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VALIDATED UV SPECTROPHOTOMETRIC AND HPTLC METHOD FOR DETERMINATION OF ATORVASTATIN CALCIUM, ASPIRIN, RAMIPRIL AND METAPROLOL TARTARATE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid and precise, UV spectrophotometric and High Performance Thin Layer Chromatographic (HPTLC) methods were developed and validated for quantitative determination of Atorvastatin calcium, Aspirin, Ramipril and Metaprolol tartarate in bulk and pharmaceutical formulations. In UVspectrophotometric method, estimation of Fosamprenavir calcium was carried out using 0.5N hydrochloric acids as solvent at 291.5nm, 247nm, 242.5nm and 229.5nm respectively. The drug obeyed Beer–Lambert's law in the concentration range of 3-21µg/mL, 10-70 µg/mL, 10-70 µg/mL and 10-70 µg/mL and for all drugs coefficient of correlation (R2) is 0.999. In the HPTLC method, the chromatographic development was carried out on HPTLC plates precoated with silica gel 60 F254 using Methanol as mobile phase. Detection was carried out at 270 nm. The calibration curve was linear over a range of 100-600ng/spot, 10-60ng/spot, 20-120ng/spot and 75-4500ng/spot with a regression coefficient of 0.999. The Rf value for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin were found to be 0.14 ± 0.01, 0.26 ± 0.01, 0.40 ± 0.01 and 0.99 ± 0.01, respectively. Both the methods were validated as per ICH guideline with respect to linearity, accuracy, precision, robustness etc. The methods can be adopted in routine analysis of Atorvastatin calcium, Aspirin, Ramipril and Metaprolol tartarate in tablet dosage form.

INTRODUCTION:

Atorvastatin Calcium is a white to off-white, crystalline powder, chemically (βR, 8R) -2- (4-fluorophenyl)-α, δ-dihydroxy-5-(1 methylethyl)-3-phenyl-4-(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid trihydrate (Figure 1). Atorvastatin is in a group of drugs called HMG CoA reductase inhibitors, or "statins." Atorvastatin reduces levels of "bad" cholesterol (low-density lipoprotein, or

LDL) and triglycerides in the blood, while increasing levels of "good" cholesterol (high-density lipoprotein, or HDL). It is administered 10 mg or 20 mg orally once a day. It is freely soluble in methanol; slightly soluble in ethanol; very slightly soluble in acetonitrile; distilled water, phosphate buffer pH 7.4; insoluble in aqueous solution of pH 4 and below[1,6]. Aspirin, or acetylsalicylic acid (ASA), is commonly used as a pain reliever for minor aches and pains and to reduce fever. It is also an anti-inflammatory drug and can be used as a blood thinner and chemically it is known as 2-(Acetyloxy) benzoic acid (Figure 2). Aspirin is one of the most widely used medications in the world and it is colourless or white crystals, crystalline powder; odourless or almost odourless[2,3, 6]. Ramipril is a 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid derivative. It is a white, crystalline substance soluble in polar organic solvents and buffered aqueous solutions. Ramiprilat, the diacid metabolite of ramipril, is a non-sulfhydryl ACE inhibitor. Ramipril is converted to ramiprilat by hepatic cleavage of the ester group. Ramipril chemically is (2S.3aS.6aS)-1-[(S)-N-[(S)-1-Carboxy-3-phenylpropyl] alanyl] ctahydrocyclopenta[b]pyrrole-2-carboxylic acid. 1-ethyl ester (Figure 3). Metoprolol Tartrate is a beta-blocker that affects the heart and circulation (blood flow through arteries and veins). Metoprolol Tartrate is used to treat angina (chest pain) and hypertension (high blood pressure). It is also used to treat or prevent heart attack. It is chemically standing for (RS)-1-(isopropylamino) -3 -p - (2-methoxyethyl) phenoxypropan-2-ol (2R, 3R) - tartrate (Figure 4). It is **v**ery soluble in water, soluble in ethanol and chloroform; practically insoluble in ether[5,6]. The literature review revealed a dissolution method and an electrochemical method 1for evaluation of Atorvastatin Calcium Metoprolol Tartrate, Ramipril and Aspirin in bulk drugs and pharmaceutical dosage forms. However, some LC/MS, HPTLC, HPLC and HPLC-MS methods had been reported for estimation of individual Atorvastatin Calcium Metoprolol Tartrate, Ramipril and Aspirin drugs and in combination forms in human blood plasma and body fluids. Further, no official or draft monograph of mixture of Atorvastatin Calcium Metoprolol Tartrate, Ramipril and Aspirin drugs was published in any of the pharmacopoeia for compendia applications.

It was felt necessary to develop a simple, precise and rapid UV and HPTLC methods for the quantitative estimation of Atorvastatin Calcium Metoprolol Tartrate, Ramipril and Aspirin in pharmaceutical products and bulk drugs. The current research work deals with the development of UV and HPTLC methods and their validation as per International Conference on Harmonisation (ICH) guidelines[7,8,9,10].

MATERIALS AND METHODS

Chemicals and reagents

All the samples working standard was received as a sample from Madras Pharmaceuticals Chennai. (India). The formulations ZYCAD-4, containing Atorvastatin Calcium- 10 mg, Aspirin- 75 mg, Ramipril- 5 mg and Metoprolol Tartrate- 50 mg was purchased from Vasantha Medicals, Choolaimedu, Chennai. Acetonitrile used was of HPLC grade (Qualigens, Mumbai). Millipore water was used throughout analysis. Pharmaceutical dosage form (Fosamprenavir Calcium tablets containing 100 mg Fosamprenavir) was prepared in laboratory using Lactose Monohydrate, Microcrystalline Cellulose, Starch and Magnesium Stearate. Water was obtained from a Milli-Q UF-Plus apparatus (Millipore) and was used to prepare all solutions for the method. Other chemicals used were analytical or HPLC-grade.

The present research work was made to develop and validate simple, accurate and precise methods for the simultaneous analysis of Atorvastatin Calcium, Aspirin,

Ramipril and Metoprolol Tartrate in bulk and in pharmaceutical dosage form by UV Spectroscopy (First order derivative spectrophotometry) and HPTLC method.

UV Spectroscopy

First Order Derivative Spectrophotometry

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectra are understood by reference to a Gaussian band which represents an ideal absorption band. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order or D° spectrum. The first derivative (D_1) spectrum is a plot of the ratio of change of absorbance with wavelength against wavelength.

Selection of Solvent: The solubility of drugs was determined in a variety of polar and non-polar solvents as per specification. The common and stable solvent was found to be methanol and further dilutions were made with distilled water for the analysis of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate for the proposed method.

Preparation of Standard Stock Solutions: 15 mg of Atorvastatin Calcium was weighed accurately and transferred in to 10 ml volumetric flask. Dissolved with methanol and made up to the volume with methanol. The stock solution contains 1.5 mg/ ml of Atorvastatin Calcium. 50 mg of Aspirin, Ramipril and Metoprolol Tartrate were accurately weighed and transferred in to 50 ml volumetric flask separately. Dissolved in methanol and made up to the volume to 50 ml. The solution contains 1 mg/ ml of Aspirin, Ramipril and Metoprolol Tartrate.

Selection of Wavelength: 10 µg/ ml concentration solutions of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate were prepared from the stock solution and the spectra were recorded between 200 and 400 nm by using distilled water as blank. The zero order spectra were derivitized to first order and the spectra were overlained. From the overlain spectra, the wavelength selected for the analysis were 291.5 nm, 247 nm 242.5 nm and 229.5 nm for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively.

