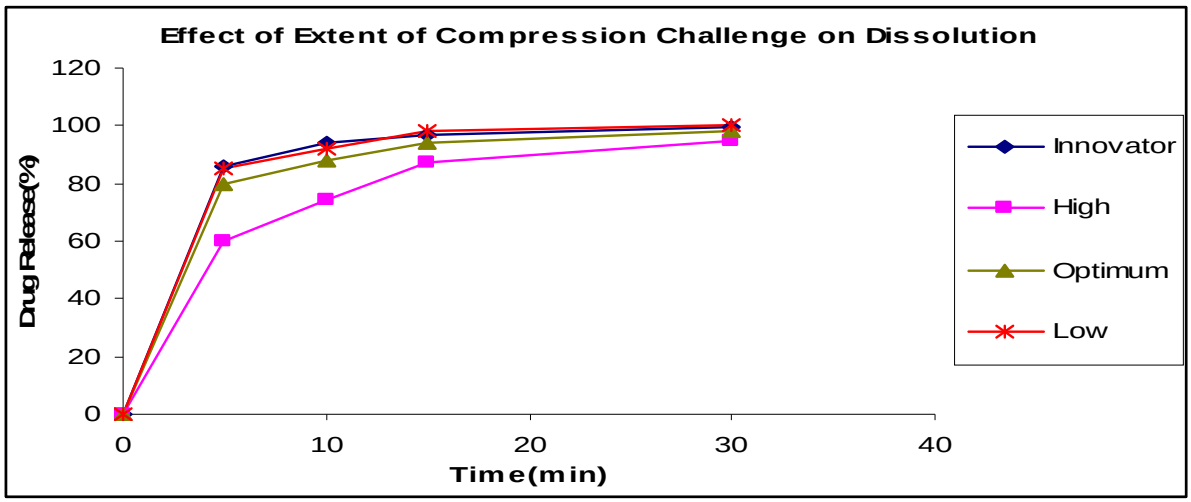

Fig. 11: Effect of Hardness on Drug Release

RESULT AND DISCUSSION

Preformulation Study

The present investigation was carried out to develop and formulate stable oral solid dosage form of class II drug Atorvastatin Calcium. The dosage form was developed as tablet and the tablets were prepared by using different excipients along with stabilizer.

Compatibility Study

From the results obtained for Drug-excipients compatibility study, it was found that the Candidate Drug is compatible with the respective excipients under evaluation based on physical observation. So chosen excipients can be used in the formulation trials.

API Characterization Study

pH Dependent Solubility Study

pH of Atorvastatin Calcium in 10% solution (water) found to slightly basic. The pH dependent solubility study carried out by wing of different pH buffer solutions ranging pH 1.2 (0.1 N HCl), pH 2.1 acid buffer, pH 4.5 acetate buffer, pH 5.5 acetate buffer and pH 6.8 phosphate buffer. Study shows solubility of Atorvastatin Calcium was more in pH 6.8 phosphate buffer i.e. 1.245 mg/ml. Therefore, pH 6.8 phosphate buffer was used as dissolution medium (It is also official in OGD).

Powder Flow Properties

The flow properties of pure drug were carried out and the results indicate that drug shows poor flow. So, it was decided to overcome this problem by converting them to granules which was done by wet granulation technique using appropriate binder to import good flow as well as compressibility.

Evaluation of Formulation Parameters

Evaluation was divided in mainly

- Pre compression Parameters and
- Post compression Parameters.

Pre Compression Parameters

Loss on Drying (LOD)

LOD of dried granules maintained in the level by drying at 105°C and optimize drying time for achieve LOD in particular limit.

Powder Flow Characteristics

The decision of choosing wet granulation method for granulation and choosing optimum amount of lubricant has eliminated the flow problem of powder blend and the flow properties of the blend were found to be satisfactory. The respective values of

- Bulk density in the range 0.41 – 0.467 gm/ml
- Tapped density in the range 0.52-0.59 gm/ml,
- Carr's Index ranging 18-26 and
- Hauser's ratio in the range 1.21-1.35

show the good flow characteristics.

Sieve Analysis

Sieve Analysis by Mechanical shaker shows there was good blend of fines and granules which result in good flow and reduces weight variation problems.

Post Compression Parameters

- **Weight Variation**
  Tablet weight was ranging 997-1009 mg for core tablets (Target wt – 1000mg/Tablet) which is less than 10% indicates that the variation in the weight of the tablets is within standard official limits. No weight variation was observed, as the blend characteristics were maintained throught the development process.

- **Thickness Evaluation**
  Thickness of tablets was observed by Vernier Caliper. The results obtained did not show any measurable deviation thickness of tablet.
- **Hardness Test**
  Hardness of the tablet was measured in 'Newton' unit in digital hardness tester. The hardness of tablets was found to be uniform within range 230-250 N for final trail.
- **Disintegration Test**
  Disintegration test was carried out in Electro lab (ED-2AL). Disintegration time for 6 tablets was found to be 2–3 min indicating that disintegration time within the specification limit.
- **Friability Test**
  The friability was carried out by using Roche Friabilator. The percentage friability of tablet was ranging 0.09% - 0.19% which was less than the standard limit of 1% indicates that the prepared tablets are mechanically stable.

Drug Content Uniformity

In each trials drug content was found to be ranging from 95% - 101.2% which is within the range of 93 – 105% for Atorvastatin. It indicates uniform distribution of drug in the tablets of each formulation.

***In-Vitro* Drug Release Studies**

Atorvastatin Calcium tablets were subjected to in vitro drug release studies pH 6.8 Phosphate Buffer for 30min. The drug release studies carried out in USP Dissolution Test Apparatus II (Paddle) using 900 ml of dissolution medium, maintained at 37°C ± 0.5°C.

