Development and application of Liquid Chromatographic method for determination of Cerivastatin in bulk and in tablet dosage forms

Dr.L.Satyanarayana*, Sumalatha. Reddi
Department Of Pharmaceutical Analysis,
Omega College Of Pharmacy, Ghatkesar, Hyderabad, T.S.500034

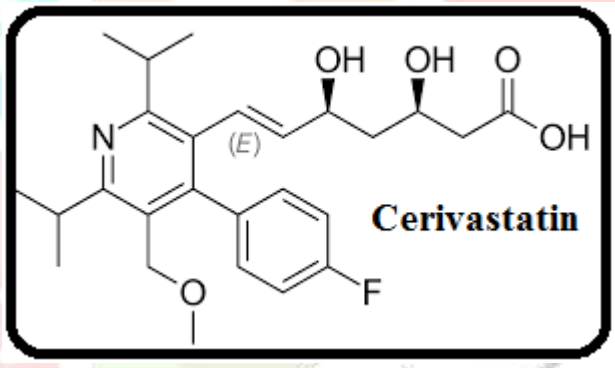
Abstract: Cerivastatin, a competitive HMG-CoA reductase inhibitor effective in lowering LDL cholesterol and triglycerides, is used to treat primary hypercholesterolemia and mixed dyslipidemia. Used as an adjunct to diet for the reduction of elevated total and LDL cholesterol levels in patients with primary hypercholesterolemia and mixed dyslipidemia (Fredrickson Types IIa and IIb) when the response to dietary restriction of saturated fat and cholesterol and other non-pharmacological measures alone has been inadequate. Developing a accurate and precise analytical method for the estimation of Cerivastatin in a tablet dosage form is very challenging, due to the formation of drug-drug and drug-excipient interactions. The present study demonstrates the applicability of chromatographic method to develop a new, sensitive, single HPLC method for the quantitative determination of antifungal agents in freeze dried powder for injection pharmaceutical dosage form. Chromatographic separation active pharmaceutical ingredient was achieved by using a isocratic elution at a flow rate of 1.0 mL/min on X-Terra RP-18 column (250mm×4.6 mm, 5µm particle size, 100Å pore size) at ambient temperature. The contents of the mobile phase were 3.48 gms of Di Potassium hydrogen *ortho*-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 3.2 with dilute *ortho*-phosphoric acid (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in a isocratic mode in the ratio of 40: 60 (v/v) of separation was used to resolute the Cerivastatin. UV detection at 254 nm was employed to monitor the analytes. A linear response was observed for Cerivastatin over the concentration range 2-24 µg/ mL. Limit of detection (LOD) and Limit of quantification (LOQ) for Cerivastatin were found to be 0.002µg/mL, and 0.006µg/mL respectively.

Key words: Cerivastatin, Isocratic-HPLC, Casporan®, Lyophilized powder for injection.

Introduction: Cerivastatin sodium is sodium [S-[R*,S*-(E)]]-7-[4-(4-fluorophenyl)-5-methoxymethyl)-2,6bis(1-methylethyl)-3-pyridinyl]-3,5-dihydroxy-6-heptenoate[1-2]. The empirical formula for cerivastatin sodium is $C_{26}H_{33}FNO_5Na$ and its molecular weight is 481.5. Cerivastatin sodium is a white to off-white hygroscopic amorphous powder that is soluble in water, methanol, and ethanol, and very slightly soluble in acetone[3-5]. Cerivastatin sodium is an entirely synthetic, enantiomerically pure inhibitor of 3-hydroxy-3-

methylglutaryl-coenzyme A (HMG-CoA) reductase [6]. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol [7-9]. Cerivastatin is a competitive inhibitor of HMG-CoA reductase, which is responsible for the conversion of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) to mevalonate, a precursor of sterols, including cholesterol. The inhibition of cholesterol biosynthesis by cerivastatin reduces the level of cholesterol in hepatic cells, which stimulates the synthesis of LDL receptors, thereby increasing the uptake of cellular LDL particles[10]. The end result of these biochemical processes is a reduction of the plasma cholesterol concentration. Cerivastatin sodium tablets are indicated as an adjunct to diet to reduce elevated Total-C, LDLC, apo B, and TG and to increase HDL-C levels in patients with primary hypercholesterolemia and mixed dyslipidemia (Fredrickson Types IIa and IIb) when the response to dietary restriction of saturated fat and cholesterol and other non-pharmacological measures alone has been inadequate [11-12].

