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A COMPARATIVE STUDY FOR PRE-FORMULATION AND STABILITY OF COMBINATION OF ATORVASTATINE AND EZETIMIBE

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ABSTRACT
Purpose: Study compatibility between ATV and EZT in suggested pre-formulation, the parameters of different SDF and effect of different manufacturing technique on the physical and chemical properties of the drug combination, then detection of expire date of the selected formula of drug combination prepared by different techniques. **Methods:** study interaction of drug combination by TLC, FTIR, DSC, and then study compatibility of drugs with excipients. The selected formula was evaluated in different dosage form by determine disintegration time, in vitro release studies, and dissolution profile. Finally, determination of expire date for the selected formula after storage for six month using HPLC procedure by applying of Arrhenius equation. **Result:** Solubility of two drugs in proved with increasing Ph above 5.5. There was probability of partial interaction between the two drugs. The selected formula (F5) was used as it increases release and solubility of the two drugs. The rate of release of two drugs in capsule was higher than tablet. **Conclusion:** Formula (F5) was preferred to use in drug combination. Capsules were recommended for drug combination then tablets. Shelf lives of drugs combination in capsules were being better then tablets. Storage for six month affect dissolution rate of drug combination.

KEY WORDS: TLC, FTIR, DSC.

INTRODUCTION
Cardiovascular disease (CVDs) are the major course of death in developed countries and also are rapidly emerging as a main cause of death in the developing world. It is estimated that almost 20 million people will die from CVDs 2015.[1]

The reduction of elevated serum total cholesterol and low-density lipoprotein cholesterol reduces the risk of coronary artery disease, resulting in a decrease in cardiovascular morbidity and mortality.[2]

Cholesterol and tri-glyceride in the diet enter in the exogenous path way of lipid transport.

Lipids produced by the body are transported through the endogenous path way. Triglycerides can be synthesized by the liver, especially in the presence of execs carbohydrates.

Hypercholesterolemia can be caused by genetic predisposition through secondary causes like underlying disease states, drugs or life style or both.

Atarvastatin calcium is a synthetic lipid-lowering agent. Atarvastatin is an inhibitor of 3-hydroy-3-methyleglutouyl-coenzyme A (HMG-CoA) reductose. This enzyme catalyzes the conversion of HMG-CoA to mevabenate, an early and rate limiting step in cholesterol biosynthesis.

Atorvatatin in rapidly absorbed after oral administration maximum plasma concentration occur within 1 to 2 hours.

The absolute bioavailability of Atorvastatin is approximately 14% and the systemic availability of HMG-CoA redactose inhibitory activity is approximately 30%, food decreases the rate and extent of drug absorption by approximately 25%. Mean plasma elimination half-life of Atorvastatin in humans is approximately 14 hr., but the half-life of inhibitory activity for HMG-CoA reductose is 20 to 30 hr. due to the contribution of active metabolites.

Less than 2% of a dose of Atorvastatin is recovered in urine following oral administration.[3]

Ezetimibe is the first selective cholesterol absorption inhibitor. It is licensed for the treatment of primary hypercholesterolemia in patients poorly controlled with a stain alone, and for homozygous familial hypercholesterolemia. Ezetimibe selectively inhibits the absorption of dietary and billiard cholesterol and related

plant sterols. It dose hot effect the absorption of fat soluble vitamins or triglycerides.[4]

Ezetimibe absorbed and extensively conjugated to active phenolic glucuronide (ezetimibe-glucuronide) after oral administration. The mean Ezetimibe plasma peak concentration after single 10 mg dose was (C max) of 3.4 to 5.5 mg/ml which attained within 4-12 hours (T max). The absolute bioavailability of ezetimibe cannot be determined, as the compound is virtually insoluble in aqueous media suitable for injection Ezetimibe has variable bioavailability, the coefficient of variation, used on inter-subject volubility was 35 to 60% for AUC values.[3] Co administration of Ezetimibe and Atorvastatin, produce significant LDL-C level reduction and favorably effected total cholesterol, HDL-c, and triglyceride levels.[5,6] The combination of 2 active ingredient eliminates side effects such as statin-associated myopathy and hepatotoxicity and Ezetimibe - induced compensatory – elevated hepatic cholesterol synthesis.[6,7]

Aim: The aim of this work to study the combination of Atorvastatin and Ezetimibe in the most stable and effective formula to achieve guideline-recommended LDL-C goals for patients with hypercholesterolemia safely.

Experimental and methodology

1. Materials

- **Atorvastatin calcium and Ezetimibe** was kindly supplied from Mayrcl Pharmaceutical Industries Company – Elobour City.

2. Equipment

- **High Performance Liquid Chromatography** (Shimadzu™LC- 20A series chromatograph with a Rheodyne injector valve with 20 µI loop and a SPD- 20A UV detector, Japan)

- **U.V-V spectrophotometer** with 1 cm quartz cuvettes, connected to IBM PC computer used for all the absorbance measurements and treatment of data and HP laser jet 1000 series printer. (Agilent, Model 8453, Germany).

- **Thin- Layer Chromatographic plates,** precoated with silica gel GF, 10 X 10 cm, 0.25 mm thickness fluorescent at 254 nm (E-Merck, Germany).

- **Fourier transforms infrared spectrophotometer** (Schimadazu IR- 345-U- 04, Japan).

- **Diffrential Scanning Colourmetry (DSC)** (DSC-50; Shimadzu - Kyoto, connected with thermal analyzer TA-501 and Deskjet 500C printer, Japan).

METHODS AND EXPERIMENT

1. Ultraviolet scanning of Atorvastatin calcium, Ezetimibe,and Combination in methanol: Atorvastatin calcium solution, Ezetimibe and mixture of them (10 mg/ml) were scanned spectrophotometrically at 200-400 nm using ultraviolet spectrophotometer, the wave lengths of maximum absorbance were determined.

2. Solubility study of combination of Atorvastatin calcium and Ezetimibe were studied at different PH: Ten mg of each drug were mixed with 100 ml phosphate buffers at different pH (4.5, 5.5, 6.5, 7.5) in 100 ml stopper glass bottles and placed in thermostatically controlled water both at 25°C±1 for 36 hrs. The samples were taken at interval times 12 , 24 and 36 hrs then samples were filtered through (0.22 mm) millipore filter. Concentration of drug was determined using UV method.

3. Partition coefficient of combination of Atorvastatin calcium and Ezetimibe using n-butanol/ distilled water: phosphate buffer of 10 ml at different pH (4.5, 5.5, 6.5, 7.5) was used distilled water as aqueous phase and (n-butanol) as oily phase was used. Ten mg of drug combination (10 mg of each drug) were dissolved in aqueous phase (10 ml), equal amount of (n-butanol) (after saturation with distilled water) placed in a (screw capped glam tube) of 50 ml capacity. Shake for 24 hrs in thermostatically controlled at 37°C ± 0.5°C. Using separating funnel, an aqueous phase and n-butanol phase was separated then take aqueous phase and measured the absorbance at wave lengths 232.5 nm and 246 nm then calculate the concentration of each drug[8], experiment was repeated three times then partition coefficient was calculated according to Nerset Equation[9]

$$K = \frac{C_{oil}}{Caqu.}$$

K: portion coefficient
Coil: concentration of drug in organic phase
Caqu.: concentration of drug in aqueous phase

4. Study of interaction of Atorvastatin calcium and Ezetimibe

4.1. Thin layer chromatography (TLC)

Study was performed an 20 cm x 20 cm aluminum plates pre-coated with silica gel 60 F. plates were washed with methanol, activated in oven at 105°C for 25 min, then left to cool at room temperature. Ten mg Atrovastatin calcium powder, Ezetimibe and physical mixture (1) were dissolved in 100ml of mobile phase (chloroform: benzene: methanol: acetic acid)(6:3:1:0.1 v/v/v/v).[10] Samples were applied to pre-washed activated plates. The plates were developed with mobile phase in glass jar perversely saturated with mobile phase vapor for 20 min.

4.2 Fourier transforms infrared spectroscopy (FTIR)

For further elucidation and confirmation of the possible interaction of Atorvastatin calcium with Ezetimibe, the IR absorption spectra were obtained for Atorvastatin calcium alone, Ezetimibe alone, and their corresponding physical mixtures (1:1 w/w). Samples (1-2 mg) were mixed with potassium bromide (IR grade).The mixture

was compressed into discs in the compressor unit under vacuum, and scanned from 4000-400 cm^{-1} with an empty pellet holder as a reference.

4.3 Differential scanning calorimetry (DSC): (DSC) used for solid material thermal properties. In the DSC analysis, the differences in power required to maintain the sample and the reference to the same temperature are recorded as a function of temperature. DSC analysis, the transisitions (first and second order) can be observed in the curve of heat flow versus temperature. Samples of Atovastatin calcium and Ezetimibe separately and physical mixtures of two drugs in ratio of (1:1) were heated in thematically sealed aluminum pans over the temperature range of 0-200°c at a constant rate of 10°c / min under nitrogen purge (30 ml/min). Atorvastatin calcium and Ezetimibe mixture were prepared to detect any interactions if ever present.[11]

5. Study of compatibility of drug-excipients by Fourier Transform Infrared Spectroscopy (FTIR)

In this study, the compatibility of each drug in suggested drug combination with certain excipients lactose monohydrate, calcium carbonate, avicel PH101, PVP K25, tween 80, aerosil 200, Ac-Di-Sol, and magnesium stearate was investigated. This was achieved by comparing IR spectrum of each drug individually and those (1:1 w/w) physical mixtures of each drug and the corresponding excipient then comparing IR spectrum of the mixture of two drugs and those (1:1 w/w) physical mixtures of this mixture and the corresponding excipient. Samples (1-2 mg) of each drug alone and the mixture of two drugs as well as physical mixtures of those with investigated excipients (1:1 w/w) prepared by simple and perfect mixing on clean waxy paper were mixed with potassium bromide IR grade. The mixture was compressed into discs under vacuum, and scanned 4000-400 cm-1 with an empty pellet holder as a reference.

6. Preparation of different formulations containing suggested drug combination containing Atorvastatin calcium and Ezetimibe R

Atorvastatin calcium	**10.82 mg equivalent to 10 mg of base / tabe**
Ezetimibe	10 mg / tab
Alkanizing agent (Calcium carbonate)	0-20 %
Diluent (Lactose monohydrate)	37 %
Diluent (Avicel 101)	18 %
Binder (PVP K_{25})	1-4 %
Surfactant (Tween 80)	0-2 %
Lubricant (Magnesium stearate)	1 %
Super disintegrant (Ac-Di-Sol)	0-5 %
Glident (Aerosil 200)	0-1 % 0-1 %

Formulas containing suggested drug combination were prepared in different dosage form to choose the best formula and best dosage form (Table1).

6.1. Tablet form by Direct Compression Technique

The formulated tablets were prepared by weighing and sifting Atorvastatin calcium powder 10.82 mg (equivalent to 10 mg of Atorvstatin base) and Ezetimibe powder 10 mg were at sieve No. 20 separately prior to preparation of tablets. Other ingredients of each tablets formulation were weighed then divided approximately into two halves except magnesium stearate.[12] Sift individually calcium carbonate, lactose monohydrate (50%), and avicel PH 101 (50%) through sieve no. 20 then mixed with Atorvastatin calcium powder by geometric dilution in the Turbido Mixer and blend for 5 min at 20 rpm for each addition of each ingredient. Half amount of PVP $_{K25}$ and tween 80 were added slowly to the powder and mixed for 5 minutes. Sift remaining quantity of lactose monohydrate, a vehicle pH 101, through sieve No. 20 then mixed with Ezetimibe by geometric dilution in the Turbido Mixer and blend for 5 min at 20 rpm for each addition of each excipicnt. Second half of PVP $_{K25}$ and tween were added slowly to the powder and mixed for 5 minutes.

Mix two previous mixtures in the Turbido Mixer for 10 min then add the disintegrant (Ac-Di-Sol) and blend for 5 min at 20 rpm then blend for another 5 minutes at slow speed after addition of magnesium staret , then compress the powdered blend into tablets. The compression force was adjusted to obtain tablets with a hardness range of 4 to7 Kg. Tablet weight was maintained constant at 173.5 mg.

6.2 Capsules form: This study included the effect of difference in dosage form on the formula of choice containing drug combination. The method of preparation was the same which used in preparation of tablets by direct compression technique. After blending of the two mixtures of Atorvastatin and Ezetimibe with the disintegrates. The capsules were filled with formulae by capsule filling machines in trique blue capsule size 3 with 235 mg of weight (the weight of filled capsule = 52mg + the weight of content = 173.5 mg).

