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RESEARCH ARTICLE

Cramer's Rule, Tri Linear Regression and RP-HPLC Method Development for the Estimation of Combined Dosage Form of Anti Diabetic Drugs

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ABSTRACT

In this study two simple, rapid, precise and accurate spectrophotometric methods and one RP – HPLC method were developed and validated for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in pure form and in tablet dosage form. Different aliquots of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol were prepared in the concentration range of 4 – 24, 3 – 15 and 1 - 5 µg/ml respectively. The percentage label claim present in tablet formulation was found to be 98.46 ± 1.2781, 102.29 ± 1.1598 and 99.67 ± 1.1832 % for Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The accuracy of the method was confirmed by recovery studies. The % RSD value, percentage recovery for Atorvastatin calcium, Metformin hydrochloride and Glimepiride were calculated.

KEYWORDS

Atorvastatin Calcium, Metformin Hydrochloride, Glimepiride, Percentage Recovery Studies, Regression, Chromatography

INTRODUCTION

Analytical chemistry is the science of making quantitative measurements. The techniques of this science are used to identify the substance which may be present in a material and to determine the exact amounts of the identical substance. To meet these needs, analytical chemistry usually emphasizes equilibrium, spectroscopic and electrochemical analysis, separations, and statistics. Analytical technique is a phenomenon that has proved useful for providing information on the composition of sample e.g.: IR, NMR. Analytical method is a specific application of a technique to solve an analytical problem. Separation by RP – HPLC is similar to the extraction of different compounds from water into an organic solvent, where more hydrophobic (non - polar) compounds extract into the non – polar phase. Most of the pharmaceutical companies are manufacturing multiple drug formulations to meet the market demand and patient compatibility. Multiple drug formulations have well pharmacological action and fewer contraindications. Very few methods are available for estimation of multiple drug formulations by simultaneous method. Estimation of multiple drug formulations have advantage that the methods are less time consuming and usage of solvent is minimized. UV and HPLC grade of solvents used for respective determinations and the solvent should be readily available and economical. The solvent should be completely extracting the active ingredient from formulation.

Atorvastatin calcium, Metformin hydrochloride and Glimepiride are drugs used for the treatment

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of hyperlipidemia and hyperglycemia in combination. But, several methods were reported for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride individually as well as in combination with some other drugs. But no methods were reported for the estimation of these drugs in combined dosage forms, without prior separation. The non-availability of any UV-spectrophotometry and RP-HPLC methods until now for simultaneous analysis of the combination made it a worth-while objective to pursue the present work. Hence in this present work, our aim is to develop a simple, precise and accurate methods for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in bulk and in combined pharmaceutical dosage form.

MATERIAL AND METHODS

Materials

Atorvastatin calcium and Glimepiride were obtained as a souvenir samples from Dr. Reddy's Laboratories Ltd., Hyderabad and Metformin hydrochloride was obtained from Glenmark Pharmaceuticals., Nasik.

Formulation Used

CD pro2 tablets (Nicholas Piramal Healthcare Ltd., Mumbai.). Each tablet containing 10 mg of Atorvastatin calcium, 2 mg of Glimepiride and 500 mg of Metformin hydrochloride, was procured from Apollo Pharmacy, Chennai.

Chemicals and Solvents Used

Distilled water, Methanol (AR grade), Methanol (HPLC grade), Water (HPLC grade), Acetonitrile (HPLC grade) were purchased from Qualigens India Pvt. Limited and Loba Chemie India Limited. Double distilled water was prepared and used.

Specifications of Instruments

Shimadzu AUX- 220 Digital Balance (Shimadzu Instruction Manual)

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

Double Beam UV- Visible Spectrophotometer (Shimadzu Instruction Manual)

Model: Shimadzu, UV- 1700; Cuvettes: 1 cm quartz cells.

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment mechanism
Monochromator	Aberration- correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10g/l) 0.04% or less (340 nm: $NaNo_2$ 50g/l)
Measurement wavelength range	190~ 1100 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism
Recording range	Absorbance: -3.99 ~ 3.99 Abs Transmittance: 0 - 100%
Photometric	± 0.004 Abs (at 1.0 Abs), ±

accuracy	0.002 Abs (at 0.5 Abs)
Operating Temperature/ Humidity	Temperature range: 15 to 35° C Humidity range: 35 to 80% (15 to below 30° C) 35 to 70% (30 to below 35° C)

Shimadzu HPLC (Shimadzu Instruction Manual)

Detector Specifications	
Light source	Deuterium arc lamp
Wavelength range	190 to 700 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20 µl
Operating temperature range	4 to 40°C (39 to 104°F)
Recording range	0.0001 to 4.000 AUFS
Operating Temperature/ Humidity	4 to35°C/ 75 %
Pump Specifications	
Pump type	Double reciprocating plunger pump
Pumping methods	Constant flow delivery and constant pressure delivery
Suction filter	45 µm
Line filter	5 µm mesh
Operating temperature	4 to 40°C

Methods

In the present work an attempt was made to develop and validate simple, precise and accurate methods for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in pure form and in combined tablet dosage form by UV spectrophotometry and RP – HPLC.

1. UV Spectrophotometry

a) Cramer's Rule Solution Method (CRS)

b) Tri-linear Regression Calibration Method (TLRC)

2. RP – HPLC method

Cramer's Rule Solution (Crs) Method

In pharmaceutical science the quantitation of those compounds in the mixture has a big importance and there are several techniques available for the quantitation of those compounds were in use. Advances in spectrophotometric methods with various mathematical algorithms allow a wide application of UV absorption in the drug analysis. In the presence of closely overlapping spectra, the quantitative multi-resolution of ternary mixtures of three active compounds Atorvastatin calcium, Metformin hydrochloride and Glimepiride in tablets, without using pre-treatment such as separation step and graphical procedure of spectra can be determined by this method. The data which are required for the construction of Cramer's solution method are,

Selection of Solvent

The solubility of Atorvastatin calcium, Metformin hydrochloride and Glimepiride was determined in a variety of solvents as per I.P standards. Solubility tests were carried out in polar and non polar solvents. The common

solvent to solubilise all the three drugs were found to be methanol and the same is used for the analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride through the proposed method.

Preparation of Standard Stock Solution

25 mg of Atorvastatin calcium and Glimepiride raw materials were weighed and transferred into 25 ml standard volumetric flasks separately and 25 mg of Metformin hydrochloride was transferred into 50 ml standard volumetric flask and dissolved in methanol and made up to the volume with methanol. These solutions were observed to contain 1000 µg/ml of Atorvastatin calcium, Glimepiride and 500 µg/ml of Metformin hydrochloride.

Preparation of Working Standard Solution

From the standard stock solutions 2, 1 and 3ml of Atorvastatin calcium, Glimepiride and Metformin hydrochloride was transferred in to three separate 50 ml standard flasks and the solutions were made up to the mark with methanol. The final solution contains 40, 10 and 30 µg/ml of Atorvastatin calcium, Glimepiride and Metformin hydrochloride respectively.