Figure-1: Chemical structures of Cerivastatin



A survey of literature has revealed only one analytical method for the determination of Cerivastatin in biological fluids. These include; high-performance liquid chromatography (HPLC. On the contrary, to the best of our knowledge, there is no method reporting the determination of Cerivastatin in pharmaceutical formulation. In this paper, we report the simple precise and accurate RP-HPLC method for the assay of Cerivastatin acetate for Intravenous (IV) Infusion in sterile lyophilized powder for injection dosage form. The new method is capable of separating active ingredient present in the Intravenous (IV) Infusion. Validation of the current method will be performed according to the requirements of USP for assay determination which include accuracy, precision, selectivity, linearity and range.

Experimental:

Chemicals and reagents: Cerivastatin was obtained as kind gift sample from Grand pharma Ltd, Hyderabad. Potassium dihydrogen ortho-phosphate, acetonitrile and *ortho*-phosphoric acid were obtained from Merck,

Mumbai, India. All the solutions were prepared in Milli Q water (Millipore, USA). Test samples composed of Lipobay® 0.2 mg film coated tablets, Bayer, India contains 0.2 mg of Cerivastatin, is obtained from local market.

HPLC Instrumentation and Chromatographic conditions: Quantitative HPLC was performed on the Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing. Waters Alliance 2695 separation module (Waters Corporation, Milford, USA) equipped with 2489 UV/visible detector or 2998 PDA detector with Empower 2 software was used for the analysis. Flow rates from 50 uL/ min to 5 mL/min can be generated for use with 2.1 mm ID columns and larger. The auto-sampler has a maximum capacity of 120 vials (12x32, 2-mL) with programmable temperature control from 4 to 40°C. A heated column compartment provides temperatures from 5 degrees above ambient to 65°C. The detector is a photodiode array (model 2996) with a wavelength range of 190-800 nm and sensitivity settings from 0.0001-2.0000 absorbance units The HPLC system was equipped with a column compartment with temperature control and an on-line degasser. X-Terra RP-C18 Column (250x4.6 mm i.d; particle size 5 µm) was used for separation of Cerivastatin. The contents of the mobile phase were 3.48 gms of Di Potassium hydrogen *ortho*-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 3.2 with dilute *ortho*-phosphoric acid (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in a isocratic mode in the ratio of 40: 60 (v/v) of separation was used to resolute the Cerivastatin. They were filtered before use through a 0.45 µm membrane filter and degassed by sonication. The flow was adjusted at 1.0 ml/min flow rate and 20 µL injection load volumes were maintained. The eluted compounds were monitored at 254 nm. The column oven temperature was maintained at 25 ◦C. Data acquisition, analysis, and reporting were performed by Empower2 (Waters) chromatography software.

Preparation of Solutions:

Standard and stock solutions: Standard solution of the active pharmaceutical ingredient was prepared in the following manner: Transfer 20 mg of Cerivastatin working standard into a 100 ml volumetric flask, dissolve and dilute with Acetonitrile and water in the ratio of 50:50 v/v as diluent. 5 ml of the resulting solution is further diluted up to 50 ml in volumetric flask with diluents. The resulting solution contains 20 µg/mL of Cerivastatin as working standard solutions. The prepared stock solutions were stored at 4 ^{0}C and protected from light.

