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"FORMULATION, DEVELOPMENT AND EVALUATION OF ATORVASTATIN, ASPIRIN AND CLOPIDOGREL TABLETS IN CAPSULES FORM"

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ABSTRACT

The present work was done to formulation, development and evaluation of immediate release tablets of Atorvastatin calcium, Clopidogrel bisulphate and delayed release tablet of Aspirin in a capsule. The Aspirin and Clopidogrel bisulphate used as anti-platelate agent and Atorvastatin calcium is HMG Co-A reductase inhibitor which lowers the plasma concentration of cholesterol. The combination of drugs used in Chronotheraphy. The tablets were prepared by direct compression method. The pure drug and granules were evaluated for angle or repose, bulk density, tapped density, compressibility index, Hausner's ratio and characterized by FT-IR, DSC spectrum were evaluated. Immediate release Atorvastatin calcium and Clopidogrel bisulphate was prepared and using different concentration of Croscarmellose sodium and Crospovidone super-disintegrant by direct compression method. Delayed release Aspirin were prepared direct compression method. Atorvastatin calcium, Clopidogrel bisulphate were substituted for aqueous film coating to mask the spotting from both drugs and protection from light. Aspirin were substituted for enteric coating to mask the gastric problem in acid pH. The tablets coating as to avoid any interaction with three drugs. The core and coated tablets were subjected to weight variations, diameter, thickness, hardness, friability, disintegration time, drug content by assay and in-vitro dissolution studies were evaluated. All the tablet formulations showed acceptable pharmaceutical properties. From dissolution profile of ATF7 Atorvastatin calcium gives the release within 30 minutes required value i.e - 103.12% respectively. CLT7 Clopidogrel bisulphate gives the release

within 30 minutes required value i.e – 102.14% respectively. AT4 Aspirin gives the release with 2 hrs required value I,e – 104.21% respectively. The optimized formulations showed the f2 (f2=64, 62 & 65) values. The similarity factor f2 was applied between the optimized formulations and the theoretical dissolution profile. The result of stability studies of batch ATF7, CT7 and AT4 indicate that it is stable at 40°C / 75% ± 0.5 % relative humidity as there was no significant differences observe for physical, weight variations, diameter, thickness, hardness, friability, disintegration time, drug content by assay and in-vitro dissolution studies were evaluated. The formulations were found to be stable for after 3 months of accelerated stability studies.

KEYWORDS: Atorvastatin calcium, Aspirin, Clopidogrel bisulphate, Chronotheraphy.

INTRODUCTION

The convenient oral drug delivery has been known for decades is the most widely utilized route of administration among all the routes. It remains the preferred route of administration in the discovery and development of new drug candidates. The popularity of oral route is attributed to patient acceptance, ease of administration, accurate dosing, cost effective manufacturing methods and generally improve the shelf life of the product.[1] Immediate release tablets are designed to disintegrate and release the drug in absence of any controlling features such as coating or other formulation techniques. Despite a rising interest in controlled-release drug delivery systems, the most common tablets are those intended to be swallowed whole, disintegrating and releasing their medicaments rapidly in the gastrointestinal tract. A Disintegrant is a substance in a tablet formulation that enables the tablet to break up into smaller fragments upon contact with gastrointestinal fluids. Such a rapid rupture of the tablet matrix increases the surface area of the tablet particles, Thereby increasing the rate of absorption of the active ingredient and producing the desired therapeutic action.[2] The proper choice of Disintegrant and its consistency of performance are critical to formulation development of immediate release tablets. In the past, starch was one of the most widely used, Inexpensive, and effective tablet disintegrants. A high concentration of starch is required to bring about effective disintegration. Scientist's search for disintegrating agents with efficient disintegrating properties at relatively low concentrations has led to the development of some new compounds with excellent disintegrating properties is called superdisintegrant. These systems are based on pH dependent drug release mechanism of similar to conventional enteric-coated formulations,

but they differ in target site for delivery and therefore type of enteric polymers. Most commonly used polymers are derivatives of acrylic acid and cellulose. These polymers have ability to withstand from low pH end several hours. In pharmaceutical practice several approaches exist for administration of drug to the patient. If the drug is given in conventional dosage form, it has to be administered several times to produce designed therapeutic effect. Because of frequent dosing fluctuation in plasma drug level occur. Fluctuation resulting from the conventional dosage form it minimize by delayed release dosage form. Drug concentration can be controlled within narrow therapeutic range by use delayed release system. The delayed release tablets of Aspirin were prepared by using direct compression method. Different formulations were prepared with varying concentration of disintegrating agent and lubricant and optimized formulation was to be found in this present study. This delayed release of the optimized formulation was expected to increase the bioavailability. In pharmaceutical practice several approaches exist for administration of drug to the patient. If the drug is given in conventional dosage form, it has to be administered several times to produce designed therapeutic effect. Because of frequent dosing fluctuation in plasma drug level occur. Fluctuation resulting from the conventional dosage form it minimize by delayed release dosage form. Drug concentration can be controlled within narrow therapeutic range by use delayed release system. Combination of three drugs gives synergistic action to reduce the myocardial infarction. So that such combination tablet prepared.[3-5]

MATERIALS AND METHODS

Materials

Atorvastatin Calcium, Aspirin and Clopidogrel bisulphate was received as a gift sample from Caplin Point Research Laboratory. Calcium Carbonate, Lactose DCL-11, MCC pH-102 and Mannitol (DC grate) was gifted by FMC Bio-polymer (India). Croscarmellose sodium, Cross povidone and Sodium Starch Glycolate and Sodium Lauryl Sulphate was gifted by Chetan & Chetan (India). Purified Talc, Aerosil, Calcium Stearate and magnesium stearate was gifted by Cabot Sanmer (India).

IMPURITYPROFILE

Single and total impurities present in Active pharmaceutical ingredient (API) were measured by HPLC. The results are shown in Table. No: 18-20 & Figure. No: 24-6.

ASSAY: In house HPLC based method of assay was developed or both API's. The sample of drug solution was prepared and suitably diluted with mobile phase. Each sample was run and

chromatograms were obtained. The concentration of drug was calculated as.

Concentration of sample=Peak area of sample x Concentration of reference standard Peak area of reference standard

The results are shown in Table. No:18-20 & Figure. No: 4-6

SPECTRAL IDENTIFICATION[6]

Excipients are integral components of almost all pharmaceutical dosage forms. The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients, which are added to facilitate administration, to promote the consistent release and bioavailability of the drug and protect it from degradation.

Infra red spectroscopy 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. The results are shown in Figure. No: 7-12.

DIFFERENTIAL SCANNING CALORIMETER STUDIES [7]

The sample of plain drug was scanned in beginning. Than physical mixtures of drug with excipients kept for one month, were scanned. Both the drug was scanned from50 $^{\circ}$C to 250 $^{\circ}$C. The results are shown in Figure. No: 13-16

COMPATIBILITY STUDIES [8]

Drugs–Excipients compatibility was performed using HPLC method and by physical observation. The results are shown in Table. No: 21-24.

Table.no:1 Innovator Tablet Parameters to be Evaluated (Atorvastatin Calcium, Aspirin and Clopidogrel Bisulphate)

S.NO.	PARAMETERS EVALUATED FOR
1	Strength
2	Label Claim
3	Tablet Color
4	Tablet Shape
5	Description
6	Dimensions
7	Average Weight
8	Hardness

9	Dissolution Study
10	Uniformity Of Dosage Units
11	Impurity-A
12	Any Other Impurity
13	Total Impurities
14	Assay

The results are shown in Table. No: 25-31.

PREFORMULATION STUDIES OF PURE DRUG AND EXCIPIENTS [9-10]:

Preformulation study relates to pharmaceutical and analytical investigation carried out proceeding and supporting formulation development efforts of the dosage form of the drug substance. Preformulation yields basic knowledge necessary to develop suitable formulation for the toxicological use. It gives information needed to define the nature of the drug substance and provide frame work for the drug combination with pharmaceutical recipients in the dosage form. Hence, the following Preformulation studies were performed on the obtained sample of drug.

The results are shown in Table. No: 32-34.

TABLET MANUFACTURING

Manufacturing of Atorvastatin Calcium

Manufacturing Procedure - Atorvastatin calcium tablets using direct compression

(A) The corresponding amount of drug (Atorvastatin Calcium) was screened using screen #40, and Lactose DCL-11 accurately weighted & screened using screen # 40. The screened powder was transferred into the poly bag in 1:10 ratio and mixed for 3 minutes. Pass it every time through #40, further mix for 2 minutes. Geometric mixing with remaining Lactose DCL is done in same proportion. MCC pH-102 pass through # 40, mix well for 3 minutes with A & pass it through #40. Calcium Carbonate pass through screen #40, was transferred into the cage blender and mixed for 5 minutes. Super-disintegrants and lubricants is accurately weighed & screen # 60 is then mixed in the poly bag or cage blender for 3 minutes. The mixture was compressed into tablets using an instrumented tablet press with 6mm punches for 100mg weight at 7-8kp hardness and tablets were collected during compression for in-process testing (weight, friability and hardness).

Table. No:2 Formulation of Atorvastatin Calcium Tablet

Batch. No	ATF1	ATF2	ATF3	ATF4	ATF5	ATF6	ATF7
Ingredient	**mg/tablet**						
Atorvastatin Calcium	10.35	10.35	10.35	10.35	10.35	10.35	10.35
Calcium Carbonate	11	11	11	11	11	11	11
Lactose DCL -11	54	52	50	54	52	50	44
MCC pH-102	19.85	19.85	19.85	19.85	19.85	19.85	19.85
Croscarmellose Sodium	2	4	6	-	-	-	6
Cross Povidone	-	-	-	2	4	6	6
Talc	1	1	1	1	1	1	1
Sodium Lauryl Sulphate	1	1	1	1	1	1	1
Calcium Stearate	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Total	100mg	100mg	100mg	100mg	100mg	100mg	100mg

Table. No:3 Film Coating For Atorvastatin Calcium IR Tablets

S.NO	Ingredients	Quantity(mg)
Ingredients	**For One tablet**	
1	Erythrocin Supra	0.05
2	Protectab – HP	1.8
3	Polysorbate – 80	0.8
4	Purified Water	Qs

Table. No:4 Optimized Parameters for Film Coating for Atorvastatin IR Tablets

Conditions	Pre-heating	Coating	Drying
Inlet air temperature (°C)	55-60	60-65	50
Product temperature (°C)	55-60	50-55	55-60
Outlet air temperature (°C)	35-60	55-60	50-55
Spray rate (ml/min)	-	1-2	-
Atomizing air pressure (psi)	-	*20*	
Pan speed (rpm)	35-37	35-37	35-37

Manufacturing Procedure

Manufacturing Procedure - Aspirin tablets using direct compression: The corresponding amount of drug (Aspirin) was screened using screen #40, and MCC pH-102 pass through #40, mix well for 3 minutes. Super disintegrant was pass through #80, mix well for 3 minutes. Aerosil was pass through #30 and Magnesium stearate was pass through #60, and then mixed in the poly bag or cage blender for 3 minutes. The mixture was compressed into tablets using an instrumented tablet press with 6mm punches for 100mg weight at 7-8kp hardness and tablets were collected during compression for in-process testing (weight, friability and hardness).

Table. No:5 Formulation of Aspirin Tablets

Batch. No	AF1	AF2	AF3	AF4
Ingredients	**mg/tablet**			
Aspirin	75	75	75	75
MCC pH-102	20.5	18.5	16.5	14.5
Sodium Starch Glycolate	2	4	6	8
Talc	1	1	1	1
Aerosil	0.5	0.5	0.5	0.5
Magnesium Stearate	1	1	1	1
Total	100mg	100mg	100mg	100mg

Table. No:6 Sub Coating For Aspirin DR Tablets

S.NO	Ingredients	Quantity(mg)
Ingredients	**For One tablet**	
1	Ethylcellulose	2.0
2	Isopropyl Alcohol	Qs
3	Methylene Chloride	Qs

Table. No:7 Enteric Coating For Aspirin DR Tablets

S.NO	Ingredients	Quantity(mg)
Ingredients	**For One tablet**	
1	Kolicoat MAEP – 100	12.0
2	Sunset Yellow Lake	1.2
3	Purified Talc	1.0
4	Titanium Dioxide	1.0
5	Diethyl Phthalate	5.0
6	Isopropyl Alcohol	Qs
7	Methylene Chloride	Qs

Table. No:8 Optimized Parameters for Sub Coating for Aspirin DR Tablets

Conditions	Pre-heating	Coating	Drying
Inlet air temperature (°C)	55-60	60-65	50
Product temperature (°C)	55-60	50-55	55-60
Outlet air temperature (°C)	35-60	55-60	50-55
Spray rate (ml/min)	-	1-1.5	-
Atomizing air pressure (psi)	-	*20*	
Pan speed (rpm)	55-57	55-57	55-57

Table. No:9 Optimized Parameters for Enteric Coating for Aspirin DR Tablets

Conditions	Pre-heating	Coating	Drying
Inlet air temperature (°C)	55-60	60-65	50
Product temperature (°C)	55-60	50-55	55-60
Outlet air temperature (°C)	35-60	55-60	50-55
Spray rate (ml/min)	-	2-3	-
Atomizing air pressure (psi)	-	*30*	
Pan speed (rpm)	55-57	55-57	55-57

MANUFACTURING OF CLOPIDOGREL BISULPHATE IR TABLETS

Manufacturing Procedure

Clopidogrel bisulphate tablets using direct compression: The corresponding amount of drug (Clopidogrel bisulphate) was screened using screen #40, and MCC pH-102 pass through #40, mix well for 3 minutes. Mannitol DC grate was pass through #30, mix well for 3 minutes. Super disintegrants was pass through #80, mix well for 3 minutes. Aerosil was pass through #30 and Magnesium stearate was pass through #60 and then mixed in the poly bag or cage blender for 3 minutes. The mixture was compressed into tablets using an instrumented tablet press with 6mm punches for 100mg weight at 7-8kp hardness and tablets were collected during compression for in-process testing (weight, friability and hardness).