7. Evaluation of prepared tablets and capsules of different formula

7.1. Disintegration time: The disintegration time of tablets were measured using Erweka disintegration tester in which distilled water kept at 37°C was used as a medium and the basket was raised and lowered at a constant frequency of 30 cycles/min, six tablets were evaluated from each batch according to USP NF 2010, the disintegration test was carried without auxiliary discs.[13]

7.2. *In Vitro* release studies: This validated method of dissolution study was performed using USP Type II (paddle type) instrument using a single tablet or capsule

per dissolution jar. The dissolutions were carried out using phosphate buffer with pH 7.4 and 0.05% w/v sodium lauryl sulfate (SLS) as dissolution medium. All the dissolution experiments were carried out with 900 ml of dissolution medium at a rotation speed 75 rpm and the dissolution medium was maintained at 37 ±0.5°C throughout the study. Samples were withdrawn at time interval 5, 10, 20, 30, 45, 60 min. filtered through Millipore filter (0.45 µm), then the solution of samples was analyzed by using high performance liquid chromatography (HPLC) method using stationary phase column ACE C 18 and mobile phase containing 0.05 M ammonium acetate buffers by adjusting the pH with acetic acid or ammonium hydroxide to 6.5 then detection at λ_{max} 232.5 nm for Atorvastatin calcium and 246nm for Ezetimibe using phosphate buffer as a blank. The results were the mean of values of three runs.

7.3. Dissolution profile of selected formula: The dissolution profile studies were performed as described in but it was differed in using two different dissolution media with pH (7.4 and 8). The two dissolution media were prepared from 0.05 M ammonium acetate buffers containing 0.05% w/v sodium lauryl sulfate (SLS). The adjustment of pH of dissolution media was achieved by adding acetic acid or ammonium hydroxide to reach required PH. All the dissolution experiments were carried out using 900 ml dissolution medium at 75 rpm and at 37 ± 0.5°C taking samples at different time intervals 5, 10, 15, 30, 45, 60, and 120 min replacing the dissolution media with fresh buffer equilibrated to maintain a constant volume after each sample. The absorbance's of the collected samples were measured by high performance liquid chromatography method using stationary phase column ACE Cl8 and mobile phase then detection by UV at $\lambda_{max.}$ for Atorvastatin calcium and Ezetimibe using buffer as a blank . The results were the mean of values of three runs. The percentage of each drugs released from different formulations was calculated from samples which were taken in 10 minutes intervals as seen. The percent of drug release was calculated and plotted against time for all the formulations to determine the release profile.

8. Stability study

Selected formula was chosen for stability study

8.1 Storage conditions: Samples of (direct compression) tablets and capsules of the selected formula were packed in Al/Al strips and packed in thermostatically controlled stability cabinets at different temperatures (30°C±2°C, 40°C±2°C and 50°C±2°C) with relative humidity 75% ± 5%.

8.2 Sampling protocol: The tablets and capsules of the selected formula were evaluate the Physical stability and In vitro release at 0, 1, 2, 3, 4, 5 and 6 months.

8.3 Physical stability: The tablets and capsules were evaluated for their changes in their physical such as % friability, disintegration, hardness and content uniformity for tablets and capsules as well as the in vitro release.

8.4 In vitro release study: Dissolution study was performed using USP Type II (paddle type) instrument, at PH 7.4 using a single tablet or capsule per dissolution jar.(As mention before in 7.2.).

9. Accelerated stability study of the selected formula for Atorvastatin and Ezetimibe combination prepared by direct compressed tablets and capsules

The drugs content of the stored tablets and capsules containing the drug combination in the selected formula were determined at different time intervals (0, 1, 2, 3,4,5,6 months at different temperatures 30, 40 and 50°C and 75% RH) using HPLC procedure (As mentioned before 7.2), content for Atorvastatin calcium and Ezetimibe was analyzed at different time interval. The order of chemical degradation was determined and the rate constant K was estimated. The logarithm of rate constant K was then plotted against 1/T according to **Arrhenius equation**. The K_{25} was obtained from the extrapolation of the plot and the t_{90}% was estimated.

RESULTS AND DISCUSSION

1. Ultraviolet scanning of each drug and there combination: Ultraviolet scanning of each drug alone and there mixture in methanol show that two absorbance maxima were observed at 232.5 and 246 nm. Figure (1) shows the ultraviolet scanning of Azetimibe and Ezetimibe and there combination.

2. Solubility profile at different pH for the combination of Atorvastatin calcium and Ezetimibe

According to results of solubility profile of mixture of two drugs at time interval Fig.(2), it was found that Atorvastatin calcium has limited solubility in aqueous phosphate buffers and distilled water but the solubility improved with increasing pH values. The higher solubility of Atorvastatin calcium was found at phosphate buffer, pH 7.5 (0.091 mg/ml) after 12 hrs and the lower solubility of the drug was found at pH 4.5(0.0039 mg/ml), so Atorvastatin calcium is considered as slightly soluble according to bio-pharmaceutics classification system. The low solubility of Atorvastatin calcium may be due to chemical structure of Atorvastatin from which it can be found that pKa of Atorvastatin terminal carboxyl group is 4.5, so the solubility of drug improved with pH equal to or greater than pH 4.5.[14] Ezetimibe was found to be less soluble than Atorvastatin calcium but also slightly improved with increasing pH values. The higher solubility of Ezetimibe was found at phosphate buffer, pH 7.5 (0.0015 mg/ml) after 12 hrs and the lower solubility of the drug was found at distilled water (0.00042 mg / ml). Ezetimibe is considered as very slightly soluble according to bio-pharmaceutics classification system.

3. Study of Partition coefficient of combination of Atorvastatin calcium and Ezetimibe: The partition

coefficient of Atorvastatin calcium using n-butanol/water system or n-butanol/phosphate buffer with different PH were approximately 1.4. The partition coefficient of Ezetimibe using n-butanol/water and other phosphate buffer was approximately equal to 4.5[15] From the obtained results, it was evident that Atorvastatin calcium as well as Ezetimibe had extremely lipophilic nature. From the previous results and results of solubility profile and partitioning, it was found that the two drugs are belonged to class II of bio-pharmaceutics classification system (BCS) which was low solubility - high permeability drugs (FDA, 1997).

4. Study of compatibility of Atorvastatin calcium and Ezetimibe by different techniques

4.1 Thin layer chromatography (TLC): The results of TLC study of Atorvastatin calcium and Ezetimibe and mixture of two drugs (1:1) are shown in Fig.(3). The R*f* value is the "retardation factor" or the "ratio-to-front" value expressed as a decimal fraction. The R*f* is calculated by dividing the distance traveled by the drug to the distance traveled by the solvent. The R*f* value can be calculated as

$$Rf = \frac{\text{Distance spot travels}}{\text{Distance solvent travels}}$$

It was found that principle spot of Ezetimibe sample with R*f* equal 0.53 cm ± 0.04 and principle spot of Atorvastatin calcium sample with R*f* equal 0.3cm ± 0.04 and in the mixture of two drugs we found the same two principle spots of individual Ezetimibe and Atorvastatin calcium (0.53 and 0.3 ± 0.04 respectively). It can be concluded that no interaction between Atorvastatin calcium and Ezetimibe.

4.2 Fourier infrared spectroscopy (FTIR): In order to investigate the possibility of interaction between the two drug Atorvastatin calcium and Ezetimibe, FTIR analysis was employed as more advanced technique than TLC. The FTIR spectra of Atorvastatin calcium alone, Ezetimibe alone and mixture of two drugs (1:1 w/w) are shown in Fig. (4). The results showed that the characteristic peaks related to Atorvastatin calcium alone showed characteristic peaks at 2955.15 cm^{-1} (C-H - stretching), 1313.56 cm^{-1} (C-N - stretching), 3059.15 cm^{-1} (C-HO - stretching alcoholic group), 1564.97 cm^{-1} (C=O -stretching amidic group), 3403.27 cm^{-1} (N-H - stretching), 1656.97 cm^{-1} (C=C - bending), 751.62 cm^{-1}, 696.95 cm^{-1} (C-F - stretching), 1104.39 cm^{-1} (O-H - bending).[16] Ezetimibe structure showed characteristic peaks at C=O tension bands of the carbonyl group at 1716. 7 cm^{-1}, C=C resonance double bond tension bands that belong to thearmatic chain at 1511.7 cm^{-1}[17], strong C-O tension bands of the ester group at 1104.39 cm^{-1}, strong aliphatic tension bands at 2958.9 cm^{-1}, expanded O-H tension bands of the carboxylic acid group at 3273 cm^{-1}, C-F tension bands at 1220 cm^{-1}, *p*- substituted benzene tension bands at 800-1000 cm^{-1} as indicated in Figure(4). The spectra of Atorvastatin calcium alone and Ezetimibe were equivalent to the spectra obtained by the mixture of two drugs as indicated in Figure (4). There is a small difference; it may be the possibility of intermolecular hydrogen bonding between Atorvastatin calcium and Ezetimibe molecules. This indicated that there is partial interaction occurred with a solid dispersion of two drugs. But in general, the results revealed no considerable changes in the IR peaks of Atorvastatin calcium and Ezetimibe, when mixed together in physical mixture.

5.3 Differential scanning colormetry (DSC): In this study, the thermal behavior of each drug individual then the possibility change in this behavior in the mixture of two drugs. The DSC thermo-gram of Atorvastatin calcium and Ezetimibe individual were compared to DSC thermo-gram of the corresponding physical mixture of two drugs as indicated in Fig.(5). Atorvastatin calcium shows sharp endothermic at temperatures 156.62°C (an endothermic event corresponding to melting point of Atorvastatin calcium. Ezetimibe showed a melting peak at 162.62°C (an endothermic event corresponding to melting point) and there was another peak at 71.36°C may be due to loss of water from hydrated molecule. In the physical mixture of two drugs thermo-grams, peaks Atorvastatin calcium and Ezetimibe were observed at the same position or slightly shifted but sharp endothermic peak of Atorvastatin calcium become not sharp or slightly disappeared. Peak of Ezetimibe in mixture was present at 161.96°C i.e. near to 162.62°C and another peak of water was present at 67.95°C i.e. near to 71.36°C. Weak peak of Atorvastatin calcium in mixture was present at 154.63°C i.e. near to 156.62°C. Some broadening of peaks leading to changes in peak temperature occurs simply due to mixing of the components without indicating any significant interaction.[18] These results demonstrated that there is no interaction between Atorvastatin calcium and Ezetimibe after treatment of drugs or on physical mixture of two drugs.

5. Study of compatibility of drug-excipients by Fourier transform infra red spectroscopy (FTIR)

Infra red absorbance spectra were done for pure Atorvastatin calcium and its corresponding physical mixture with the investigated excipients (1:1 w/w), pure Ezetimibe and its corresponding physical mixtures with the investigated excipients (1:1 w/w) and finally, the IR absorbance spectra of pure suggested drug combination (physical mixture of two drugs (1:1 w/w)) after treatment and its physical mixtures (1:1 w/w) with the utilized excipients.

Figure (6) show the IR band positions of pure Atorvastatin calcium and its corresponding physical mixture with the tested excipients (1:1 w/w). The results showed that the characteristic peaks related to Atorvastatin calcium alone are not considerably changed after comparison of IR spectrum of Atorvastatin calcium

with the spectra of the physical mixture. The main characteristic peaks of Atorvastatin calcium indicated are slightly changed for physical mixture of investigated excipients.

Figure (7) show the IR band positions of pure Ezetimibc and its corresponding physical mixture with the investigated excipients (l:1 w/w). The results showed that the characteristic peaks related to Ezetimibe alone are not considerably changed after comparison of the IR spectrum of Ezetimibe with the spectra obtained for the investigated physical mixture. The main characteristic peaks of Ezetimibe as previous indicated are slightly changed after physical mixture of investigated excipients. The IR band positions of suggested drug combination (The physical mixture of two drugs (l:1 w/w)) and its corresponding physical mixture with the investigated excipients (1:1 w/w). The results showed that the characteristic peaks related to two drugs in suggested drug combination alone are not considerably changed after comparison of the IR spectra obtained for the utilized physical mixture. The main characteristic peaks of pure suggested drug combination as previous indicated are slightly changed after preparation of physical mixture of utilized excepients Figure(8).

This indicated absence of chemical interaction between Atorvastatin calcium alone or in suggested drug combination and their investigated excepients and so the absence of chemical interaction between Ezetimibe alone or in suggested drug combination.