Preparation of the Sample solution: Lipobay® (cerivastatin sodium tablets) is supplied as tablets containing 0.2, 0.3, 0.4 or 0.8 mg of cerivastatin sodium, for oral administration. Active Ingredient: cerivastatin sodium. Inactive Ingredients: mannitol, magnesium stearate, sodium hydroxide, crospovidone, povidone, iron oxide yellow, methylhydroxypropylcellulose, polyethylene glycol, and titanium dioxide. 20 Lipobay® 0.2 mg film coated tablets were collected, their average weight was recorded. Then they are crushed to fine homogenous

powder, uniformly blended and a quantity equivalent to 2 mg was weighed and transferred in to a 10-mL volumetric flask, extracted in diluent by sonication, and filtered through Whatman no. 41 filter paper. The filtrate (5 mL) was quantitatively transferred to a 50-mL volumetric flask, and solution was diluted to volume with the diluents. The resulting solution contains 20 µg/mL of Cerivastatin as working sample solutions. The prepared stock solutions were stored at 4 ^{0}C and protected from light.

Solutions for validation study:

Calibration and Quality control samples: Calibration standards (2–24 µg/ mL of Cerivastatin were prepared from working standard solutions by appropriate dilution with Acetonitrile and water in the ratio of 50:50 v/v as diluents. Quality control (QC) samples for accuracy studies were prepared at three concentrations of the linearity range (16 µg/ mL, 20 µg/ mL and 24 µg/ mL) for Cerivastatin were prepared from the standard solutions.

Method Validation: The developed chromatographic method was validated for selectivity, linearity, precision, accuracy, sensitivity, robustness and system suitability.

Specificity: The terms selectivity and specificity are often used interchangeably. The specificity of the developed LC method for quantification of active pharmaceutical ingredient was determined the presence of excipients present in pharmaceutical products. In specificity study, interference between drugs and excipients usually employed in film coated tablets were evaluated from the comparison of spectral purity obtained from the analysis for the standard solutions and sample solutions.

System suitability: The system suitability was assessed by six replicate analyses of the drugs at concentrations of 20 µg/ mL for Cerivastatin. The acceptance criterion was ±2% for the RSD for the peak area and retention times for all four analytes. The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between peak and peaks of the other three analytes were defined.

Linearity: Linearity of the method was evaluated at seven equi-spaced concentration levels by diluting the standard solutions to give solutions over the ranges 10–120% target concentration for main analyte of interest. The calibration curves were constructed at seven concentrations between 2–24 µg/ mL for Cerivastatin. These were injected in triplicate and the peak areas were inputted into a Microsoft Excel® spreadsheet program to plot calibration curves. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The peak areas of the analyte to concentration of analyte were used for plotting the linearity graph. The linearity data is reported in Table-3.

Table-3: Linearity Data for Cerivastatin

Precision: Precision was evaluated in terms of intra-day repeatability and inter-day reproducibility. The intra-day repeatability was investigated using six separate sample solutions prepared, as reported above, from the

freshly reconstructed tablet formulations at 100% of the target level. Each solution was injected in triplicate and the peak areas obtained were used to calculate means and RSD% values. The inter-day reproducibility was, by preparing and analyzing in triplicate sample solutions from the reconstructed formulations at the same concentration level of intra-day repeatability; the means and RSD% values were calculated from peak areas. (Table-4)

Table-4: Intra-day and inter-day precision data for for Cerivastatin

Accuracy: The accuracy of the method was determined by measuring the recovery of the drug by the method of standard additions. Quality control (QC) samples for accuracy studies were prepared at three concentrations of the linearity range (16 µg/ mL (80% dilution), 20 µg/ mL (100% dilution) and 24 µg/ mL (120% dilution) for Cerivastatin were prepared from the standard solutions. Known amounts of 10 % dilution of drug (2 µg/mL of Cerivastatin) was added to corresponding to 80%, 100%, and 120% of the target test concentrations were added to a placebo mixture to determine whether the excipients present in the formulation led to positive or negative interferences. Each set of additions was repeated three times at each level. Extraction sample preparation procedure is followed and assayed against qualified reference standard. The accuracy was expressed as the percentage of the analytes re-covered by the assay. (Table-5)

Table-5: Accuracy: recovery data for Cerivastatin

Sensitivity: Limits of detection (LOD) and quantification (LOQ) were estimated from the signal- to-noise ratio. The detection limit was determined as the lowest concentration level resulting in a peak area of three times the baseline noise. The limit of detection was determined, by injecting progressively low concentrations of analyte of interest. The quantification limit was determined as the lowest concentration level that provided a peak area with signal-to-noise 10.