Table. No:10 Formulation of Clopidogrel Bisulphate Tablets

Batch. No	**CF1**	**CF2**	**CF3**	**CF4**	**CF5**	**CF6**	**CF7**	**CF8**
Ingredient	**mg/tablet**							
Clopidogrel Bisulphate	97.875	97.875	97.875	97.875	97.875	97.875	97.875	97.875
MCC pH-102	12.725	11.225	9.225	12.725	11.225	9.225	8.225	6.225
Mannitol (DC Grate)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	13.0
Croscarmellose Sodium	1.5	3	5	-	-	-	3	5
Cross povidone	-	-	-	1.5	3	5	3	5
Colloidal Silicon Dioxide	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Talc	1	1	1	1	1	1	1	1
Magnesium Stearate	1	1	1	1	1	1	1	1
Total	130mg	130mg	130mg	130mg	130mg	130mg	130mg	130mg

Table. No:11 Film Coating For Clopidogrel Bisulphate IR Tablets

S.NO	**Ingredients**	**Quantity(mg)**
Ingredients	**For One tablet**	
1	Iron oxide Supra	0.05
2	Protectab – HP	1.8
3	Polysorbate – 80	0.8
4	Purified Water	Qs

Table.No:12Optimized Parameters for Film Coating for Clopidogrel Bisulphate IR Tablets

Conditions	**Pre-heating**	**Coating**	**Drying**
Inlet air temperature (°C)	55-60	60-65	50
Product temperature (°C)	55-60	50-55	55-60
Outlet air temperature (°C)	35-60	55-60	50-55
Spray rate (ml/min)	-	1-2	-
Atomizing air pressure (psi)	-	*20*	
Pan speed (rpm)	35-37	35-37	35-37

POST COMPRESSION PARAMETERS [11-14]

a) Weight Variation Test: Twenty tablets were selected randomly from each batch and weighed individually to check for weight variation. A little variation was allowed in the weight of a tablet according to U.S. Pharmacopoeia. The following percentage deviation in weight variation was allowed.

Average weight of a tablet	Percentage deviation
130 mg or less	± 10
>130 mg and <324 mg	± 7.5
324mg or more	± 5

The results are shown in Table. No: 35-37.

b) Tablet Dimensions: Thickness and diameter were measured using calibrated Vernier calipers. Five tablets of each formulation were picked randomly and thickness and diameter was measured individually.

The results are shown in Table. No: 35-37.

c) Thickness: The thickness of the tablets was determined by Vernier calipers. Five tablets from each batch were used and the average values were calculated. The results are shown in Table. No: 15-16.

d) Hardness: Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the tablets was determined using Monsanto hardness tester. It is expressed in kg/cm2. Five tablets were randomly picked and hardness of the tablets was determined. The results are shown in Table. No: 35-37.

e) Friability test: The friability of tablets was determined by using Roche friabilator. It is expressed in percentage (%). Twenty tablets were initially weighed (Wt) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 100revolutions. The tablets were weighed again (WF). The % friability was then calculated by-

$$\%F = \frac{W\ (initial)-W\ (final)}{W\ (initial)} \times 100$$

The results are shown in Table. No: 35-37.

f) Disintegration test: The disintegration time for immediate release layer was determined using the disintegration apparatus. One tablet was placed in each of six tubes placed in a beaker containing 1000 ml of purified water maintained at 37 ± 20 C and the apparatus was operated. The time taken for the tablets to disintegrate and pass through the mesh was noted. The results are shown in Table. No: 35-37.

METHOD OF ANALYSIS [15-19]

IN VITRO DISSOLUTION STUDY

For Atorvastatin Calcium

Apparatus : Dissolution Apparatus USP Type I (Paddle)
Medium : 6.8 Phosphate buffer
Medium Volume : 900ml
Speed : 75 RPM
Time : 30 Minutes
Time intervals : 5, 10, 15, 20& 30 Minutes
Temperature : 37 ± 0.5°C.

Chromatographic Conditions

Apparatus : High Performance Liquid Chromatography system (HPLC)
Column : C18, 4.6mm × 250 cm. 5µ
Wavelength : 246nm
Detector : UV/PDA
Injection volume : 20µl.
Flow rate : 1.0ml/min
Sample cooler temp. : 30°C
Run Time : 10 minutes
Elution : Isocratic

Calculations

Dissolution of Atorvastatin in mg/tablet

$$= \frac{\text{SPL Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{100} \times \frac{5}{50} \times \frac{5}{20} \times \frac{900}{1\ \text{Tablet}} \times \frac{99.75}{100} \times \frac{0.9653}{100} \times 100$$

The results are shown in Table. No: 38-39 & Figure. No: 17-18.

For Aspirin

Apparatus : Dissolution Apparatus USP Type II (Basket)

Medium : 0.1N Hydrochloric acid

Volume : 1000mL

Speed : 100 RPM

Time :2 Hours.

Time intervals : 30 Minutes, 1 & 2 Hours.

Temperature : 37 ± 0.5°C.

Chromatographic Conditions

Apparatus : High Performance Liquid Chromatography system (HPLC)

Column : C18, 150 × 4.6, 5µ (Inertsil)

Wavelength : 265nm

Detector : UV/PDA

Injection volume : 20µl.

Flow rate : 1.0ml/min

Sample cooler temp. : 30°C

Run Time : 10 minutes

Elution : Isocratic

Calculations

From the Standard and Sample Chromatogram, calculated the percentage of the labeled amount of Aspirin ($C_9H_8O_4$) percentage release of the Tablets taken by the following formula,

Dissolution of Aspirin in mg/tablet

$$= \frac{\text{SPL Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{100} \times \frac{5}{50} \times \frac{1000}{1\ \text{Tablet}} \times \frac{99.85}{100} \times \frac{1}{100} \times 100$$

The results are shown in Table. No:40 Figure. No: 19

BUFFER MEIDA

Apparatus : Dissolution Apparatus USP Type I (Paddle)

Medium : Buffer pH-6.8

Volume : 1000mL

Speed : 100 RPM
Time : 90 Minutes.
Time intervals : 15, 30, 45, 60 & 90 Minutes.
Temperature : 37 ± 0.5°C.

Chromatographic Conditions

Apparatus : High Performance Liquid Chromatography system (HPLC)
Column : C18, 150 × 4.6, 5µ (Inertsil)
Wavelength : 265nm
Detector : UV/PDA
Injection volume : 20µl.
Flow rate : 1.0ml/min
Sample cooler temp. : 30°C
Run Time : 15 minutes
Elution : Isocratic

Calculations: From the Standard and Sample Chromatogram, calculated the percentage of the labeled amount of Aspirin (C9H8O4) percentage release of the Tablets taken by the following formula,

Dissolution of Aspirin in mg/tablet

$$= \frac{\text{SPL Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{100} \times \frac{5}{50} \times \frac{1000}{1\ \text{Tablet}} \times \frac{99.75}{100} \times \frac{1.305}{100} \times 100$$

The results are shown in Table. No:41-42 & Figure. No: 20-21.

For Clopidogrel Bisulphate

Dissolution study of Immediate release of different tablet formulations and marked tablets were carried out separately.

Apparatus : Dissolution Apparatus USP Type I (Paddle)
Medium : pH – 2 Hydrochloric acid Buffer
Medium Volume : 1000mL
Speed : 75 RPM
Time : 30 Minutes
Time intervals : 5, 10, 15, 20 & 30 Minutes
Temperature : 37 ± 0.5°C.

Calculation

$$= \frac{\text{CCSpl Abs}}{\text{STD Abs}} \times \frac{\text{STD wt}}{200} \times \frac{5}{250} \times \frac{1000}{1} \times \frac{50}{5} \times \frac{100}{\text{Label Claim}} \times 100$$

The results are shown in Table. No:43-44 Figure. No: 22-23.

ASSAY

For Atorvastatin Calcium

Chromatographic Conditions

Apparatus : High Performance Liquid Chromatography system (HPLC)

Column : C18, 4.6mm × 250 cm. 5µ

Wavelength : 246nm

Detector : UV/PDA

Injection volume : 20µl.

Flow rate : 1.0ml/min

Sample cooler temp. : 30°C

Run Time **:** 10 minutes

Elution **:** Isocratic

Calculations

Assay of Atorvastatin in mg/tablet

$$= \frac{\text{SPL Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{100} \times \frac{1}{50} \times \frac{50}{\text{SPLwt1}} \times \frac{50}{100} \times \frac{99.56}{100} \times \frac{0.9653}{1} \times 100$$

The results are shown in Table. No: 45.

For Aspirin

Chromatographic Conditions

Apparatus : High Performance Liquid Chromatography system (HPLC)

Column : C18, 150 × 4.6, 5µ (Inertsil)

Wavelength : 265nm

Detector : UV/PDA

Injection volume : 20µl.

Flow rate : 1.0ml/min

Sample cooler temp. : 30°C

Run Time : 10 minutes

Elution : Isocratic

Calculations

From the Standard and Sample Chromatogram, calculated the percentage of the labeled amount of Aspirin percentage release of the Tablets taken by the following formula,

Assay of Aspirin in mg/tablet

$$= \frac{\text{SPL Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{100} \times \frac{5}{50} \times \frac{100}{\text{SPLwt}} \times \frac{50}{5} \times \frac{99.76}{100} \times \frac{1}{100} \times 100$$

The results are shown in Table. No:46.

For Clopidogrel Bisulphate

Chromatographic Conditions

Apparatus : High Performance Liquid Chromatography system (HPLC)

Column : C18, 250 × 4.6, 5µ (Inertsil)

Wavelength : 240nm

Detector : UV/PDA

Injection volume : 20µl.

Flow rate : 1.5ml/min

Sample cooler temp. : Ambient(25°C)

Run Time : 10 minutes

Elution : Isocratic

Calculations

$$= \frac{\text{SPL Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{100} \times \frac{5}{50} \times \frac{100}{\text{SPLwt}} \times \frac{50}{5} \times \frac{99.76}{100} \times \frac{1.305}{100} \times 100$$

The results are shown in Table. No:47

STABILITY STUDIES [20-22]

Stability testing forms an integral part of formulation development. It is important to assess the effect of temperature and humidity on stability of drug and in-vitro drug release rate. It helps to generate information for predicting the shelf life of the product and recommended storage conditions. Stability data is required to be submitted as part of the dossier submitted to the regulatory agencies.

Protocol For stability studies

Formulation was selected on the basis of in-vitro drug release profile which was comparable to that of the IR or DR formulation under reference i.e. optimized formula for both Atorvastatin, Clopidogrel& Aspirin batches. Optimized formula Batch.no:ATF7 for Atorvastatin IR (10mg), in Alu Blister Pack. Optimized formula Batch.no:AT4for Aspirin DR(75mg),in Alu Blister Pack. Optimized formula Batch.no: CT7 for Clopidogrel bisulphate IR (75mg), in strip pack was tested for stability under two conditions for a period of three months. The conditions for stability are as mentioned in Table. No: 13.

Table. No:13 Stability Condition For Atorvastatin, Clopidogrel & Aspirin Tablet

Study	Storage condition	Time Period Covered
Room Temperature (RT)	25℃ ± 2℃/60%RH± 5%RH	3months **Testing :**If accelerated condition tablet is passed
Accelerated	40℃ ± 2℃/75%RH± 5%RH	3 months Testing:1,2,3month

- These were evaluated for their physicochemical characteristics, drug content, assay and in-vitro release profile of Atorvastatin, Clopidogrel& Aspirin Tablet
- In–vitro release and content of active ingredients was estimated at one month interval during to rage period.

Atorvastatin, Clopidogrel & Aspirin Tablet and Capsule Stability Study

(A Capsule with both)

After optimizations of three formulations i.e. Atorvastatin Calcium IR (10mg), Clopidogrel Bisulphate IR (75mg) & Aspirin DR (75mg) Tablet individually, three formulations of Atorvastatin, Clopidogrel& Aspirin Tablet are put in a size one capsule and kept for three months stability as per ICH in strip pack. Each month sampling was done and all the parameters of tablet are analyzed.

Table. No: 14 Stability Condition for Atorvastatin, Clopidogrel & Aspirin Capsule

Study	Storage condition	Time Period Covered
Room Temperature (RT)	25℃ ± 2℃/60%RH± 5%RH	3months **Testing :**If accelerated condition tablet is passed
Accelerated	40℃ ± 2℃/75%RH± 5%RH	3 months Testing:1,2,3month

The results are shown in Table. No: 48-53;

Figure. No: 24-32.