6. Evaluation of the prepared tablets and capsules

6.1. Disintegration: The disintegration time of prepared tablets was found to be within the pharmacopeia limits except formula F1. The disintegration time of the prepared formulations were in the range of 5.82 ± 2.19 to 17.2 ± 1.7 min as indicated in table (2). The results of the disintegration test for tablets containing drug combination containing different concentrations of Ac-Di-Sol show that, increasing Ac-Di-Sol concentration from 0 to 4% resulted in gradual decrease in the disintegration time from 17.2 to 5.8 min. Further increases in the super disintegrante concentration showed insignificant change in disintegration time in presence or absence of calcium carbonate.

The effect of Ac-Di-Sol concentration on disintegration time of prepared tablets performed in table (2) and Figure (9).The results show that incorporation of Ac-Di-Sol in a concentration ranged from 0 to 5% resulted in decrease in the disintegration time. The results indicate that 4% Ac-Di-Sol is a critical concentration of that super disintegrante.[19] This may be due to the high uptake of liquids during disintegration process leading to decreasing the adhesion and binding forces between tablet powder and fast disintegration.[20] The smallest disintegration time is detected at 4% and no significant change with further increasing of Ac-Di-Sol concentration.

6.2. *In Vitro* release of drugs from the prepared, formulations: The results of drugs release of from different formulations are illustrated in Figure (10). Dissolution profile of F1 showed that the Atorvastatin calcium and Ezetimibe releases are slow, which may be due to slow disintegration of the tablet in absence of croscarmellose sodium or calcium carbonate. The obtained results showed that there is just small amount of increase in drugs release. In case of F3, the concentration of super disintegrant was increased and tween 80 as surfactant was added to improve the drug release.

The prepared formulations F4, F5 and F6 were found to be the higher release rates and this may be due to the presence of calcium carbonate which was able to provide sufficient basic environment to dissolve the suggested drug combination. The formulations F5 and F6 increase the concentration of super disinstegrant, the release of two drugs were improved in presence of alkalizer. The formulation F5 was found to be the much better than F6 with the higher rate of release of two drugs as indicated in Figure (10). This result indicated that formulation of this suggested drug combination need the presence of calcium carbonate for increasing the solubility of the drug combination.[21] In addition to tween 80 as surfactant affect the solubility.

On the basis of obtained results, F5 was selected to see the effect of different PH medium on the dissolution profile of drug combination prepared in direct compression tablet form and capsule.

6.3. Effect of different PH on the dissolution profile of drug combination from the selected formula (tablets and capsules): In this study, the tablets which are prepared by direct compression and capsules which containing suggested drug combination are subjected to study of dissolution profile of two drugs in two different dissolution media with different pH 7.4 and 8 to determine circumstance of release of the suggested drug combination .Also study carried out to determine the suitable dosage form and suitable manufacturing technique for the suggested drug combination. Figure (11) Illustrate the results of dissolution profile of suggested drug combination in dissolution medium with pH 7.4 which show that Atorvastatin calcium release increase gradually with time up to 60 min then the release decline after 120 min. The percentage of Atorvastatin calcium release was ranged from 41.3% to 91.2% in case of tablets prepared by direct compression and from 47.3% to 97.8% from capsules. The percentage of Ezetimibe release was ranged from 36.24% to 89.34% from tablets prepared by direct compression and from 48.19% to 98.67% from capsules. These results investigate that the maximum release time of the two drugs in dissolution medium with pH 7.4 after 60 min. The results of dissolution profile of suggested drug combination using dissolution medium with pH 8 as illustrated in Figure (12). The obtained results show that Atorvastatin calcium release increase gradually with time

up to 60 min then the release decline decrease after 120 minutes. The percentage of Atorvastatin calcium release was ranged from 51.3% to 94.8% in tablets prepared by direct compression and from 57.3% to 99.8% in capsule form. The percentage of Ezetimibe release was ranged from 46.24% to 89.34% in direct compression tablets and from 58.19% to 98.67% from capsules. These results reveled that the maximum release time of the two drugs in dissolution medium with pH 7.4 and pH 8 after 60 min. From the previous results, it can be concluded that the release of two drugs from capsule were more than tablets prepared by direct compression. Also, the selected formula has improved dissolution profile in dissolution media with pH8.

7. Stability study

7.1 Physical stability of tablets and capsules: The tablets and capsules showed no change in their physical appearance along the storage period as in tables (4-5) shows the effect of six months storage on the physical characteristics of tablets stored at different temperatures: 30°C, 40°C and 50°C.

7.1.1 Tablet friability: The results obtained in tables (4-5), show that the percent friability of tablets slightly decreased upon storage of tablets especially for those stored at 50°C where it reached 0.39% after six months.

7.1.2 Tablet hardness: From the results obtained in tables (4-5), it was found that, there was a noticeable increase in hardness of tablets prepared by direct compression upon storage at different temperatures, in which the hardness were increased from 7.56 to 12.18, 11.91 and 12.06 for tablets stored at 30°C, 40°C and50^{0}C.

The increase in tablet hardness through storage may be explained on the basis of the increase of the inter particulate bonding between particles of the prepared tablets. As a result of increasing tablet hardness upon storage, the tensile strength of tablets also increased and well correlated with the decrease in the percent friability.[22,23]

7.1.3 Disintegration time: From the results obtained in table (4-5), it was found that there was a noticeable increase in disintegration time of the tablets prepared by direct compression and capsules upon storage at different temperatures. Where the disintegration increased from 2.85 to 4.68min., 15.33 and 17.96min for capsules stored at 30°C, 40°C and 50°C respectively and increased from 10.85 to 17.68min, 17.33 and 25.96 min stored at 30°C, 40°C and 50°C respectively in case of direct compression tablets.

The increase in disintegration time through storage in case of direct compression tablets may be explained on the basis of the increase of hardness values but in case of capsules, the increase of disintegration time may be explained on the basis of increase of the moisture content.[24]

7.1.4 Content uniformity: The three batches prepared for stability test fulfilled the pharmacopoeias requirements for drug content uniformity (BP 2012) as obtained in tables (4-5) which indicate that all formulas were within limit where the amount of drug in each of the assayed tablets and capsules lied between 85% and 115% of the average content (±15).

8. *In vitro* drug release studies: Figures (13-14) and tables (4-5) show the effect of storage at different temperatures and different relative humidity for six months on the release pattern of two drugs from the selected tablets prepared by direct compression and capsules. From the obtained results, it was found that the rate of drugs release of all prepared formulations, were markedly decrease for both capsules and tablets prepared by direct compression and stored at stress conditions at different temperatures. Elevated temperatures and the higher humidity conditions during storage time markedly decrease the amount released of two drugs from tablets prepare by direct compression and capsules.

From the results which illustrated in table (4-5) and plotted graphically in Figures (13-14), it was found that the prepared capsules were the slowest release of the two drugs.

Storage conditions caused a marked decrease in dissolution, especially at 50°C, the prepared capsules and the tablets prepared by direct compression cannot pass the in house dissolution criteria.[25,26,27]

This occurs due to Atorvastatin calcium and Ezetimibe in capsule dosage form was readily converted to the insoluble acid form in the presence of humidity, which could account dramatic decrease in dissolution, especially in the higher humidity condition after storage. Moisture would enter the capsule from outside converting a layer of Atorvastatin calcium and Ezetimibe at capsule shell surface to the insoluble Atorvastatin and Ezetimibe acid, there by further retarding dissolution of the remaining Atorvastatin calcium and Ezetimibe inside content of capsule.

The altered dissolution behavior of capsules may be due to the moisture in the capsule gelatin shells which act as a plasticizer to impart flexibility to hard gelatin capsules. Variations in the moisture content of a capsule shell as the storage conditions change may lead to undesired physical properties such as brittleness and stickiness. Conversely, moisture can move from the shell to the capsule contents during storage, especially for deliquescent and hygroscopic ingredients. Moisture transfer between shell and contents can be one of the reasons for a change in the properties of gelatin when stored at 40°C, 50°C and 75% RH.[24]

It was reported that directly-compressed tablets were much susceptible to change caused by humidity during storage .It was found that the tablets absorbed moisture

at 50% and 75% RH (relative humidity) but lost moisture at 30% RH.[28]

9. Evaluation of Expiry date for Drug combination

The samples were analyzed for the remaining two drugs content after storage for 0, l, 2, 3, 4, 5 and 6 months at different temperatures 30, 40 and 50°C and 75% RH using high performance chromatography (HPLC) . No significant interference from impurities was detected under the chromatographic conditions described.

Table (6,7) shows the percent remained of Atorvastatin calcium and Ezetimibe in (direct compression) tablets and Table(8,9)for capsules, stored at different temperatures for six months. The percent remaining of the Atorvastatin calcium and Ezetimibe after six months were found.

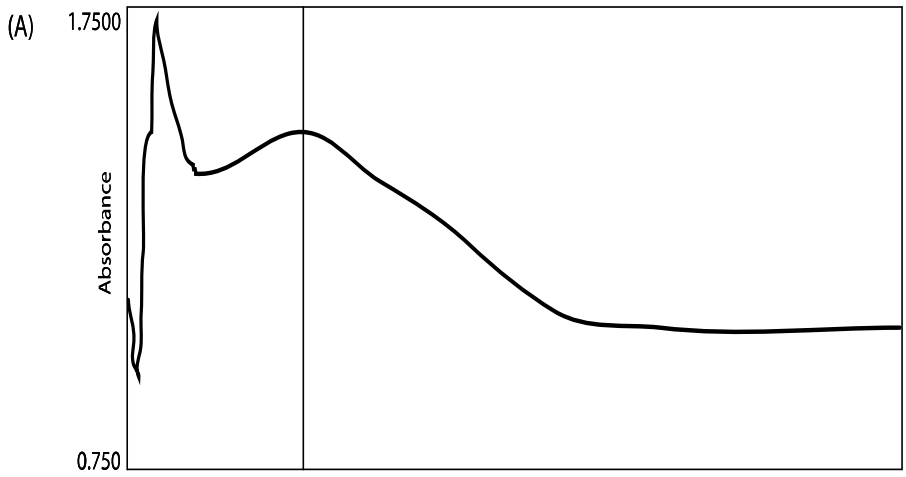
Figure (15-17) represent the HPLC chromatograms of the suggested drug combination content in the tablets prepared by direct compression stored for 6 months at different temperatures 30,40and 50°C and 75% RH. Figures (22-24) represent the HPLC chromatograms of the suggested drug combination content in the prepared capsules stored for 6 months at different temperatures 30, 40 and 50 °C and 75% RH.

From the above results the chromatograms of two drugs show single peak for each drug with the same retention time. This indicates the absence of any degradation products after storage for 6 months at elevated temperature and humidity.

The rate constant (K) and the log K was plotted versus 1/T and a straight line was calculated from the plots in Figure (18-21) and table (6-7) for direct compression tablets and the predicted shelf life of the drug was 1.85 years for Atorvastatin calcium and 3.3 years for Ezetimibe in case of direct compression tablets as indicated in table (10). In case of capsules, The kinetic analysis of the data revealed first order degradation mechanism with higher correlation coefficient. The rate constant (K) and the log K was plotted versus 1/T and a straight line was calculated from the plots in Figure(25-28) and table (8 ,9) and the predicted shelf life was 2.75 years for Atorvastatin calcium and 3.5 years for Ezetimibe in capsules as indicated in table(10).

In case of tablets prepared by direct compression, the percent remaining of the Atorvastatin calcium after six months were found to be 99.15, 93.09 and 90.39% stored at 30°, 40° and 50°C respectively. The percent remaining of Ezetimibe after six months were found to be 99.85, 97.75 and 92.89% stored at 30°, 40° and 50°C respectively. The kinetic analysis of the obtained data revealed first order degradation mechanism with higher correlation coefficient.