Robustness: To determine the robustness of the developed method, experimental conditions were deliberately changed and the relative standard deviation for replicate injections of Cerivastatin and the USP resolution factor between and the other two peaks were evaluated. The mobile phase flow rate was 1.0 mL/min. This was changed by ±0.2 units to 0.8 and 1.2 mL/min. The effect of stationary phase was studied by the use of LC columns from different batches at 25°C. The effect of buffer pH was studied at pH 3.0 and 3.4 (± 0.2 units). The chromatographic variations were evaluated for resolution between and the other three analytes in a system suitability solution with respect to retention time RT and % assay of drugs.

Table-6: Robustness data for Cerivastatin

Solution stability: To assess the solution stability, standard and test solutions were kept at 25°C (laboratory temperature) for 24 h. These solutions were compared with freshly prepared standard and test solutions.

RESULTS AND DISCUSSION:

HPLC method development: The API solution of analyte of interest i,e., Cerivastatin was prepared in diluent at a concentration of 20 µg/mL and scanned in UV-Visible spectrometer; and the Cerivastatin was found to have UV maxima at around 254 nm. Hence detection at 254 nm was selected for method development purpose. Some important parameters, pH of the mobile phase, concentration of the acid or buffer solution, percentage and type of the organic modifier, etc. were tested for a good chromatographic separation. The main analytical challenge during development of a new method was obtaining adequate retention of the polar compound Cerivastatin. Trials showed that acidic mobile phase with reverse phase column gives symmetric and sharp peaks. For this reason, potassium dihydrogen phosphate buffer with pH-3.0 was adjusted with *o*-phosphoric acid was preferred as acidic buffer solution. Acetonitrile and buffer in the ratio of 60:40 (v/v) were chosen as the organic modifier because it dissolves drugs very well. Mobile phase composition in isocratic mode at a flow rate of 1.0 mL per minute was observed for a good resolution. Then method was optimized to separate the active ingredient by changing to isocratic mode. The satisfactory chromatographic separation, with good peak shapes were achieved on X-Terra RP-18-C18 (250 × 4.6) mm with 5 µm particles, using the column temperature as maintained at 35°C and the detection was monitored at a wavelength of 254 nm. The injection volume was 20 µL. Acetonitrile and water in the ratio of 50:50 v/v) were used as diluent. In the optimized isocratic conditions, Cerivastatin was well separated with a resolution (Rs) of greater than 2 and the typical retention time of about 2.835 minutes, the typical chromatogram of System suitability shown in Figure 2.

Method validation:

The developed method was validated, as described below, for the following parameters: system suitability, selectivity, linearity, precision, accuracy and LOD/LOQ.

Selectivity: Selectivity of the current method was demonstrated by good separation of the active ingredients. Furthermore, matrix components, e.g. excipients, do not interfere with the four analytes as they have no absorbance. The representative chromatogram (Fig. 5) of the tablet dosage form solution containing excipients showed no peak interfering with analytes; moreover the adjacent chromatographic peak was separated with resolution factors >3. Overall, these data demonstrated that the excipients did not interfere with the active ingredients peaks, indicating selectivity of the method

System suitability: The RSD values of peak area and retention time for the analytes are within 2% indicating the suitability of the system.

Figure-2: System suitability chromatogram of working standard solution contains 20 µg/mL of Cerivastatin.