RESULT AND DISCUSSION

Table.no:15 Standard Calibration Curve of Atorvastatin Calcium

S.No	Concentration in ppm	Area
1	10	1827247.667
2	20	3618371.667
3	50	8959876.000
4	100	18118982.333
5	120	21555409.333
6	160	29532241.333
7	200	36224779.667

*Mean±SD n=3

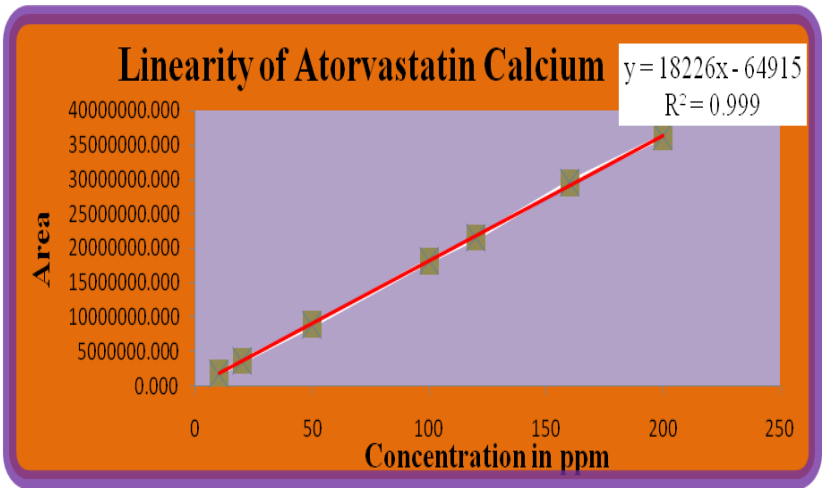

Fig. no:1 Standard Calibration Curve of Atorvastatin Calcium

Table.no: 16 Standard Calibration Curve of Aspirin

S.No	Concentration in ppm	Area
1	10	25.8885
2	20	54.8114
3	50	140.4123
4	100	298.1254
5	120	375.2312
6	160	497.0895
7	200	607.1886

*Mean±SD n=3

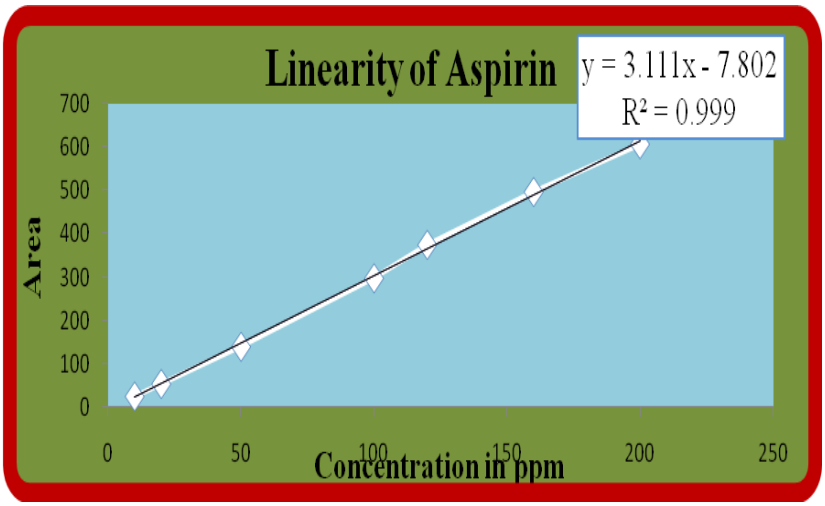

Figure.no: 2 Standard Calibration Curve of Aspirin

Table.no:17 Standard Calibration Curve of Clopidogrel bisulphate

S.No	Concentration in ppm	Area
1	10	135.4085
2	20	268.4221
3	50	750.8499
4	100	1546.109
5	120	1877.405
6	160	2511.711
7	200	3121.033

*Mean±SD n=3

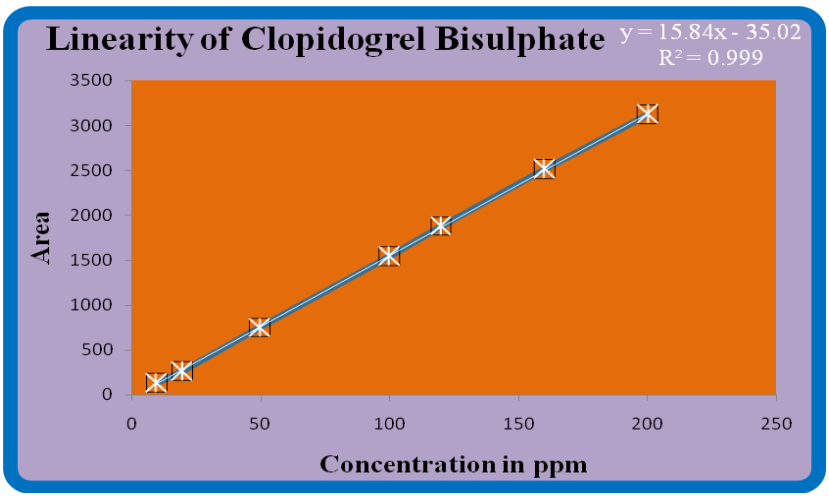

Figure.no:3 Standard Calibration Curve of Clopidogrel bisulphate

IMPURITY PROFILE AND ASSAY

FOR ATORVASTATIN CALCIUM

Table. No: 18 Impurity Profile and Assay of Atorvastatin Calcium API

Impurity A	0.04%
Impurity B	Not Detected
Impurity C	Not Detected
Impurity D	0.07%
Any Other Impurity	Not Detected
Total Impurity	0.35%
Assay	99.75%
Conversional factor	1.0359

*Mean±SD (n=6)

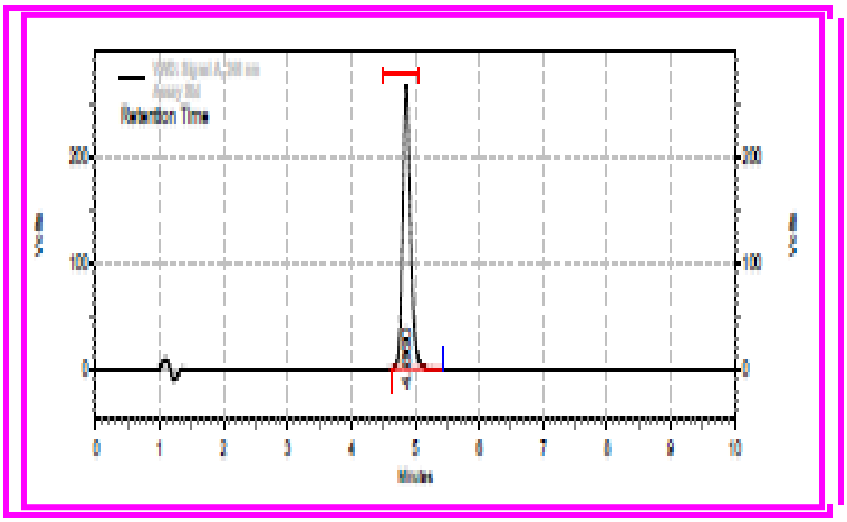

Figure. No:4 Atorvastatin calcium Assay Chromatogram

FOR ASPIRIN

Table. No:19 Impurity Profile and Assay of Aspirin API

Impurity A	0.05%
Impurity B	Not Detected
Impurity C	Not Detected
Impurity D	0.06%
Any Other Impurity	Not Detected
Total Impurity	0.25%
Assay	99.85%
Conversional factor	1

*Mean±SD (n=6)

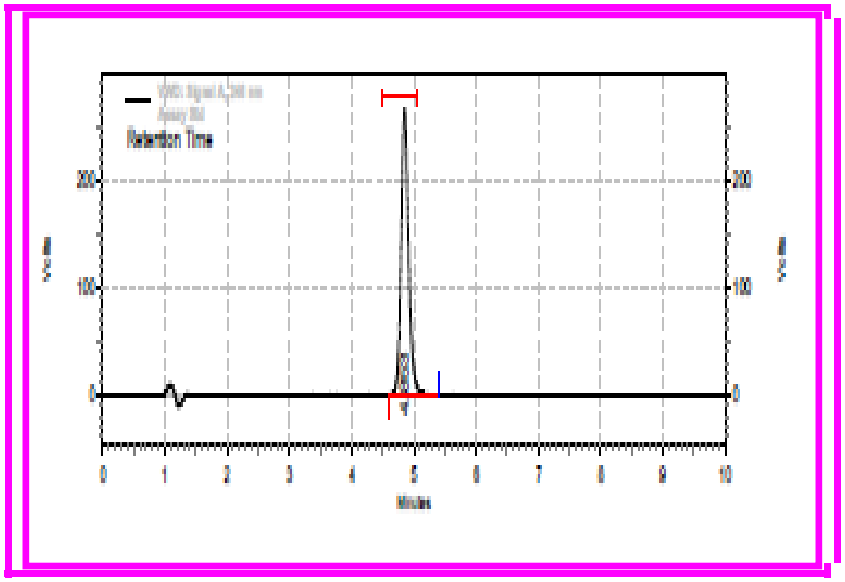

Figure. No: 5 Aspirin Assay Chromatogram

FOR CLOPIDOGREL BISULPHATE

Table. No:20 Impurity Profile and Assay of Clopidogrel bisulphate API

Impurity A	0.03%
Impurity B	Not Detected
Impurity C	Not Detected
Impurity D	0.08%
Any Other Impurity	Not Detected
Total Impurity	0.45%
Assay	99.75%
Conversional factor	1.3048

*Mean±SD (n=6)

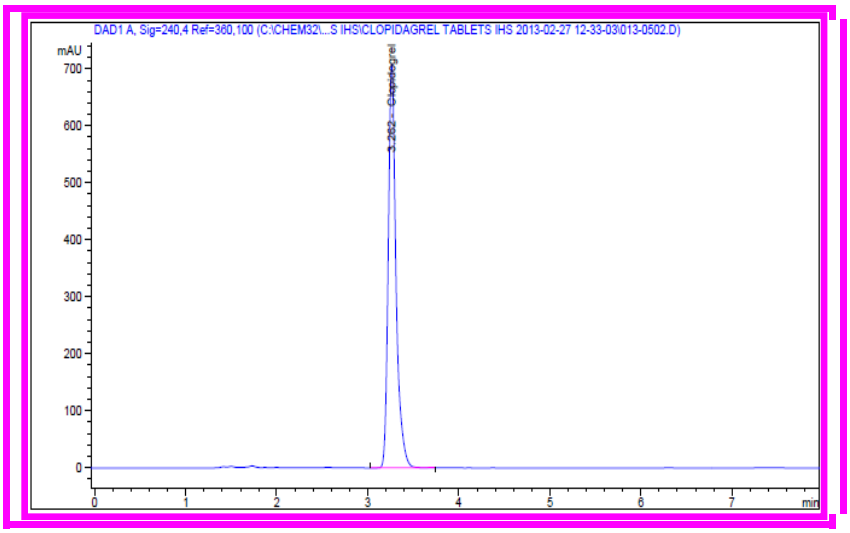

Figure. No:6 Clopidogrel bisulphate Assay Chromatogram

FT-IR SPECTROSCOPY

The result of FT-IR study for Atorvastatin calcium and their excipients are shown in Figure. No:7-12

FOR ATORVASTATIN CALCIUM

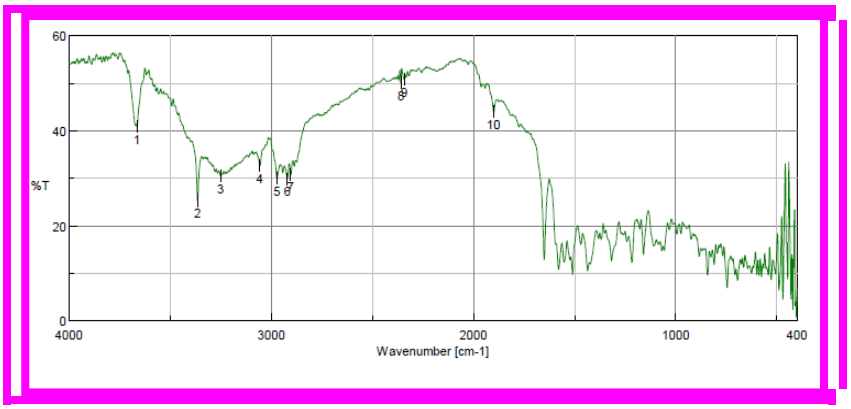

Figure.No:7 FTIR Spectrum of Pure Atorvastatin Calcium

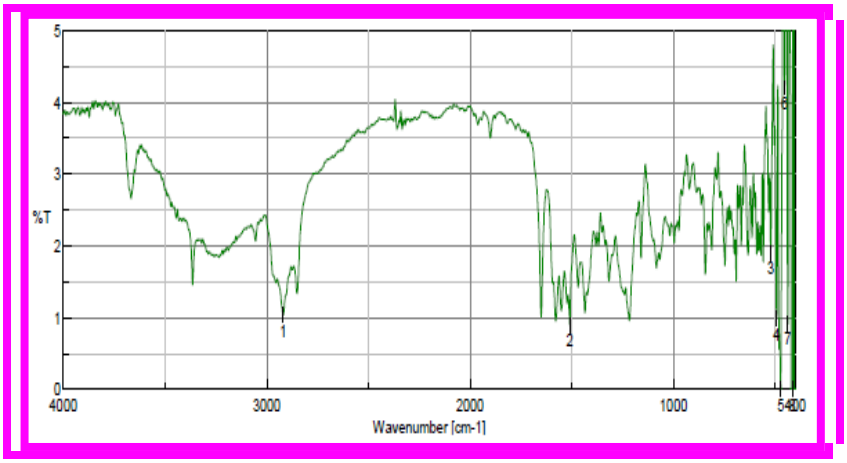

Figure.No:8 FTIR Spectrum of Atorvastatin Clacium + All excipients

FOR ASPIRIN

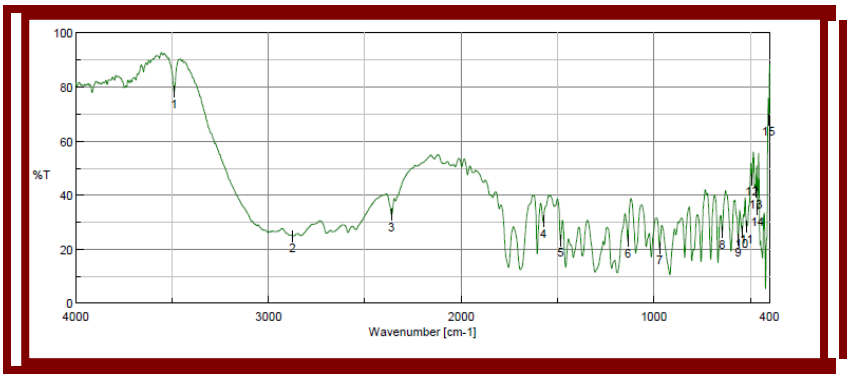

Figure.No:9 FTIR Spectrum of Aspirin Pure

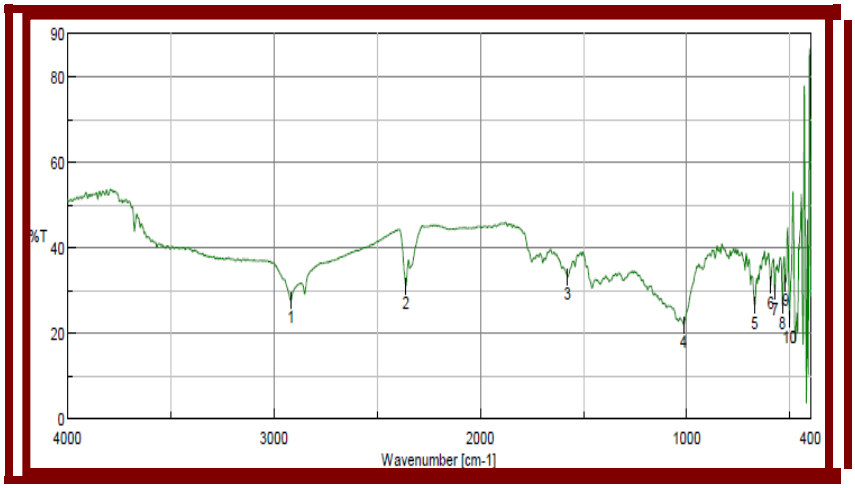

Figure.No:10 FTIR Spectrum of Aspirin + All excipients (Tablet)