Stability Studies: The stability of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate were checked using 10 µg/ml solutions at the selected wavelengths in different time intervals. It was found that Atorvastatin Calcium was stable for up to 3 hours, Aspirin up to 3 hours and 30 minutes, Ramipril up to 1 hour and 30 minutes, and Metoprolol Tartrate up to 1 hour.

Linearity and Calibration: 5ml of Atorvastatin Calcium stock solution was pipetted out in to 50 ml volumetric flask and made up to 50 ml with distilled water. From that 1 – 7 ml were transferred in to a series of 50 ml volumetric flasks and made up to the volume with distilled water to get concentrations of 3 to 21 µg/ ml. 1 – 7 ml of the stock solutions of Aspirin, Ramipril and Metoprolol Tartrate were transferred in to series of 100 ml volumetric flask individually and made up to the volume with distilled water to get the concentrations of 10 – 70 µg/ ml of Aspirin, Ramipril and Metoprolol Tartrate.

Synthetic Mixture: 4 µg/ ml of Atorvastatin Calcium and 30 µg/ ml of Aspirin, Ramipril and Metoprolol Tartrate were prepared individually from their corresponding stock solutions. 1 to 5 ml were pipetted out from each stock solution into a series of six100 ml volumetric flasks and made up to 100 ml with distilled water to get a mixture of Atorvastatin Calcium, Aspirin, Ramipril, and Metoprolol Tartrate in the concentration of 4 to 20 µg/ ml for Atorvastatin Calcium

and 30 to 70 µg/ ml for Aspirin, Ramipril, and Metoprolol Tartrate. The absorbances of the prepared synthetic mixtures were measured at the selected wavelengths. The amount of drugs in the prepared synthetic mixture was calculated.

Quantification of Formulation: Twenty capsules (ZYCAD-4, containing Atorvastatin Calcium – 10 mg, Aspirin – 75 mg, Ramipril – 5 mg, Metoprolol Tartrate – 50 mg) were accurately weighed and the average weight was calculated. The capsules containing powder were crushed and made in to a fine powder. The mixed contents of capsule powder equivalent to 25 mg of Aspirin was accurately weighed and transferred in to a series of 25 ml volumetric flasks and added 15 mg/ ml solution of Ramipril. Dissolved in methanol and sonicated for15 minutes. The volume was made up to 25 ml with methanol and was centrifuged for 15 minutes at 2000 rpm. The solution was filtered through whatmann filter paper No.41. 3 ml of the stock solution was further diluted to 100 ml volumetric flask and made up to with distilled water to get the theoretical concentrations of 4 µg/ml, 30 µg/ml, 20µg/ml and 20 µg/ml of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively. The absorbances of solutions were measured at 291.5 nm, 247 nm, 242.5 nm and 229.5 nm. The procedure was repeated for six times. The amounts of these drugs were calculated.

Recovery Studies

Preparation of Raw Material and Standard Stock Solution

10 mg of Atorvastatin Calcium raw material was weighed accurately in to a 10 ml volumetric flask, dissolved with methanol and made up to 10 ml with methanol to get a concentration of 1 mg/ml of Atorvastatin Calcium. Similarly 100 mg of Aspirin, Ramipril and Metoprolol Tartrate raw material were weighed accurately and transferred in to 10 ml volumetric flasks separately, dissolved with methanol and made up to 10 ml with methanol to get concentrations of 10 mg/ml of Aspirin, Ramipril and Metoprolol Tartrate.

Recovery Procedure: The recovery study was done by adding a specified quantity of the drug to the pre-analyzed formulation. The tablet powder equivalent to 25mg of Aspirin was weighed accurately and added 15 mg/ ml solution of Ramipril in to three separate 25 ml volumetric flasks. To this 2 ml, 3 ml, 4 ml of Atorvastatin Calcium, 2.0 ml, 2.5 ml, 3.0 ml of Aspirin, 1.0 ml, 1.5 ml, 2.0 ml of Ramipril and Metoprolol Tartrate stock solutions were added. Dissolved in methanol, sonicated for 15 minutes and made up to 25 ml with methanol. The solution was centrifuged for 15 minutes at 2000 rpm and was filtered through whatmann filter paper No.41. From each solution, 3 ml was transferred to three 100 ml volumetric flasks separately and made up to the volume with distilled water. The absorbances of the solutions were measured at 291.5 nm, 247 nm, 242.5 nm and 229.5 nm. The amount of drug recovered was calculated. The procedure was repeated three times for each concentration.

Validation of Developed Methods

Linearity: A calibration curve was plotted with concentration versus the absorbance value. The linearity was checked for Atorvastatin Calcium in the concentration range of 3 to 21 µg/ ml, Aspirin, Ramipril and Metoprolol Tartrate in the concentration range of 10 to 70 µg/ ml. The drugs were found to be linear in the specified concentration ranges.

Precision: The repeatability of the method was confirmed by repeated analysis of the formulation for six times with the same concentration. The amount of drug present in the formulation was calculated. The percentage RSD value was calculated. Confidence Interval was also calculated. The intermediate precision was confirmed by the

intraday and inter day analysis i.e. the analysis was performed three times on the same day and on three successive days. The amount of drug present in the formulation was calculated. The percentage RSD values were calculated. Confidence Interval was also calculated.

Ruggedness: Ruggedness of the method was confirmed by the analysis of formulation in different instrument and by different analyst. The amount of drug was calculated and the percentage RSD values were also calculated. Confidence Interval was also calculated.

Accuracy: Accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation a known quantity of the standard drug solutions were added and the amount of drug recovered was calculated. The percentages RSD values were calculated. Confidence Interval was also calculated.

LOD and LOQ: The linearity study was carried out for six times. The LOD and LOQ were calculated based up on the calibration curve method. The LOD and LOQ were calculated using the average of slope and standard deviation of intercept.

HPTLC

HPTLC instrumentation and chromatographic conditions: HPTLC plates pre-coated with silica gel GF254 on aluminum plate, (20.0 x 10.0 cm), Merck, were used for the analysis. Densitometry was carried out with a CAMAG TLC Scanner 3, fitted with win CATS 1.4.0 planar chromatography manager software. Samples were applied to the HPTLC plates using the spray-on technique of CAMAG Linomat 5 under nitrogen gas flow and developed in a CAMAG 20.0 x 10.0 cm twin trough chambers. Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate reference standard solution was prepared using methanol as solvent. Solutions of 0.2 µL was applied to the HPTLC plates as spot bands of 8mm using LINOMAT V. The development chamber was left for saturation with mobile phase (methanol) vapors for 5 minutes before each run. Development of the plate was carried out by the ascending technique to a migration distance of 8 cm. After development, the plates were air dried. All the analyses were carried out at room temperature. Densitometry scanning was done in absorbance mode at 270 nm using a deuterium lamp.

Preparation of Standard Stock Solution: 100 mg of Metoprolol Tartrate, 10 mg of Ramipril, 20 mg of Atorvastatin Calcium, and 75 mg of Aspirin raw materials were accurately weighed in to 100 ml volumetric flask dissolved in methanol and made up to the volume with methanol. This solutions contains 1 mg/ ml of Metoprolol Tartrate, 100 µg/ ml of Ramipril, 200 µg/ ml of Atorvastatin Calcium and 750 µg/ ml of Aspirin.

Linearity and Calibration curve: 1 ml of the stock solutions was pipetted out in to 10 ml volumetric flasks individually and made up to the volume with methanol. 1 ng/ µl – 6 ng/ µl were spotted on TLC plates and to get the concentration range of 100 – 600 ng/ µl, 10 – 60 ng/ µl, 20 – 120 ng/ µl and 75 – 450 ng/ µl of Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively.