Selection of Wavelengths for Estimation and Stability Studies

For the selection of wavelengths for estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, a suitable diluted stock solution containing 10 µg/ml of each and the solutions were scanned between 200 and 400 nm by using methanol as blank. From the overlain spectra, the spectral characteristics of Atorvastatin calcium, Metformin hydrochloride and Glimepiride were observed. The wavelengths selected were 228, 236 and 246 nm which is the λ max of Glimepiride, Metformin hydrochloride and Atorvastatin calcium, respectively. Hence, for the analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, the respective λ max of the drugs were selected. The stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol were stable for more than 3 hours at all the selected wavelengths.

Preparation of Calibration Graph

Different aliquots of 1-6ml of 40µg/ml solution of Atorvastatin calcium and 1-5ml of 30µg/ml solution of Metformin hydrochloride and 10 µg/ml solution of Glimepiride was transferred into 10 ml volumetric flasks separately and made up to the volume with methanol. 1-5 ml of 30 µg/ml solution was transferred into 10 ml volumetric flask separately and made up to the volume with methanol. 1-5 ml of 10 µg/ml solutions was transferred into 10 ml volumetric flask separately and made up to the mark with methanol. The absorbance of different concentration solutions were measured at three selected wavelengths. The calibration curve was plotted against concentration Vs absorbance. Atorvastatin calcium, Metformin hydrochloride, Glimepiride was found to be linear in the concentration range of 4-24, 3-15 and 1 – 5 µg/ml at all the three wavelengths.

Quantification of Formulation (By Standard Addition Method)

Quantification Procedure

Twenty tablets of formulation (**CD pro2** each tablet containing 10 mg of Atorvastatin calcium, 500mg of Metformin hydrochloride, and 2 mg of Glimepiride) were weighed accurately. The average weight of the tablets were found and powdered. The tablet powder equivalent to 25 mg of Metformin hydrochloride was weighed and transferred into 50 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and added 5 ml of standard addition solutions of Atorvastatin calcium and Glimepiride in to the same flask and made up to the volume with the methanol. The solution was sonicated for 15 minutes, centrifuged for 15 minutes at 3000 rpm and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 5 ml to 25 ml with methanol to obtain 100 µg/ml solution of Metformin hydrochloride. Furthermore, 1 ml of solution was transferred in to 10 ml standard volumetric flask

and diluted to mark obtain 10 µg/ml of Metformin hydrochloride, 8.2 µg/ml of Atorvastatin calcium and 2.04 µg/ml of Glimepiride theoretically. The absorbance measurements were made on 6 replicates for the formulation at 228, 236 and 246 nm. By using absorptivity values of three drugs at three wavelengths, the amount of Atorvastatin calcium, Metformin hydrochloride and Glimepiride could be determined by using Cramer's rule solution method.

Recovery Studies

The recovery experiment was done by adding known concentrations of Atorvastatin calcium, Metformin hydrochloride and Glimepiride solution to the pre-analyzed formulation. To 25 mg equivalent of Metformin hydrochloride in formulation added 1,2 and 3 ml stock solutions which contains 1000 µg/ml of Glimepiride, 2500 µg/ml of Metformin hydrochloride and 4000 µg/ml of Atorvastatin calcium into a series of 50 ml volumetric flasks and dissolved with methanol and made up to the mark with the methanol. The solutions were sonicated for 15 minutes. After sonication, centrifuged for 15 minutes at 3000 rpm, the solutions were filtered through Whatmann filter paper No. 41. From each solution, 5 ml of clear filtrate was transferred into a series of 25 ml volumetric flasks and made up to the volume with methanol. Furthermore, 1 ml was transferred in to 10 ml standard flasks. The absorbances of the resulting solutions were measured at their selected wavelengths for determination of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The amount of each drug recovered from the formulation was calculated for all the drugs by using Cramer's rule solution method. The procedure was repeated for three times for each concentration.

Validation of Developed Method

Linearity

A calibration curve was plotted against concentration Vs absorbance. Metformin hydrochloride, Atorvastatin calcium and Glimepiride showed linearity in the concentration range of 3 – 15 µg/ml, 4 - 24 µg/ml and 1 – 5 µg/ml at 228, 236 and 246 nm respectively.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation, a known volume of standard stock solutions were added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

The repeatability of the method was confirmed by the analyzing the formulation for 6 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intra day and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs present in the formulation was determined and percentage RSD also calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation was done by the different analysts. The amount and % RSD were calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

Trilinear Regression Calibration Method

In the recent years the simultaneous analysis of binary mixtures using a numerical method has carried out. The modification of this method was applied to the analysis of ternary mixtures is called as Tri-linear regression calibration (TLRC) method**.**

TLRC method is based on the finding of the best three wavelengths in the spectrum. TLRC model requires the application of matrix mathematics to three linear regression equations at three

wavelength point. TLRC technique uses slope values to determine the three drugs whereas CRS technique uses absorptivity values to determine the three drugs.

Selection of Solvent

The solubility of Atorvastatin calcium, Metformin hydrochloride and Glimepiride was determined in a variety of solvents as per I.P standards. Solubility tests were carried out in polar and non polar solvents. The common solvent to solubilise all the three drugs were found to be methanol and the same is used for the analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride through the proposed method.

Preparation of Standard Stock Solution

25 mg of Atorvastatin calcium and Glimepiride raw materials were weighed and transferred into 25 ml standard volumetric flasks separately and 25 mg of Metformin hydrochloride was transferred into 50 ml standard volumetric flask and dissolved in methanol and made up to the volume with methanol. These solutions were observed to contain 1000 µg/ml of Atorvastatin calcium, Glimepiride and 500 µg/ml of Metformin hydrochloride.

Preparation of Working Standard Solution

From the standard stock solutions 2, 1 and 3ml of Atorvastatin calcium, Glimepiride and Metformin hydrochloride was transferred in to three separate 50 ml standard flasks and the solutions were made up to the mark with methanol. The final solution contains 40, 10 and 30 µg/ml of Atorvastatin calcium, Glimepiride and Metformin hydrochloride respectively.

Selection of Wavelengths for Estimation and Stability Studies

For the selection of wavelengths for estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, a suitable diluted stock solution containing 10 µg/ml of each and the solutions were scanned between 200 and 400 nm by using methanol as blank. From the overlain spectra, the spectral characteristics of Atorvastatin calcium, Metformin hydrochloride and Glimepiride were observed and 11 wavelengths were selected (218, 222, 224, 228, 230, 234, 236, 238, 242, 246 and 247 nm respectively and analysed for their linearity and sensitivity, finally three wavelengths were selected. The wavelengths selected were 228, 236 and 246 nm which is the λ max of Glimepiride, Metformin hydrochloride and Atorvastatin calcium, respectively. Hence, for the analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, the respective λ max of the drugs were selected. The stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol were stable for more than 3 hours at all the selected wavelengths.

Preparation of Calibration Graph

Different aliquots of 1-6 ml of 40µg/ml solution of Atorvastatin calcium and 1-5ml of 30µg/ml solution of Metformin hydrochloride and 10 µg/ml solution of Glimepiride was transferred into 10 ml volumetric flasks separately and made up to the volume with methanol. 1-5 ml of 30 µg/ml solution was transferred into 10 ml volumetric flask separately and made up to the volume with methanol. 1-5 ml of 10 µg/ml solutions was transferred into 10 ml volumetric flask separately and made up to the mark with methanol. The absorbance of different concentration solutions were measured at three selected wavelengths. The calibration curve was plotted against concentration Vs absorbance. Atorvastatin calcium, Metformin hydrochloride, Glimepiride was found to be linear in the concentration range of 4-24, 3-15 and 1 – 5 µg/ml at all the three wavelengths.