FOR CLOPIDOGREL BISULPHATE

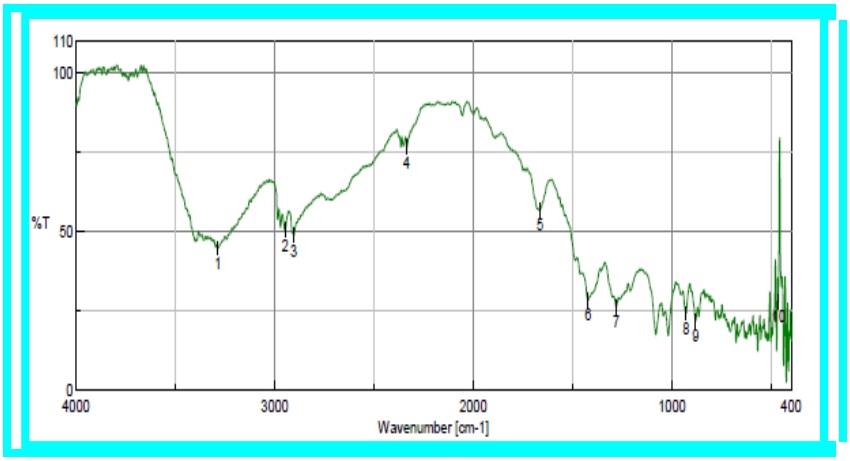

Figure.No:11 FTIR Spectrum of Clopidogrel Bisulphate Pure

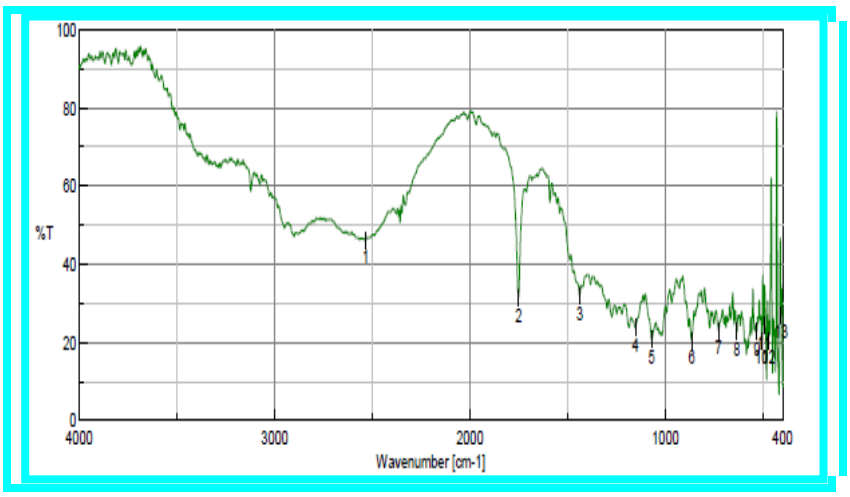

Figure.No: 12 FTIR Spectrum of Clopidogrel bisulphate + All Excipients (tablet)

DIFFERENTIAL SCANNINGCALORIMETERSTUDIES:

FOR ATORVASTATIN CALCIUM

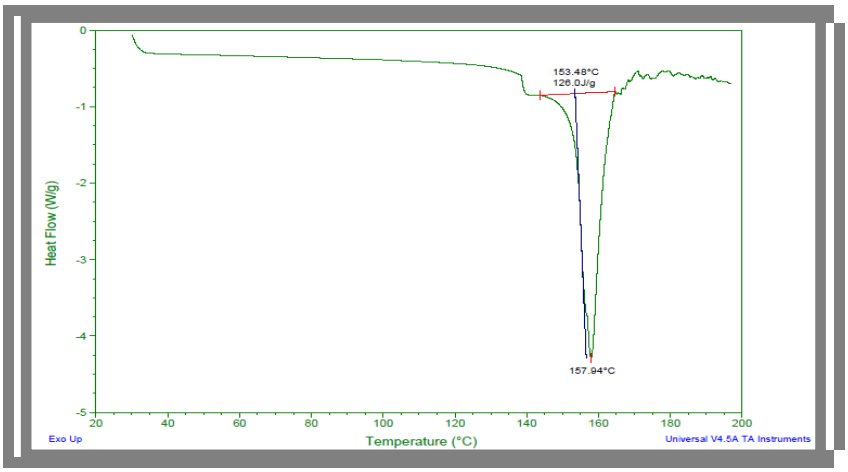

Figure. No: 13 DSC Graph of Atorvastatin calcium

DISCUSSION

From this figure. No:15 it can be seen that peak value of Atorvastatin Calcium was found to be 157.94°C in DSC thermogram. This value matches with that given in the literature and confirm the purity of API.

FOR ASPIRIN

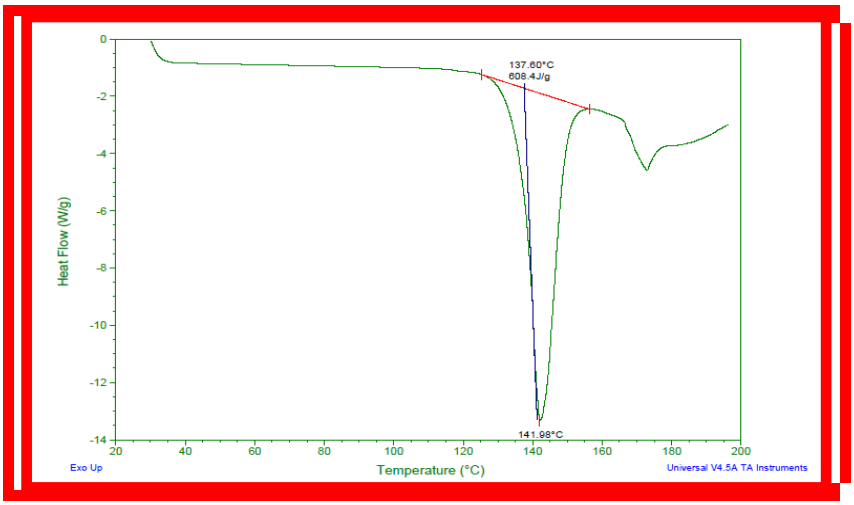

Figure.No: 14 DSC Graph of Aspirin

DISCUSSION

From this figure. No:13 it can be seen that peak value of Aspirin was found to be 141.98°C in DSC thermogram. This value matches with that given in the literature and confirm the purity of API.

FOR CLOPIDOGREL BISULPHATE

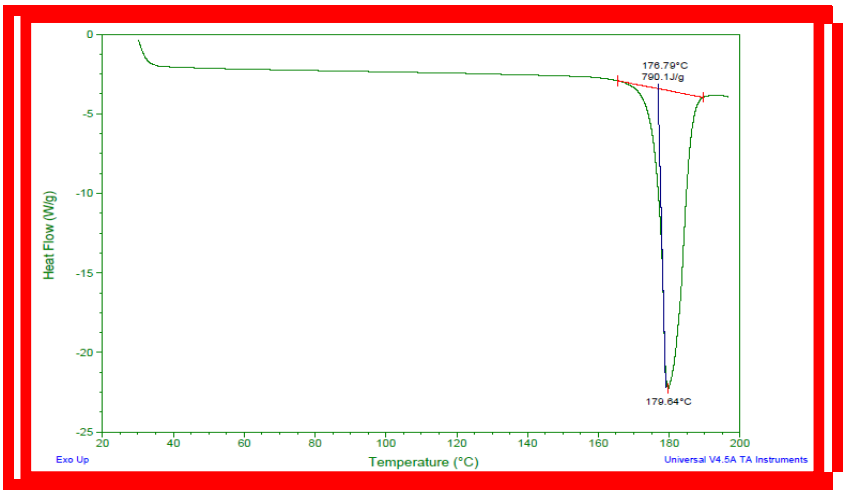

Figure.No:15 DSC Graph of Clopidogrel bisulphate

DISCUSSION

From this figure. No:11 it can be seen that peak value of Clopidogrel Bisulphate was found to be 179.54°C in DSC thermogram. This value matches with that given in the literature and confirm the purity of API.

FOR ATORVASTATIN CALCIUM, ASPIRIN AND CLOPIDOGREL BISULPHATE

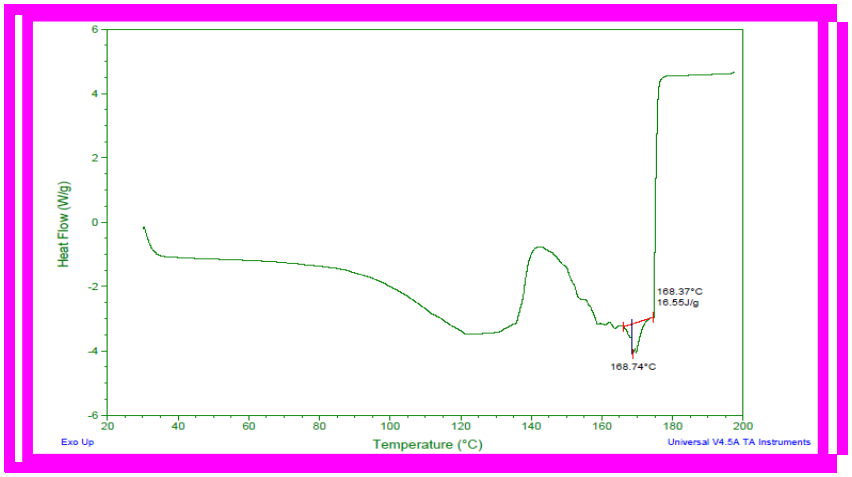

Figure.No: 16 DSC Graph of Atorvastatin Calcium, Aspirin and Clopidogrel bisulphate

DISCUSSION

From this figure. No:50 it can be seen that peak value of Atorvastatin Calcium was found to be 168.74°C in DSC thermogram. This value matches with that given in the literature and confirm the purity of API.

COMPATABILITY STUDIES

FOR ATORVASTATIN CALCIUM

Table. No: 21 Compatibility study of Atorvastatin calcium with Excipients

The RS Data of Aspirin (By HPLC) of 1 month excipients Compatability @ 40°C-75% RH

Ingredient	Ratio	Description		
		Related substance %w/w	1Month 25°C/60 %RH	1 Month 40°C/75 %RH
Atorvastatin Calcium	1	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : Calcium carbonate	1:1	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : Lactose DCL-11	1:1	White to pale yellow, granular FF powder	*	*
Atorvastatin Calcium : MCC pH-102	1:1	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : Croscarmellose Sodium	1:1	White to Greyish white , granular powder	*	*
Atorvastatin Calcium : Cross povidone	1:1	White to Grayish white , granular powder	*	*
Atorvastatin Calcium : Purified Talc	1:3	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : Sodium Lauryl Sulphate	1:3	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : Calcium Stearate	1:3	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : Erythrocin Supra	1:0.5	White to pale yellow, granular FF powder	*	*
Atorvastatin Calcium : HPMC E-15	1:0.5	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : HPC	1:0.5	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : Titanium dioxide	1:0.5	White to pale yellow, granular powder	*	*
Atorvastatin Calcium : All excipients	1:1	White to pale yellow, granular powder	*	*

Result: * Indicated That No Change Was Observed

FOR ASPIRIN

Table. No: 22 Compatibility study of Aspirin with Excipients

The RS Data of Aspirin (By HPLC) of 1 month excipients Compatability @ 40°C-75% RH

Ingredient	Ratio	Description		
		Related substance %w/w	1 Month 25°C/60% RH	1 Month 40°C/75% RH
Aspirin	1	White to pale yellow, granular powder	*	*
Aspirin : MCC pH-102	1:1	White to pale yellow, granular powder	*	*
Aspirin : Sodium Starch Glycolate	1:1	White to Grayish white, granular FF powder	*	*
Aspirin : Purified Talc	1:3	White to pale yellow, granular powder	*	*
Aspirin : Colloidal Silicon Dioxide	1:3	White to pale yellow, granular powder	*	*
Aspirin : Magnesium Stearate	1:3	White to pale yellow, granular powder	*	*
Aspirin : Ethylcellulose	1:0.5	White to pale yellow, granular powder	*	*
Aspirin : Kolicoat maaep-100	1:1	White to pale yellow, granular powder	*	*
Aspirin : Titanium Dioxide	1:0.5	White to pale yellow, granular powder	*	*
Aspirin : Sunset Yellow Lake	1:0.5	White to Grayish white, granular FF powder	*	*
Aspirin : All Excipients	1:1	White to pale yellow, granular powder	*	*

Result: * Indicated That No Change Was Observed

FOR CLOPIDOGREL BISULPHATE

Table. No: 23 Compatibility study of Clopidogrel Bisulphate with Excipients

The RS Data of Aspirin (By HPLC) of 1 month excipients Compatability @ 40°C-75% RH

