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DESIGN AND *IN-VITRO* EVALUATION OF BILAYER TABLET CONTAINING TWO INCOMPATIBLE ANTIHYPERTENSIVE DRUGS.

Tekade B. W.,* Jadhao U. T., Thakare V.M., Chaudhri Dipali G. and Patil V.R.

Department of Pharmaceutics, TVES's Honorable Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, (India).

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***Corresponding Author**
Dr. Bharat W. Tekade
Department of Pharmaceutics, TVES's Honorable Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, (India).

ABSTRACT

The main goal of this study was to develop tablet formulation for two incompatible antihypertensive drugs Amlodipine besilate and Losartan potassium as an immediate release bi layer tablet and to evaluate its dissolution studies. The formulation development work was initiated with wet granulation for losartan potassium layer and direct compression for amlodipine besilate layer. The prepared tablets were evaluated for weight variation, hardness, thickness, friability, % drug content, disintegration time, *in vitro* drug release. Formulation F8 showed a highest drug release of 96.56% for Amlodipine and 94.23 % for Losartan potassium in 30 minutes which was selected as optimized formulation and considered for further studies. The stability studies, shown that the formulation F8 was stable enough at 40°C / 75 % RH for a period of 30 days.

KEYWORDS: Amlodipine Besilate, Losartan Potassium, bi-layer tablet, Anti-hypertensive.

INTRODUCTION

The term "Hypertension" literally means and an abnormally raised arterial blood pressure. Hypertension is the most common cardiovascular disorder. Essential hypertension is characterized by a sustained systolic blood pressure of greater than 140 mm Hg and a diastolic blood pressure at greater than 90 mm Hg. Complications of hypertension include myocardial infarction, peripheral vascular diseases, heart failure, renal insufficiency and stroke. Hypertension is widely associated with modern lifestyle, dietary habits and stress conditions. Most conventional oral drug products, such as tablets and capsules, are

formulated to release the active drug immediately after oral administration, to obtain rapid and complete systemic drug absorption.

Bi layer tablets are prepared with one layer of drug for immediate release while second layer designed to release drug later, either as second dose or in an extended release manner.[1, 2] Bi layer tablet is suitable for sequential release of two drugs in combination, separate two incompatible substances, and also for sustained release tablet in which one layer is immediate release as initial dose and second layer is maintenance dose or both the layers can be immediate release.[3-5] Hypertension is among the most common diseases of adults in industrialized countries and is one of the important modifiable risk factors for cardiovascular and renal disease. The goal of losartan potassium and amlodipine besylate (anti-hypertensive drug) is to prevent complications of hypertension. Specially used for the treatment of patients with stage 1 and 2; essential hypertension in comparison with monotherapy regimens of a calcium channel antagonist or an angiotensin II receptor blocker. The main goal of this study was to develop as tablet formulation for two incompatible antihypertensive drugs Amlodipine besilate and Losartan potassium as an immediate release bi layer tablet and to evaluate its dissolution studies.

MATERIALS AND METHODS

Materials: Losartan Potassium & Amlodipine Besilate was obtained as a gift sample from Cipla. Calcium hydrogen Phosphate, Crosscormellose sodium obtained from Colorcon Asia Pvt. Ltd., All the reagents and materials were of analytical or pharmacopoeia grade.

Preparation of Bilayer Tablet.

Preparation of Losartan Potassium immediate release granules: Sift the weighed quantity of losartan potassium, microcrystalline cellulose, calcium hydrogen phosphate, croscormellose sodium, feric oxide red, through mesh 30#. Transfer the sifted material and binder solution to granulation. And prepared granules of that mixture dry the granules at 50º for 15 min. sift talc and aerosil through mesh 40#. Load sifted dried granules along with the above sifted material & into blender and blends it for 10 min. Finally sift Magnesium Stearate through Mesh 60# and mix for 2 minutes.[6]

Preparation of Amlodipine besilate layer blend.

Sift the weighed quantities of Amlodipine Besilate, calcium hydrogen phosphate, Micro crystalline cellulose, croscormellose sodium, and all the sifted material pass through Mesh

30#.After that above material sifted with talc an aerosil through mesh 40#. Load sifted dry material into blend and blend it for 10 min. finally Sift Magnesium Stearate through Mesh 60# and add to the above mixture in the blender and mix for another 2 minutes.[7]

Table No.1: Composition of tablet formulations.