Quantification of Formulation (By Standard Addition Method)

Preparation of Standard Addition Solution

For standard addition method, 25 mg of Atorvastatin calcium and 100 mg of Glimepiride was transferred in to separate 25 ml standard flask and made up to the volume with methanol to obtain 1000 µg/ml of Atorvastatin calcium and 4000 µg/ml of Glimepiride respectively.

Quantification Procedure

Twenty tablets of formulation (**CD pro2** each tablet containing 10 mg of Atorvastatin calcium, 500mg of Metformin hydrochloride, and 2 mg of Glimepiride) were weighed accurately. The average weight of the tablets were found and powdered. The tablet powder equivalent to 25 mg of Metformin hydrochloride was weighed and transferred into 50 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and added 5 ml of standard addition solutions of Atorvastatin calcium and Glimepiride in to the same flask and made up to the volume with the methanol. The solution was sonicated for 15 minutes, centrifuged for 15 minutes at 3000 rpm and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 5 ml to 25 ml with methanol to obtain 100 µg/ml solution of Metformin hydrochloride. Furthermore, 1 ml of solution was transferred in to 10 ml standard volumetric flask and diluted to mark obtain 10 µg/ml of Metformin hydrochloride, 8.2 µg/ml of Atorvastatin calcium and 2.04 µg/ml of Glimepiride theoretically. The absorbance measurements were made on 6 replicates for the formulation at 228, 236 and 246 nm. By using absorptivity values of three drugs at three wavelengths, the amount of Atorvastatin calcium, Metformin hydrochloride and Glimepiride could be determined by Tri-linear regression calibration method.

Recovery Studies

The recovery experiment was done by adding known concentrations of Atorvastatin calcium, Metformin hydrochloride and Glimepiride solution to the pre-analyzed formulation. To 25 mg equivalent of Metformin hydrochloride in formulation added 1,2 and 3 ml stock solutions which contains 1000 µg/ml of Glimepiride, 2500 µg/ml of Metformin hydrochloride and 4000 µg/ml of Atorvastatin calcium into a series of 50 ml volumetric flasks and dissolved with methanol and made up to the mark with the methanol. The solutions were sonicated for 15 minutes. After sonication, centrifuged for 15 minutes at 3000 rpm, the solutions were filtered through Whatmann filter paper No. 41. From each solution, 5 ml of clear filtrate was transferred into a series of 25 ml volumetric flasks and made up to the volume with methanol. Furthermore, 1 ml was transferred in to 10 ml standard flasks. The absorbances of the resulting solutions were measured at their selected wavelengths for determination of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The amount of each drug recovered from the formulation was calculated for all the drugs by using Tri-linear regression calibration method. The procedure was repeated for three times for each concentration.

Validation of Developed Method

Linearity

A calibration curve was plotted against concentration Vs absorbance. Metformin hydrochloride, Atorvastatin calcium and Glimepiride showed linearity in the concentration range of 3 – 15 µg/ml, 4 - 24 µg/ml and 1 – 5 µg/ml at 228, 236 and 246 nm respectively.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation, a known volume of standard stock solutions were added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

The repeatability of the method was confirmed by the analyzing the formulation for 6 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intra day and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs present in the formulation was determined and percentage RSD also calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation was done by the different analysts. The amount and % RSD were calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

Reverse Phase – HPLC Method

In RP – HPLC, the retention of a compound is determined by its polarity, pK_a, molecular weight, experimental conditions, mobile phase, column and temperature. The column (typically octyl (C_8) and octadecyl (C_{18}) bonded phase) is less polar than the water – organic phase, usually an almost or entirely mobile phase. Sample molecules partition between the polar and non – polar mobile phase (C_8 and C_{18}) stationary phase and more hydrophobic (non - polar) compounds are retained more strongly. Polar compounds are less strongly held and elute from the column first and vice versa. Usually the lower the polarity of the mobile phase, higher in its elution strength. RP – HPLC columns are efficient, stable and reproducible because of the solvents used. Generally gradient and isocratic elution techniques used for elution, isocratic elution technique employed for resolution of compounds in present study.

Method Development and Optimization of Chromatographic Conditions

Selection of Mobile Phase and Detection Wavelengths

Solutions of Atorvastatin calcium, Metformin hydrochloride and Glimepiride (10 µg/ml) were prepared in the mobile phase [Phosphate buffer (p^H - 4.5): Acetonitrile (40:60% v/v)] and scanned in the UV region of 200 – 400 nm and recorded the spectrums. It was found that all three drugs have marked absorbance at 226 nm and can be effectively used for the estimation of three drugs without interference. Hence 226 nm was selected as detection wavelength for estimation of three drugs by RP – HPLC method with an Isocratic elution technique.

Stability of Sample Solutions

Atorvastatin calcium, Metformin hydrochloride and Glimepiride (10 µg/ml) solutions were prepared and checked the absorbance for their stability at 226 nm. It was found that three drugs are stable for approximately 2 hours.

Optimization of Chromatographic Conditions

Initial Separation Conditions

The following chromatographic conditions were preset initially to get better resolution of Atorvastatin calcium, Metformin hydrochloride and Glimepiride.

Mode of operation - Isocratic

Stationary phase - C_{18} column (150 mm × 4.6 mm i.d. 5µ)

Mobile phase - Water: Acetonitrile

Proportion of mobile phase - 50: 50 % v/v

Detection wavelength - 234 nm

Flow rate - 1 ml/min

Temperature - Ambient

Sample load - 20 µl

Operating pressure - 102 kgf

Method - External Standard Calibration method

The mobile phase was primarily allowed to run for 30 minutes to record a sturdy baseline. Mixture of Solutions of Atorvastatin calcium, Metformin hydrochloride and Glimepiride were injected and the respective chromatogram was recorded. It was found that both Metformin was eluted with tailing effect. And Atorvastatin calcium and Glimepiride were eluted less than 2 minutes. For this reason different ratios of mobile phase with different solvents were tried to obtain good chromatogram with acceptable system suitability parameters.

From the above information, in the mobile phase of Phosphate buffer (pH – 4.5): Acetonitrile

(40:60 %v/v) these three drugs were eluted with sharp peak and better resolution. Hence it was selected as suitable mobile phase for this chromatographic method.