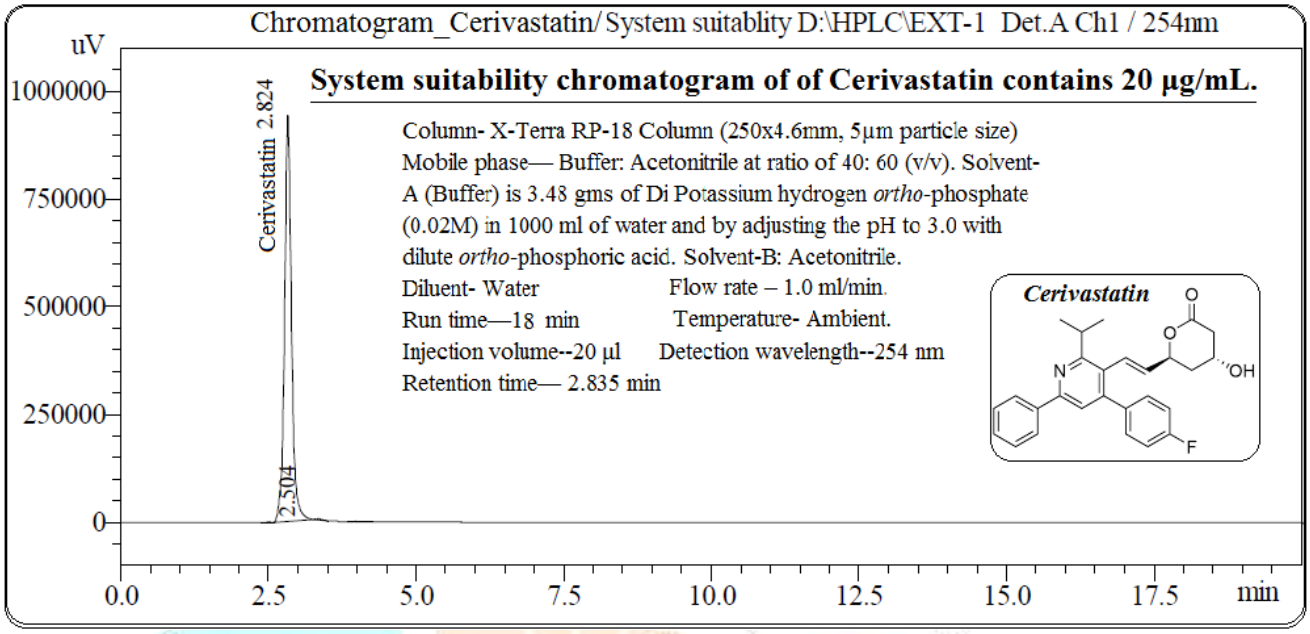


Table-2: Results of System suitability study.

Parameter	Cerivastatin
Retention time	2.824
Theoretical plates	5661.439
Tailing Factor	1.095
HETP	$4.416x10^{-5}$
USP plates/meter	22645.76
Resolution	1.686
Peak area	7746645
% of Peak area	99.683

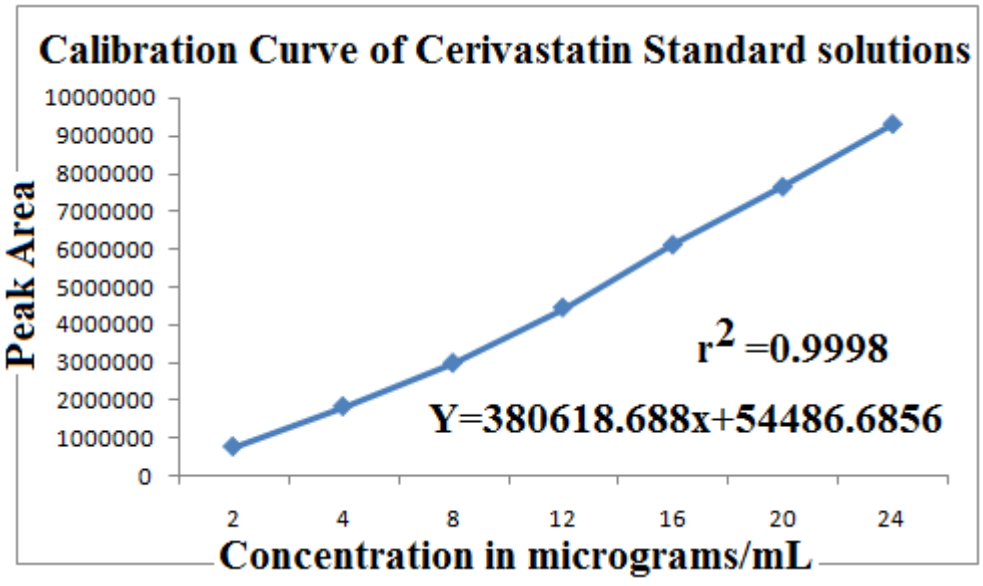
Linearity and range: Seven concentration levels within 10–120% of the target concentration range for analytes were considered to study the linearity. The calibration curves were prepared by plotting the peak area of the drug to the respective concentrations, which were linear in the range of 2–24 µg/ mL for Cerivastatin. Peak areas of the active ingredients and concentrations were subjected to least square linear regression analysis to calculate the calibration equations and correlation coefficients. The mean regression equations were found as Y=380618.688x+54486.6856 for Cerivastatin. The square of the correlation coefficient (r2 > 0.999) demonstrated a significant correlation between the concentration of analytes and detector response. The results