Sr no	Ingredients(mg)	F1	F2	F3	F4	F5	F6	F7	F8
Amlodipine Besilate Layer									
1	Amlodipine besilate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
2	Calcium hydrogen phosphate	6	9	11.70	12	12	14	15.16	15.16
3	Microcrystalline cellulse	50.55	49	49	47.63	47	45.64	46	46.32
4	Carboxy methyl cellulose sodium	4	3.46	3.15	2	2	2.25	2.75	2.75
5	Aerosil	--	--	--	0.20	0.40	0.45	0.45	0.45
6	Talc	0.62	0.50	0.40	0.32	0.32	0.32	0.32	0.32
7	Magnesium stearate	0.75	0.69	0.65	0.60	0.50	0.50	0.50	0.50
Losartan Potassium Layer									
8	Losartan	25	25	25	25	25	25	25	25
9	Calcium hydrogen phosphate	8	9	12	15	13	13.59	12	12.75
10	Ferric oxide	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
11	Microcrystalline cellulse	64.33	63.16	60.6	54	56	54	54.5	52
12	Carboxymethyl cellulose sodium	5	4	3	6	6.53	7	8	7.5
13	Aerosil	--	1.5	1.5	1.5	1.5	1.5	52.57	1.5
14	Talc	0.75	--	--	0.75	0.75	0.75	0.75	0.75
15	Magnesium stearate	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
16	Purified water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
	Total weight	170	170	170	170	170	170	170	170

Evaluation of tablets

Pre-compression study.

Flow properties of gum base and drug: excipient mixtures were determined by measurement of angle of repose, bulk density, tapped density, compressibility index (CI) and hausner's ratio.[8]

Post-compression studies.

Thickness

The thickness of the tablets was determined using a Vernier caliper. Five tablets from each type of formulation were used and average values were calculated.[9]

Hardness

The resistance of tablets to shipping, breakage, under conditions of storage, transportation and handling before usage depends on its hardness. For each formulation, the hardness of 6 tablets was determined using the Monsanto hardness tester. The tablet was held along its

oblong axis in between the two jaws of the tester. At this point, reading should be zero kg/cm^2. Then constant force was applied by rotating the knob until the tablet fractured. The value at this point was noted.

Friability: Friability is the measure of tablet strength. Roche Friabilator was used for testing the friability using the following procedure. This test subjects a number of tablets to the combined effect of shock abrasion by utilizing a plastic chamber which revolves at a speed of 25 rpm, dropping the tablets to a distance of 6 inches in each revolution. A sample of preweighed 6 tablets was placed in Roche friabilator which was then operated for 100 revolutions i.e. 4 minutes. The tablets were then dusted and reweighed. A loss of less than 1 % in weight in generally considered acceptable. Percent friability (% F) was calculated as follows**.**

$$\% F = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Weight variation test: To find out weight variation, 20 tablets of each type of formulation were weighed individually using an electronic balance, average weight was calculated and individual tablet weight was then compared with average value to find the deviation in weight.

Table No 2:- Specifications for tablets as per Pharmacopoeia of India

Sr No.	Average Weight of Tablet	% Deviation
1	80 mg or less	10
2	More than 80 mg but less that 250 mg	7.5
3	250 or more	5

Disintegration test

The in-vitro disintegration time was determined by using disintegration test apparatus. One tablet placed in each of the six tubes of the apparatus and one disc was added to each tube. The medium used was 0.1 N HCl (pH 1.2). The time in seconds taken for complete disintegration with no palpable mass in the apparatus was measured in seconds.[10]

Uniformity of drug content

Five tablets of each type of formulation were weighed and crushed in mortar and powder equivalent to 25 mg of Losartan potassium And Amlodipine losatane 2.5mg was weighed and dissolved in 100 ml of 0.1N HCl (pH 1.2). This was the stock solution from which 0.2 ml

sample was withdrawn and diluted to 10 ml with 0.1N HCl. The absorbance was measured at wavelength 226 nm and wavelength 239 nm using double beam UV-Visible spectrophotometer.

Content uniformity was calculated using formula

% Purity = 10 C (Au / As)

Where, C - Concentration,

Au and As - Absorbance's obtained from unknown preparation and standard preparation respectively.