Quantification of Formulation: Twenty capsules were weighed accurately and the average weight of each capsule was determined. The capsule containing powder is crushed well in to a fine powder. The capsule powder equivalent to 75 mg of Aspirin was weighed in to a series of six 100 ml volumetric flasks. Dissolved in a methanol and sonicated for 15 minutes. The volume was made up to 100 ml with methanol and was centrifuged for 15 minutes at 2000 rpm. The solution was filtered through whatmann filter paper No.41. The filtrate was further diluted to get a concentration of 100 ng/ µl, 10 ng/ µl, 20 ng/ µl and 150 ng/ µl of Metoprolol Tartrate , Ramipril, Atorvastatin Calcium and Aspirin theoretically. 1 µl spots were placed on the plates and the chromatogram was

developed in the twin through chamber. From the peak area and amount of drugs were calculated. The procedure was repeated for six times

Recovery Studies: To the capsule powder equivalent to 75 mg of Aspirin, 80, 100 and 150 mg of Metoprolol Tartrate, 8, 10 and 12 mg of Ramipril 16, 20 and 24 mg of Atorvastatin Calcium, and 120, 150 and 180 mg of Aspirin were added in to 50 ml volumetric flasks. Added about 45 ml 0f methanol and sonicated for 15 min. The solution was then made up to the mark with methanol. The solution was centrifuged for 15min at 2000 rpm, and filtered through the Whatmann filter paper No.41. Further, 1 ml of the stock solution was diluted to 10 ml. From this solution, 1µl quantity of sample was spotted and the chromatogram was recorded. From the peak area, the amounts of drug recovered were calculated for each concentration and repeated for three times.

Validation of Developed Method

Linearity: A calibration curve was plotted with concentration versus the peak area. The linearity range was checked for in the concentration range of 100 – 600 ng/ µl, 10 – 60 ng/ µl, 20 – 120 ng/ µl and 75 – 450 ng/ µl of Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively. The drugs were found to be linear in the specified concentration ranges.

Precision: The repeatability of the method was checked by repeated analysis of the formulation for six times with the same concentration. The amount of drug present in the formulation was calculated. The percentage RSD value was calculated. Confidence Interval was also calculated. The intermediate precision was confirmed by the intraday and inters day analysis i.e. the analysis was performed three times on the same day and on three successive days. The amount of drug present in the formulation was calculated. The percentage RSD values were calculated. Confidence Interval was also calculated.

Accuracy: Accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation a known quantity of the standard drug solutions were added and the amount of drug recovered was calculated. The percentages RSD values were calculated. Confidence Interval was also calculated.

LOD and LOQ: The linearity study was carried out for three times. The LOD and LOQ were calculated based up on the calibration curve method. The LOD and LOQ were calculated using the average of slope and standard deviation of intercept.

RESULTS AND DISCUSSION

UV spectrophotometric method

Selection of Wave Length

UV spectrum of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate had shown λmax at 291.5 nm, 247 nm, 242.5 nm and 229.5 nm respectively. Hence they were selected for the analysis (Figure 5).

Stability studies: The stability of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate were checked at the selected wavelengths using methanol and distilled water as solvent. It was found that Atorvastatin Calcium stable for about 3 hours, Aspirin for about 3 hours and 30 minutes, Ramipril for about 1 hour and 30 minutes and Metoprolol Tartrate for about 1 hour. Various aliquots of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate were prepared in the concentration range of 3 - 21 µg /ml, 10 – 70 µg/ ml, 10 to 70 µg/ ml and 10 to 70 µg/ ml, respectively. The absorbances of these solutions were measured at the selected wavelengths. The calibration curve was constructed using concentration Vs absorbance. The preparation of calibration curve was repeated for six times for each drug at their selected wavelengths. The optical

parameters like Molar absorptivity, Sandell's sensitivity, Correlation coefficient, Slope, Intercept, LOD and LOQ were calculated. The correlation coefficient for all the four drugs was found to be above 0.999. Hence all the drugs obey Beer's law and the concentrations were found to be linear. The calibration graph for Atorvastatin Calcium at 291.5 nm. The calibration curves for Atorvastatin Calcium and Aspirin at 247 nm, respectively. The calibration curves for Atorvastatin Calcium, Aspirin and Ramipril at 242.5 nm, respectively. At 229.5 nm, the calibration curves for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate are shown in figure 6 respectively. The optical characteristics of the drugs at their selected wavelengths are shown in table 1 respectively. The developed method was applied and validated for the analysis of synthetic mixture the amount of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate were found to be in the range of 98.00 - 100.87%, 98.16 - 100.36%, 99.22 - 100.84% and 100.23 – 101.21%, respectively. The results are listed in table 2. The amount found was good agreement with the expected concentration. Hence it was planned to apply for the analysis of formulation. The percentage purity of the drugs in the formulation was found to be 102.76 ± 1.4487, 99.00 ± 1.5795, 99.95 ± 1.7250 and 98.66 ± 0.8496 for Atorvastatin Calcium, Aspirin, Ramipril, and Metoprolol Tartrate, respectively. The results are listed in table 3.The confidence interval for drugs was found to be in the range of 101.23 – 104.28, 97.34 – 100.65, 98.13 – 101.76 and 97.76 – 99.55 for Atorvastatin Calcium, Aspirin, Ramipril, and Metoprolol Tartrate, respectively.

Precision

The precision of the method was confirmed by the repeated analysis of formulation for six times. The percentage RSD values were found to be 1.4097, 1.5954, 1.7257 and 0.8612 for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively. Further the precision of the method was confirmed by intraday and inter day analysis. Intraday and inter day analysis of formulation was done on three times on same day and one time on three consecutive days. The percentage RSD for the intraday and inter day precision was found to be 0.1528 and 0.0680 for Atorvastatin Calcium, 0.8116 and 0.3145 for Aspirin, 1.9063 and 1.1612 for Ramipril and 1.7204 and 1.0513 for Metoprolol Tartrate, respectively. The results are listed in table 4. The low percentage RSD values indicated that the precision of the method was confirmed. The confidence interval of intraday analysis were found to be in the range of 101.48 – 102.25, 97.15 – 101.14, 96.06 – 105.61 and 95.25 – 103.76 for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively. The confidence interval of inter day analysis were found to be in the range of 101.72 – 102.07, 98.57 – 100.12, 98.25 – 104.08 and 96.24 – 101.41 for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively.

Ruggedness

The ruggedness of the method was validated by using different analysts and different instruments. The percentage RSD for analyst 1 and analyst 2 were found to be 1.3842 and 0.2887 for Atorvastatin Calcium, 1.5954 and 1.8396 for Aspirin, 1.7256 and 1.8602 for Ramipril and 0.8611 and 0.8367 for Metoprolol Tartrate, respectively. The percentage RSD for instrument 1 and instrument 2 were found to be 1.1602 and 1.5153 for Atorvastatin Calcium, 0.6684 and 0.4462 for Aspirin, 1.9820 and 1.0782 for Ramipril and 1.2887 and 0.8223 for Metoprolol Tartrate, respectively. The results are listed in table 5. The Confidence interval of different analyst 1 was found to be in the range of 101.23 -104.22, 97.34 –

100.65, 98.13 – 101.76 and 97.76 – 99.55 for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively. The Confidence interval of different analyst 2 was found to be in the range of 101.63 - 102.24, 97.60 – 101.45, 98.03 – 101.94 and 98.20 – 99.93 for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively. The Confidence interval of different instrument 1 was found to be in the range of 101.87 -102.22, 98.70 – 100.09, 99.00–103.21 and 97.63 – 100.30 for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively. The Confidence interval of different instrument 2 was found to be in the range of 98.37 - 101.54, 98.54 – 99.47, 98.67 – 100.92 and 98.17 – 99.88 for Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively.