show that there is an excellent correlation between the peak area ratios and the concentrations of drugs in the range tested.

Table-3: Linearity data for the Lipobay®- 0.2 mg- Film coated tablets.

Concentration	Peak Area	Parameter	Cerivastatin
2 µg/ mL	770615	Concentration Range	2-24 µg/ mL
4 µg/ mL	1836560	Regression equation	Y=380618.688x+54486.6856
8 µg/ mL	2984355	Correlation Coefficient	0.999
12 µg/ mL	4452582	0.95 Confidence interval	Lower-Limit-0.993/ Upper Limit-1
16 µg/ mL	6113994	0.95 Confidence interval	Lower-Limit-0.987/ Upper Limit-1
20 µg/ mL	7646282	Limit of Detection(LOD)	0.002 µg/ mL
24 µg/ mL	9310299	Limit of Quantification(LOQ)	0.006 µg/ mL

Figure-3: Calibration Curve of Lipobay®- Film coated tablets.



Precision: Precision of this method was determined by injecting the standard solution of the three analytes six times. The R.S.D. of peak area of six replicates was found to be less than 2. The results obtained are shown in Table 4. In all instances the %RSD values were less than 2%.

Table-4: Intra-day and inter-day precision data for Cerivastatin

Precision data of Cerivastatin	**Inter-day precision**	**Intra-day precision**

Analyte-conc. (20 µg/ml)	Retention time in min.	Peak Area	Retention time in min.	Peak Area
Cerivastatin injection-1	2.819	7698622	2.812	7661994
Cerivastatin injection-2	2.818	7698577	2.810	7655525
Cerivastatin injection-3	2.817	7693136	2.810	7636160
Cerivastatin injection-4	2.813	7683915	2.810	7624722
Cerivastatin injection-5	2.813	7670573	2.809	7641083
Cerivastatin injection-6	2.812	7668883	2.809	7644532
Mean	2.815	7685618	2.810	7644002
% RSD.	0.108	0.175	0.038	0.175
Std. Deviation	0.003	13438	0.001	13405

Accuracy: Percentage recovery of the active ingredient using this method was determined using Lipobay® 0.2 mg is (cerivastatin sodium tablets) is supplied as tablets containing 0.2, 0.3, 0.4 or 0.8 mg of cerivastatin sodium, for oral administration. The results of accuracy studies from standard solution and excipient matrix were shown in Table 5; recovery values demonstrated that the method was accurate within the desired range.

Table-5: Accuracy study and recovery data for Cerivastatin

S. No	Recovery at 80% dilution Level Peak areas		Recovery at 100% dilution Level Peak areas		Recovery at 120% dilution Level Peak areas	
	Standard	Spiked	Standard	Spiked	Standard	Spiked
1	5906572	6804782	7670239	8429009	9284253	10331455
2	5937767	6808106	7669595	8424708	9287667	10304172
3	5909404	6802275	7668384	8422411	9305486	10307276
Avg	5917914	6805054	7669406	8425376	9292468.7	10314301
Std.Dev	17251	2925	942	3349	11402	14937
%RSD	0.292	0.043	0.012	0.040	0.123	0.145
% Recovery	109.2		99.5		116	