***In-vitro* Dissolution**

The dissolution study was carried out using USP Type II (Paddle type) dissolution apparatus. The dissolution was carried out in 900 ml of 0.1 N HCl (pH 1.2) maintained at 37°C at 100 rpm. 10 ml aliquots of samples were taken at 5 min. time intervals which were replaced with same volume of fresh 0.1 N HCl (pH 1.2) maintained at 37°C. The withdrawn samples were analyzed by an UV spectrophotometer at 226 nm and 239 nm using 0.1N HCl as a blank. Drug content in dissolution sample was determined using calibration curve.

Infrared spectroscopy

The FTIR of pure drug and physical mixture of formulation ingredients of optimized batch was measured using Fourier transform infrared spectrophotometer (Model FTIR- 8400S, Shimadzu, Japan). The amount of each formulation ingredient in the physical mixture was same as that in the optimized batch. The pure drug and physical mixture were then separately mixed with IR grade KBr. This mixture was then scanned over a wave number range of 4000 to 400cm-1.[11-13]

Stability Studies

Stability testing of formulation batch was carried out to determine the stability of drug and carrier and also to determine the physical stability of formulation under accelerated storage condition at 45ºC/70%RH. The prepared tablets were placed in borosilicate screw capped glass containers. The samples were kept at condition of 45°C/70% RH and were analyzed at 7^{th}, $14^{th,}$ $21^{st}$and 28^{th} days for drug content, hardness and *in-vitro* dissolution study.[14]

RESULT AND DISCUSSION

Pre-compression study.

The bulk density obtained for all the formulations in the range of 0.555±0.01 to 0.625±0.01 (g/ml) for Amlodipin and 0.571±0.01 to 0.571±0.01(g/ml) for Losartan. The tapped density in the range of 0.625±0.01 to 0.741±0.02 (g/ml) for Amlodipin and 0.645±0.02 to 0.714±0.02 for Losartan. The Angle of repose of the powder blend of all the formulations was found in range of 22.55° to 26.41° for Amlodipin and 23.44° to 25.10° for Losartan. which is in the good or in the acceptable range means showing the good flowability necessary for proper flow of powder blend into the die cavity. The Carr's index of the powder blend of all the formulations was found in the range of 11.20±1.13 to 16.64±0.48 % for Amlodipin and 9.07±0.58 to 16.41±0.58 % for Losartan which is good or in the acceptable range means showing good or fair flowability for proper flow of powder blend. The Hausner's ratio was found to be in the range of 1.126±0.003 to 1.199±0.007 for Amlodipin and 1.103±0.006 to 1.220±0.004 for Losartan. All these results indicated that, the powder mixture possess good flow of powder blend into the die cavity and compressibility properties.

Table No.3: Physical parameters of powder blend

Parameter / Formulation		Bulk Density (g/ml)	Tapped Density (g/ml)	Angle of Repose (°)	Carr's Index (%)	Hausner's Ratio
F1	A	0.619±0.02	0.699±0.04	23.29±0.89	11.44±0.23	1.129±0.04
	L	0.606±0.01	0.714±0.02	24.61±1.18	15.12±0.46	1.178±0.006
F2	A	0.625±0.01	0.741±0.02	26.41±0.49	15.65±0.48	1.185±0.007
	L	0.626±0.03	0.691±0.03	25.10±0.51	9.40±0.49	1.103±0.006
F3	A	0.555±0.01	0.625±0.01	24.31±0.85	11.20±1.13	1.126±0.003
	L	0.601±0.03	0.661±0.04	23.44±1.56	9.07±0.58	1.099±0.007
F4	A	0.583±0.02	0.682±0.03	22.55±0.85	14.51±0.64	1.169±0.008
	L	0.571±0.01	0.645±0.02	25.02±0.76	11.47±0.90	1.129±0.004
F5	A	0.672±0.02	0.784±0.04	23.29±0.89	14.28±0.23	1.166±0.04
	L	0.653±0.01	0.771±0.02	24.61±1.18	15.30±0.46	1.128±0.006
F6	A	0.646±0.01	0.775±0.02	26.41±0.49	16.64±0.48	1.199±0.007
	L	0.670±0.03	0.792±0.03	25.10±0.51	15.35±0.49	1.182±0.006
F7	A	0.630±0.01	0.749±0.01	24.31±0.85	15.88±1.13	1.188±0.003
	L	0.606±0.03	0.725±0.04	23.44±1.56	16.41±0.58	1.196±0.007
F8	A	0.588±0.02	0.682±0.03	22.55±0.85	13.78±0.64	1.159±0.008
	L	0.602±0.03	0.735±0.02	25.02±0.76	15.78±0.90	1.220±0.004

Post-compression studies.