In case of capsules dosage form, the percent of the Atorvastatin calcium remaining after six months were found to be 99.15, 99.5 and 97.34% stored at 30°, 40° and 50°C respectively. The percent remaining of Ezetimibe after six months were found to be 108.85, 96. 75 and 103.89% stored at 30°, 40° and 50°C respectively. The kinetic analysis of the data revealed first order degradation mechanism with higher correlation coefficient. The percents remaining of two drugs are within the pharmacopeial limits of drug content (USP NF2012). The expiration dates (t90%) of the two drugs were calculated from K25 of the first order reaction using the equation: t90% == 0.105/K.[29]



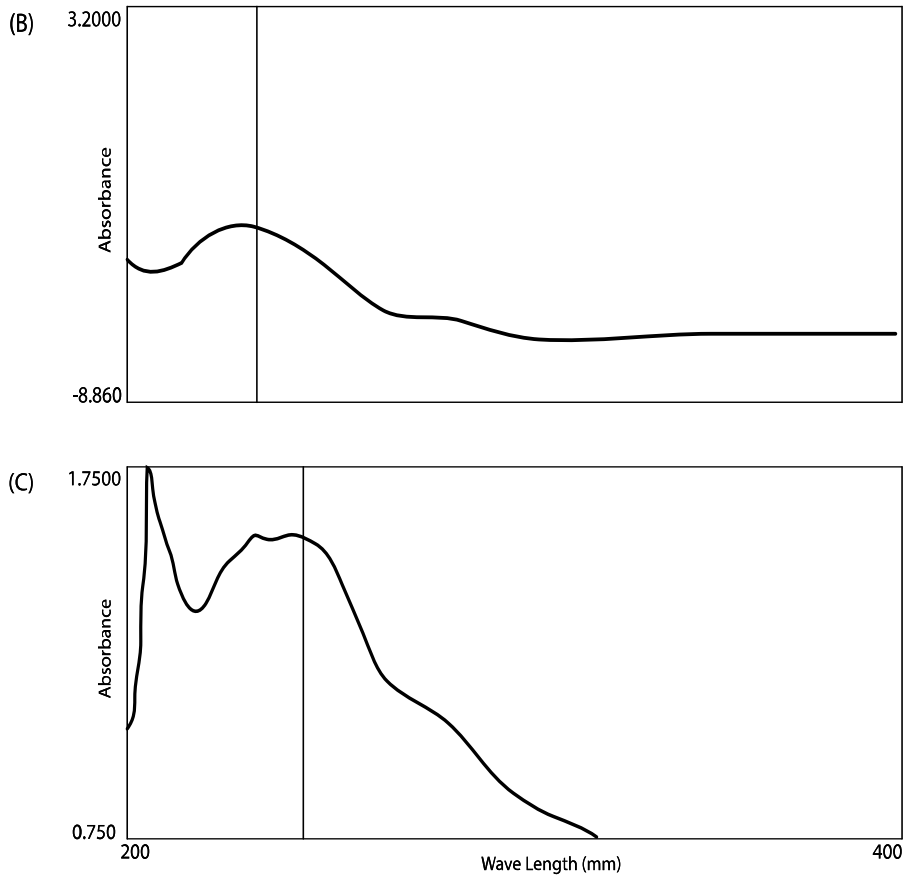


Fig. (1): Ultraviolet scanning of (A) Atorvastatin calcium in methanol, (B) Ezetimibe in methanol, (C) Atorvastatin calcium and Ezetimibe in mixture of two drugs in methanol.

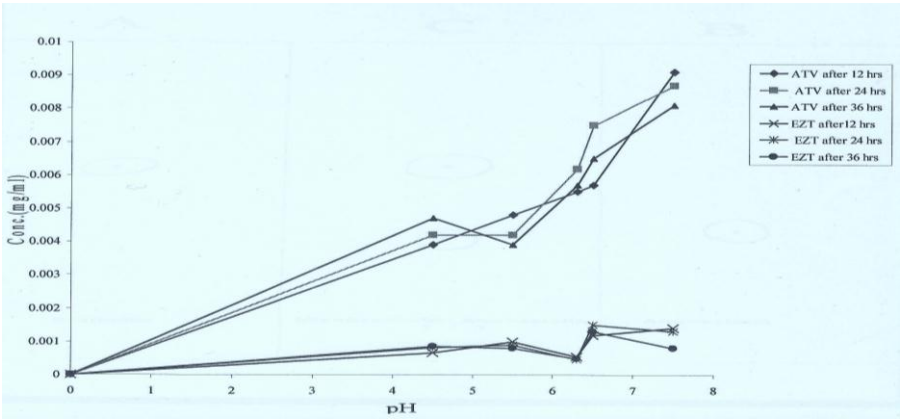


Fig. (2): Solubility of Atorvastatin calcium and Ezetimibe at different PH

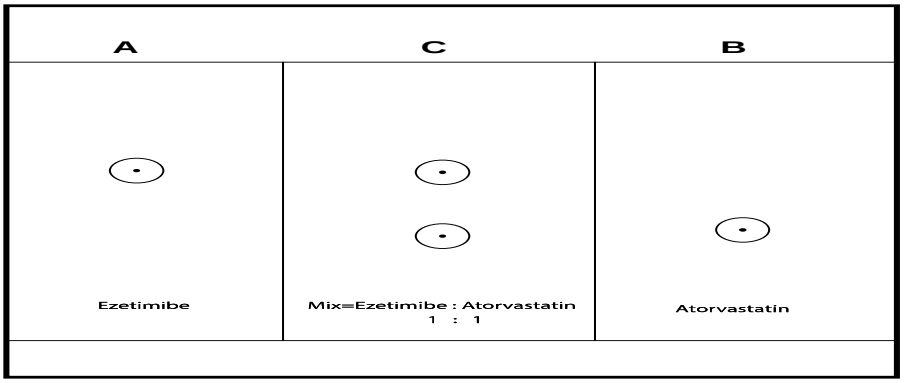
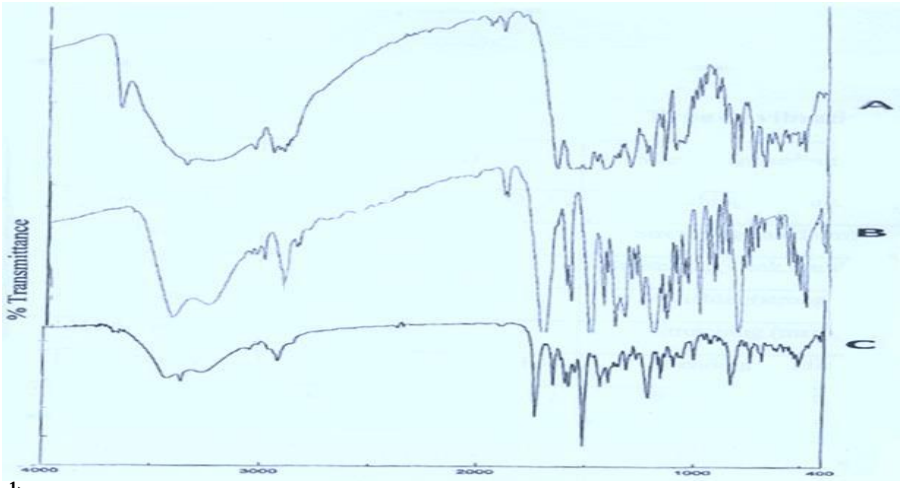


Fig. (3): Thin layer chromatography of (A) Ezetimibe, (B) Atorvastatin calcium and (C) two drugs mixture.



Wave length (cm^{-1})

Fig. (4): FTIR scanning of (A) Atorvastatin calcium, (B) Ezetimibe and (C) physical mixture of the two drugs (1:1).

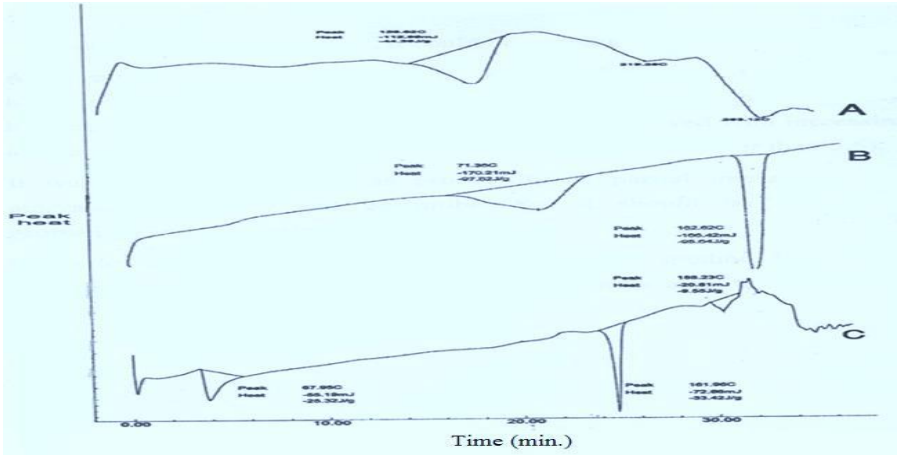


Fig. (5): DSC scanning of (A) Atorvastatin calcium, (B) Ezetimibe and (C) physical mixture (l:l w/w) of Atorvastatin calcium and Ezetimibe

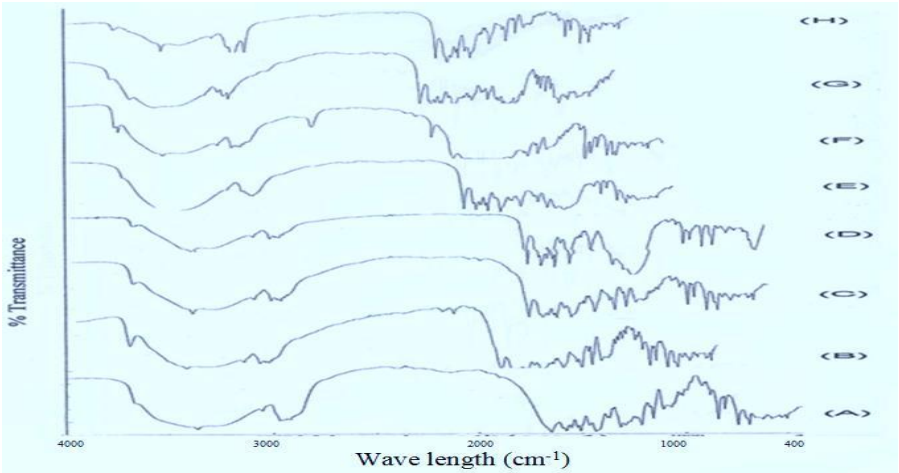


Fig. (6): FTIR scanning of (A) Atorvastatin calcium alone, and its physical mixtures (1:1 w/w) of

(B) Atorvastatin calcium and Avicel 101,
(C) Atorvastatin calcium and aerosil 200,
(D) Atorvastatin calcium and calcium carbonate,
(E) Atorvastatin calcium and lactose monohydrate,
(F) Atorvastatin calcium and tween 80,
(G) Atorvastatin calcium and PVP $_{K25}$,
(H) Atorvastatin calcium and magnesium stearate.

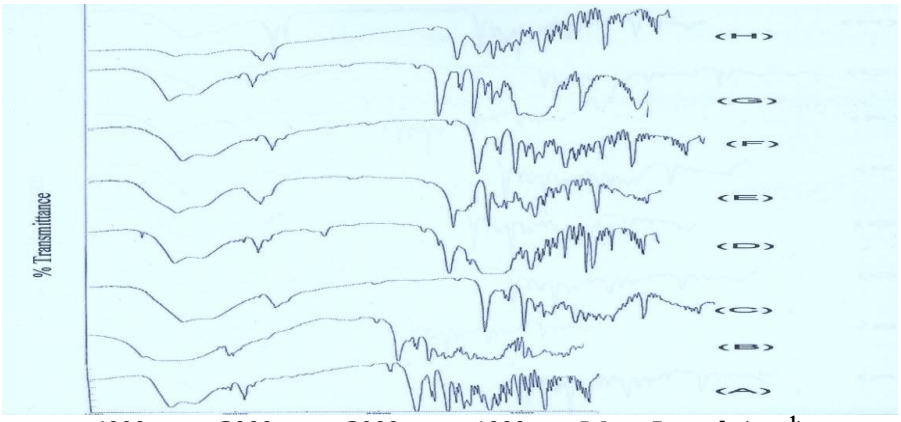


Fig. (7): FTIR scanning of (A) Ezetimibe alone, & its physical mixtures (1:1 w/w) of

(B) Ezetimibe and Avicel 101,
(C) Ezetimibe and aerosil 200,
(D) Ezetimibe and calcium carbonate,
(E) Ezetimibe and lactose monohydrate,
(F) Ezetimibe and tween 80,
(G) Ezetimibe and PVP $_{K25}$,
(H) Ezetimibe and magnesium stearate.

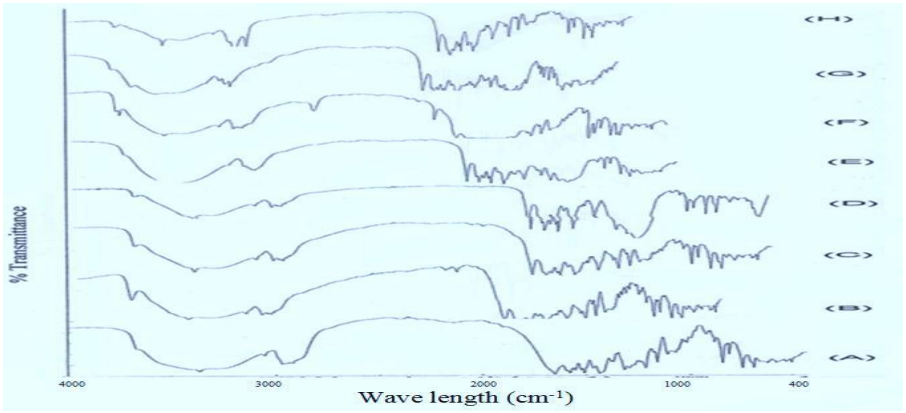


Fig. (8): FTIR scanning of (A) Physical mixture of two drugs alone, and its physical mixtures (1:1 w/w) of

(B) Physical mixture of two drugs and Avicel 101,
(C) Physical mixture of two drugs and aerosil 200,
(D) Physical mixture of two drugs and calcium carbonate,
(E) Physical mixture of two drugs and lactose mono hydrate,
(F) Physical mixture of two drugs and tween 80,
(G) Physical mixture of two drugs and PVP $_{K25}$,
(H) Physical mixture of two drugs and magnesium stearate.