Ingredient	Ratio	Description		
		Related substance %w/w	1Month 25°C/60 %RH	1 Month 40°C/75 %RH
Clopidogrel Bisulphate	1	White to pale yellow, granular powder	*	*
Clopidogrel Bisulphate : MCC pH-102	1:1	White to pale yellow, granular powder	*	*
Clopidogrel Bisulphate : Mannitol DC Grate	1:1	White to Grayish white, granular FF powder	*	*
Clopidogrel Bisulphate : Croscarmellose Sodium	1:1	White to Grayish white, granular powder	*	*
Clopidogrel Bisulphate : Cross povidone	1:1	White to Grayish white, granular powder	*	*
Clopidogrel Bisulphate : Aerosil	1:3	White to bluish yellow, granular powder	*	*
Clopidogrel Bisulphate :	1:3	White to pale yellow, granular powder	*	*

Magnesium stearate				
Clopidogrel Bisulphate : Talc	1:3	White to pale yellow, granular powder	*	*
Clopidogrel Bisulphate : Iron oxide Supra	1:0.5	White to pale yellow, granular powder	*	*
Clopidogrel Bisulphate : HPMC E-15	1:0.5	White to Grayish white, granular FF powder	*	*
Clopidogrel Bisulphate : HPC	1:0.5	White to pale yellow, granular powder	*	*
Clopidogrel Bisulphate : Titanium dioxide	1:0.5	White to pale yellow, granular powder	*	*
Clopidogrel bisulphate : All excipients	1:1	White to pale yellow, granular powder	*	*

Result: * Indicated That No Change Was Observed

Table. No: 24 Compatibility study of Atorvastatin calcium, Aspirin &Clopidogrel Bisulphate : The RS Data of Clopidogrel bisulphate (By HPLC) of 1 month excipients Compatability @ 40°C-75% RH

Ingredient	**Ratio**	**Description**		
		Related substance %w/w	**1Month**	**1 Month**
			25°C/60%RH	**40°C/75%RH**
Atorvastatin Calcium: Clopidogrel Bisulphate	1:1	White to pale yellow, granular powder	*	*
Atorvastatin Calcium: Aspirin	1:1	White to pale yellow, granular powder	*	*
Clopidogrel bisulphate : Aspirin	1:1	White to pale yellow, granular powder	*	*
Atorvastatin Calcium: Aspirin : Clopidogrel bisulphate	1:1:1	White to pale yellow, granular powder	*	*

Result: * Indicated That No Change Was Observed

INNOVATOR PRODUCTS CHARATERIZATION

FOR ATORVASTATIN CALCIUM

Table. No: 25 Atorvastatin calcium Innovator Tablet Characterization

Brand Name	INNOVATOR
Strength	10 mg TABLET
Label Claim	Each tablet contains Atorvastatin 10 mg
Tablet Color	Sunset yellow Colour
Tablet Shape	Round Shape
Description	Debbosed with ‘z’ on one side & ‘sz’ on other side, Film Coated tablets
Dimensions	**DIAMETER :** 5.50-5.55mm & **THICKNESS :** 3.40-3.80mm
Average Weight	201.8
Hardness	7-8kp
Uniformity of Dosage Unity	MEAN : 102.12; SD: 2 & RSD :2
Assay	102.67%(10.56mg/Tablet)

*Mean±SD (n=6)

FOR ASPIRIN

Table.no: 26Aspirin Innovator Tablet Characterization

Brand Name	INNOVATOR
Strength	75mg TABLET
Label Claim	Each tablet contains Aspirin 75 mg
Tablet Color	Sunset Yellow Colour
Tablet Shape	Round Shape
Description	Debbosed with 'z' on one side& 'sz' on other side, Enteric Coated Tablets
Dimensions	**DIAMETER:**5.69-5.72mm & **THICKNESS**: 3.40- 4.48mm
Average Weight	122.23mg
Hardness	6-7kp
Uniformity of Dosage Unity	**MEAN: 102.12; SD: 1.8 & RSD: 1.8**
Assay	102.14% (76.12mg/Tablet)

*Mean±SD (n=6)

FOR CLOPIDOGREL BISULPHATE

Table.no:27 Clopidogrel bisulphate Innovator Tablet Characterization

Brand Name	INNOVATOR
Strength	75mg TABLET
Label Claim	Each Tablet Contains Clopidogrel Bisulphate 75mg
Tablet Color	Iron oxide yellow
Tablet Shape	Round Shape
Description	Debbosed with 'z' on one side & 'sz' on other side, Film Coated Tablets
Dimensions	**DIAMETER:**5.56 – 5.58 & **THICKNESS:**3.43 – 3.85
Average Weight	202.56 mg
Hardness	7-8kp
Uniformity of Dosage Unity	MEAN: 101.56
Assay	101.56% (75.87mg/Tablet)

*Mean±SD (n=6)

FOR ATORVASTATIN CALCIUM

Table. No: 28 Dissolution Profile of the Atorvastatin Calcium IR Innovator Tablets 10mg (Phosphate Buffer pH-6.8)

Dissolution Media (900mL Media, at 75RPM)	**Number of Units Used 6**	**Percentage of Drug Dissolved in Minutes**				
		5	**10**	**15**	**20**	**30**
Phosphate Buffer pH-6.8	**Mean**	68.4	85.7	98.8	99.8	101.3
	±SD	1.7	1.8	1.8	1.8	1.9
	±RSD	1.7	1.8	1.8	1.8	1.9

*Mean±SD (n=6)

FOR ASPIRIN

Table. No:29 Dissolution profile of the Aspirin Innovator Tablet (0.1N Hydrochloric acid)

Dissolution Media (1000mL Media, at 100RPM)	Number of Units Used 6	Percentage of Drug Dissolved in Minutes		
		30	60	120
0.1 N Hydrochloric acid	Mean	0	0.75	1.2
	±SD	0	0	0
	±RSD	0	0	0

*Mean±SD (n=6)

Table. No: 30 Dissolution Profile of the Aspirin Innovator Tablets 75mg (pH-6.8 Phosphate Buffer)

Dissolution Media (1000mL Media, at 100RPM)	Number of Units Used 6	Percentage of Drug Dissolved in Minutes				
		10	30	45	60	90
Phosphate Buffer pH-6.8	Mean	17.79	67.98	86.43	97.67	102.14
	±SD	1.6	1.7	1.6	1.7	1.8
	±RSD	1.6	1.7	1.6	1.7	1.8

*Mean±SD (n=6)

FOR CLOPIDOGREL BISULPHATE

Table. No: 31 Dissolution Profile of the Clopidogrel bisulphate IR Innovator Tablet

Dissolution Media (900mL Media, at 75RPM)	Number of Units Used 6	Percentage of Drug Dissolved in Minutes				
		5	10	15	20	30
pH – 2 Hydrochloric acid Buffer	Mean	17.79	67.98	86.43	97.67	102.14

*Mean±SD (n=6)

DISCUSSION

The dissolution was found to be rapid with more than 85% drug being released in15 minutes under moderate agitation (75rpm) in 6.8 Phosphate Buffer (Table. No: 12). The dissolution was found to be rapid with more than 85%drugbeingreleasedin15 minutes under moderate agitation (75rpm)inpH-2 Hydrochloric acid buffer(Table. No:12). From this figure. No:16 it can be seen that amount of Aspirin DR dissolved in 10 & 90 Minutes is NLT 75% respectively. So, the above criteria as acceptance limit. From this figure. No:13 it can be seen that amount of Atorvastatin calcium & Clopidogrel bisulphate IR dissolved in 5 & 30 Minutes is NLT 75% respectively. So, the above criteria as acceptance limit.

FOR ATORVASTATIN CALCIUM

Table No: 32 Preformulation Study of the blend (ATORVASTATIN CALCIUM)

Batch Code	Bulk Density*	Tapped Density*	Angle of repose*	% Compressibility*	Hausner Ratio*	Loss on Drying*
ATF1	0.41	0.47	24.58	12.76	1.15	2.1
ATF2	0.44	0.52	25.91	15.38	1.18	1.9
ATF3	0.44	0.51	26.86	13.72	1.16	1.8
ATF4	0.47	0.54	24.43	12.96	1.14	1.7
ATF5	0.45	0.50	24.10	12.00	1.06	1.6
ATF6	0.46	0.53	24.77	13.20	1.15	1.7
ATF7	0.47	0.52	25.42	9.61	1.11	1.5

*Mean±SD (n=6)

The physical parameters of drug as well as blends concluded that these were considerably good to formulate the tablet using direct compression technique.

FOR ASPIRIN

Table No 33 Preformulation Study of the blend (ASPIRIN)

Batch Code	Bulk Density*	Tapped Density*	Angle of repose*	% Compressibility*	Hausner Ratio*	Loss on Drying*
AF1	0.41	0.47	24.58	12.76	1.15	1.9
AF2	0.44	0.52	25.91	15.38	1.18	1.8
AF3	0.45	0.51	26.86	13.72	1.16	1.6
AF4	0.46	0.53	24.75	13.24	1.15	1.4

*Mean±SD (n=6)

The physical parameters of drug as well as blends concluded that these were considerably good to formulate the tablet using direct compression technique.

FOR CLOPIDOGREL BISULPHATE

Table No 34 Preformulation Study of the blend (CLOPIDOGREL BISULPHATE)

Batch Code	Bulk Density*	Tapped Density*	Angle of repose*	% Compressibility*	Hausner Ratio*	Loss on Drying*
CF1	0.41	0.47	24.58	12.76	1.15	1.8
CF2	0.42	0.52	24.81	15.38	1.18	1.7
CF3	0.44	0.50	25.68	13.72	1.16	1.6
CF4	0.43	0.53	24.34	12.96	1.14	1.9
CF5	0.44	0.48	25.13	12.00	1.06	1.6
CF6	0.46	0.53	24.77	13.20	1.15	2.1
CF7	0.47	0.56	25.18	13.31	1.13	1.4
CF8	0.45	0.55	24.34	13.32	1.17	1.6

*Mean±SD (n=6); The physical parameters of drug as well as blends concluded that these were considerably good to formulate the tablet using direct compression technique.

FOR ATORVASTATIN CALCIUM

Table No: 35 Evaluation of Atorvastatin calcium Film Coated-Tablets

Batch No	Weight variation (mm)**	Diameter (mm)*	Thickness (mm)*	Hardness (kg/cm^2)*	Disintegration Time*
ATF1	105±6.5	5.69±0.01	3.42±0.03	4.15±0.21	1 mts 23 sec
ATF2	104±7.5	5.68±0.02	343±0.04	4.17±0.20	1 mts 33 sec
AFT3	104±6.6	5.69±0.02	3.44±0.04	3.75±0.14	1 mts 33 sec
AFT4	105±7.5	5.57±0.01	3.45±0.05	3.87±0.13	1 mts 35 sec
AFT5	104±7.8	5.68±0.02	3.46±0.04	3.87±0.12	1 mts 32 sec
AFT6	104±6.8	5.69±0.01	3.48±0.05	3.76±0.11	1 mts 31 sec
AFT7	104±7.8	5.69±0.03	3.45±0.06	3.87±0.15	1 mts 12 sec

*Mean±SD (n=6) **Mean±SD (n=20)

FOR ASPIRIN

Table No: 36 Evaluation of Aspirin Enteric Coated-Tablets (pH-6.8 phosphate buffer)

Batch No	Weight variation (mm)*	Diameter (mm)*	Thickness (mm)*	Hardness (kg/cm^2)*	Disintegration Time
AF1	112±6.4	5.89±0.01	3.82±0.03	4.15±0.21	2 mts 23 sec
AF2	113±7.6	5.88±0.02	383±0.04	4.18±0.20	2 mts 34 sec
AT3	112±6.6	5.83±0.02	3.84±0.04	3.75±0.14	2 mts 42 sec
AT4	114±7.5	5.87±0.01	3.85±0.05	3.87±0.13	2 mts 53 sec

*Mean±SD (n=6) **Mean±SD (n=20)

FOR CLOPIDOGREL BISULPHATE

Table No: 37 Evaluation of Clopidogrel bisulphate Film Coated-Tablets

Batch No	Weight variation (mm)**	Diameter (mm)*	Thickness (mm)*	Hardness (kg/cm^2)*	Disintegration Time*
CF1	133±6.3	5.69±0.01	4.41±0.03	4.15±0.18	1 mts 43 sec
CF2	134±6.5	5.68±0.02	4.43±0.04	4.18±0.17	1 mts 53 sec
CF3	134±5.6	5.69±0.02	4.44±0.04	3.66±0.13	1 mts 43 sec
CF4	133±6.5	5.57±0.01	4.45±0.05	3.77±0.14	1 mts 55 sec
CF5	134±5.8	5.68±0.02	4.46±0.04	3.67±0.13	1 mts 52 sec
CF6	134±6.9	5.69±0.01	4.48±0.05	3.78±0.15	1 mts 51 sec
CF7	134±6.8	5.69±0.03	4.45±0.06	3.88±0.16	1 mts 52 sec
CF8	131±7.4	5.36±0.02	4.35±0.07	3.78±0.17	1 mts 52 sec

*Mean±SD (n=6) & **Mean±SD (n=20)

FOR ATORVASTATIN CALCIUM

Table.No:38 Dissolution Profile of the Atorvastatin calcium IR Tablets AFT1-AFT7

% Cumulative Amount of Drug Release							
Time (Minutes)	**ATF1**	**ATF2**	**ATF3**	**ATF4**	**ATF5**	**ATF6**	**ATF7**
5	58.34	59.13	60.54	59.78	60.67	61.89	65.19
10	78.98	79.45	80.56	79.67	80.19	81.89	84.12
15	92.14	94.45	95.19	91.78	93.18	94.87	97.01
20	93.45	95.76	96.87	93.17	95.48	96.67	100.17
30	97.45	98.13	98.78	96.67	97.87	98.89	103.12

*Mean±SD (n=6)