Selection of Mobile Phase

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded, they include the following

S. No	Mobile phase	Observation
1	Water: Acetonitrile (50: 50 % v/v)	Atorvastatin and Glimepiride were merged
2	Water: Acetonitrile (40: 60 % v/v)	Metformin eluted less than 2 min
3	Water: Acetonitrile (30: 70 % v/v)	Resolution between Atorvastatin, Glimepiride and Metformin is less
4	10 mM Phosphate buffer pH-3: Acetonitrile (30: 70 % v/v)	Metformin eluted less than 2 min and Atorvastatin and Glimepiride eluted as broad peak
5	10 mM Phosphate buffer pH-4: Acetonitrile (40: 60 % v/v)	Metformin eluted less than 2 min
6	25 mM Phosphate buffer pH-4: Acetonitrile (40: 60% v/v)	Metformin eluted less than 2 min
7	30 mM Phosphate buffer pH-4: Acetonitrile (40: 60 % v/v)	Metformin eluted less than 2 min
8	50 mM Phosphate buffer pH-4: Acetonitrile (40: 60 % v/v)	Metformin eluted less than 2 min
9	50 mM Phosphate buffer pH-4.5: Acetonitrile (40: 60 % v/v)	Metformin eluted less than 2 min
10	50 mM Phosphate buffer pH-5: Acetonitrile (40: 60 % v/v)	Atorvastatin and Glimepiride not complies
11	50 mM Phosphate buffer pH-3: Acetonitrile (40: 60 % v/v)	Glimepiride not complies
12	50 mM Phosphate buffer pH-4.5: Acetonitrile (35: 65 % v/v)	Metformin eluted less than 2 min
13	50 mM Phosphate buffer pH-4.5: Acetonitrile (45:55 % v/v)	Metformin eluted less than 2 min
14	Water: Methanol: Acetonitrile (20: 50: 30 % v/v)	Atorvastatin and Metformin were merged
15	Water: Methanol: Acetonitrile (30: 50: 20 % v/v)	Atorvastatin and Metformin were merged

From the above information, in the mobile phase of 50 mM Phosphate buffer pH-4.5: Acetonitrile, these three drugs were eluted with sharp peak and better resolution.

Hence this mobile phase was used to optimize the chromatographic conditions.

Effect of Ratio of Mobile Phase

The different ratios of Water: Acetonitrile (In the ratio of 50: 50, 40: 60, 30: 70 % v/v) and Phosphate buffer: Acetonitrile (In the ratio of 40: 60, 35: 65, 45: 55 % v/v) were tried.

At Phosphate buffer: Acetonitrile in the ratio of 60: 40 % v/v, the peaks obtained were very sharp with better resolution.

Hence this ratio was selected for further analysis.

Effect of pH of Phosphate Buffer

The Phosphate buffer of different pH (pH-3.0, 4.0, 4.5 and 5.0) were tried. At pH-4.5 the peaks were eluted with better resolution. Hence this pH was selected for further analysis.

Optimized Chromatographic Conditions

The following optimized conditions were employed for analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride by Isocratic RP – HPLC method.

Mode of operation - Isocratic

Stationary phase - C_{18} column (150 mm × 4.6 mm i.d. 5µ)

Mobile phase - Phosphate buffer (p^H - 4.5): Acetonitrile

Proportion of mobile phase - 40:60 % v/v

Detection wavelength - 226 nm

Flow rate - 1 ml/min

Temperature - Ambient

Sample load - 20 µl

Operating pressure - 94 kgf

Method - External Standard Calibration method

Preparation of Standard Atorvastatin Calcium Solution

25 mg of Atorvastatin calcium was weighed accurately and transferred into 25 ml volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (1000 µg/ml).

Preparation of Standard Metformin Hydrochloride Solution

50 mg of Metformin hydrochloride was weighed accurately and transferred into 25 ml volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (2000 µg/ml).

Preparation of Standard Glimepiride Solution

25 mg of Glimepiride was weighed accurately and transferred into 25 ml volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (1000 µg/ml).

Preparation of Working Standard Solution of Atorvastatin calcium

1 ml of standard stock solution of Atorvastatin calcium was transferred into 10 ml standard flask to acquire 100 µg/ml solution.

Preparation of Working Standard Solution of Glimepiride

1 ml of standard stock solution of Glimepiride was transferred into 10 ml standard flask to acquire 100 µg/ml solution.

Preparation of Mixture of Working Standard Solution

5 ml from the working standard solution of Atorvastatin calcium and 8 ml from the standard stock solution of Metformin hydrochloride and 1 ml from working standard solution of Glimepiride was transferred in to a 100 ml volumetric flask and made up to the mark with the mobile phase to obtain 5 µg/ml of Atorvastatin calcium, 160 µg/ml of Metformin hydrochloride and 1 µg/ml of Glimepiride**.**

Preparation of Calibration Graph

In this progression, the aliquots of mixture of working standard solution of Atorvastatin calcium, Metformin hydrochloride and Glimepiride (1 – 5 ml) individually were transferred into five 10 ml volumetric flasks and made up to the mark with mobile phase, to obtain the concentration range of 0.5 – 2.5 µg/ml, 16 - 80 µg/ml and 0.1 - 0.5 µg/ml of Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively. All the solutions were injected and the chromatograms were recorded at 226nm. The above concentration range was found to be linear and obeys Beer's law. The procedure was repeated for three times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in Tablet Formulation

Estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in tablet formulation by RP – HPLC was carried out using optimized chromatographic conditions. Twenty tablets of formulation (CD pro2 each containing 10 mg of Atorvastatin calcium, 2 mg of Glimepiride and 500 mg of Metformin hydrochloride) were weighed accurately. The average weight of tablets was found and powdered.

The tablet powder equivalent to 100 mg of Metformin hydrochloride was weighed and transferred into 100 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and made up to the volume with the same (1000 µg/ml).

The solution was sonicated for 15 minutes, centrifuged at 3000 rpm for 15 minutes and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 0.5 ml into 10 ml with mobile phase to obtain 50 µg/ml solution of Metformin hydrochloride, which also contains 1 and 0.2 µg/ml of Atorvastatin calcium and Glimepiride respectively. This solution is used for further analysis.

Assay Procedure

A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 30 minutes, six test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery Experiments

Preparation of Standard Recovery Stock Solution of Atorvastatin calcium, Metformin hydrochloride and Glimepiride

An accurately weighed quantity of 25 mg of Metformin hydrochloride was transferred into 50 ml volumetric flask and added sufficient methanol to dissolve the substance and made up to the mark with the same (500 µg/ml). An accurately weighed quantity of 25 mg of Glimepiride and Atorvastatin calcium was transferred separately in 25 ml volumetric flask (1000 µg/ml) and made up to the mark with methanol. Further, 1 ml of each solution was transferred in to 10 ml volumetric flask separately to obtain each recovery stock solution, 2.5 ml was transferred in to 10 ml standard flask and made up to the mark with the mobile phase (25 µg/ml). From the Glimepiride recovery stock solution, 1 ml was transferred in to 10 ml standard flask and made up to the mark with the mobile phase (10 µg/ml).

Preparation of Working Recovery Solution

From the standard recovery stock solution 2.5 ml and 1 ml of Atorvastatin calcium and Glimepiride was transferred in to 10 ml standard flask separately and made up to the mark with mobile phase to get 25 µg/ml and 10 µg/ml of Atorvastatin calcium and Glimepiride respectively.

Preparation of Mixture of Working Recovery Standard Solution

The mixture of working recovery standard solution was prepared by pipetting 4 ml from standard recovery stock (500 µg/ml) solution of Metformin hydrochloride, 5 ml and 2.5 ml from

working recovery solution of Atorvastatin calcium and Glimepiride were transferred in to 25 ml volumetric flask and made up to the mark with the mobile phase.