Table (1): The prepared formulas containing suggested drug combination.

Ingredients	Function	F1	F2	F3	F4	F5	F6
Core tablet							
Active constituents							
Atorvastatin ca.	Active ingred.	10.82 mg	10.82 mg	10.82 mg	10.82 mg	10.82 mg	10.82 mg
Ezetimibe	Active ingred.	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg
In active constituents							
Lactose monohyd.	Diluent	38 %	38 %	38 %	38 %	38 %	38 %
Avicel 101	Diluent	18 %	18 %	18 %	18 %	18 %	18 %
Ca Co_3	Alkalizing agent	.....	.....	.....	20 %	20 %	20 %
PVP K_{25}	Binder	2 %	3 %	4 %	2 %	4 %	4 %
Tween 80	Surfactant	.....	.....	1 %	.....	1 %	.....
Ac-Di-Sol	Superdisintegrant	.....	1 %	2 %	3 %	4 %	5 %
Aerosil 200	Tableting aid	.....	1 %	.....	.....	1 %	1 %

Mag. stearate	Lubricant	1 %	1 %	1 %	1 %	1 %	1 %
Total		**173 mg**	**173 mg**	**173 mg**	**173 mg**	**173 mg**	**173 mg**

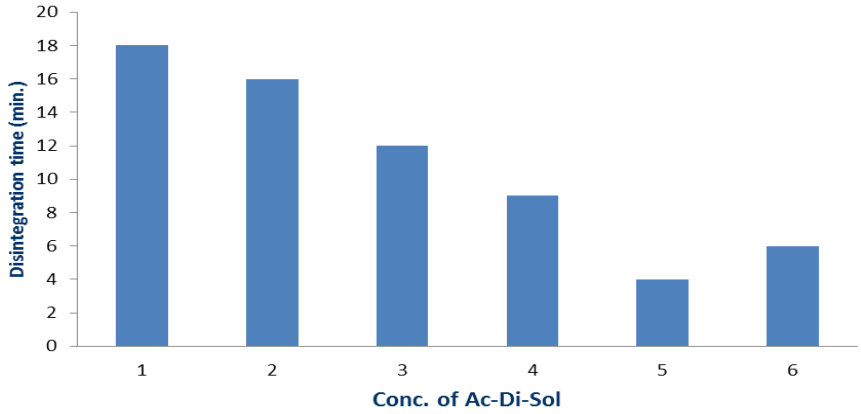
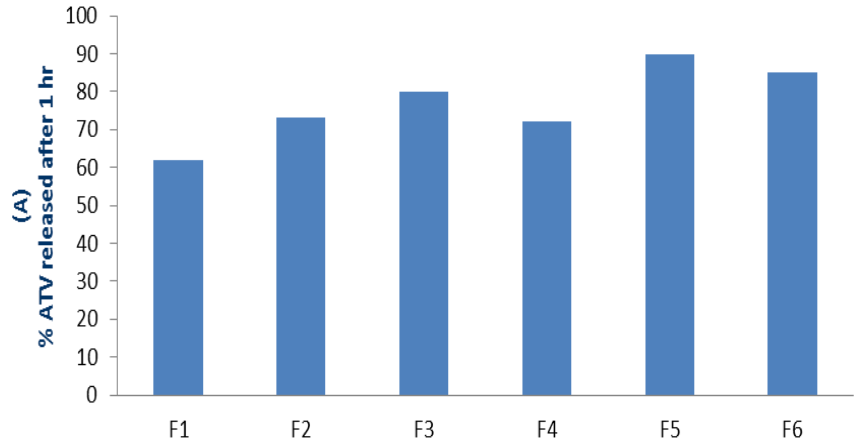


Fig. (9): Effect of different cone. of Ac-Di-Sol on disintegration time of prepared different formulae containing suggested drug combination
(1): The formula prepared with 0% Ac-Di-Sol.
(2): The formula prepared with 1% Ac-Di-Sol.
(3): The formula prepared with 2% Ac-Di-Sol.
(4): The formula prepared with 3% Ac-Di-Sol.
(5): The formula prepared with 4 % Ac-Di-Sol.
(6): The formula prepared with 5% Ac-Di-Sol.

Table (2): Disintegration time of formulated tablets containing suggested drug combination of Atorvastatin calcium and Ezetimibe.

Formula	Disintegration (min.) ± SD
F1	17.2 ± 1.7
F2	14.2 ± 1.1
F3	10.2 ± 1.03
F4	7.2 ± 2.09
F5	5.82 ± 2.19
F6	7.12 ± 2.09

*All values of different parameters are expressed as a mean of three determinations ± SD for n = 3



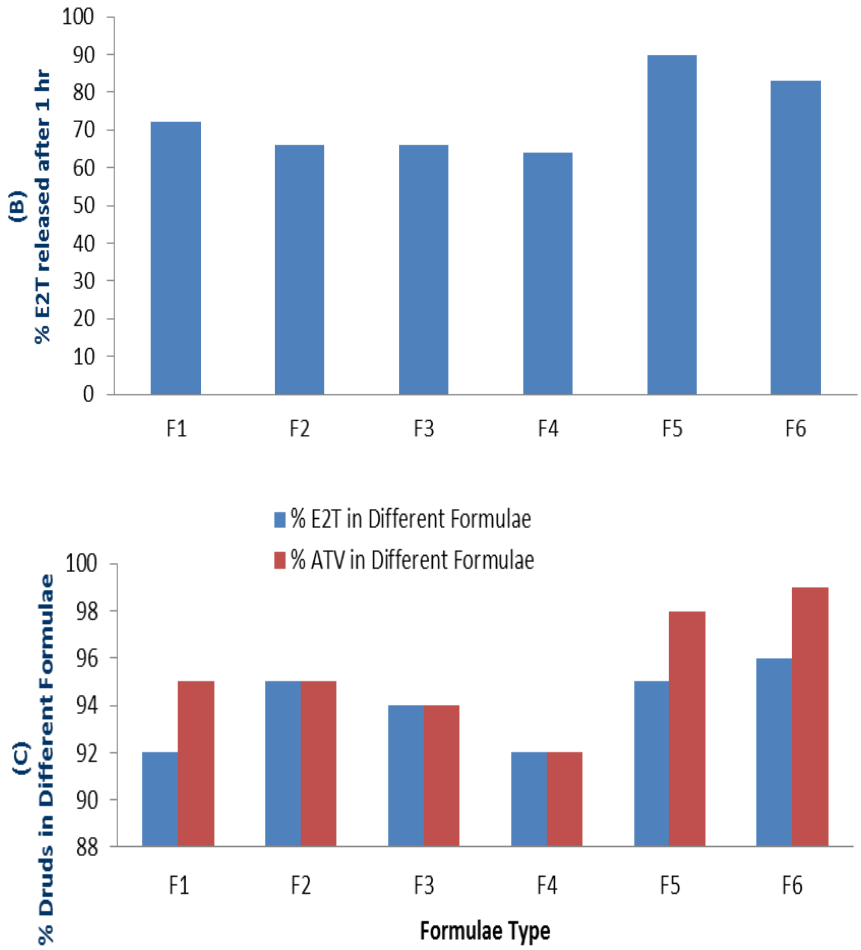


Fig. (10): Release of (A) Atorvastatin calcium, (B) Ezetimibe, (C) Atorvastatin calcium and Ezetimibe (physical mixture) from different formulations containing suggested drug combination.

F1: The formula prepared without (calcium carbonate, tween80, Ac-Di-Sol, aerosil 200) and with 2% PVP K_{25} and 1% mag. stearate.
F2: The formula prepared without (calcium carbonate, tween80,) and with 3% PVP K_{25}, 1% Ac-Di-Sol, 1% aerosil 200 and 1% mag. stearate.
F3: The formula prepared without (calcium carbonate, aerosil 200) and with 4% PVP K_{25}, 1% tween80, 2% Ac-Di-Sol and 1% mag. stearate.
F4: The formula prepared without (tween80, aerosil 200) and with 20% calcium carbonate, 2% PVP K_{25}, 3% Ac-Di-Sol and 1% mag. stearate.
F5: The formula prepared with 20% calcium carbonate, 1% tween80, 4% PVP K_{25}, 1% aerosil 200, 4% Ac-Di-Sol and 1% mag. stearate.
F6: The formula prepared without (tween80) and with 20% calcium carbonate, 4% PVP K_{25}, 1% aerosil 200, 4%Ac-Di-Sol and 1% mag. stearate.

Table (3): Atorvastatin calcium and Ezetimibe dissolved after dissolution of different formulations containing drug combination.

Formula	Content uniformity		Dissolution (% of the drug after 1 hr) ± SD	
	ATV	EZT	ATV	EZT
Direct compression tablets (A)	103.5 ± 0.73	101.98 ± 0.33	89.3 ± 1.52	89 ± 1.65
Capsule dosage form (B)	109 ± 1.38	105.9 ± 0.25	90 ± 0.99	92 ± 1.25

*All values are expressed as a mean +SD, n = 3
(A): the selected formula was prepared by direct compression technique
(B): the selected formula was formulated in capsule dosage form.

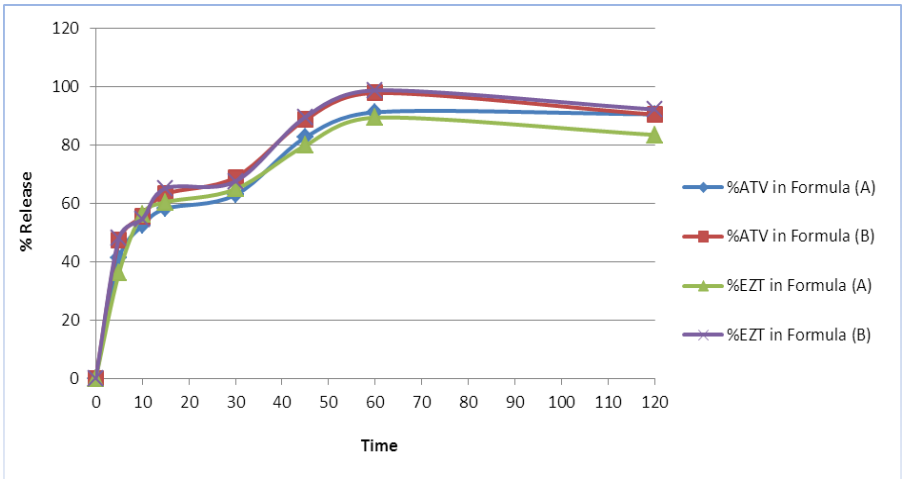


Fig. (11): Dissolution profile of Atorvastatin calcium and Ezetimibe in suggested drug combination released from prepared direct compression tablets (A) and prepared capsules (B) in phosphate buffer pH 7.4.

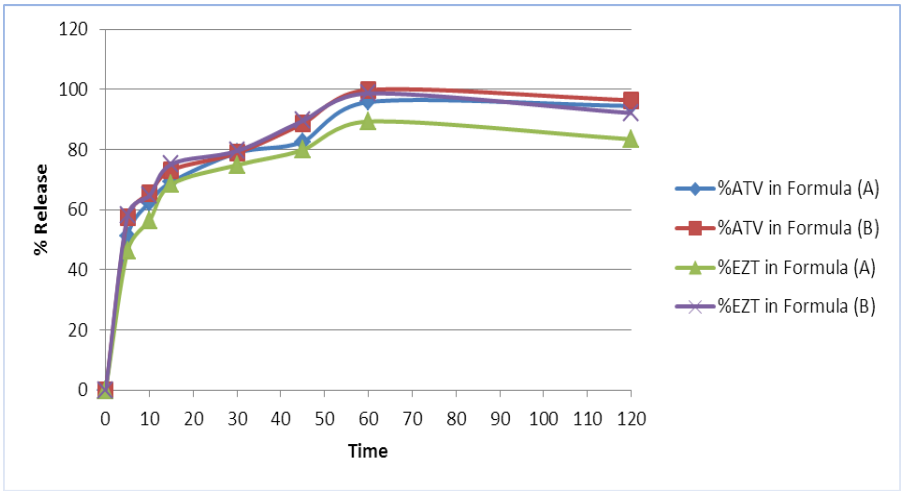


Fig. (12): Dissolution profile of Atorvastatin calcium and Ezetimibe in suggested drug combination released from prepared by direct compression tablets (A) and prepared capsules (B) in phosphate buffer pH 8.