The hardness of tablets was found to be 2.8±0.90 to 4.8±0.72 kg/cm2. Thicknesses of all tablets were found to be in the range of 2.02±0.9 to 2.25±0.3 mm. All the tablets shows % friability in the range of 0.45±0.64 to 0.79±0.7 % which is within the limit. All the

formulations passes the weight variation test as all tablets within the pharmacopoia limit of 7.5% of the weight. The disintegration time of the all batches were found to between 10 min to 32 sec.

Table No.4: Physical parameters of Tablets.

Parameters / Formulations	Hardness ($kg/cm^2\pm$)	Thickness (mm)	% Friability	Wt. Variation	Disintegration time
F1	3.3±0.52	2.04±0.3	0.63±0.56	171±1.75	10:32±00.50
F2	3.0±0.82	2.20±0.1	0.61±0.99	170.25±2.6	09:26±00:35
F3	2.8±0.90	2.15±0.2	0.75±0.62	170±2.25	07:03±00:40
F4	2.9±0.73	2.02±0.9	0.79±0.7	170.12±1.98	07:17±00:25
F5	3.1±0.41	2.07±0.6	0.62±0.36	170.56±1.81	06:42±00:16
F6	3.7±0.20	2.04±0.4	0.58±0.45	170.36±2.55	05:18±00:20
F7	3.4±0.13	2.25±0.3	0.51±0.32	170.11±0.85	05:25±00:10
F8	4.8±0.72	2.21±0.6	0.45±0.64	170.09±0.55	04:21±00:12

Tablets from each batch of amlodipine besylate showed uniformity of content in the range 91.18% to 101.38% and losartan potassium showed uniformity of content in the range 95.85% to 103.33 which is within pharmacopoeial specifications. All the formulations complies the test for uniformity of content were found to be within the limit of 90- 110%.

Table No.5:- Uniformity content of formulations F1 to F8

Formulations	Uniformity of Content ± SD (%)	
	AMLODIPINE	LOSARTAN
F1	96.23±0.67	98.36±0.47
F2	91.18±0.46	102.72±0.64
F3	94.26±0.16	103.33±0.59
F4	101.38±0.82	101.96±0.43
F5	97.35±0.79	95.85±0.31
F6	93.12±0.45	100.8±0.28
F7	91.66±0.14	96.17±0.37
F8	98.53±0.34	97.90±0.19

***In-vitro* Dissolution**

In-vitro dissolution studies of bi-layer tablets were conducted for 30 minutes. *In- vitro* drug release profile from all formulations (F1-F8) showed fast and immediate release of Losartan potassium and amlodipine besylate over a period of 30 minutes. The maximum drug release was found for the optimize formulation F8 shows least disintegration time and better dissolution properties compared to the other batches.

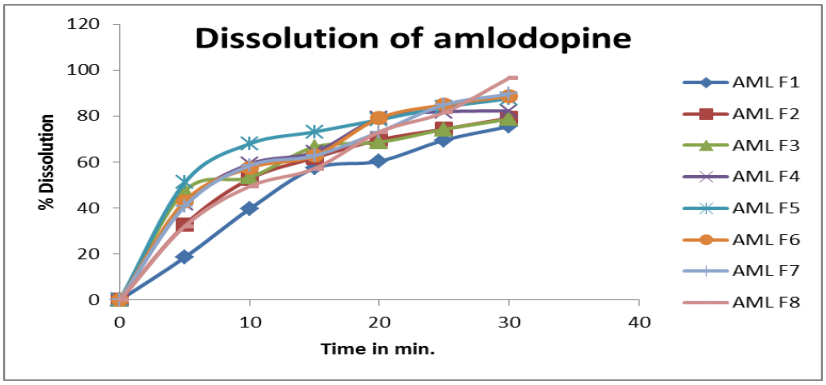

Figure No.1-In-vitro Dissolution Profile of Formulation Batch F1 to F8.