Recovery Procedure

To 1 ml of pre-analyzed formulation solution added 1, 2 and 3 ml of mixture of raw material working recovery standard solution (contains 80, 5 and 1 µg/ml of Metformin hydrochloride, Atorvastatin calcium and Glimepiride respectively) into 10 ml volumetric flasks separately and made up to the mark with mobile phase. The procedure was repeated as per analysis of formulation. The quantity of drug recovered was calculated by using slope and intercept values from the calibration graph.

System Suitability Studies

The system suitability studies conceded as per ICH guidelines and USP. The parameters like capacity factor, tailing factor, asymmetry factor and number of theoretical plate and resolution were calculated.

RESULTS AND DISCUSSION

Estimation of multiple drug formulations have advantage that the methods are less time consuming and usage of solvent is minimized.

Two simple, rapid, precise and accurate spectrophotometric methods and an isocratic RP – HPLC method were developed and validated for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in pure form and in combined tablet dosage form.

The methods employed for the analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride were,

UV-Spectrophotometric Methods

1. Cramer's Rule Solution (CRS) Method
2. Tri-Linear Regression Calibration (TLRC) Method

RP – HPLC Method

Cramer's Rule Solution (Crs) Method

The solubility of Atorvastatin calcium, Metformin hydrochloride and Glimepiride was determined as per Indian Pharmacopoeia. The numerous polar and non – polar solvents were tried to dissolve the drugs. From the solubility profile methanol was chosen as a common solvent for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride. The solubility data is shown in Tables 1, 2 and 3 for Metformin hydrochloride, Atorvastatin calcium and Glimepiride respectively.

The sample solutions of 10 µg/ml of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol was prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank. The overlain spectrum of Atorvastatin calcium, Metformin hydrochloride and Glimepiride was recorded as shown in Figure 1. From the spectrum, the corresponding λ max of three drugs i.e 228 nm, 236 nm, 246 nm was selected for the estimation of Glimepiride, Metformin hydrochloride and Atorvastatin calcium simultaneously. Absorptivity A_1^1 (1%, 1 cm) were calculated by using the absorbances measured at 228 (λ_1), 236 (λ_2) and 246 (λ_3) for zero order spectra for each of the compounds in the ternary mixture. Three linear equation functions were obtained and this linear equation system was resolved by using Cramer's rule for the prediction of Glimepiride, Metformin hydrochloride and Atorvastatin calcium present in pure form in mixture and in formulation.

Different aliquots of Metformin hydrochloride, Atorvastatin calcium and Glimepiride in methanol were prepared in the concentration range of 3 - 15, 4 – 24 and 1 – 5 µg/ml respectively. The absorbances of these solutions were measured at 228, 236 and 246 nm respectively. The calibration graph was plotted at three respective wavelengths are shown in Figure 2 – 4, 5 – 7, and 8 – 11 for Metformin hydrochloride, Atorvastatin calcium and Glimepiride respectively. The preparation of calibration curve was repeated for six times for each drug at their selective wavelengths. The optical parameters like, Sandell's sensitivity, molar absorptivity, correlation coefficient, slope,

intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for all the three drugs was found to be 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The optical characteristics of three drugs at their selective wavelengths are shown in Table 4 - 6 for Metformin hydrochloride, Atorvastatin calcium and Glimepiride.

CD pro2 tablets (Nicholas Piramal Healthcare Pvt Ltd., Mumbai.) each tablet containing 10 mg of Atorvastatin calcium, 2 mg of Glimepiride and 500 mg of Metformin hydrochloride was selected for analysis. The Quantification of formulation was done by Standard Addition Method. The nominal concentration of Metformin hydrochloride from linearity (10 µg/ml) was prepared and also contains 0.2 µg/ml of Atorvastatin calcium and 0.04 µg/ml of Glimepiride in the actual formulation solution. Then the stock solution of Atorvastatin calcium and Glimepiride were added to the formulation solution to get a final concentration of formulation as Metformin hydrochloride (10 µg/ml), Glimepiride (2.04 µg/ml) and Atorvastatin calcium (8.2 µg/ml) and to improve the sensitivity of the method. The absorbances of the solution were measured at their respective wavelengths. The percentage label claim present in tablet formulation was found to be 98.46 ±1.2781, 99.67±1.1832 and 102.29±1.1598% for Atorvastatin calcium, Glimepiride and Metformin hydrochloride respectively. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.0129, 0.0119 and 1.1330 for Atorvastatin calcium, Glimepiride and Metformin hydrochloride, respectively. The low % RSD values indicate that the method has good precision. The results of analysis are shown in Table 7.

Further the precision of the method was confirmed by Intra day and Inter day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intra day and inter day analysis were found to be 0.0111 and 0.0068, 0.0063 and 0.0025 and 0.0118 and 0.0026 for Atorvastatin calcium, Glimepiride and Metformin hydrochloride respectively. The results of analysis are shown in Table 8. The results showed that the precision of the method was confirmed.

The developed method was validated for ruggedness. In the present work it was confirmed by different analysts. The % RSD value by analyst 1 and 2 were found to be 0.6370 and 0.9271, 0.7523 and 0.9047 and 0.0015 and 0.0029 for Atorvastatin calcium, Glimepiride and Metformin hydrochloride, respectively. The low % RSD values indicate that the developed method was more rugged. The results were shown in Table 9.

The accuracy of the method was performed by recovery studies. To the pre-analyzed formulation, a known quantity of Atorvastatin calcium, Metformin hydrochloride and Glimepiride raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery and % RSD was found to be 99.22 to 100.39 % and 0.0066, 99.27 to 100.78 % and 0.0081 and 99.00 to 101.00 % and 0.0087 for Atorvastatin calcium, Glimepiride and Metformin hydrochloride respectively. The low % RSD value for three drugs indicates that this method is very accurate. The recovery data is shown in Table 10.

Tri-Linear Regression Calibration (TLRC) Method

A simple, accurate, rapid and precise Tri-linear regression method was developed and validated. Methanol was chosen as a common solvent for estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride. The sample solutions of 10 µg/ml of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol was prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank. The overlain spectrum of mixture of Atorvastatin calcium, Metformin hydrochloride and Glimepiride were recorded.

From the spectrum, 11 wavelengths were selected for the determination of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively without any interference. At the selected 11 wavelengths the three drugs were checked for its linearity. From these wavelengths, three wavelengths which are sensitive wavelengths to determine three drugs were selected for the analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The three sensitive wavelengths selected are 228, 236 and 246 nm respectively. Different aliquots of Atorvastatin calcium, Metformin hydrochloride and Glimepiride were prepared in the concentration range of 4 – 24, 3 – 15 and 1 – 5 µg/ml. The absorbance of these solutions was measured at 228, 236 and 246 nm respectively. The plotted calibration graphs are shown in Figure 5 -7, 8 - 10 and 11 – 13 for Metformin hydrochloride, Atorvastatin calcium and Glimepiride respectively.

The preparation of calibration curve was repeated for six times for each drug at their selected wavelength. The calibration curve was plotted using concentration against absorbance. The optical parameters like, Sandell's sensitivity, molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated for all the three drugs. The correlation coefficient for all the three drugs was found to be above 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The results are shown in Table 4, 5 and 6 for Metformin hydrochloride, Atorvastatin calcium and Glimepiride respectively.