Table (4): The physicochemical characteristics of tablets prepared by direct compression stored at different temperatures for six months.

Storage temp. (°C)	Time (months)	% Friability	Hardness (Kp) mean±SD	Disint. Time (min)	Percent of two drugs release %		Content uniformity	
					ATV	EZT	ATV	EZT
30	0	0.65	7.56 ± 0.42	10.85	89.45	79.65	Conf.	Conf.
	1	0.45	11.5 ± 0.66	16.54	85.5	78.6	Conf.	Conf.
	2	0.43	12 ± 1	17.26	79.8	74.3	Conf.	Conf.
	3	0.43	11.5 ± 1.14	16.47	75.2	73.9	Conf.	Conf.
	4	0.45	12.13 ± 0.62	17.67	72.7	72.5	Conf.	Conf.
	5	0.46	11.75 ± 0.25	16.53	68.7	70.9	Conf.	Conf.
	6	0.40	12.18 ± 1.2	17.68	65.4	69.4	Conf.	Conf.
40	1	0.46	12.0 ± 0.25	22.23	82.5	73.4	Conf.	Conf.
	2	0.43	12.08 ± 0.94	23.38	75.6	65.3	Conf.	Conf.
	3	0.42	11.14 ± 0.38	16.64	68.2	64.34	Conf.	Conf.
	4	0.41	11.75 ± 0.25	17.06	63.9	59.89	Conf.	Conf.
	5	0.44	11.6 ± 1.47	16.73	58.5	54.9	Conf.	Conf.
	6	0.41	11.91 ± 0.52	17.33	54.7	46.3	Conf.	Conf.
50	1	0.45	11.33 ± 0.38	21.38	83.5	72.3	Conf.	Conf.
	2	0.44	12.58 ± 0.14	18.14	73.6	66.4	Conf.	Conf.
	3	0.44	11.68 ± 0.55	20.65	68.2	59.3	Conf.	Conf.
	4	0.45	12.01 ± 0.39	19.76	67.9	55.9	Conf.	Conf.

	5	0.42	12.44 ± 1.29	23.35	56.2	59.6	Conf.	Conf.
	6	0.39	12.06 ± 1.19	25.96	51.3	45.5	Conf.	Conf.

Table (5): The physicochemical characteristics of capsules stored at different temperatures for six months.

Storage temp. (°C)	Time (months)	Disintegration time (min)	Percent of two drugs release %		Content uniformity	
			ATV	EZT	ATV	EZT
30	0	2.85	88.5	81.25	Conform	Conform
	1	3.54	86.5	78.9	Conform	Conform
	2	3.26	79.7	73.4	Conform	Conform
	3	4.47	77.8	69.8	Conform	Conform
	4	2.67	75.6	67.5	Conform	Conform
	5	3.53	71.4	65.9	Conform	Conform
	6	4.68	67.7	64.3	Conform	Conform
40	1	9.23	85.5	72.4	Conform	Conform
	2	10.38	76.6	62.3	Conform	Conform
	3	13.61	69.2	51.34	Conform	Conform
	4	14.06	65.9	49.89	Conform	Conform
	5	14.73	57.5	42.9	Conform	Conform
	6	15.33	43.5	34.6	Conform	Conform
50	1	16.38	82.5	71.3	Conform	Conform
	2	18.14	73.6	66.4	Conform	Conform
	3	16.65	68.2	56.3	Conform	Conform
	4	17.76	57.9	45.9	Conform	Conform
	5	18.35	46.2	39.6	Conform	Conform
	6	17.96	36.8	31.6	Conform	Conform

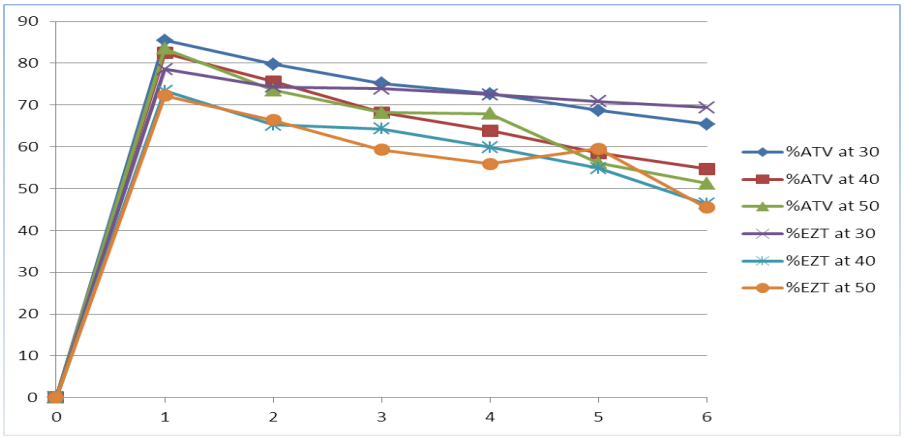


Fig. (13): Effect of six months storage at different temperatures on the release of Atotvastatin calcium and Ezetimibe from tablets prepared by direct compression.

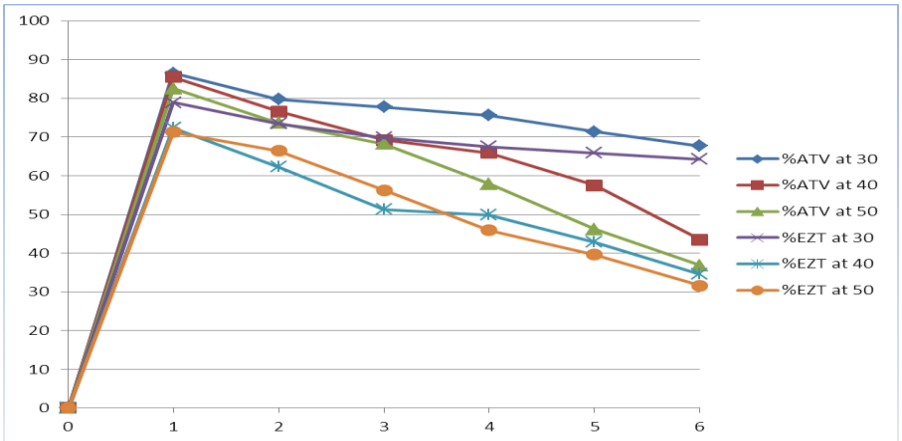


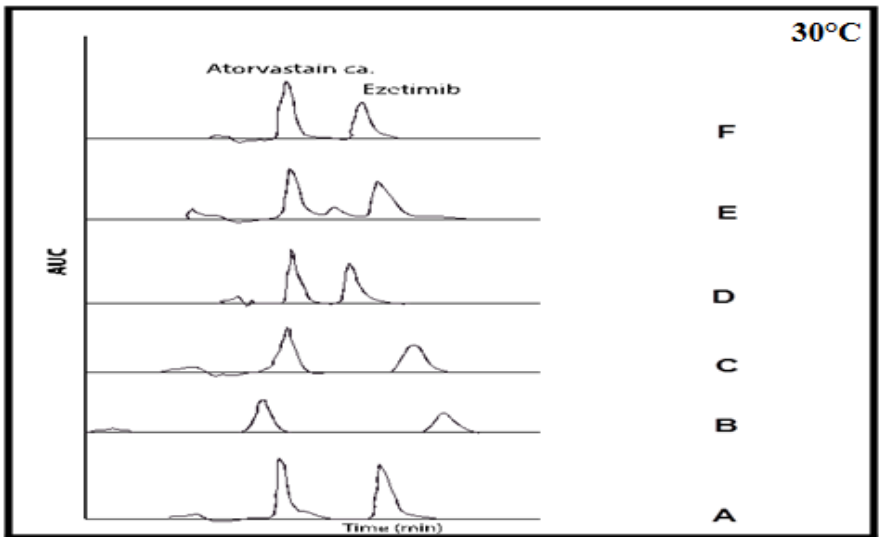
Fig. (14): Effect of six months storage at different temperatures on the release of Atorvastatin calcium and Ezetimibc from capsules.

Table (6): Percent remained of Atorvastatin calcium in tablets prepared by direct compression stored at different temperatures for six months.

Storage period (months)		% Remaining of atorvastatin calcium (mean ± SD)		
		Storage temperature (°C)		
		30	40	50
0		99.96 ± 3.4	100.86 ± 3.4	99.49 ± 3.4
1		99.96 ± 0.81	100.86 ± 2.62	97.3 ± 1.93
2		98.53 ± 2.61	100.04 ± 2.73	92.68 ± 1.06
3		97.9 ± 1.51	100.59 ± 1.29	95.73 ± 1.91
4		97.44 ± 2.82	95.64 ± 1.42	95.55 ± 0.63
5		87.38 ± 1.09	97.99 ± 2.18	91.84 ± 0.34
6		99.25 ± 0.83	93.84 ± 0.25	90.82 ± 1.23
First order	r^2	0.9881	0.9916	0.9845
	Intercept	1.9979	1.9978	1.9969
	Slope	-0.002	-0.0026	-0.003
	K	4.6×10^{-3}	5.98×10^{-3}	6.91×10^{-3}

Table (7): Percent remained of Ezetimibe in tablets prepared by direct compression stored at different temperatures for six months.

Storage period (months)		% Remaining of ezetimibe (mean ± SD)		
		Storage temperature (°C)		
		30	40	50
0		99.49 ± 3.4	99.49 ± 3.4	99.49 ± 3.4
1		98.3 ± 0.81	102.93 ± 2.62	100.1 ± 1.93
2		98.52 ± 2.61	101 ± 2.73	92.56 ± 1.06
3		98.1 ± 1.51	99.73 ± 1.29	91.64 ± 1.91
4		100.77 ± 2.82	97.72 ± 1.42	91.83 ± 1.91
5		98.41 ± 1.09	99.22 ± 2.18	92.70 ± 0.63
6		99.94 ± 0.83	97.68 ± 0.25	92.89 ± 0.34
First order	r^2	0.9881	0.9916	0.9845
	Intercept	1.9979	1.9978	1.9969
	Slope	-0.002	-0.0026	-0.003
	K	4.6×10^{-3}	5.98×10^{-3}	6.91×10^{-3}



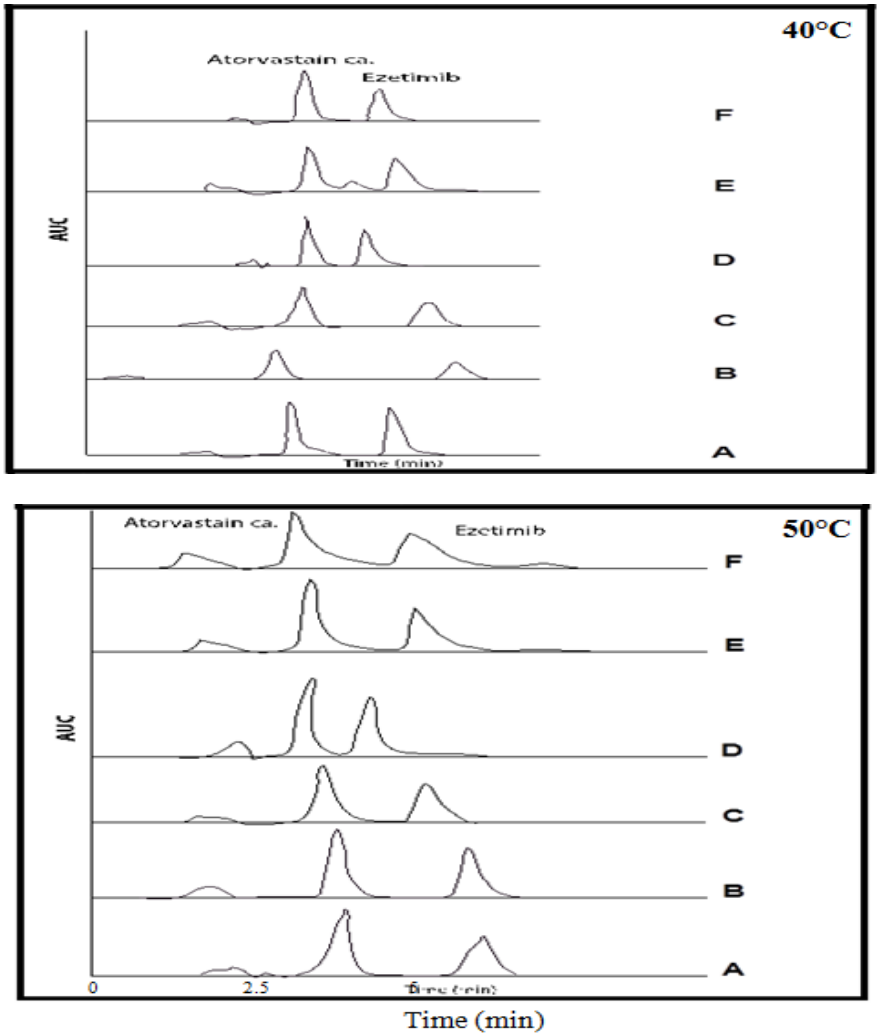


Fig. (15): HPLC chromatograms of atorvastatin calcium and Ezetimibe tablets prepared by direct compression at 30°C, 40°C, 50°C for six months

(A) HPLC chromatogram of direct compression tablets at 1st month.
(B) HPLC chromatogram of direct compression tablets at 2nd month.
(C) HPLC chromatogram of direct compression tablets at 3rd month.
(D) HPLC chromatogram of direct compression tablets at 4th month.
(E) HPLC chromatogram of direct compression tablets at 5th month.
(F) HPLC chromatogram of direct compression tablets at 6th month.