Lipobay® sterile, lyophilized product for intravenous (IV) infusion working sample solution was spiked
-at 80% level (16 µg/ml was spiked with 10% of mixed standard solution of API's(2 µg/ml)
-at 100% level (20 µg/ml was spiked with 10% of mixed standard solution of API's(2 µg/ml)
-at 120% level (24 µg/ml was spiked with 10% of mixed standard solution of API's(2 µg/ml)

Sensitivity: Limit of detection (LOD) for Cerivastatin was 0.001µg/mL and limit of quantification (LOQ) for Cerivastatin was 0.003µg/mL. The results of LOD and LOQ were indicating a high sensitivity of the method.

Robustness: The HPLC parameters were deliberately varied from normal procedural conditions including the mobile phase flow rate was 1.0 mL/min. This was changed by ±0.2 units to 0.8 and 1.2 mL/min. The effect of stationary phase was studied by the use of LC columns from different batches at 35°C. Under these variations, all analytes were adequately resolved and elution orders remained unchanged. The testing solution maintained a signal-to-noise ratio over 10 in all varied conditions. The peak resolution was all larger than 1.5 under each variation.

Table-5: Robustness study of Lipobay® 0.2 mg Film coated tablet solution at 100 % level (20 µg/mL):

Parameter	**Cerivastatin** in Flow increase study		**Cerivastatin** in Flow decrease study		**Cerivastatin** in Variable column Study	
	Run time	Peak Area	Run time	Peak Area	Run time	Peak Area
Injection-1	2.561	7171521	3.122	8676086	2.809	7767794
Injection-2	2.560	7199787	3.118	8675465	2.808	7728248
Injection-3	2.560	7193828	3.123	8674522	2.808	7755779
Mean	2.560	7188379	3.121	8675358	2.808	7750607
% RSD	0.020	0.207	0.082	0.009	0.029	0.262
Std. Dev	0.001	14900	0.003	788	0.001	20274

Analysis of the fixed dose combination tablet:

Lipobay® (cerivastatin sodium tablets) is supplied as tablets containing 0.2, 0.3, 0.4 or 0.8 mg of cerivastatin sodium, for oral administration. Active Ingredient: cerivastatin sodium. Inactive Ingredients: mannitol, magnesium stearate, sodium hydroxide, crospovidone, povidone, iron oxide yellow, methylhydroxypropylcellulose, polyethylene glycol, and titanium dioxide. 20 Lipobay® 0.2 mg film coated tablets were collected, their average weight was recorded. Then they are crushed to fine homogenous powder, uniformly blended to obtain fine tablet powder. An amount of the homogenous powder equivalent to 5 mg was transferred into a 100ml volumetric flask, added 40 ml of diluents (Acetonitrile and water in the ratio of 50:50 v/v), sonicated for 30 min, diluted to 100 ml with diluents. 50ml sample taken from this solution was centrifuged at 3000 rpm for 15 min. A 5-ml aliquot from supernatant was then decanted to another 50-ml volumetric flask. Test solutions were then made up to volume with the diluent. The filtrate (5 mL) was quantitatively transferred to a 50-mL volumetric flask, and solution was diluted to volume with the diluents.