DISCUSSION

Above results showed that Bulk density of API is very less therefore, to improve the flow of granules wet granulation technique was tried in **Trial 1.** Use of sodium hydroxide was done as alkalizer. This trial was carried out to check the feasibility.

Dissolution report of **Trial 1** shows that, overall drug release is so much less. It was also found that, the disintegration time is more though it is within limit. It took time to disintegrate the tablet into granules and therefore to release drug.

The **Trial 2** was taken by using the Magnesium hydroxide. The result shows that there is decrease in the drug release. But there is improvement in reduction of D.T.

In **Trial 3**, the use of calcium hydroxide to improve the drug release. The result shows that the drug release was slightly increased and not 100% at 30 min which may be due excess concentration of binder which was retarding the drug release.

Trial 4 was taken by adding Sodium carbonate. Drug release was increased upto an acceptable level. But still not 100 %.

Trial 5 was taken by using different disintegrant sodium starch glycolate. There was slight decrease in the drug release, and also DT was increase.

Trial 6 was taken by using crospovidone XL-10 as disintegrant and it was found that there is decrease in the DT but there was slight decrease in the drug release.

In **Trial 7,** Increase in the solubilizer concentration was done for the purpose of increasing drug release. The results shown the increase in drug release, decrease in DT, and also the blend properties were good.

Trial 8 was taken by decreasing the concentration of alkalizer but there was decrease in the drug release, flow properties were also slightly decreased.

Trial 9 was taken by increasing the concentration of binder it has shown the increase in DT slightly. And slight decrease in the drug release.

Trial 10 decrease in the disintegrant concentrantion caused the decreased in DT and drug release.

Factorial batches

All the factorial trials shown the good drug release but trial F5 was Found to be the best one because drug release was upto acceptable level,

Factorial design was done for the purpose of finding the possible interation of formulation factors affecting the response variable i.e. % Drug release in 30 mins. ANOVA is also established for analyzing the effects statistically. After analyzing the effects it was found that there is no significant interaction between the

sodium carbonate, polysorbate 80 and croscarmellose sodium which can affect the response factor. Also it was found that all three factors were showing the positive effect over the % drug release.

F_2 Value

Similarity factor (F2) was calculated between innovator formulation and in-house formulation. Similarity factor value in the range of 50-100 indicates that there is Similarity in the release profile of the formulations.
Among all Trials, **Trial 7** shows highest F2 value 88 in pH 6.8 phosphate buffer, and **Trial 9** and **Trial 10** shown the F2 value as 55 and 51 resply
All the factorial trials were shown F2 Values within 50 to 100 which is good, but from that **Trial F5** was showing the F2 value 96 which is very close to innovator.

Test for Related Substances

Test for related substances (degradation product and process related impurities) was carried out by HPLC method.
Individual impurity profile for trial F5 was established for initial and after 80°C for 2 days.

Exposure Study

Exposure studies were carried out of selected trial. In exposure study, in house formulation and innovator formulation was subjected to different environmental stress conditions like 80° for 2 days and in autoclave at 121°C for 15 min. The result shows similar behavior between our trial and innovator in different conditions.

Stability Study

The stability studies of final trial F5 was done for 3 months by packing in HDPE container in humidity chamber (40°C/75% RM)
The result given in table for 1 month, 2 months and 3 months shows that all parameters of formulation including physical parameters, impurity profile, content uniformity or dissolution profile were within specification limit. So it indicates optimized formulation is stable.

Worst Case Study

Worst case study for final formulation was performed to optimize the critical stages during the formulation process. In this case dry mixing, granulation and compression force were considered as critical stages which may cause problem if the set parameters vary.

Granulation Challenge

High granulation time shows slow initial release and is not satisfactory up to limit. Low granulation show some what faster release initially but matches with innovator drug release. So, high or low granulation may causes problem in formulation for our trial.
The optimized granulation time is said to be 7 min.

Compression Force Challenge

Compression force challenge study was carried out at three different compression forces. Their higher and lower extreme level was selected by considering good physical appearance at constant machine speed 18 rpm.
The dissolution profiles for optimum and low compression force were found to be satisfactory. But the higher compression force has retarded the initial drug release. Hence the calculated F2 value was less. Also the low compression force tablets were susceptible to friability. So, compression force does have effect on dissolution and strength of tablets. The optimized compression is said to be within the range of 250-300N.
In these different steps of formulation and development study of Atorvastatin tablet which were successfully accomplished and results were found satisfactory and Comparable with innovators formulation.

CONCLUSION

Before going to preformulation a detailed literature review was carried out to know about the innovator i.e. type of dosage form available in the market, its dimensions, shape and size, excipients used and all other physical parameters. The patent status of the drug is thoroughly monitored. Preformulation study and drug excipient compatibility study was done initially and the results obtained directed the way to method of

formulation. With the data obtained from Literature review, Preformulation and drug excipient compatibility study, prototype formulation trials were started for the highest dose of Atorvastatin calcium (80 mg) and optimized to get the final formula. Atorvastatin calcium (Amorphous) is highly susceptible to heat and it has very poor flow property so dry granulation and direct compression method was avoided. Granules were evaluated for tests such as LOD, Bulk density, Tapped density, Compressibility index and Hausner ratio and sieve analysis before compression. tablets were tested for weight variation, thickness, hardness, friability, and dissolution. In vitro dissolutions were performed and F1 and F2 values were calculated. Dissolution profile of final trial batch was matched perfectly with innovator and F2 value was found to be excellent. Also stability result of final trial batch was found to be excellent. Factorial design was used for the understanding of possible interaction amongst the excipients as sodium carbonate, polysorbate 80 and croscarmellose sodium. Results shown that there is no significant interaction between these factors.

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