Accuracy

The accuracy of the method was confirmed by the recovery studies. To the pre-analyzed formulation, a known quantity of raw material was added and the percentage recovery was calculated. The percentage of raw material added was 60%, 90%, and 120% for Atorvastatin Calcium, Ramipril and Metoprolol Tartrate and 80%, 100% and 120% for Aspirin. The percentage recovery was found to be in the range of 99.76 - 100.45% for Atorvastatin Calcium, 100.38 - 101.08% for Aspirin, 98.58 - 101.64% for Ramipril and 98.33 - 101.69% for Metoprolol Tartrate. The percentage RSD value was found to be 0.3508 for Atorvastatin Calcium, 0.3506 for Aspirin, 1.5580 for Ramipril and 1.8860 for Metoprolol Tartrate. The low percentage RSD indicated there was no interference due to excipients used in formulation. Hence, the accuracy method was conformed. The results are listed in table 6. The confidence interval for drugs was found to be in the range of 99.52 – 101.27, 99.82 – 101.57, 96.40 – 104.17 and 94.85 – 104.18 for Atorvastatin Calcium, Aspirin, Ramipril, and Metoprolol Tartrate, respectively.

HPTLC

The initial separation was based up on the solubility of drugs, the different mobile phase were tried to get the better resolution. The different mixtures of the mobile phases were tried and choosen benzene: toluene: methanol: glacial acetic acid, all the drugs were well separated in the ratio of 7.5: 1.0: 1.5: 0.1, and UV spectra of all the drugs were recorded and overlained. From the overlain spectra, at 224 nm all the drugs showed marked absorbance. With the optimized chromatographic conditions, the chromatograms were recorded and shown in figures 7, 8, 9 and 10 for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively. The Rf value for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin were found to be 0.14 ± 0.01, 0.26 ± 0.01, 0.40 ± 0.01 and 0.99 ± 0.01, respectively.

Linearity

The linearity range was fixed as 100 – 600 ng/ µl for Metoprolol Tartrate, 10 – 60 ng/ µl for Ramipril, 20 – 120 ng/ µl for Atorvastatin Calcium and 75 – 450 ng/ µl for Aspirin in methanol The calibration graph was recorded using peak area and concentrations and these are shown in figure 11. The correlation coefficients were found to be 0.9999, 0.9998, 0.9998 and 0.9998 for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively. The optical characteristics such as the Correlation coefficient, Slope, Intercept, LOD and LOQ and were calculated and shown in tables 7. The correlation coefficient values indicated that the selected concentration was linear. The capsule dosage form ZYCAD-4 was selected for the analysis.

Table 1: Optical characteristics of Atorvastatin Calcium, Aspirin, Ramipril, and Metoprolol Tartarate

Parameters	Atorvastatin Calcium	Aspirin	Ramipril	Metoprolol Tartarate
Wave length	AT 291.5 nm	AT 247 nm	AT 242.5 nm	AT 229.5 nm
Beers law limit (µg/ ml)	3 – 21	10 - 70	10 - 70	10 – 70
Molar absorptivity (L $mol^{-1}cm^{-1}$)	740.67	117.52	66.66	672.118
Sandell's sensitivity (µg/cm^2/0.001 A.U)	1.5960	1.0017	6.1907	0.3742
Correlation coefficient (r)	0.9999	0.9968	0.9996	0.9998
Regression equation (Y=mx+c)	Y = 0.0006x +(-) 0.0001	Y = 0.0009x+ (-) 0.0035	Y = 0.0002x+ (-) 0.0001	Y = 0.0027x+ (-) 0.0016
Slope (m)	0.0006	0.0009	0.0002	0.0027
Intercept (c)	(-) 0.0001	(-) 0.0035	(-) 0.0001	(-) 0.0016
LOD (µg/ ml)	0.6176	0.1432	1.6587	0.1310
LOQ (µg/ ml)	1.8714	0.4343	5.0263	0.3969
Standard Error	0.00003	0.00002	0.00002	0.00003

Table 2 Analysis of synthetic mixtures

Drug	Concentration prepared (µg/ ml)	Amount Found (µg/ ml)*	Percentage Purity	Average (%)	S.D	RSD	S.E
ATR	4 8 12 16 20	3.92 8.07 12.06 15.89 20.04	98.00 100.87 100.50 99.31 100.20	99.78	1.1481	1.1506	0.0459
ASP	30 40 50 60 70	30.11 39.60 49.30 58.90 69.74	100.36 99.00 98.60 98.16 99.62	99.15	0.8646	0.8720	0.0346
RAM	30 40 50 60 70	30.16 39.77 50.42 60.20 69.46	100.53 99. 42 100.84 100.33 99.22	100.07	0.7101	0.7096	0.0284
MET	30 40 50 60 70	30.07 40.37 50.43 60.73 70.24	100.23 100.92 100.86 101.21 100.34	100.71	0.4135	0.4106	0.0165

(* Mean of six observations)

Table 3 Quantification of Formulation (ZYCAD-4)

Drug	Label Claim (mg/tab)	Amount Found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	RSD	S.E
ATR	10	10.1849	101.84	102.76	1.4487	1.4097	0.0402
	10	10.1747	101.74				
	10	10.4575	104.57				
	10	10.4692	104.69				
	10	10.1911	101.91				
	10	10.1826	101.82				
ASP	75	73.9410	98.58	99.00	1.5795	1.5954	0.0438
	75	72.3707	96.49				
	75	74.2635	99.01				
	75	75.8524	101.11				
	75	73.9855	98.64				
	75	75.1467	100.19				
RAM	5	5.0974	101.94	99.95	1.7250	1.7257	0.0479
	5	4.9926	99.85				
	5	4.9648	99.29				
	5	4.8815	97.63				
	5	5.1005	102.01				
	5	4.9515	99.03				
MET	50	49.2274	98.45	98.66	0.8496	0.8612	0.0236
	50	49.0784	98.15				
	50	49.0568	98.11				
	50	49.0374	98.07				
	50	49.4569	98.91				
	50	50.1394	100.27				

Table 4 Intraday and Inter Day Analysis of Formulation (ZYCAD-4)

Drug	Amount labeled (mg/tab)	Percentage Obtained*		S.D		%RSD	
		Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
ATR	10	102.02	101.82	0.1557	0.0693	0.1528	0.0680
	10	101.71	101.94				
	10	101.89	101.94				
MEAN		101.87	101.90				
ASP	75	98.75	98.99	0.8048	0.3124	0.8116	0.3145
	75	100.08	99.55				
	75	98.63	99.51				
MEAN		99.15	99.35				
RAM	5	101.61	102.52	1.9222	1.1748	1.9063	1.1612
	5	102.26	100.38				
	5	98.65	100.61				
MEAN		100.84	101.17				
MET	50	98.62	98.58	1.7119	1.0391	1.7204	1.0513
	50	98.42	99.98				
	50	101.48	97.95				
MEAN		99.51	98.83				