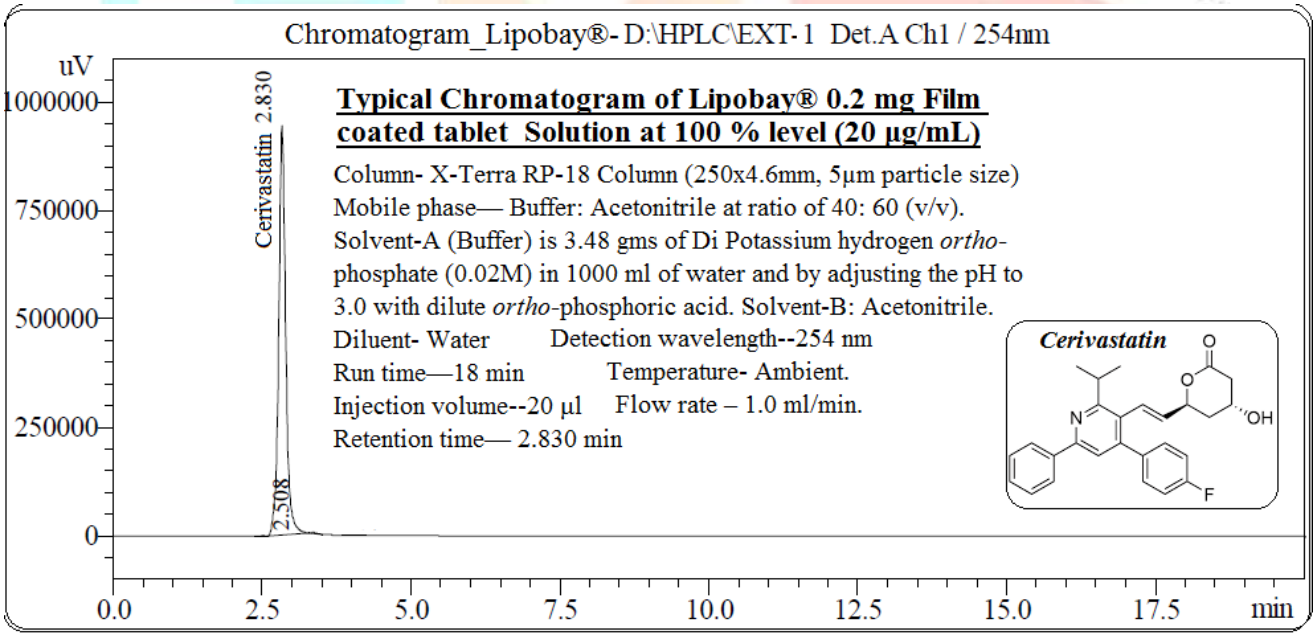
The resulting solution contains 20 µg/mL of Cerivastatin as working sample solutions. The prepared stock solutions were stored at 4 ^{0}C and protected from light. The amount of Cerivastatin in standard mixtures or dosage forms were individually calculated using the related linear regression equations.

On the basis of above results, the proposed method was applied to the determination of antifungal agent Cerivastatin present in tablet dosage forms. Figure-3 shows representative chromatograms obtained from the analysis of Lipobay® 0.2 mg (Cerivastatin sodium tablets). The differences between the amount claimed and those assayed were very low and the R.S.D. values were within the acceptable range mentioned by pharmacopoeias. The mean percentage recoveries obtained after six repeated experiments were found between 98 and 108.2 (Table 6), indicating that the results are accurate and precise and there is no interference from the common excipients used in the pharmaceutical dosage forms.

Table-6: Assay results of Lipobay®- 0.2 mg- Film coated tablets.

Formulation	Label Claim (mg/tablet)	Amount found in (mg/tablet)
Tablet	20	19.9

Figure-5: Typical Chromatogram of Lipobay®- 0.2 mg- Film coated tablets.



Conclusion: In this study, a validated simple and reliable RP-HPLC-PDA procedure was described for the assay of a Lipobay®-0 .2 mg film coated tablets that contains a Cerivastatin, which is indicated is indicated as empirical therapy for presumed fungal infections in febrile, neutropenic adult and pediatric patients. To our present knowledge, no attempts have yet been made to estimate these tablets by analytical procedure. The active pharmaceutical ingredient was successfully resolved and quantified using X-Terra RP-18 Octadecyl

column (250×4.6mm, 5µm) in a relatively short run time of 18 minutes in isocratic mode s chromatographic method. The proposed method provides a good resolution between active ingredients. The developed method reported herein was validated by parameters as described in ICH-Q2B guideline. System suitability, specificity, linearity, LOD, LOQ values, within- and between-day precision and accuracy of the proposed technique were obtained during the validation studies. The proposed method has the advantages of simplicity, repeatability, sensitivity and requires less expensive reagents.

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