CD pro2 tablets (Nicholas Piramal Healthcare Pvt Ltd., Mumbai.) each tablet containing 10 mg of Atorvastatin calcium, 500 mg of Metformin hydrochloride and 2 mg of Glimepiride was selected for analysis. The Quantification of formulation was done by Standard Addition Method. The nominal concentration of Metformin hydrochloride from linearity (10 µg/ml) was prepared and also contains 0.2 µg/ml of Atorvastatin calcium and 0.04 µg/ml of Glimepiride in the actual formulation solution. Then the stock solution of Atorvastatin calcium and Glimepiride were added to the formulation solution to get a final concentration of formulation as Metformin hydrochloride (10 µg/ml), Glimepiride (2.04 µg/ml) and Atorvastatin calcium (8.2 µg/ml) and to improve the sensitivity of method. The absorbance of these solutions was measured at 228 nm, 236 nm and 246 nm. The amount present in six test solutions was determined. The percentage label claim in tablet formulation was found to be 100.25±1.7444, 101.98±1.1676 and 99.81±0.6043 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.0174, 0.0114 and 0.0060 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The results of analysis are shown in Table 11. The low % RSD values indicate that the method has good precision.

Further the precision of the method was confirmed by Intra day and Inter day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of Intra day and Inter day analysis are 0.0074 and 0.0086, 0.0016 and 0.0019 and 0.0081 and 0.0050 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The results of analysis are shown in Table 12. Hence the precision was confirmed. The results showed that the precision of the method was further confirmed.

The developed method was validated for ruggedness. In the present work it was confirmed by different analysts. The % RSD value by analyst 1 and 2 were found to be 0.9409 and 1.4111, 0.7051 and 0.5878 and 0.8775 and 1.0167 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table 9.

The accuracy of the method was performed by recovery studies. To the pre-analyzed

formulation, a known quantity of Atorvastatin calcium, Metformin calcium and Glimepiride raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery and % RSD was found to be 99.93 to 99.94 % and 0.00007, 98.50 to 101.0 % and 0.01602 and 99.37 to 99.88 % and 0.0026 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively. The low % RSD value for three drugs indicates that this method is very accurate. The recovery data is shown in Table 11.

RP – HPLC Method

An exercise has been made for a simple, rapid, accurate and precise method for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in pure form and in formulation by an isocratic RP – HPLC method. The solutions of 10 µg/ml of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in mobile phase (Phosphate buffer (p^H – 4.5): Acetonitrile in the proportions of 40:60 % v/v) were prepared and the solutions were scanned in the range of 200 – 400 nm. It was found that all three drugs have marked absorbance at 226 nm and can be effectively used for estimation of three drugs without interference. Therefore 226 nm was selected as detection wavelength for estimation of three drugs by RP – HPLC method with an isocratic elution technique and it was found that three drugs are stable for approximately two hours.

The optimization was done by changing the composition of mobile phase ratio. The mobile phase consists of Water: Acetonitrile (50:50 % v/v) was initially tried and chromatograms were recorded. These are shown in Figure 14. The mobile phase consists of Water: Acetonitrile (40:60, 30:70 % v/v), Phosphate buffer: Acetonitrile (pH-3.0, 4.0, 4.5 and 5.0) were tried and the chromatograms are shown in Figure 15 – 24. The system suitability parameters were calculated. After calculating all system suitability parameters, Phosphate buffer (p^H – 4.5): Acetonitrile with the ratio of 40:60 %v/v at a flow rate of 1 ml/min was selected and the optimized chromatogram is shown in Figure 25. Finally the mobile phase consists of Phosphate buffer (p^H – 4.5): Acetonitrile with the ratio of 40:60 % v/v was selected for this method. The retention time of Metformin hydrochloride, Atorvastatin calcium and Glimepiride were found to be 1.85, 5.20 and 7.99, respectively. The retention time between three drugs indicate that the drugs were separated with better resolution of 11.16 between Metformin Hydrochloride and Atorvastatin Calcium and 7.97 between Atorvastatin calcium and Glimepiride. The system suitability parameters for the optimized chromatogram are shown in Table 12.

With the optimized chromatographic conditions, stock solutions of Atorvastatin calcium, Metformin hydrochloride and Glimepiride were prepared in mobile phase and prepared the mixture of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in the concentration range of 0.5 – 2.5, 16 – 80 and 0.1 – 0.5 µg/ml of Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively. 20 µl of each solution was injected and recorded the chromatograms at 226 nm. The chromatograms are shown in Figure 18 – 21. The calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation co – efficient was found to be above 0.999 for all three drugs. The calibration graph of Metformin hydrochloride, Atorvastatin calcium and Glimepiride are shown in Figure 23, 24 and 25 respectively. The optical characteristics of Atorvastatin calcium, Metformin hydrochloride and Glimepiride are shown in Table 13.

The tablet dosage form (CD pro2) was selected for the analysis. The ostensible concentration 50 µg/ml of Metformin hydrochloride, which also contains 1 µg/ml of Atorvastatin calcium and 0.2 µg/ml of Glimepiride in the mobile phase was prepared. 20 µl of each solution was injected and chromatograms were recorded. The percentage purity was found to be 99.18±1.3556, 106.33±1.0552 and 98.90±0.4016 % for Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively. The precision of

the method was confirmed by repeatability of formulation for six times and the chromatograms are shown in Figure 27 – 28. The percentage RSD was found to be 0.0136, 0.0099 and 0.0040 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively. It indicates that the method has good precision. The data is shown in Table 13.

The accuracy of the method was performed by recovery studies. To the pre-analyzed formulation, a known quantity of Atorvastatin calcium, Metformin hydrochloride and Glimepiride raw material solutions were added at different levels, injected the solutions. The chromatograms were recorded as shown in the Figure 29 – 30.

The percentage recovery and %RSD were found to be in the range of 97.62 to 100.46 % and 0.0147, 99.47 to 103.77 % and 0.0161 and 99.10 to 100.60 % and 0.0078 for Atorvastatin calcium, Metformin and Glimepiride respectively. The low % RSD values for recovery indicates that the method was found to be accurate. The values are given in the Table 14. The high percentage recovery revealed that no interference produced due to the excipients used in formulation. Therefore, the developed method was found to be accurate.

All the above parameters with the ease of operation ensure that the projected methods could be applied for the routine analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in pure form and in tablet dosage forms.

CONCLUSION

Two simple, rapid, precise and accurate spectrophotometric methods and one RP – HPLC method were developed and validated for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in pure form and in tablet dosage form.

The methods employed for the analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride were,

UV- Spectrophotometric Methods

1. Cramer's Rule Solution (CRS) Method
2. Tri-linear Regression Calibration (TLRC) Method

RP – HPLC method

Cramer's Rule Solution (CRS) Method

From the solubility profile methanol was chosen as a common solvent for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride. The sample solutions of 10 µg/ml of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol were prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank. The overlain spectrum of mixture of Atorvastatin calcium, Metformin hydrochloride and Glimepiride was recorded. From the spectrum, 228 nm, 236 nm and 246 nm were selected for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride without any interference.