Table 5 Ruggedness Study

Drug	Condition	% Obtained	S.D	%RSD	S.E
ATR	Analyst 1	102.73	1.4218	1.3842	0.0394
	Analyst 2	101.94	0.2943	0.2887	0.0081
	Instrument 1	102.05	0.1635	0.1602	0.0045
	Instrument 2	99.96	1.5147	1.5153	0.0420
ASP	Analyst 1	99.00	1.5795	1.5954	0.0438
	Analyst 2	99.53	1.8311	1.8396	0.0509
	Instrument 1	99.40	0.6644	0.6684	0.0184
	Instrument 2	99.01	0.4419	0.4463	0.0128
RAM	Analyst 1	99.95	1.7249	1.7256	0.4791
	Analyst 2	99.99	1.8625	1.8602	0.0517
	Instrument 1	101.11	2.0040	1.9820	0.0557
	Instrument 2	99.80	1.0762	1.0782	0.0299
MET	Analyst 1	98.66	0.8496	0.8611	0.0236
	Analyst 2	99.07	0.8289	0.8367	0.0230
	Instrument 1	98.97	1.2756	1.2887	0.0354
	Instrument 2	99.03	0.8143	0.8223	0.0226

(* Mean of three observations)

Table 6 Recovery Analysis of Formulation (ZYCAD-4)

Drug	Amount Present (µg/tab)	Amount Added (µg/tab)*	Amount Estimated (µg/tab)*	Amount Recovered (µg/tab)*	Percentage Recovered*	S.D	%RSD	S.E
ATR	4.1333 4.1333 4.1333	2.4799 3.7199 4.9599	6.6244 7.8615 9.0816	2.4911 3.7282 4.9483	100.45 100.22 99.76	0.3513	0.3508	0.0390
				MEAN	100.40			
ASP	29.8667 29.8667 29.8667	23.8933 29.8667 35.8400	53.8515 60.0584 65.9412	23.9848 30.1917 36.0745	100.38 101.08 100.65	0.3530	0.3506	0.0392
				MEAN	100.70			
RAM	20.1010 20.1010 20.1010	12.0606 18.0909 24.1212	31.9905 38.4895 44.3816	11.8895 18.3885 24.2806	98.58 101.64 100.66	1.5626	1.5580	0.1736
				MEAN	100.29			
MET	19.8492 19.8492 19.8492	11.9095 17.8642 23.8190	31.5875 37.4166 44.0712	11.7383 17.5674 24.2220	98.56 98.33 101.69	1.8770	1.8860	0.2086
				MEAN	99.52			

Table 7 Optical Characteristics of Metoprolol Tartrate, Ramipril, Atorvastatin Calcium, Aspirin (HPTLC Method)

PARAMETERS	METOPROLOL TARTRATE	RAMIPRIL	ATORVASTATIN CALCIUM	ASPIRIN
Detection Wavelength	224 nm	224 nm	224 nm	224 nm
Beers law limit (ng/ µl)	100 - 600	10 - 60	20 to 120	75 to 450
Correlation coefficient (r)	0.9999	0.9998	0.9998	0.9998
Regression equation (Y=mx+c)	Y = 2.2297x+ 0.9164	Y = 19.043x+ 6.272	Y = 31.775x+ 5.6914	Y = 18.561x+ (-)30.8631
Slope (m)	2.2297	19.043	31.7751	18.5616
Intercept (c)	0.9167	6.272	5.6917	(-) 30.8631
LOD (ng/ µl)	0.7632	0.1025	0.1272	0.2955
LOQ (ng/ µl)	2.3128	0.3105	0.3855	0.8954
Standard Error	0.4449	0.1427	0.1471	0.0821

(*Mean of three observations)

Table 8 Quantification of Formulation (ZYCAD-4) By HPTLC

Drug	Label Claim (mg/tab)	Amount Found (mg/tab)*	Percentage Obtained	Average (%)*	S.D	RSD	S.E
MET	50	49.4905	98.98	100.24	0.8348	0.8328	0.0232
	50	49.8501	99.70				
	50	50.2296	100.45				
	50	50.0948	100.90				
	50	50.4543	100.90				
	50	50.6541	101.30				
RAM	5	4.9540	99.08	99.93	0.5910	0.5914	0.0164
	5	5.0089	100.07				
	5	5.0289	100.57				
	5	4.9840	99.68				
	5	4.9790	99.58				
	5	5.0289	100.57				
ATR	10	9.8481	98.48	99.00	0.6526	0.6593	0.0181
	10	9.8331	98.33				
	10	9.8831	98.83				
	10	9.9680	99.68				
	10	9.8731	98.73				
	10	9.9929	99.92				
ASP	75	74.8950	99.86	99.97	0.0829	0.0830	0.0023
	75	74.9948	99.99				
	75	74.9599	99.94				
	75	75.0797	100.10				
	75	74.9449	99.92				
	75	75.0498	100.01				

Table 9 Intraday and Inter Day Analysis of Formulation

Drug	Amount labeled (mg/tab)	Percentage Obtained*		S.D		%RSD	
		Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
MET	50	99.24	101.16	0.7643	1.2842	0.7639	1.2832
	50	100.14	98.66				
	50	100.76	100.42				
MEAN		100.04	100.08				
RAM	5	100.80	100.80	0.7211	1.1015	0.7197	1.1052
	5	100.40	98.60				
	5	99.40	99.60				
MEAN		100.20	99.66				
ATR	10	98.90	102.70	0.1527	2.0113	0.1547	2.0167
	10	98.70	98.50				
	10	98.60	99.40				
MEAN		98.73	100.20				
ASP	75	99.96	100.18	0.3150	0.1400	0.3158	0.1399
	75	99.93	99.90				
	75	100.04	100.04				
MEAN		99.76	100.04				

(*Mean of six observations)

Table 10 Recovery Analysis of ZYCAD-4 BY HPTLC

Drug	Amount Present (µg/ ml)	Amount Added (µg/ ml)*	Amount Estimated (µg/ ml)*	Amount Recovered (µg/ ml)*	Percentage Recovered*	S.D	%RSD	S.E
MET	100.3783	80.3026	180.6733	80.2950	99.99	0.5064	0.5087	0.0563
	100.3783	100.3786	199.7600	99.3817	99.00			
	100.3783	120.4539	220.4533	120.0750	99.68			
				MEAN	99.55			
RAM	10.0066	8.0052	18.1100	8.1034	101.26	0.7019	0.6978	0.0780
	10.0066	10.0066	20.0000	9.9934	99.86			
	10.0066	12.0079	22.0933	12.0867	100.65			
				MEAN	100.59			
ATR	19.8233	15.8586	35.5285	15.7052	98.03	1.6536	1.6383	0.1837
	19.8233	19.8233	40.0052	20.1819	101.80			
	19.8233	23.7879	44.2833	24.2600	101.98			
				MEAN	100.60			
ASP	150.1550	120.4400	268.8700	118.5150	98.40	0.1762	0.1787	0.0196
	150.1550	150.1550	298.3766	148.2216	98.71			
	150.1550	180.1860	328.0066	177.8516	98.70			
				MEAN	98.60			