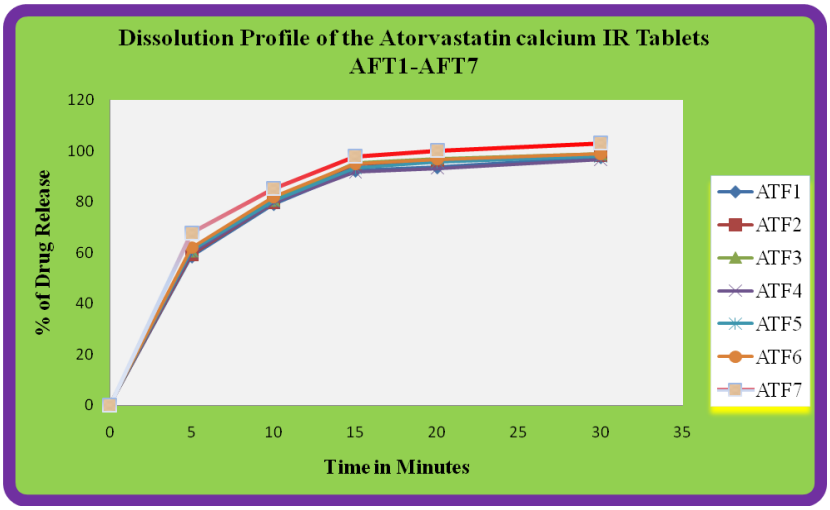

Figure.No:17 Dissolution Profile of the Atorvastatin calcium IR Tablets AFT1-AFT7

Table.No:39 Dissolution Profile of the Atorvastatin calcium IR Tablet Optimized Formulation AFT7 with Innovator Tablet

% Cumulative Amount of Drug Release		
Time in (Minutes)	**AFT7**	**INNOVATOR**
5	65.19	68.4
10	84.12	85.7
15	97.01	98.8
20	100.17	99.8
30	103.12	101.30

*Mean±SD (n=6)

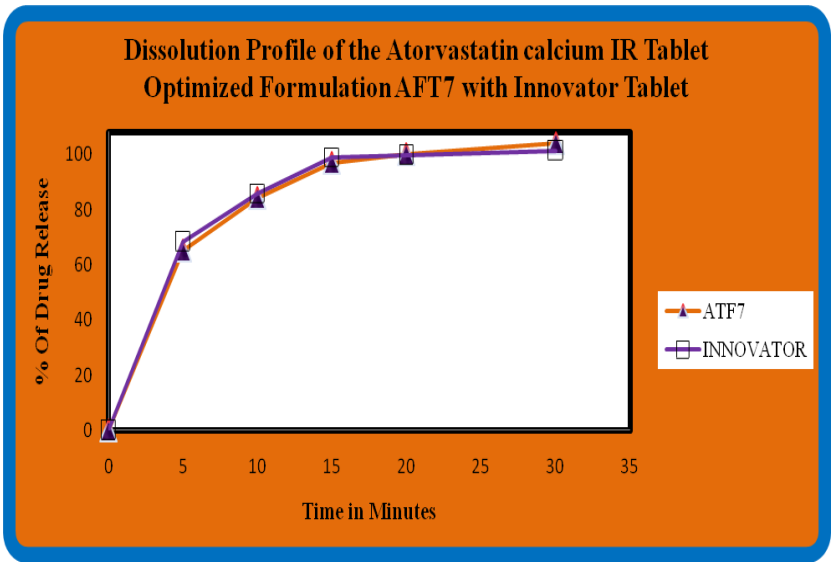

Figure. No: 18 Dissolution Profile of the Atorvastatin calcium IR Tablet Optimized Formulation AFT7 with Innovator Tablet

Discussion: From table. No:17 & figure. No:18 it can be seen that the variation of concentration of Super disintegrant and different disintegrant is affecting the release in same proportion. Different approaches were tried in batches ATF7 it was found with two super disintegrant was showing good release pattern. ATF7 shows a similar release profile to that of the Innovator with f2 value of 64. From the above results it is seen that Batch ATF7 is showing best f2 & f1 value. From Fig.no: 19 it can be inferred that release profile of Batch ATF7 matches with that of innovator product, also f1&f2 values shown in Table. No:18 are good enough to comply with the innovator's product INNOVATOR have reported similar kind of results for studies with Atorvastatin Calcium.

FOR ASPIRIN

Table.No:40 Dissolution Profile of the Aspirin DR Tablet AT1-AT4 with Innovator (0.1N Hydrochloric acid)

% Cumulative Amount of Drug Release					
Time (Minutes)	**AF1**	**AF2**	**AF3**	**AF4**	**INNOVATOR**
30	0	0	0	0	0
60	0.58	0.56	0.55	0.54	0.72
90	0.96	0.97	0.98	0.96	1.2

*Mean±SD (n=6)

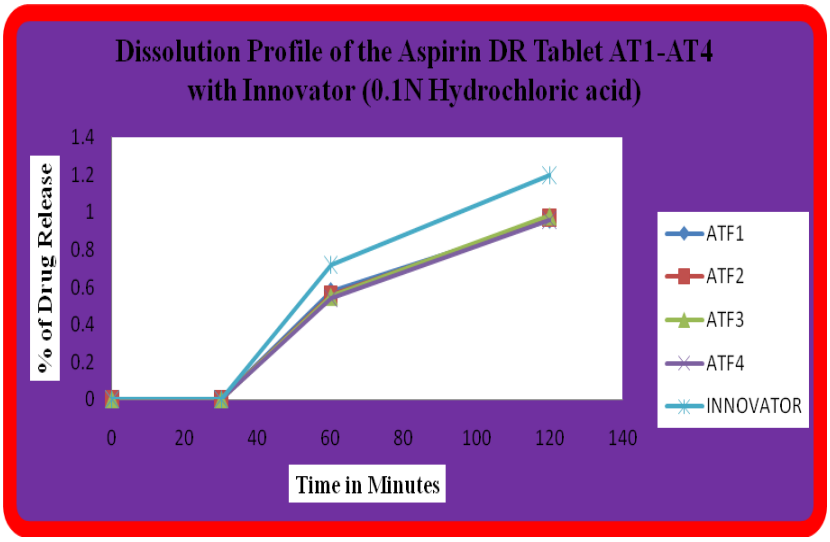

Figure. No :19 Dissolution Profile of the Aspirin DR Tablet AT1-AT4 with Innovator (0.1N Hydrochloric acid)

Table.No:41 Dissolution Profile of the Aspirin DR Tablet AT1-AT4 with Innovator (PHOSPHATE BUFFER pH-6.8)

% Cumulative Amount of Drug Release					
Time (Minutes)	**AF1**	**AF2**	**AF3**	**AF4**	**INNOVATOR**
10	14.13	14.36	14.78	15.12	17.79
30	50.45	52.34	54.67	69.12	67.98
45	72.35	74.67	77.78	89.13	86.43
60	91.87	93.87	95.57	99.89	97.67
90	93.89	95.98	98.43	104.21	102.14

*Mean±SD (n=6)

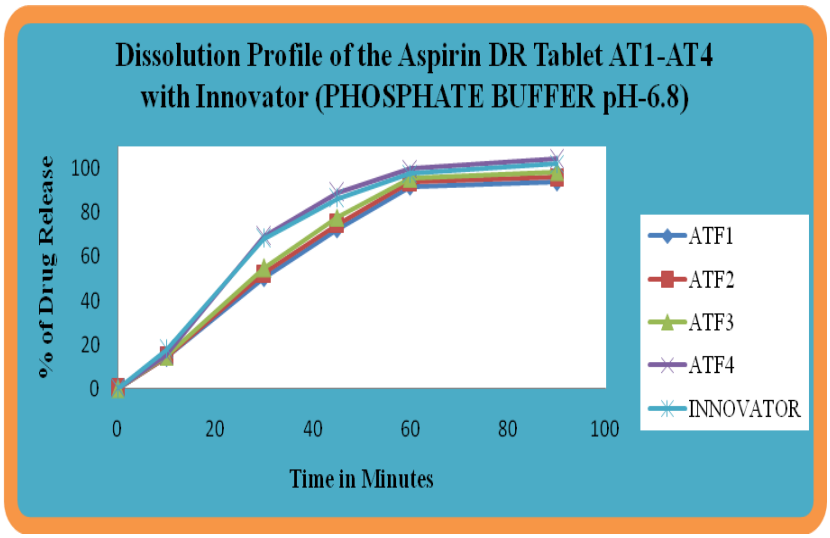

Figure. No: 20 Dissolution Profile of the Aspirin DR Tablet AT1-AT4 with Innovator (PHOSPHATE BUFFER pH-6.8)

Table.No:42 Dissolution Profile of the Aspirin DR Tablet Optimized Formulation of AT4 with Innovator (PHOSPHATE BUFFER pH-6.8)

% Cumulative Amount of Drug Release		
Time (Minutes)	**AF4**	**INNOVATOR**
10	15.12	17.79
30	69.12	67.98
45	89.13	86.43
60	99.89	97.67
90	104.21	102.14

*Mean±SD (n=6)

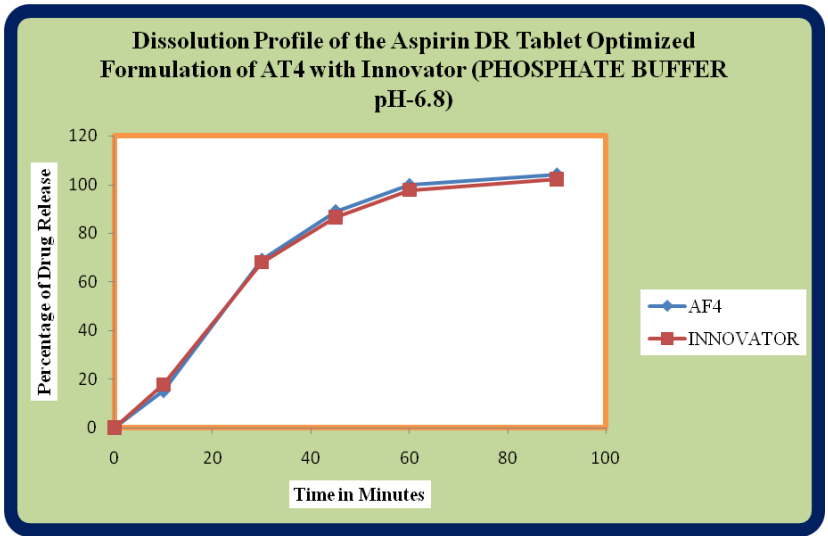

Figure. No:21 Dissolution Profile of the Aspirin DR Tablet Optimized Formulation of AT4 with Innovator (PHOSPHATE BUFFER pH-6.8)

Discussion: From table. No: 22 & figure. No:18, it can be seen that the variation of concentration of Super disintegrant is affecting the release in same proportion. Different approaches were tried in batches AF4 it was found with single disintegrants at higher concentration showing good release pattern. AF4 shows a similar release profile to that of the Innovator with f2 value of 62. From the above results it is seen that Batch AT4 is showing best f2 & f1 value. From Fig.No:19 it can be inferred that release profile of Batch AF4 matches with that of innovator product, also f1&f2 values shown in Table.No:23 are good enough to comply with the innovator's product INNOVATOR have reported similar kind of results for studies with Aspirin.

FOR CLOPIDOGREL BISULPHATE:

Table.No:43 Dissolution profile of the Clopidogrel bisulphate IR Tablet CT1-CT8

% Cumulative Amount of Drug Release								
Time (Minutes)	**CF1**	**CF2**	**CF3**	**CF4**	**CF5**	**CF6**	**CF7**	**CF8**
5	12.12	14.87	16.14	12.54	14.56	16.17	18.01	17.17
10	52.23	55.67	58.76	53.56	56.78	59.89	69.78	66.78
15	74.56	77.87	79.99	73.15	76.76	78.98	87.78	86.56
20	83.98	86.56	89.89	85.43	87.76	90.32	99.67	95.76
30	93.78	95.89	96.76	92.56	94.98	96.12	103.12	98.65

*Mean±SD (n=6)

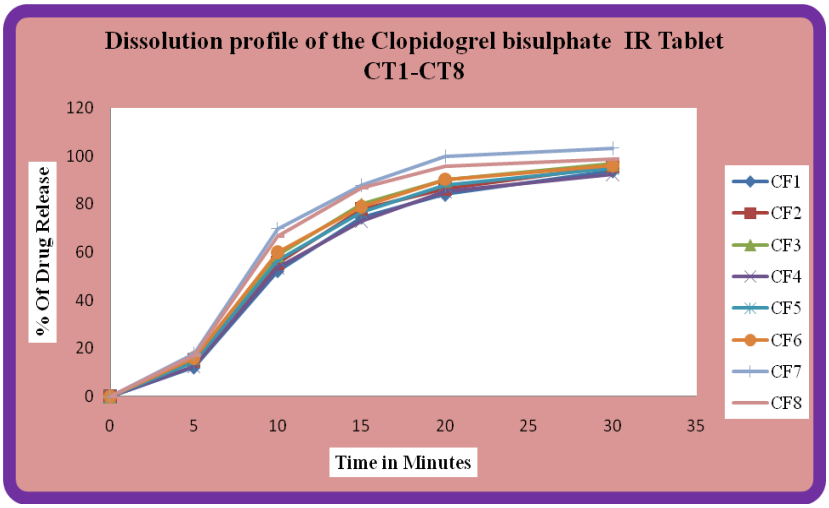

Figure.No:22 Dissolution profile of the Clopidogrel bisulphate IR Tablet CT1-CT8

Table.No:44 Dissolution Profile of the Clopidogrel bisulphate IR Optimized Formulation CT7 with INNOVATOR

% Cumulative Amount of Drug Release		
Time in Minutes	**CT7**	**INNOVATOR**
5	18.01	17.79
10	69.78	67.98
15	87.78	86.43
20	99.67	97.67
30	103.12	102.14

*Mean±SD (n=6)