Different aliquots of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol were prepared in the concentration range of 4 – 24, 3 – 15 and 1 - 5 µg/ml respectively. The absorbances of solutions were measured at 228 nm, 236 nm and 246 nm. The optical parameters like, Sandell's sensitivity, molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated for all the three drugs. The correlation coefficient for all the three drugs was found to be 0.999. CD pro2 tablets (Nicholas Piramal Healthcare Ltd., Mumbai.) each tablet containing 10 mg of Atorvastatin calcium, 500 mg of Metformin hydrochloride and 2 mg of Glimepiride was selected for analysis. The percentage label claim present in tablet formulation was found to be 98.46 ± 1.2781, 102.29 ± 1.1598 and 99.67 ± 1.1832 % for Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively.

Further the precision of the method was confirmed by Intra day and Inter day analysis. The % RSD value for intra day and inter day analysis was found to be 0.0111 and 0.0068 for

Atorvastatin calcium, 0.0118 and 0.0026 for Metformin hydrochloride and 0.0063 and 0.0025 for Glimepiride, respectively. The developed method was validated for ruggedness. The % RSD value by analyst 1 and 2 was found to be 0.6370 and 0.9271 for Atorvastatin calcium, 0.0015 and 0.0029 for Metformin hydrochloride and 0.7523 and 0.9047 for Glimepiride, respectively. The low % RSD values indicate that the developed method was more rugged. The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range of 99.22 to 100.39 % for Atorvastatin calcium, 99.00 to 101.0 % for Metformin hydrochloride and 99.27 to 100.78 % for Glimepiride. The % RSD was 0.0066, 0.0087 and 0.0081 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively.

Tri-Linear Regression Calibration (TLRC) Method

A simple, accurate, rapid and precise Tri-linear regression method was developed and validated. The common solvent used for estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride was chosen as methanol. The sample solutions of 10 µg/ml of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol was prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank. The overlain spectrum of mixture of Atorvastatin calcium, Metformin hydrochloride and Glimepiride was recorded. 228 nm, 236 nm and 246 nm were selected for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively without any interference.

Different aliquots of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in methanol were prepared in the concentration range of 4 – 24, 3 – 15 and 1 – 5 µg/ml respectively. The absorbance of the solutions was measured at 228 nm, 236 nm and 246 nm respectively. The optical parameters like, Sandell's sensitivity, molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error was calculated for all the three drugs. The correlation coefficient for all the three drugs was found to be 0.999. CD pro2 tablets (Nicholas Piramal Healthcare Pvt Ltd., Mumbai.) each tablet containing 10 mg of Atorvastatin calcium, 2 mg of Glimepiride and 500 mg of Metformin hydrochloride was selected for analysis. The percentage label claim present in tablet formulation was found to be 100.25 ± 1.7444, 101.98 ± 1.1676 and 99.81 ± 0.6043 % for Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively.

Further the precision of the method was confirmed by Intra day and Inter day analysis. The % RSD value for intra day and inter day analysis was found to be 0.0074 and 0.0086 for Atorvastatin calcium, 0.0016 and 0.0019 for Metformin hydrochloride and 0.0081 and 0.0050 for Glimepiride respectively. The developed method was validated for Ruggedness. The % RSD value by analyst 1 and 2 was found to be 0.9409 and 1.4111, 0.7051 and 0.5878 and 0.8775 and 1.0167 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively. The low % RSD values indicate that the developed method was more rugged. The accuracy of the method was confirmed by recovery studies. . The percentage recovery was found to be in the range of 99.93 to 99.94 % for Atorvastatin calcium, 98.50 to 101.0 % for Metformin hydrochloride and 99.37 to 99.88 % for Glimepiride. The % RSD was 0.00007, 0.01602 and 0.0026 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively.

RP – HPLC Method

An exertion has been made for a simple, rapid, accurate and precise method for the estimation of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in pure form and in formulation by an isocratic RP – HPLC method. The mobile phase of different ratios and different pH were tried. After calculating all system suitability parameters Phosphate buffer (p^H – 4.5): Acetonitrile with the ratio of 40:60 % v/v at a flow rate of 1 ml/min was selected for this method. The retention time of Metformin

hydrochloride, Atorvastatin calcium and Glimepiride were found to be 1.85, 5.20 and 7.99, respectively. The retention time between three drugs indicate that the drugs were separated with better resolution of 11.16 for Metformin hydrochloride and Atorvastatin calcium and 7.97 for Atorvastatin calcium and Glimepiride.

With the optimized chromatographic conditions, the drugs were linear in the concentration range of 0.5 – 2.5, 16 – 80 and 1 – 5 µg/ml for Atorvastatin calcium, Metformin hydrochloride and Glimepiride. The correlation co – efficient was found to be above 0.999 for all three drugs. The tablet dosage form (CD pro2) was selected for the analysis. The percentage purity was found to be 99.18 ± 1.3556, 106.33 ± 1.0552 and 98.90 ± 0.4016 % for Atorvastatin calcium, Metformin hydrochloride and Glimepiride respectively. The precision of the method was confirmed by repeatability of formulation for six times. The accuracy of the method was performed by recovery studies. The percentage recovery was found to be in the range between 97.62 to 100.46 % for Atorvastatin calcium, 99.47 to 103.77 % for Metformin hydrochloride and 99.10 to 100.60 % for Glimepiride. The % RSD was found to be 0.0147, 0.0161 and 0.0078 for Atorvastatin calcium, Metformin hydrochloride and Glimepiride, respectively. The low % RSD values for recovery indicated that the method was found to be accurate.

Two simple, rapid and accurate UV spectrophotometric (Cramer's Rule Solution method and Tri-linear regression calibration method) and an isocratic RP - HPLC methods were developed for the determination of Atorvastatin calcium, Metformin hydrochloride And Glimepiride in bulk and in tablet formulation by using UV spectrophotometer with methanol as a solvent and RP-HPLC with UV detection. The methods showed excellent sensitivity, reproducibility, accuracy and repeatability, which is evidenced by low percentage relative standard deviation. The results obtained in recovery studies were indicating that there is no interference from the excipients used in the formulation. By comparing three methods, UV spectrophotometric methods (Cramer's Rule Solution method and Tri-linear regression calibration method) were found to be ease and rapid when compared to RP-HPLC. Because the solvents and column used in RP-HPLC are very costly. When comparing the sensitivity of the methods, RP-HPLC method was found to be more sensitive than UV spectrophotometric method. Because the linearity range, LOD and LOQ were less in RP-HPLC method than UV spectrophotometric method. Hence it is suggested that the proposed UV spectrophotometric methods (Cramer's Rule Solution method and Tri-linear regression calibration method) and RP-HPLC methods can be effectively applied for the routine analysis of Atorvastatin calcium, Metformin hydrochloride and Glimepiride in bulk and in tablet formulation and the obtained results will be presented elsewhere.