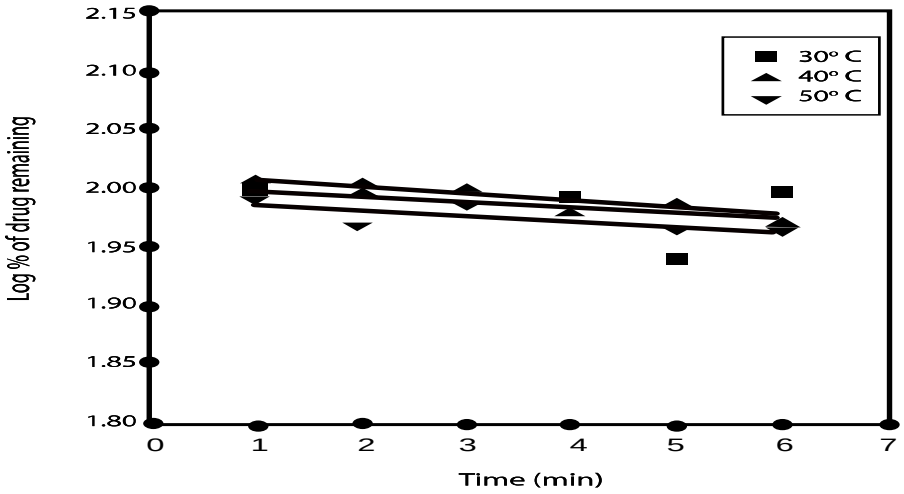


Fig. (16): First - order degradation kinetics of Atorvastatin calcium from tablets prepared by direct compression and stored for six months at different temperatures.

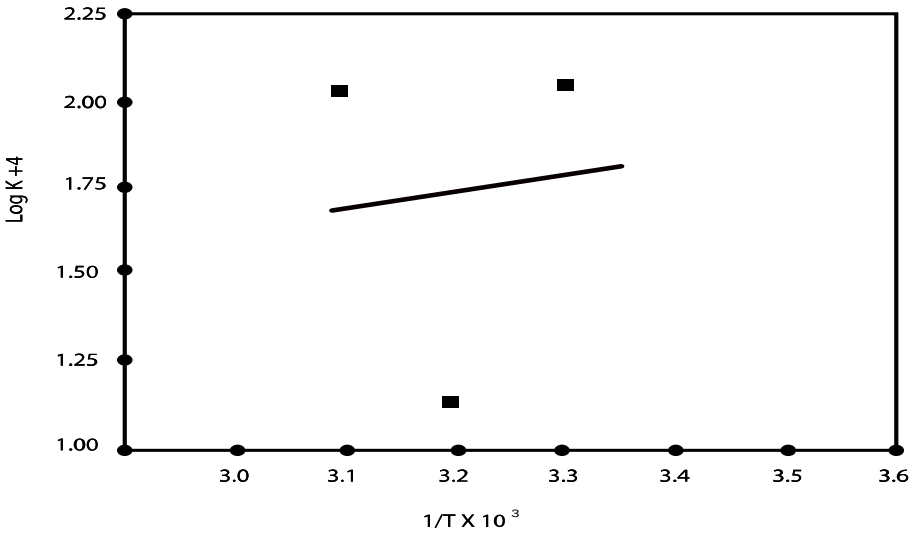


Fig. (17): Arrhenius plot for the degradation of Atorvastatin calcium from tablets prepared by direct compression.

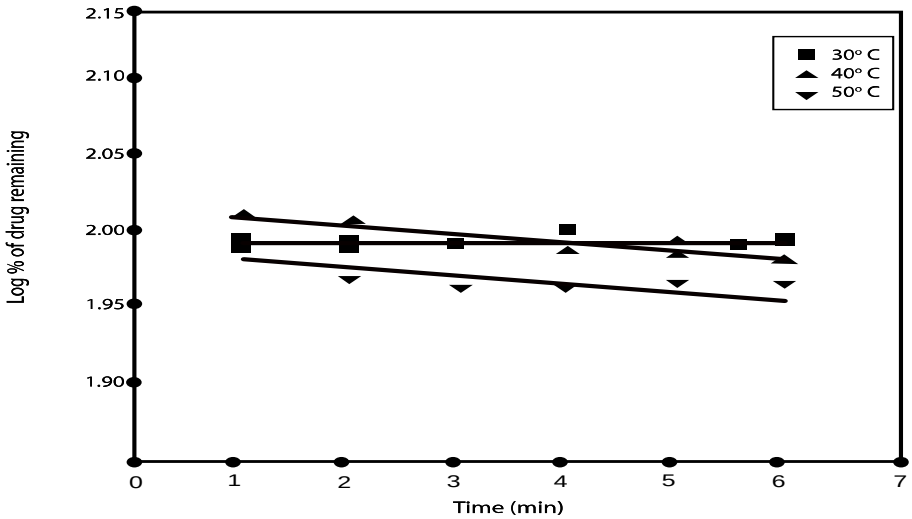


Fig. (18): First - order degradation kinetics of Ezetimibe from tablets prepared by direct compression and stored for six months at different temperatures.

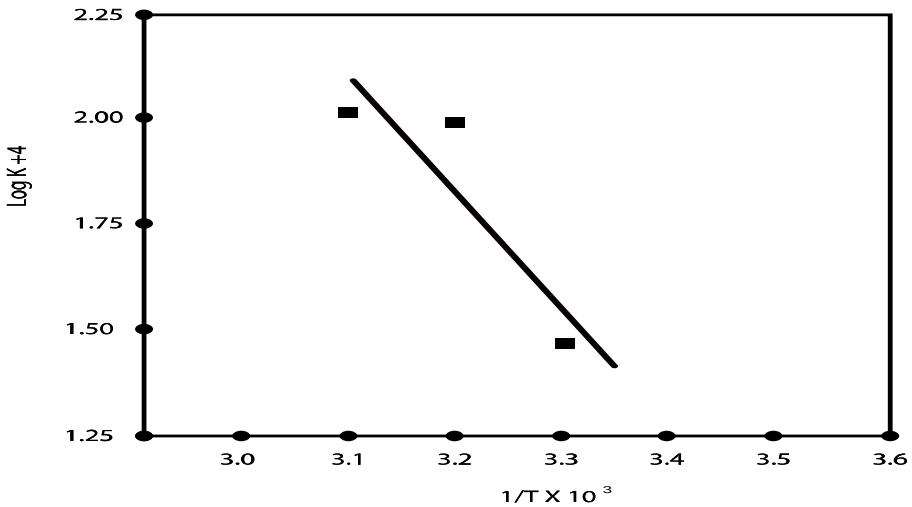


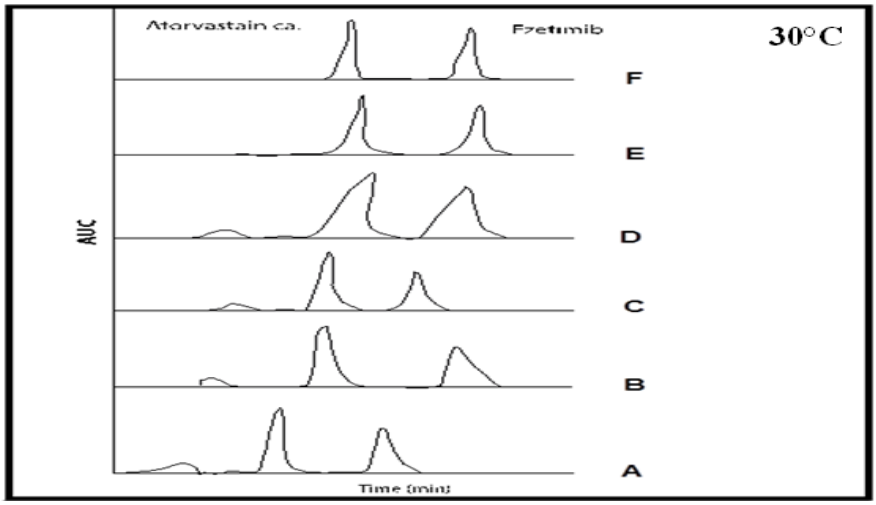
Fig. (19): Arrhenius plot for the degradation of Ezetimibe in tablets prepared by direct compression.

Table (8): Percent remained of Atorvastatin calcium in capsules stored at different temperatures for six months.

Storage period (months)		% Remaining of atorvastatin calcium (mean ± SD)		
		Storage temperature (°C)		
		30	40	50
0		99.49 ± 3.4	102.173 ± 3.4	99.49 ± 3.4
1		102.44 ± 0.81	102.173 ± 3.4	104.1 ± 1.93
2		101.25 ± 2.61	102.88 ± 2.62	103.92 ± 1.06
3		100.44 ± 1.51	100.25 ± 1.29	104.8 ± 1.91
4		99.92 ± 2.82	101.1 ± 1.42	105.48 ± 0.63
5		101.73 ± 1.09	99.35 ± 2.18	103.9 ± 0.34
6		99.41 ± 0.83	99.54 ± 0.25	97.92 ± 1.23
First order	r^2	0.9881	0.9916	0.9845
	Intercept	1.9979	1.9978	1.9969
	Slope	-0.002	-0.0026	-0.003
	K	4.6×10^{-3}	5.98×10^{-3}	6.91×10^{-3}

Table (9): Percent remained of Ezetimibe in capsules stored at different temperatures for six months.

Storage period (months)		% Remaining of ezetimibe (mean ± SD)		
		Storage temperature (°C)		
		30	40	50
0		99.49 ± 3.4	99.49 ± 3.4	99.49 ± 3.4
1		107.81 ± 0.81	108.75 ± 2.62	108.3 ± 1.93
2		106.817 ± 2.61	106.76 ± 2.73	107.54 ± 1.06
3		104.1 ± 1.51	107.53 ± 1.29	105.74 ± 1.91
4		103.87 ± 2.82	103.26 ± 1.42	105.92 ± 0.63
5		103.77 ± 1.09	97.51 ± 2.18	106.78 ± 0.34
6		108.24 ± 0.83	96.27 ± 0.25	103.06 ± 1.23
First order	r^2	0.9881	0.9916	0.9845
	Intercept	1.9979	1.9978	1.9969
	Slope	-0.002	-0.0026	-0.003
	K	4.6×10^{-3}	5.98×10^{-3}	6.91×10^{-3}