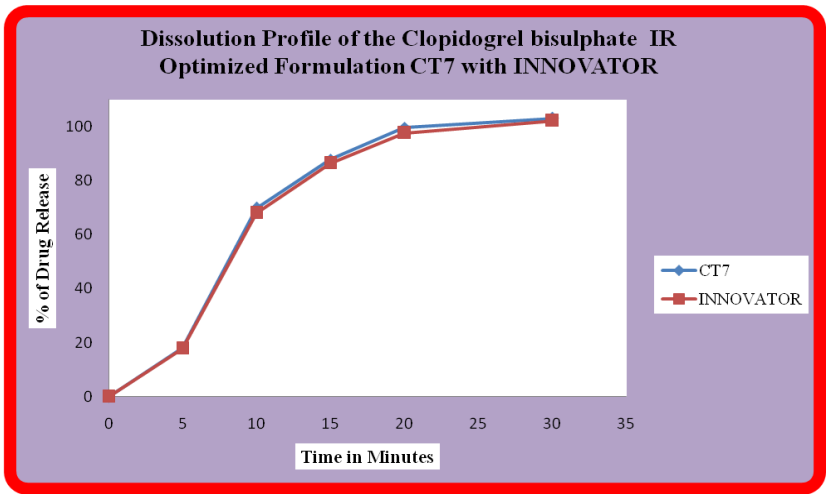

Figure. No:23 Dissolution Profile of the Clopidogrel bisulphate IR Optimized Formulation CT7 with INNOVATOR

Discussion: From table.No:18 & figure.No:15, it can be seen that the variation of concentration of Super disintegrant and different super disintegrants is affecting the release in same proportion. Different approaches were tried in batches CT7 it was found with two super disintegrants at lower concentration showing good release pattern, at higher concentration showing less release pattern. CT7 shows a similar release profile to that of the Innovator with f2 value of 65. From the above results it is seen that Batch CT7 is showing best f2 & f1 value. From Fig.No:15, it can be inferred that release profile of Batch AF4 matches with that of innovator product, also f1&f2 values shown in Table. No: 18 are good enough to comply with the innovator's product INNOVATOTR have reported similar kind of results for studies with Clopidogrel bisulphate.

ASSAY AND CONTENT UNIFORMITY

FOR ATORVASTATIN CALCIUM

Table.No: 45 Assay of the Atorvastatin Calcium

Assay	Atorvastatin Calcium Optimized (ATF7) (ALU BLISTER PACK)		
	Mean	SD	RSD
	101.56	1.7	1.7

*Mean±SD (n=6)

FOR ASPIRIN

Table. No: 46 Assay of the Aspirin

Assay	Aspirin Optimized AF4 (ALU BLISTER PACK)		
	Mean	SD	RSD
	102.46	1.6	1.6

*Mean±SD (n=6)

FOR CLOPIDOGREL BISULPHATE:

Table.No: 47 Assay of the Clopidogrel Bisulphate

Assay	Clopidogrel bisulphate (CT7) Optimized (STRIP PACK)		
	Mean	SD	RSD
	101.76	1.8	1.8

*Mean±SD (n=6)

STABILITY STUDIES

FOR ATORVASTATIN CALCIUM

Table. No:48 Stability Studies Data of the Atorvastatin Calcium Optimized Formulations (ATF7) (ALU BLISTER PACK)

Parameters	Initial	1st Month		2nd Month		3rd Month	
		RT	40°C	RT	40°C	RT	40°C
Weight variation (mm)**	104±7.8	104±7.8	103.5±7.6	103.5±7.8	103.5±7.2	103.5±7.8	103.2±7.1
Diameter (mm)*	5.69±0.03	5.68±0.03	5.67±0.03	5.68±0.03	5.67±0.02	5.67±0.03	5.66±0.01
Thickness (mm)*	3.45±0.06	3.44±0.06	3.45±0.06	3.45±0.06	3.44±0.05	3.45±0.06	3.43±0.04
Hardness (kg/cm2)*	3.87±0.15	3.86±0.15	3.86±0.14	3.86±0.15	3.86±0.12	3.86±0.15	3.85±0.10
Disintegration Time*	1 mts 12 sec	1 mts 12 sec	1 mts 11 sec	1 mts 11 sec	1 mts 11 sec	1 mts 11 sec	1 mts 10 sec

*Mean±SD (n=6) **Mean±SD(n=20)

Table. No:49 Stability Studies Data of the Assay & Dissolution Study of Atorvastatin Calcium Optimized Formulations (ATF7), Capsules With INNOVATOR (ALU BLISTER PACK) & Alu Alu Pack

Parameters	Initial	1st Month		2nd Month		3rd Month	
		RT	40°C	RT	40°C	RT	40°C
*Assay	101.267±0.435	101.260 ±0.435	101.204 ±0.421	101.260 ±0.435	101.120 ±0.435	101.260 ±0.435	101.001 ±0.435
*INNOVATOR (Assay)	100.564±0.235	100.554±0.435		100.545±0.243		100.555±0.256	
*% of Cumulative Release	103.12	102.67	102.76	102.67	102.01	102.67	101.65
*% of Cumulative Release (Capsule)	103.08	102.58	102.67	102.43	102.47	102.35	101.98
*INNOVATOR (% of Cumulative Release)	101.30	101.21		101.05		101.31	

*Mean±SD (n=6)

Discussion: Assay*Mean=Not less than 75% ; DissolutionMean = Not less than 80%.** The results indicated that the, optimized formulated tablets were within the Pharmacopeial specifications.

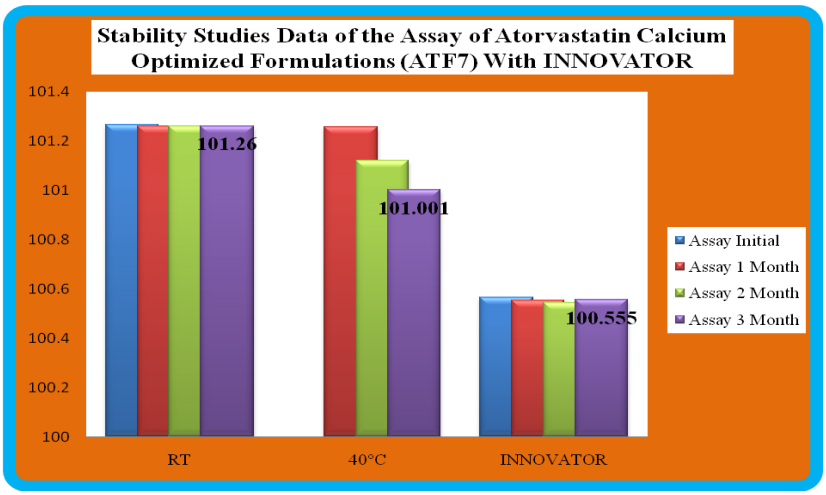

Figure. No: 24 Stability Studies Data of the Assay of Atorvastatin Calcium Optimized Formulations (ATF7) With INNOVATOR

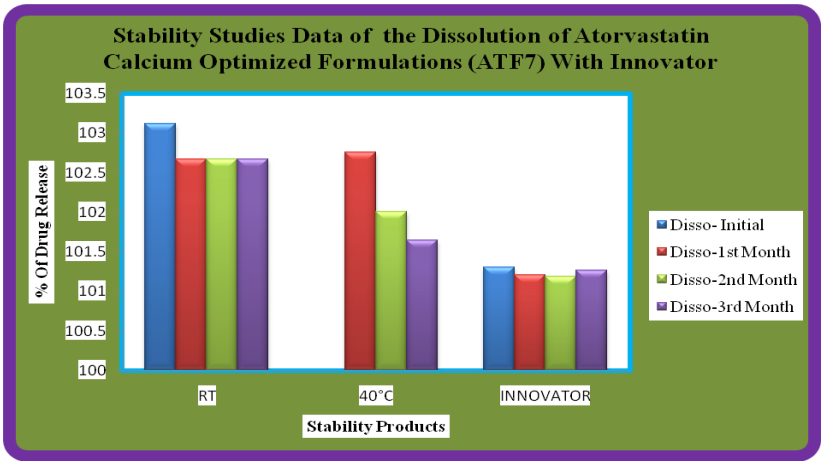

Figure. No:25 Stability Studies Data of the Dissolution of Atorvastatin Calcium Optimized Formulations (ATF7) With Innovator

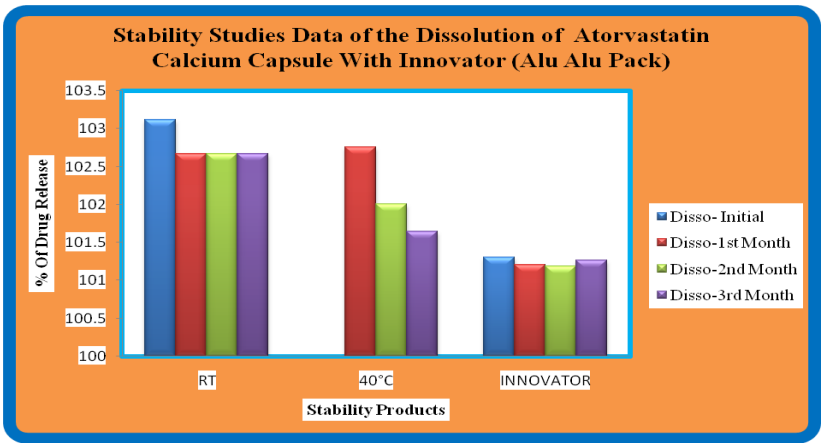

Figure. No:26 Stability Studies Data of the Dissolution of Atorvastatin Calcium Capsule With Innovator (Alu Alu Pack)

FOR ASPIRIN

Table. No:50 Stability Studies Data of the Aspirin Optimized Formulations (AF4) (ALU BLISTER PACK)

Parameters	Initial	1st Month		2nd Month		3rd Month	
		RT	40°C	RT	40°C	RT	40°C
Weight variation (mm)**	114±7.5	113.5±7.3	113.5±7.2	113.5±7.3	113.5±7.1	113.5±7.3	113.5±6.9
Diameter (mm)*	5.87±0.01	5.85±0.26	5.85±0.24	5.85±0.26	5.85±0.23	5.85±0.26	5.85±0.21
Thickness (mm)*	3.85±0.05	3.83±0.21	3.83±0.18	3.83±0.21	3.83±0.18	3.83±0.21	3.83±0.18
Hardness (kg/cm2)*	3.87±0.13	3.84±0.11	3.84±0.08	3.84±0.11	3.84±0.08	3.84±0.11	3.84±0.04
Disintegration Time*	2 mts 53 sec	2 mts 47 sec	2 mts 34 sec	2 mts 47 sec	2 mts 30 sec	2 mts 47 sec	2 mts 25 sec

*Mean±SD (n=6) **Mean±SD(n=20)

Table. No:51 Stability Studies Data Assay & Dissolution of the Aspirin Optimized Formulations (AF4), Capsules With INNOVATOR (ALU BLISTER PACK) & Alu Alu Pack

Parameters	Initial	1st Month		2nd Month		3rd Month	
		RT	40°C	RT	40°C	RT	40°C
***Assay**	101.177 ±0.478	101.128 ±0.468	101.101 ±0.418	101.128 ±0.453	101.091 ±0.418	101.128 ±0.447	101.065 ±0.389
***INNOVATOR (Assay)**	100.674 ±0.453	100.556±0.454		100.574±0.483		100.574±0.453	
***% of Cumulative Release**	104.21	103.89	103.29	103.65	102.79	103.56	102.49
***% of Cumulative Release (Capsule)**	102.98	102.78	102.69	102.64	102.23	102.47	102.01
***INNOVATOR (% of Cumulative Release)**	102.14	102.22		101.98		102.09	

*Mean±SD (n=6)

Discussion: Assay*Mean=Not less than 75% ; DissolutionMean = Not less than 80%.** The results indicated that the, optimized formulated tablets were within the Pharmacopeial specifications.