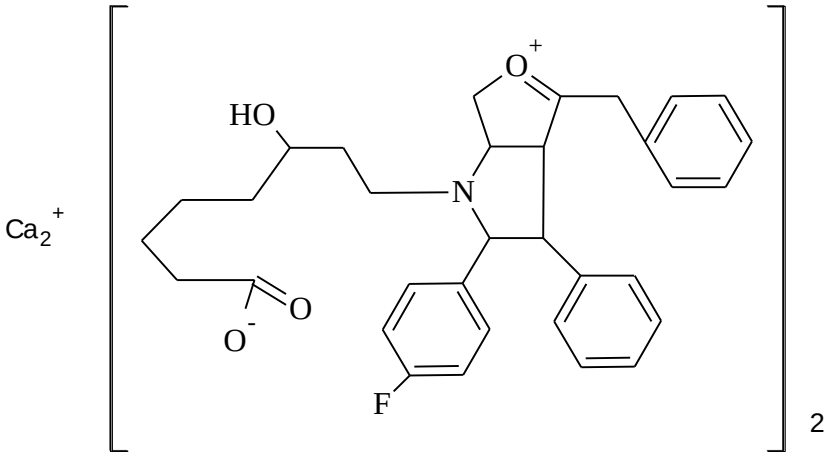


Figure 1 Atorvastatin Calcium structure

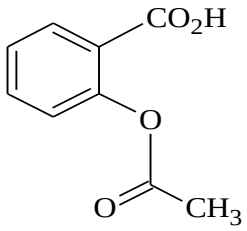


Figure 2 Aspirin structure

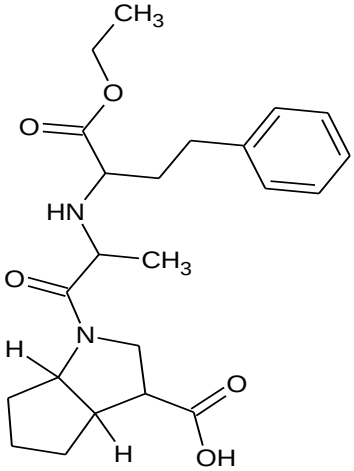


Figure 3 Ramipril structure

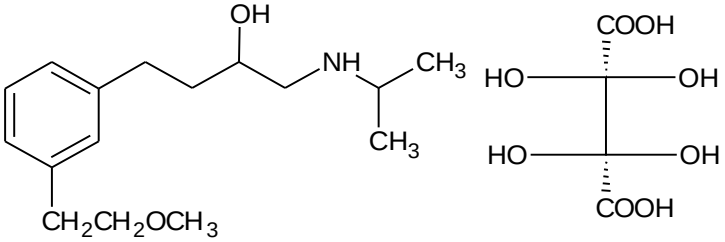


Figure 4 Metoprolol Tartrate structure

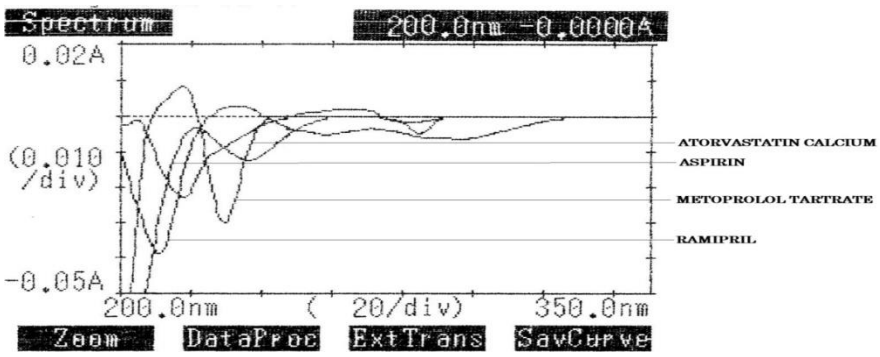
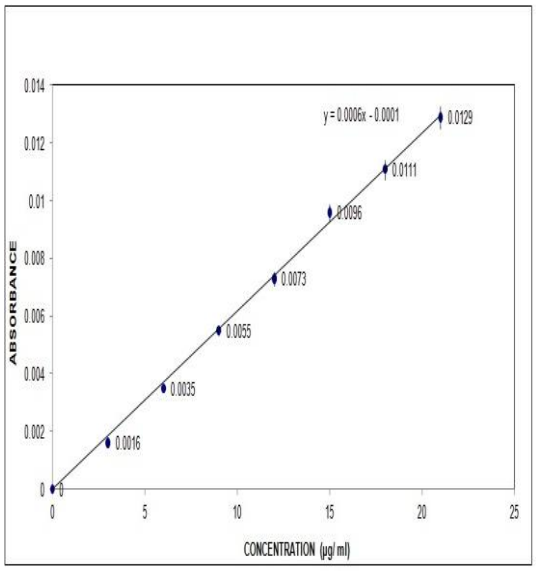
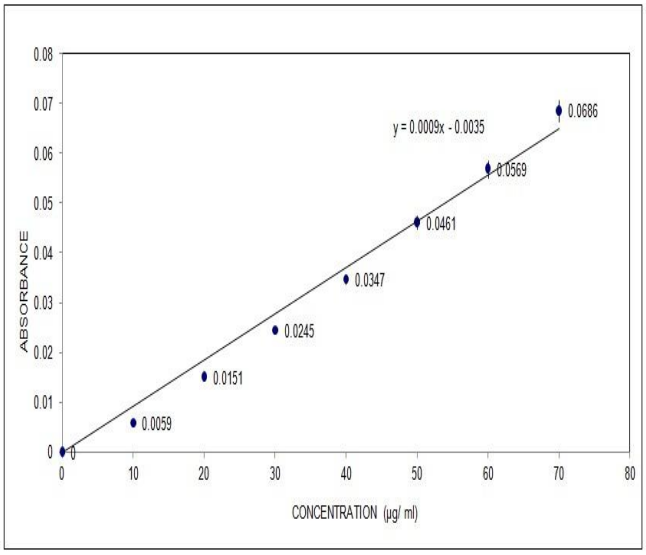
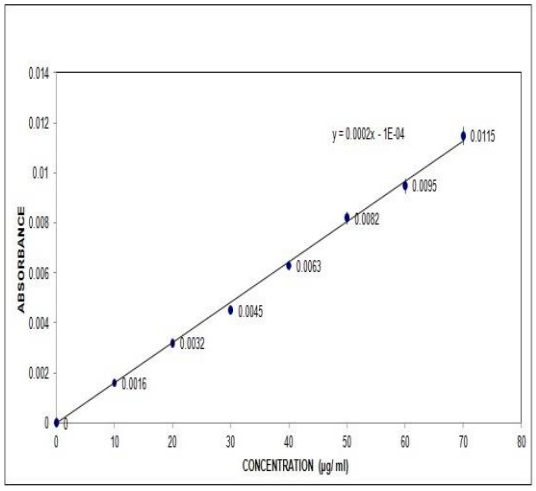


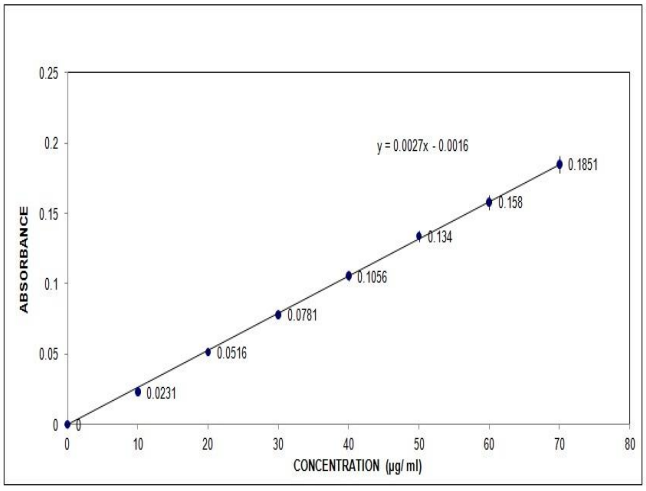
Figure 5 UV spectrum of Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin (Detection Wavelength)





CALIBRATION GRAPH FOR ATORVASTATIN **CALIBRATION GRAPH OF ASPIRIN**





CALIBRATION GRAPH OF RAMIPRIL **CALIBRATION GRAPH OF METOPROLOL TARTRATE**

Figure 6 Calibration curve for Atorvastatin (291.5nm), Aspirin (247nm), Ramipril (242.5nm), Metoprolol Tartrate (229.5nm).