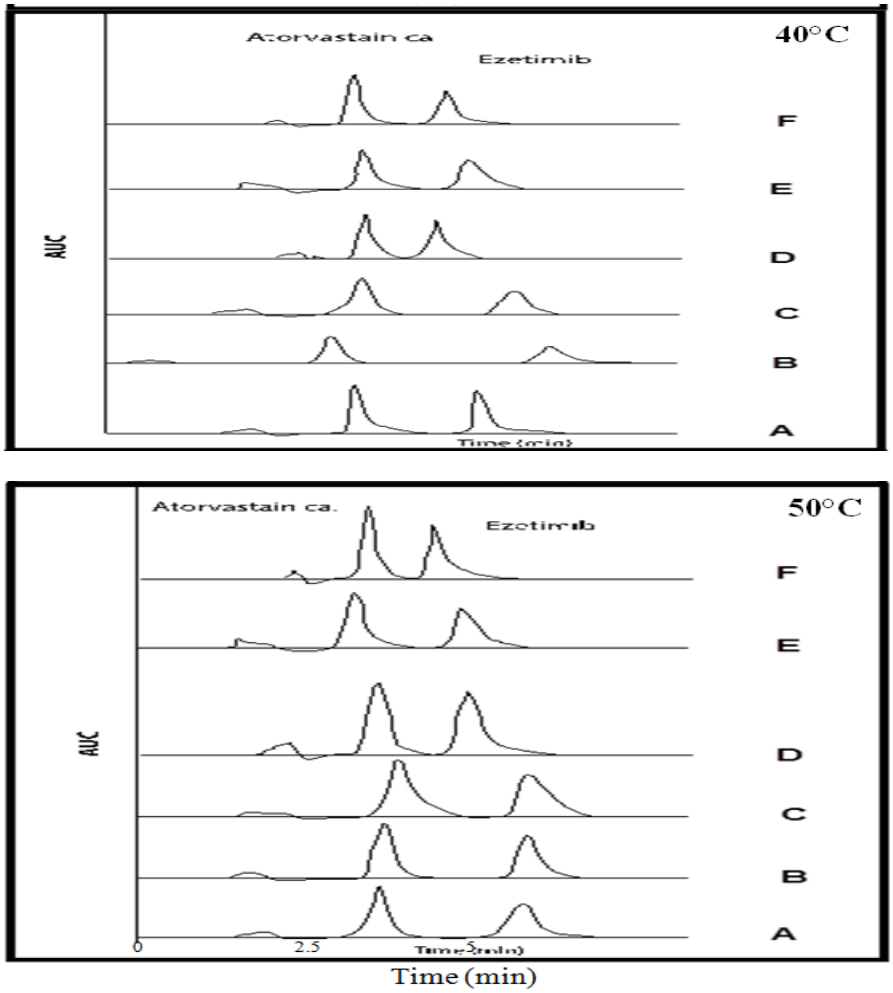


Fig. (20): HPLC chromatograms of atorvastatin calcium and Ezetimibe capsules at 30°C, 40°C, 50°C for six months:

(A) HPLC chromatogram of capsules at 1st month.
(B) HPLC chromatogram of capsules at 2nd month.
(C) HPLC chromatogram of capsules 3rd month.
(D) HPLC chromatogram of capsules at 4th month.
(E) HPLC chromatogram of capsules at 5th month.
(F) HPLC chromatogram of capsules at 6th month.

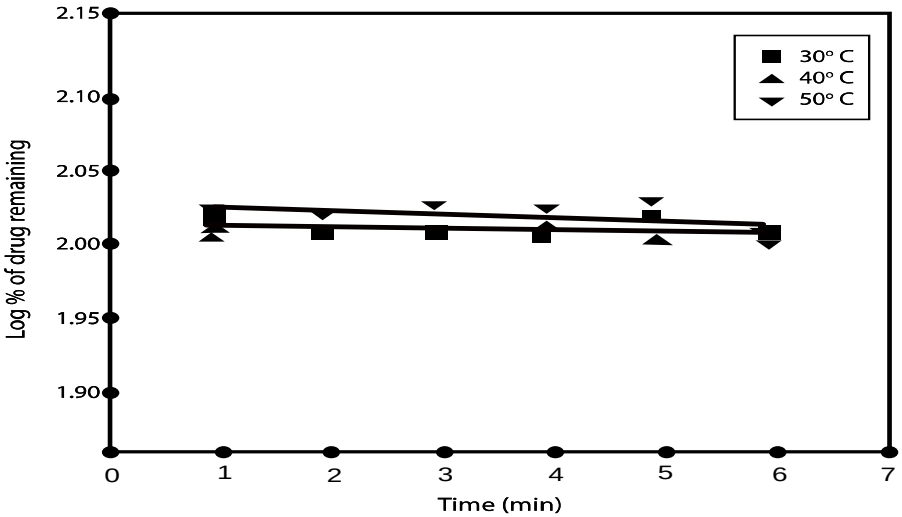


Fig. (21): First - order degradation kinetics of Atorvastatin calcium capsules and stored for six months at different temperatures.

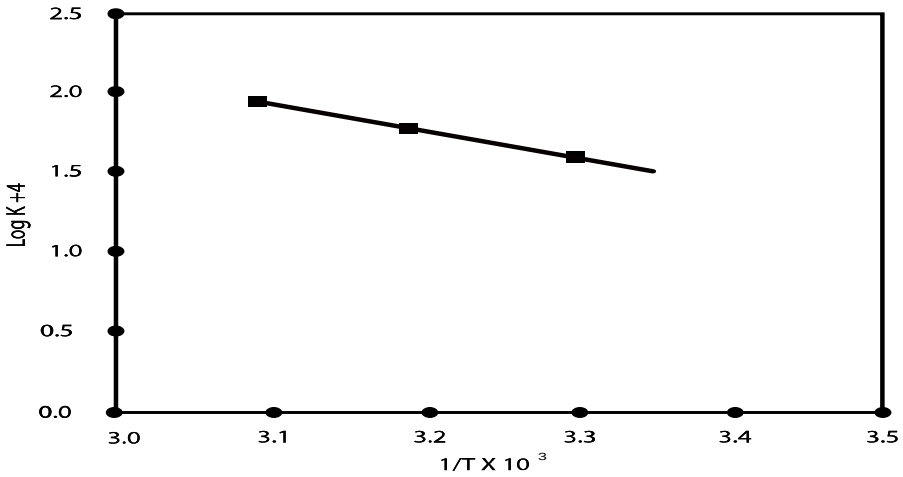


Fig. (22): Arrhenius plot for the degradation of Atorvastatin calcium from capsules.

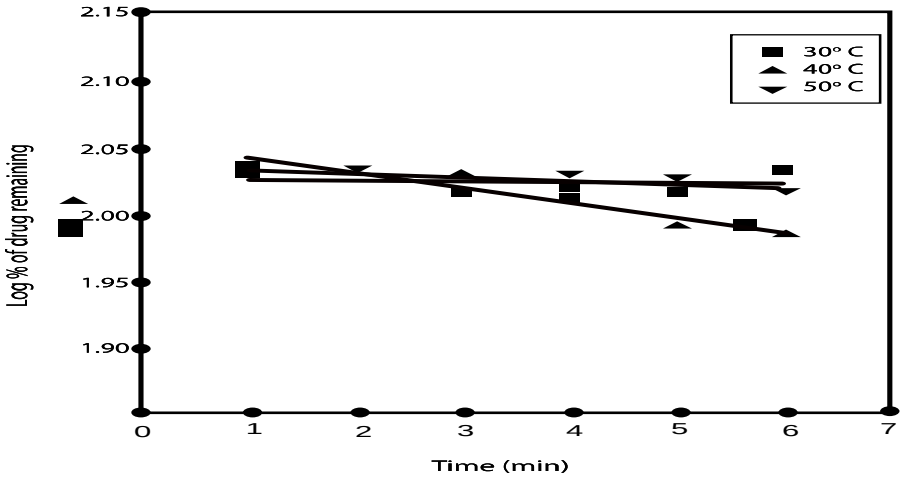


Fig. (23): First - order degradation kinetics of Ezetimibe from capsules and stored for six months at different temperatures.

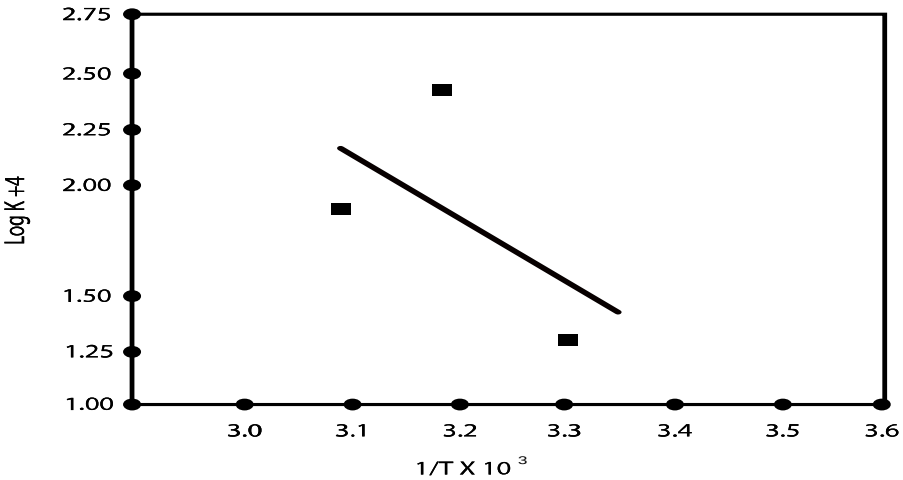


Fig. (24): Arrhenius plot for the degradation of Ezetimibe from capsules.

Table (10): The determined shelf life of atorvastatin calcium and ezetimibe in tablets and capsules.

Expiry date / Formulae	Atorvastatin calcium	Ezetimibe
(A) (Tablets prepared by direct compression)	1.85 years	3.3 years
(B) (Capsules prepared by dry mixing)	2.75 years	3.5 years

(A): The selected formula was prepared by direct compression technique
(B): The selected formula was formulated in capsule dosage form

List of Abbreviations

Abbreviations	The word
%	Percentage
°C	Degree Centigrade
µg	Microgram
µm	Micrometer
Ac-Di-Sol	Crosslinked Carboxymethylcellulose Sodium
Aerosil	Colloidal Silicon Dioxide
ATV	Atorvastatin Calcium
AUC	Area Under The Concentration Curve
Avicel PH101	Microcrystalline Cellulose
BP	British Pharmacopoeia
C_{max}	Maximum Plasma Concentration
cm	Centimeter
CVDs	Cardiovascular Diseases
Dept.	Department
Disint. Time	Disintegration Time
DSC	Differential Scanning Calorimetry
e.g.	Example
EZT	Ezetimibe
FPO6U	Faculty of Pharmacy, October 6 University
FDA	Food And Drug Administration
Fig.	Figure
FTIR	Fourier Transform Infrared Spectroscopy
g	Gram
HDL	High Density Lipoproteins
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxy Propyl Methyl Cellulose
HPMC4000	Hydroxy Propyl Methyl Cellulose 4000
hrs	Hours
K	Procedural Constant
Kg	Kilogram
LDL	Low Density Lipoproteins
LDL-C	Low-Density Lipoprotein Cholesterol
M.W.	Molecular Weight
mg	Milligram
Mg stearate	Magnesium Stearate
ml	Milliliter
mm	Millimeter
mm.	Minute
nm	Nanometer
PEG4000	Polyethylene Glycol 4000
pH	Hydrogen Ion Concentration
PVP_{K25}	Polyvinyl Pyrrolidone $_{K25}$
PVP K30	Povidone K30
Q.S.	Quantity Sufficient
r.p.m	Rotation Per Minute
R*f*	Retardation Factor
RH	Relative Humidity
RPM	Revolutions Per Minute
SD	Standard Deviation
SDF	Solid Dosage Form
Sec.	Second
SLS	Sodium Lauryl Sulfate
Tab	Tablet
TLC	Thin Layer Chromatography
T_{max}	Time At The Maximum Concentration In Serum.
T_{max}	Time Of Maximum Plasma Concentration.

Abbreviations	The word
USP	United States Pharmacopoeia
UV	Ultra Violet
v	Volume
W/W.	Weight / Weight
$\lambda_{max.}$	Maximum Wavelength

CONCLUSION

From the obtained results, it could be concluded that:

1. Absence of chemical interaction between Atorvastatin calcium alone or in suggested drug combination and their utilized excipients and so the absence of chemical interaction between Ezetimibe alone or in suggested drug combination.
2. The suitable concentrations of the super disintegrants were 4% for Ac-Di-Sol.
3. The presence of calcium carbonate exhibited increase of the release rate of two drugs.
4. The presence of aerosil 200 and tween80 increase release of two drug
5. Dissolution profile in case of capsules is higher than dissolution profile in case of tablets prepared by direct compression.
6. The hardness of the selected formula (F5) containing the drug combination in direct compression tablets was greatly increased at the different temperatures and hence the tensile strength increased. As a result of increasing hardness, the percent friability decreased and so, the disintegration time of direct compression tablets was increased.
7. The six months storage in case of direct compression tablets and capsules have a marked decrease in the dissolution of the drug combination.
8. The percent remained of two drugs in suggested drug combination was within the pharmacopeial limits upon storage for 6 months at different temperatures.
9. The kinetic analysis of drug content revealed that the drug decomposition followed the first order reaction.

The determined shelf life of the two drugs was 1.85 years for Atorvastatin calcium and 3.3 years for Ezetimibe in case of tablets prepared by direct compression and 2.75 years for Atorvastatin calcium and 3.5 years for Ezetimibe in case of capsules.

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