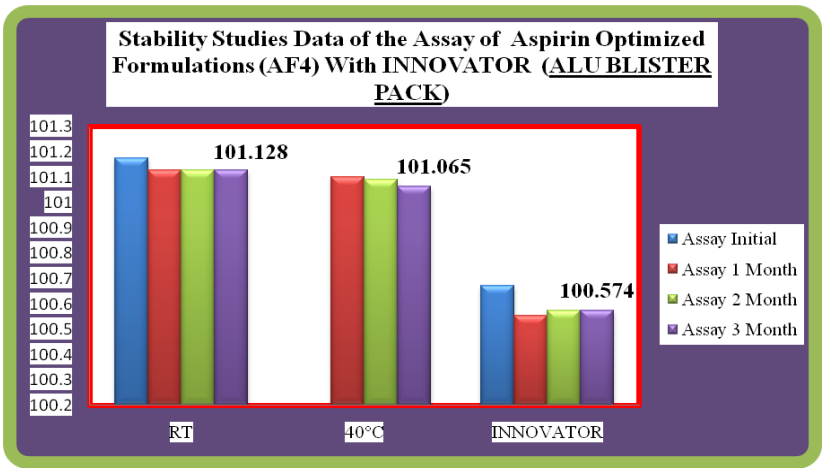

Figure. No:27 Stability Studies Data of the Assay of Atorvastatin Calcium Optimized Formulations (ATF7) With INNOVATOR

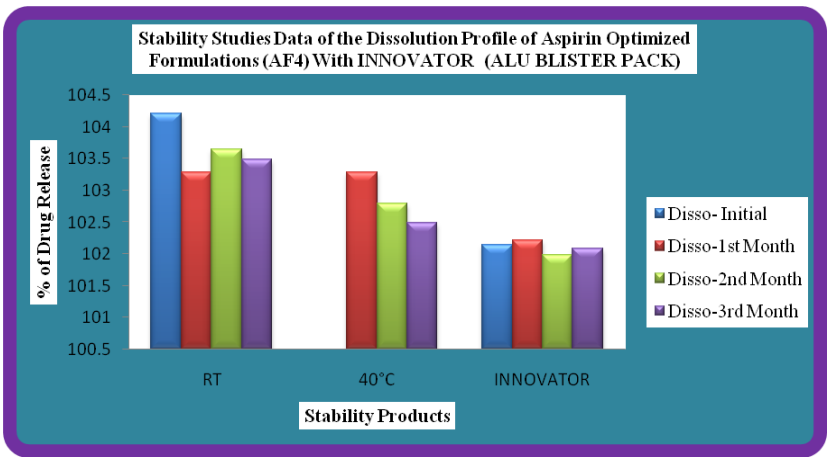

Table. No:28 Stability Studies Data of the Dissolution Profile of Aspirin Optimized Formulations (AF4) With INNOVATOR (ALU BLISTER PACK)

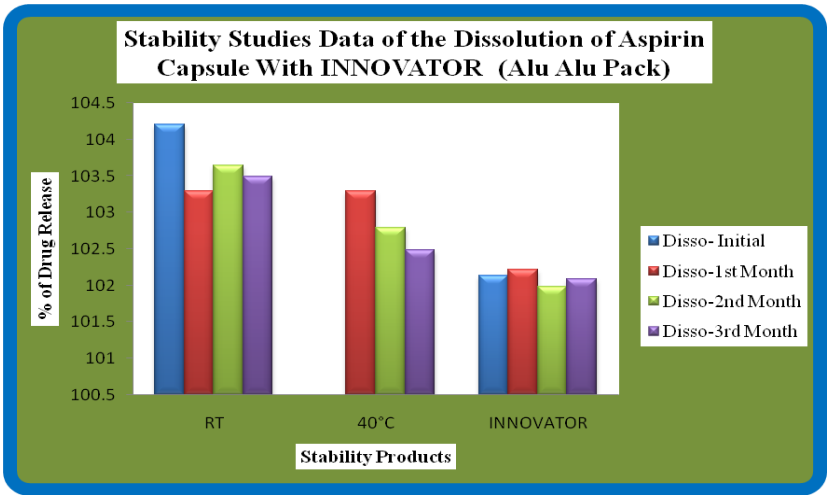

Table. No:29 Stability Studies Data of the Dissolution of Aspirin Capsule With INNOVATOR (Alu Alu Pack)

FOR CLOPIDOGREL BISULPHATE

Table. No:52 Stability Studies Data of the Clopidogrel bisulphate Optimized Formulations (CF7) (STRIP PACK)

Parameters	Initial	1st Month		2nd Month		3rd Month	
		RT	40°C	RT	40°C	RT	40°C
Weight variation (mm)**	134±6.8	133.5±6.6	133.5±6.7	133.4±6.7	133.4±6.6	133.4±6.7	133.4±6.4
Diameter (mm)*	5.69±0.03	5.67±0.13	5.66±0.93	5.66±0.33	5.65±0.63	5.66±0.23	5.64±0.93
Thickness (mm)*	4.45±0.06	4.44±0.14	4.44±0.04	4.44±0.24	4.43±0.97	4.44±0.04	4.43±0.67
Hardness (kg/cm2)*	3.88±0.16	3.79±0.12	3.76±0.22	3.80±0.12	3.75±0.67	3.80±0.12	3.74±0.87
Disintegration Time*	1 mts 52 sec	1 mts 43 sec	1 mts 33 sec	1 mts 38 sec	1 mts 23 sec	1 mts 33 sec	1 mts 18 sec

*Mean±SD (n=6) **Mean±SD(n=20)

Table. No:53 Stability Studies Data of the Assay & Dissolution of Clopidogrel bisulphate Optimized Formulations (CF7), Capsules With INNOVATOR (STRIP PACK) & Alu Alu Pack

Parameters	Initial	1st Month		2nd Month		3rd Month	
		RT	40°C	RT	40°C	RT	40°C
*Assay	101.177 ±0.347	101.165 ±0.352	101.105 ±0.341	101.156 ±0.332	100.895 ±0.052	100.896 ±0.332	100.405 ±0.520
*INNOVATOR (Assay)	100.544 ±0.346	100.44±0.987		100.448±0.654		100.32±0.646	
*% of Cumulative Release	103.12	102.87	102.54	102.84	102.24	102.76	102.09
*% of Cumulative Release (Capsule)	102.98	102.78	102.69	102.64	102.23	102.47	102.01
*INNOVATOR (% of Cumulative Release)	102.14	102.05		102.21		102.01	

*Mean±SD (n=6)

Discussion: Assay*Mean=Not less than 75% ; DissolutionMean = Not less than 80%.**

The results indicated that the, optimized formulated tablets were within the Pharmacopeial specifications.

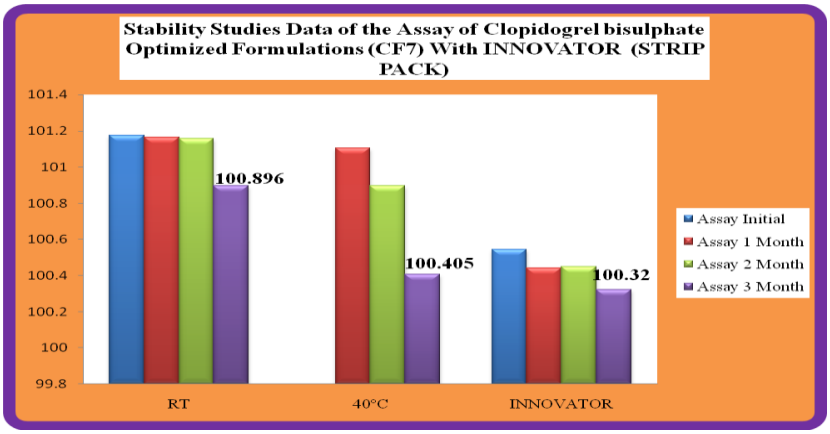

Figure. No:30 Stability Studies Data of the Assay of Clopidogrel bisulphate Optimized Formulations (CF7) With INNOVATOR (STRIP PACK)

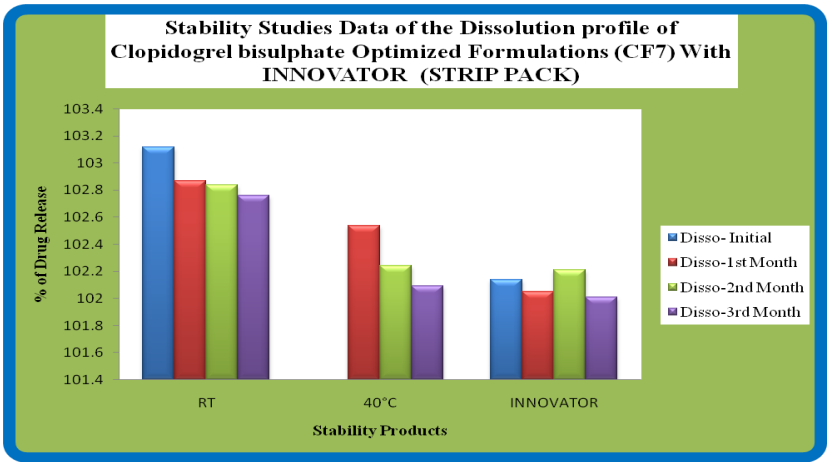

Figure. No:31 Stability Studies Data of the Dissolution profile of Clopidogrel bisulphate Optimized Formulations (CF7) With INNOVATOR (STRIP PACK)

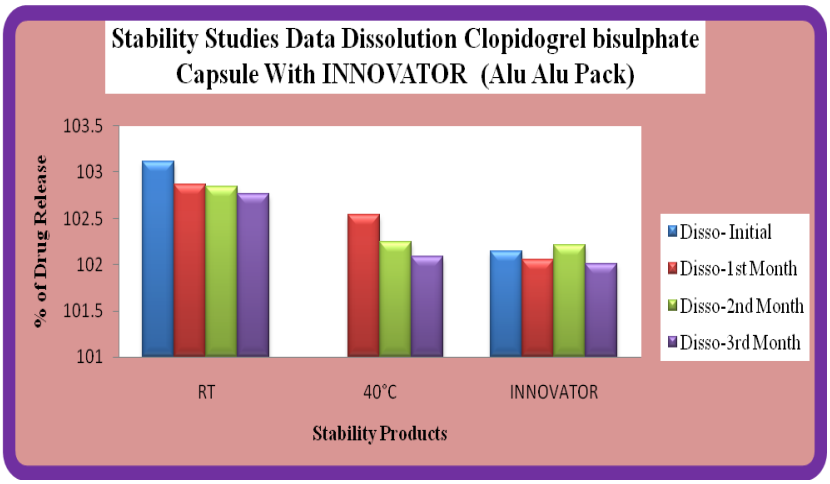

Figure. No:32 Stability Studies Data of the Dissolution Profile of Clopidogrel bisulphate Capsule With INNOVATOR (Alu Alu Pack)

DISCUSSION

From Table and Figure , it was seen that Atorvastatin calcium IR Tablets Batch. No: ATF7, Aspirin DR Tablets Batch. No: AT4, Clopidogrel bisulphate IR tablet Batch. No: CT7 and Capsule was showing good stability for three months accelerated condition @ 40^0C &75%RH. It was found that dissolution and assay value are not affected for the batch, and total impurity is also less than 1%.

SUMMARY AND CONCLUSION

The research work was aimed with formulation, development and evaluation of immediate release tablet of Atorvastatin calcium. The Assay and Impurity drug were carried out by HPLC method. The drug powders were subjected to Preformulation studies. The Preformulation characteristics are within the Pharmacopeial specifications. The Preformulation studies were carried out and the results were found to be satisfactory. The drugs and excipients compatibility were carried out by FT-IR studies and DSC. The spectra showed that there was no interaction between them. The drugs and excipients compatibility were carried out by HPLC method and by physical observation showed that there was no interaction between them. The drugs Assay and impurity were carried out by HPLC method.

Special care was taken for Atorvastatin calcium processing in low humidity condition and geometric mixing is applied to avoid content uniformity and segregation. The flow properties of the powdered blend for all the batches were found to be good and free flowing. The weight variation, hardness and friability of all the formulated tablets within the specified requirements. The disintegration times for the formulated tablets are within the range of USP. For Atorvastatin calcium IR tablets direct granulation was method of choice. Optimization was done and it was found that release profile was found to be best with two super disintegrants i.e. Croscarmellose sodium and Crospovidone. Film coating of Protectab HP-1 Erythrocin Supra aqueous coating 3%w/w was done on Atorvastatin calcium tablets as to avoid any interaction with Aspirin and Clopidogrel bisulphate. Results found that release profile of batch no.ATF7 matches with Innovator product IR Tablet. The Percentage cumulative drug release of batch. No. ATF7 was found at 30 Minutes 103.12%. From results it can be inferred that release profile of Batch. No: ATF7matches with that of innovator product, also f1&f2 values are good enough to comply with the innovator's product have reported similar kind of results for studies with Atorvastatin calcium.

The flow properties of the powdered blend for all the batches were found to be good and free flowing. The weight variation, hardness and friability of all the formulated tablets within the specified requirements. The disintegration times for the formulated tablets are within the range of USP. For Aspirin DR tablets direct granulation was method of choice. Optimization was done and it was found that release profile was found to be best with disintegrant i.e. sodium starch glycolate. Enteric coating of Protectab HP-1 Sunset yellow Lake IPA coating 10%w/w was done on Aspirin tablets as to avoid any interaction with Atorvastatin calcium and Clopidogrel bisulphate. Results found that release profile of batch no.AF4 matches with Innovator product DR Tablet. The Percentage cumulative drug release of batch. No. AF4 was found at 90 Minutes 104.21%. From results it can be inferred that release profile of Batch. No: AF4matches with that of innovator product, also f1&f2 values are good enough to comply with the innovator's product have reported similar kind of results for studies with Aspirin.

The flow properties of the powdered blend for all the batches were found to be good and free flowing. The weight variation, hardness and friability of all the formulated tablets within the specified requirements. The disintegration times for the formulated tablets are within the range of USP. For Clopidogrel bisulphate DR tablets direct granulation was method of choice. Optimization was done and it was found that release profile was found to be best with two super disintegrants i.e. Croscarmellose sodium and Crospovidone. Film coating of Protectab HP-1 Iron oxide Supra aqueous coating 3%w/w was done on Clopidogrel bisulphate tablets as to avoid any interaction with Aspirin and Atorvastatin calcium. Results found that release profile of batch no.CF7 matches with Innovator product IR Tablet. The Percentage cumulative drug release of batch. No. CF7 was found at 30 Minutes 102.14%. From results it can be inferred that release profile of Batch. No: CF7matches with that of innovator product, also f1&f2 values are good enough to comply with the innovator's product have reported similar kind of results for studies with Clopidogrel bisulphate.

Finally, the optimized formulations were subjected to accelerated stability studies and at room temperature (RT) as per ICH guidelines. The result obtained showed that there were no significant changes in tablet parameters such as appearance, hardness, friability, weight variation, drug content uniformity, and in-vitro drug release profile. Thus, it was concluded that the optimized formula (Batch. No: ATF7) for Atorvastatin calcium IR tablets, (Batch. No: AT4) for Aspirin DR tablets and (Batch. No: CT7) Clopidogrel bisulphate IR

tablets are stable under accelerated conditions of temperature and humidity. The formulations were found to be comparable with innovator (reference) formulations, with respect to physicochemical parameters having better in-vitro release profiles. All optimized tablets ATF7, AT4 and CT7 it was filled into one size capsules. The final formulation was filled in a capsule of one size consisting of Atorvastatin calcium IR tablet 10mg, Aspirin DR tablet 75mg and Clopidogrel bisulphate IR tablet 75mg to be packed in alu strip of 15 capsules per strip. It is used in case of chronictherapy. Once daily giving maintence of plasma drug concentration at therapeutic level, useful for lowering plasma cholesterol level, myocardial infarction cardiovascular.

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