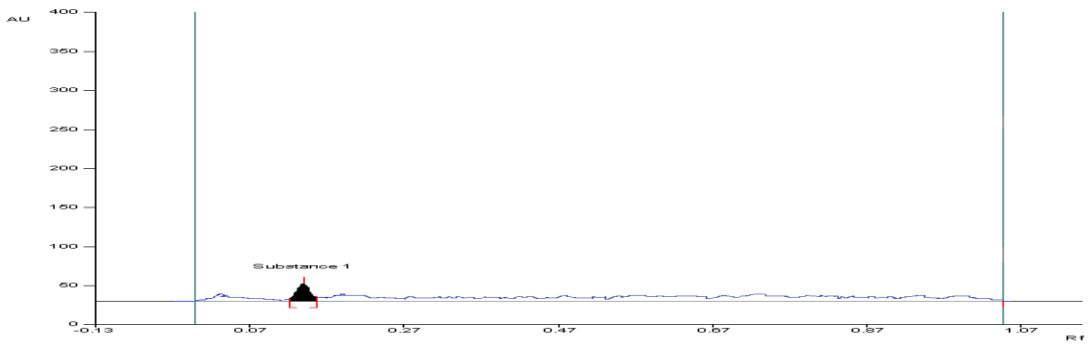


Figure 7 CHROMATOGRAM FOR METOPROLOL TARTRATE BY HPTLC (100 ng/ µl)

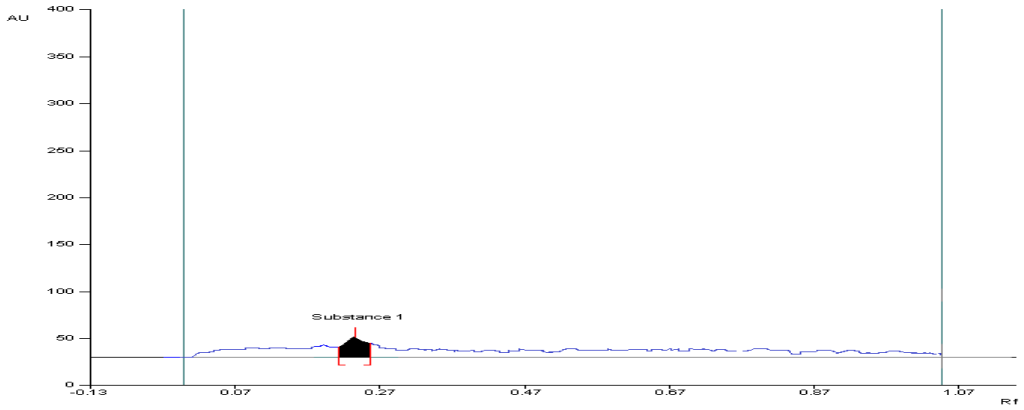


Figure 8. Chromatogram for Ramipril by HPTLC 10ng/l

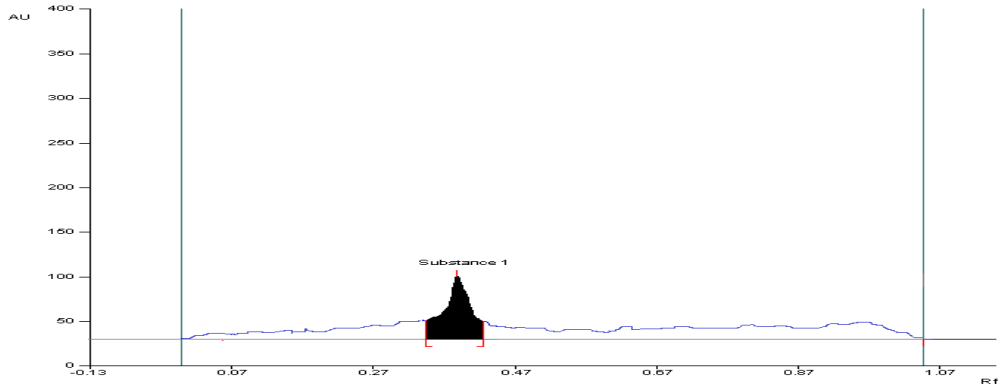


Figure 9. Chromatogram for Atorvastatin Calcium by HPTLC (20 ng/ µl)

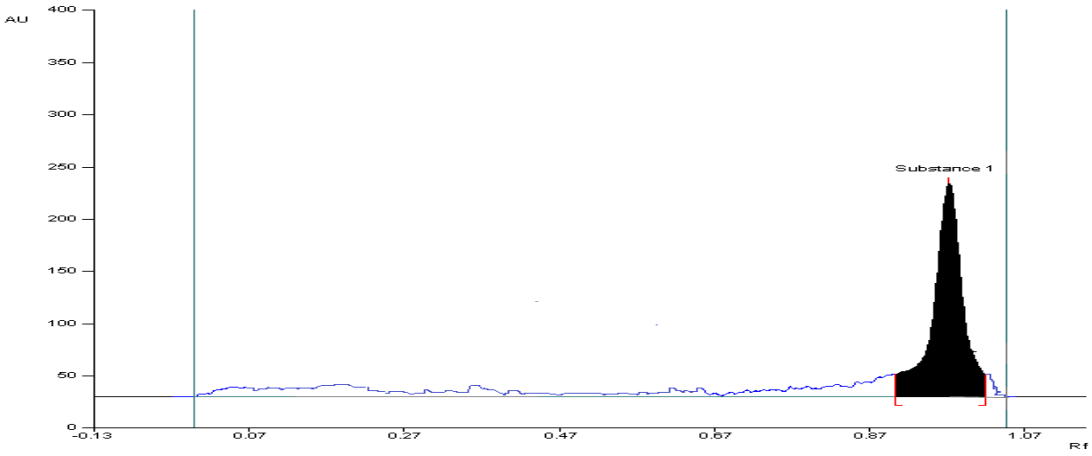


Figure 10. Chromatogram for Aspirin by HPTLC (75 ng/ µl)