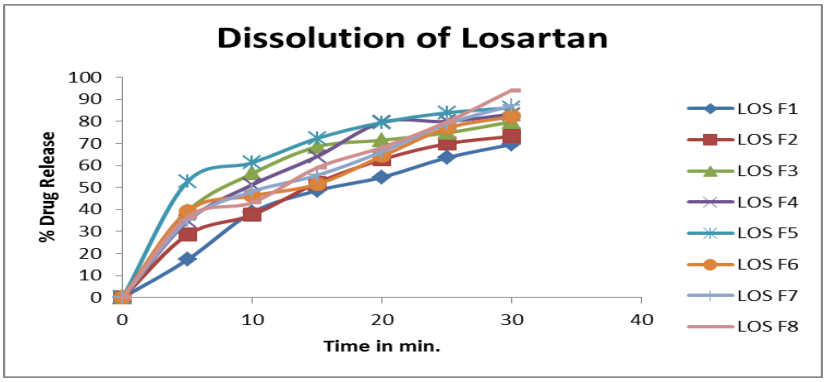

Figure No.2-In-vitro Dissolution Profile of Formulation Batch F1 to F8.

Infrared spectroscopy: It was carried out to check for the possible Drug-Excipients interaction. The IR absorption band in cm-^{1} of the drug and excipients used in the study were similar. The IR spectrum did not show presence of any additional peaks for new functional groups indicating no chemical interaction between Losartan potassium, Amlodipin Besylate & the used polymers. From the IR spectrum of the Drug and polymer it was found that there is no or negligible change is observed in the spectrum (which shown in above figures) so, there was no chemical interaction observed between drug and excipients (polymers). In this way compatibility was studied between drug and excipient and they are stable.

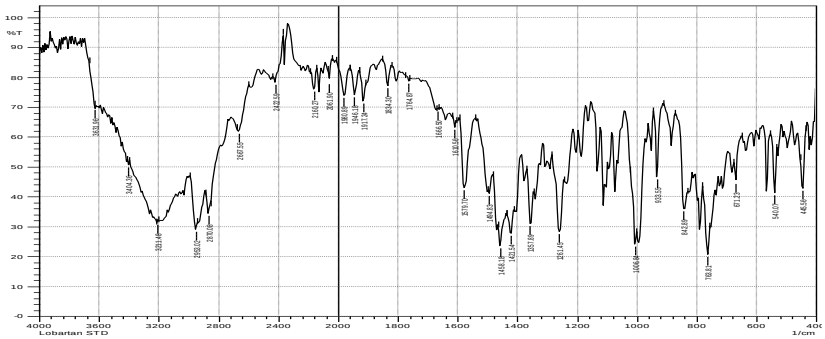

Figure No. 3:- FT-IR spectrum of Losartan potassium.

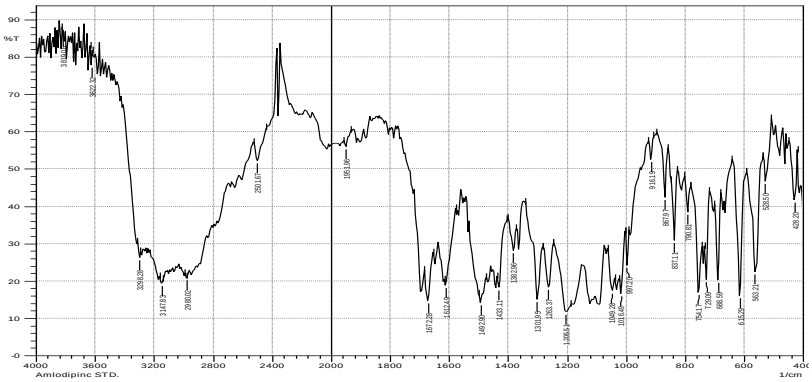

Figure No. 4:- FT-IR spectrum of Amlodipine Besilate.

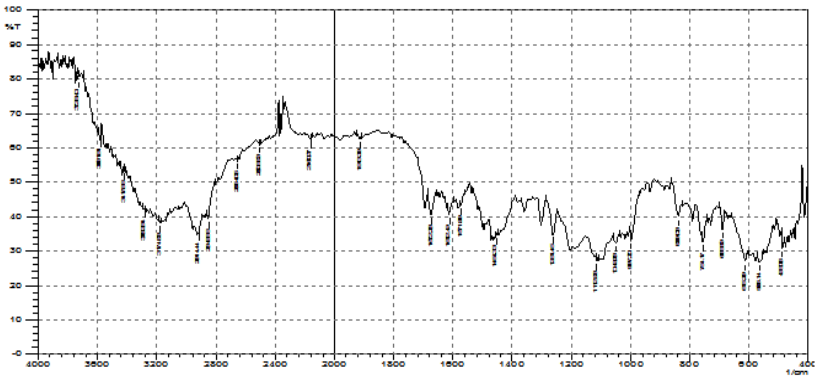

Figure No. 5:- FT-IR spectrum of Physical Mixture.

Accelerated stability studies (AST) was carried for optimized formulation F8 by exposing it to 40°C/75% RH for one month and analyzed the sample at the interval of 7,14,21,28 days. The sample was analyzed for drug content, hardness and cumulative percentage drug release.

Table No. 6- Evaluation of optimized batch F12 during stability studies.

Parameters		Days				
		0	7	14	21	28
Hardness		4.8±0.72	4.60±0.13	4.60±0.1	4.60±0.13	4.4±0.10
Drug content (%)	A	98.53±0.34	98.18±0.79	97.65±0.34	97.46±0.89	97.39±0.41
	L	97.90±0.19	97.78±0.23	97.55±0.43	97.18±0.61	96.77±.0.14
In-vitro dissolution study	A	96.56±0.16	96.33±0.62	96.00±0.62	94.93±0.42	94.42±0.39
	L	94.23±0.19	94.01±0.62	93.33±0.62	93.93±0.42	92.16±0.39

FT-IR spectrums of optimized formulation F8 after 28 days. The drug, Losartan potassium, Amlodipin besylate present in the formulation was confirmed by FT-IR spectra. The characteristics peaks due to -OH, C=O, C-Cl, C=N and N=N groups present in Losartan and C-Cl, N-H, -OH, C-H, C=O, C-N, C-S groups present in Amlodipin appeared in tablet spectra (Formulation), without any remarkable change in their position, indicating no chemical interaction between drug and Excipients.

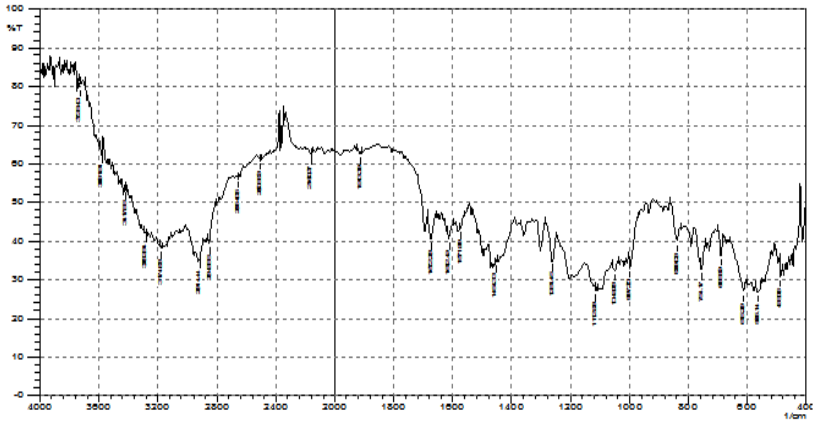

Figure No. 6:- Pre-stability study of FT-IR spectrum of formulation.

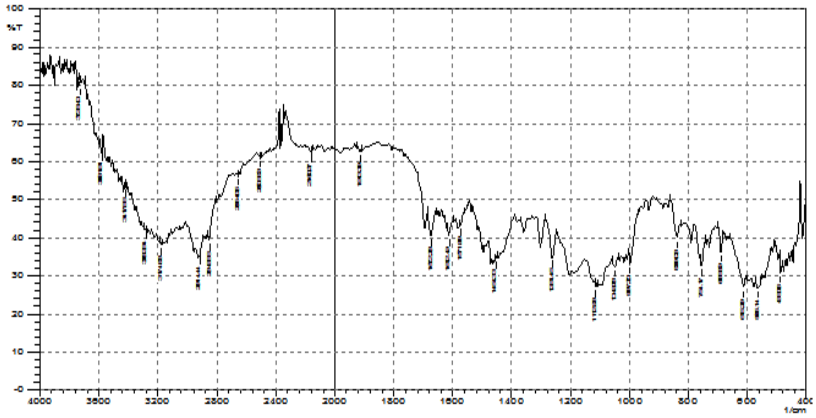

Figure No. 7:- Post-stability study of FT-IR spectrum of formulation F8 after 28 days.

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