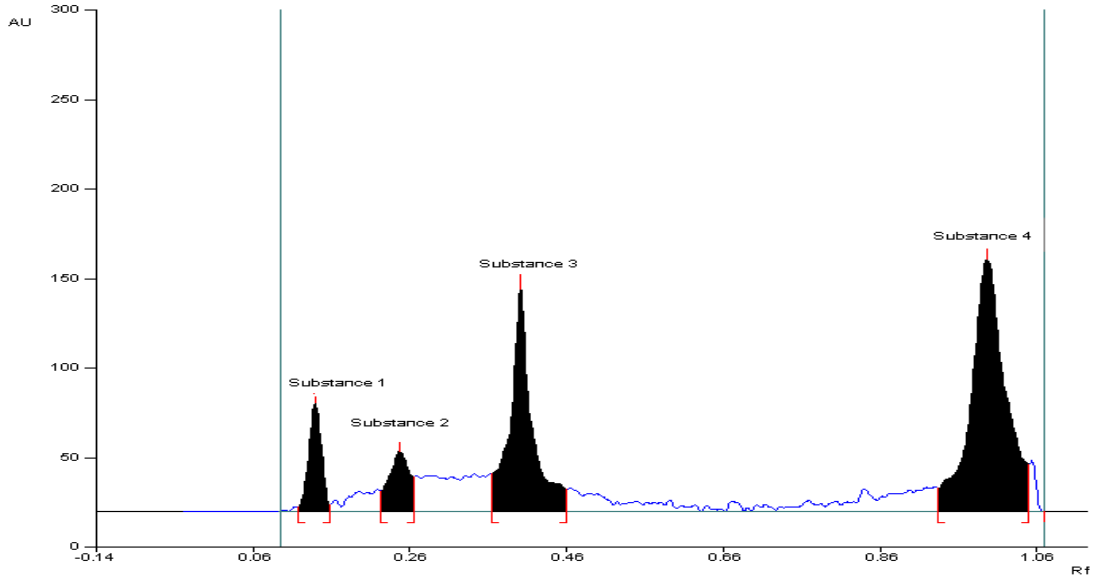
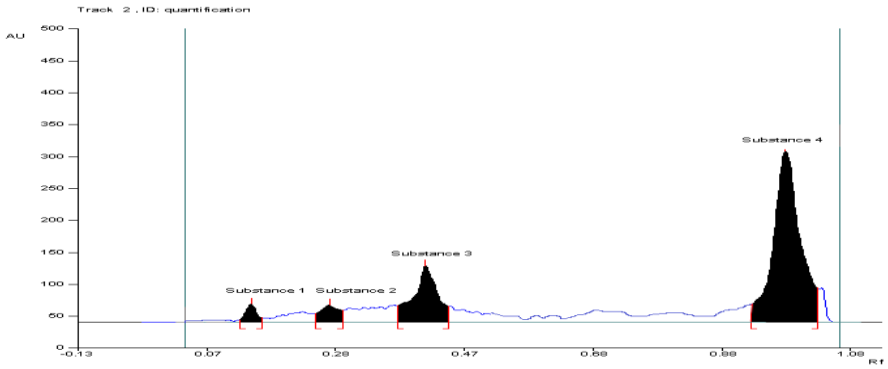


Figure 11 Linearity Chromatogram for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin By HPTLC

Figure 12



CHROMATAGRAM FOR FORMULATION ANALYSIS BY HPTLC REPEATABILITY 1

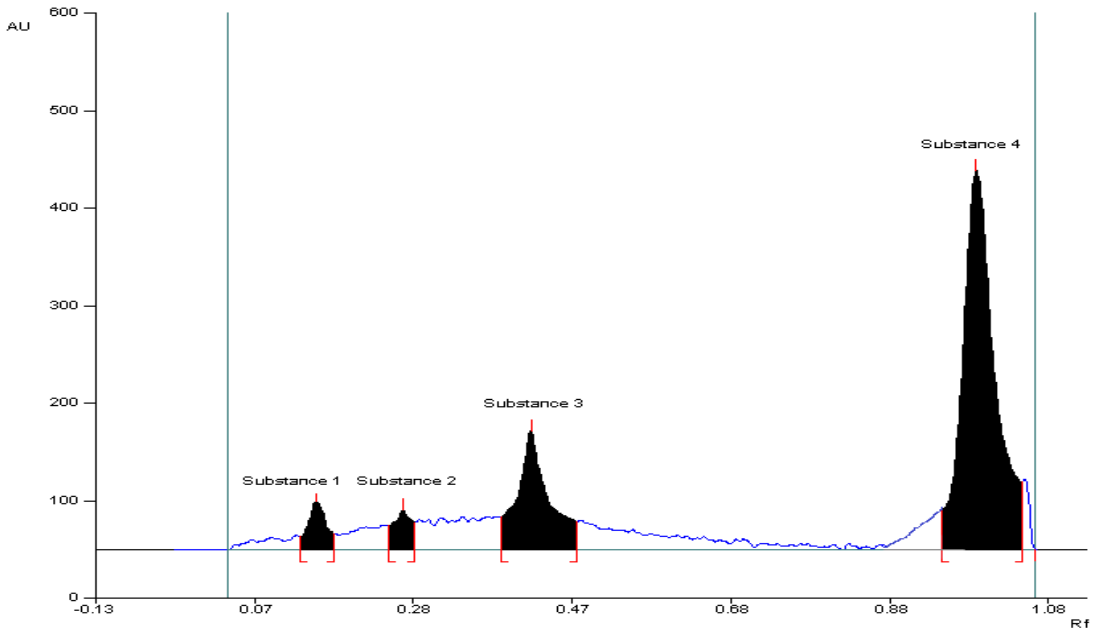


Figure 13 Chromatogram for the recovery analysis of formulation recovery

The percentage purity of Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin were found to be 100.24 ± 0.8348, 99.93 ± 0.5910, 99.00 ± 0 .6526 and 99.97 ± 0.0829, respectively. The results of analysis are shown in table 8. The confidence interval for drugs was found to be in the range of 99.36 – 101.11, 99.30 – 100.55, 98.31 – 99.68 and 99.88 – 100.05 for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively.

Precision: Precision of the method was confirmed by repeated analysis of formulation for six times. The percentage RSD values were found to be 0.8328, 0.5914, 0.6593 and 0.0830 for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively. Further the precision of the method was confirmed by intraday and inter day analysis. Intraday and inter day analysis of formulation was done on three times on same day and one time on three consecutive days. The percentage RSD values for the intraday and inter day precision was found to be 0.7639 and 1.2832 for Metoprolol Tartrate, 0.7197 and 1.1052 for Ramipril, 0.1547 and 2.0167 for Atorvastatin Calcium and 0.3158 and 0.1399 for Aspirin. The results of analysis are shown in table 9 and figure 12.. The confidence interval of intraday analysis were found to be in the range of 98.14 – 101.93, 98.40 – 101.99, 98.35 – 99.10 and 98.97 100.54 for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively. The confidence interval of inter day analysis were found to be in the range of 96.88 – 101.27, 96.92 – 102.39, 95.20 – 105.19 and 99.69 – 100.38 for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively.

Accuracy: The accuracy of the method was confirmed by the recovery studies. To the pre-analyzed formulation, a known quantity of raw material was added and the percentage recovery was calculated. The percentage of raw material added was 80%, 100% and 120% for all four drugs. The chromatograms for the recovery analysis are shown in figures 13 The percentage recovery was found to be in the range of 99.00 - 99.99%, for Metoprolol Tartrate

99.86 - 101.26% for Ramipril, 98.03 - 101.98 for Atorvastatin calcium and 98.40 - 98.71 for Aspirin. The percentage RSD values were found to be 0.5087, 0.6978, 1.6383 and 0.1787 for Metoprolol Tartrate, Ramipril, Atorvastatin calcium and Aspirin, respectively. The low percentage RSD value indicates that there was no interference due to the excipients used in formulation during the analysis. The data of recovery analysis are listed in table 10. The confidence interval for drugs was found to be in the range of 98.29 – 100.80, 98.84 – 102.33, 96.49 – 104.70 and 98.16 – 99.03 for Metoprolol Tartrate, Ramipril, Atorvastatin Calcium and Aspirin, respectively.

CONCLUSION:

A simple, rapid and reliable UV and HPTLC method has been developed and successfully validated for estimation of Atorvastatin calcium, Aspirin, Ramipril and Metoprolol tartarate in bulk and pharmaceutical dosage form. The results of the validation tests indicated that the method was accurate, precise, robust, and stability indicating. The proposed UV and HPTLC method is suitable for routine analysis in pharmaceutical formulation in quality control laboratories, where economy and time are essential.

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