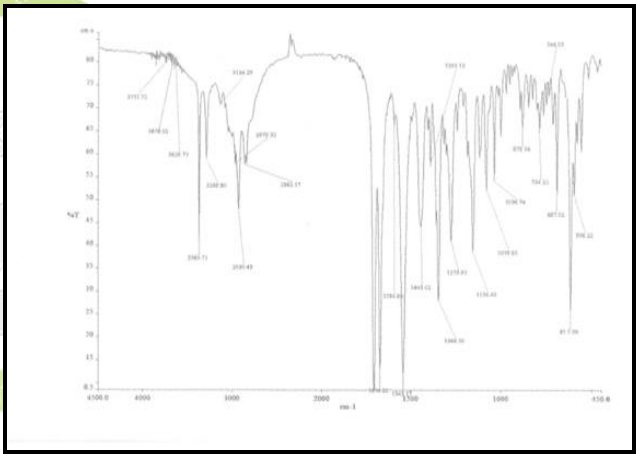


Figure 1: IR Spectrum of Glimepiride

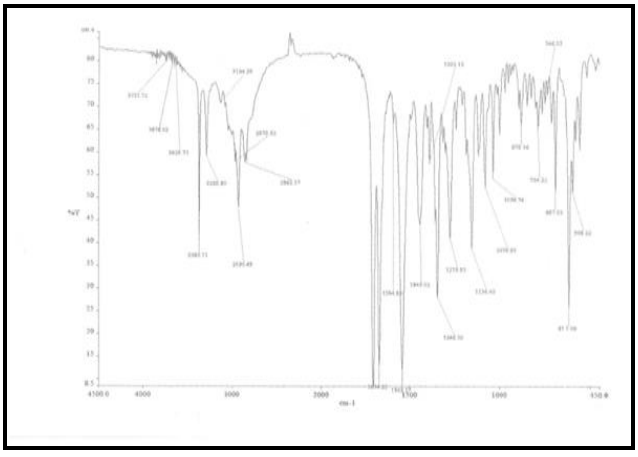


Figure 2: IR Spectrum of Metformin Hydrochloride

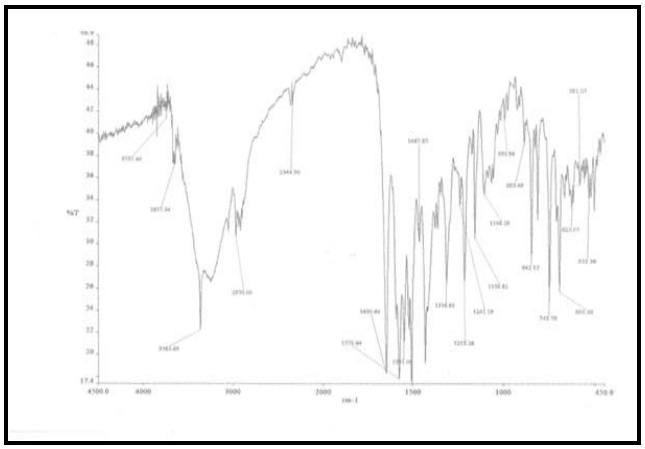


Figure 3: IR Spectrum of Atorvastatin Calcium

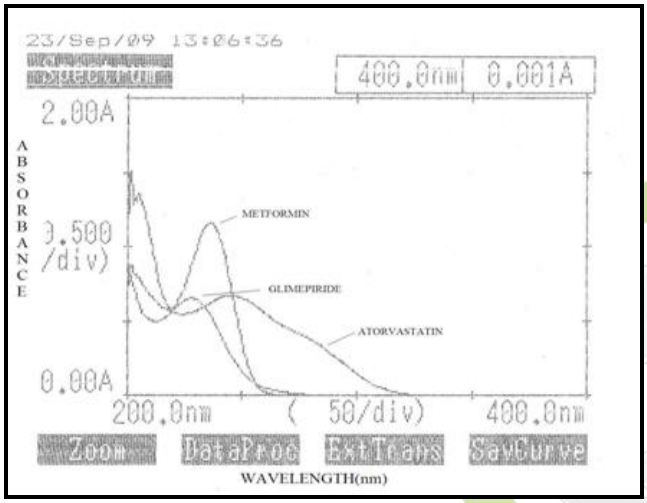


Figure 4: UV Spectrum of Atorvastatin Calcium, Metformin Hydrochloride and Glimepiride

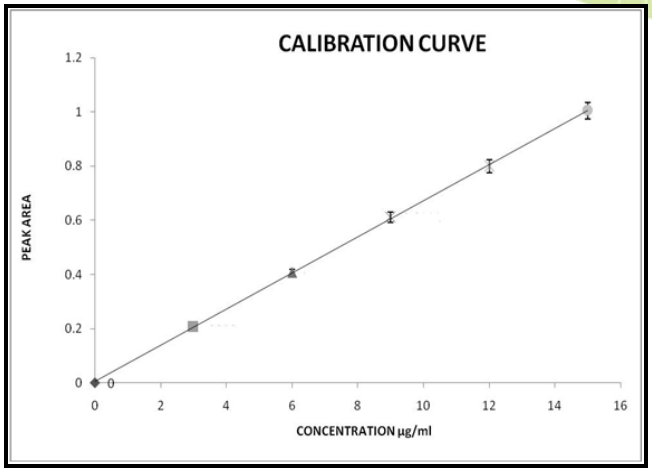


Figure 5: Calibration Curve of Metformin Hydrochloride in Methanol at 228 nm (Cramer's Rule Solution Method & Trilinear Regression Calibration Method)

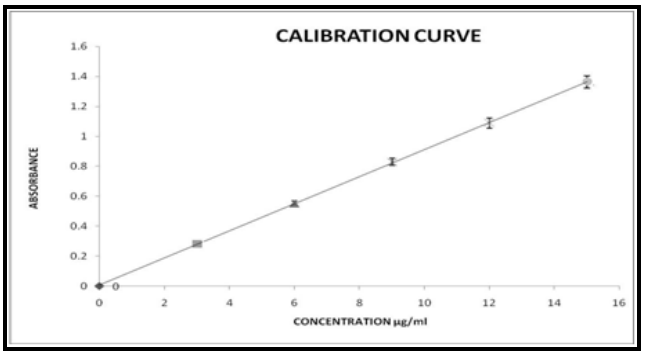


Figure 6: Calibration Curve of Metformin Hydrochloride in Methanol at 236 nm (Cramer's Rule Solution Method & Trilinear Regression Calibration Method**)**

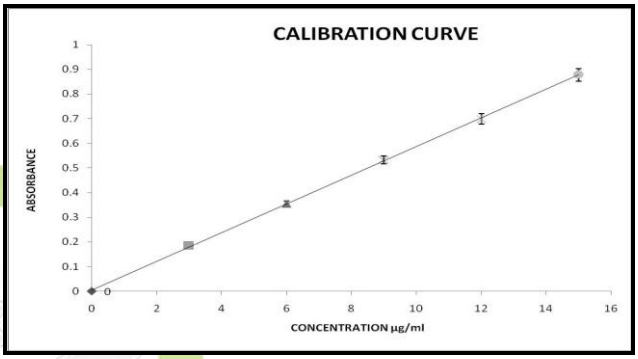


Figure 7: Calibration Curve of Metformin Hydrochloride in Methanol at 246 nm (Cramer's Rule Solution Method & Trilinear Regression Calibration Method)

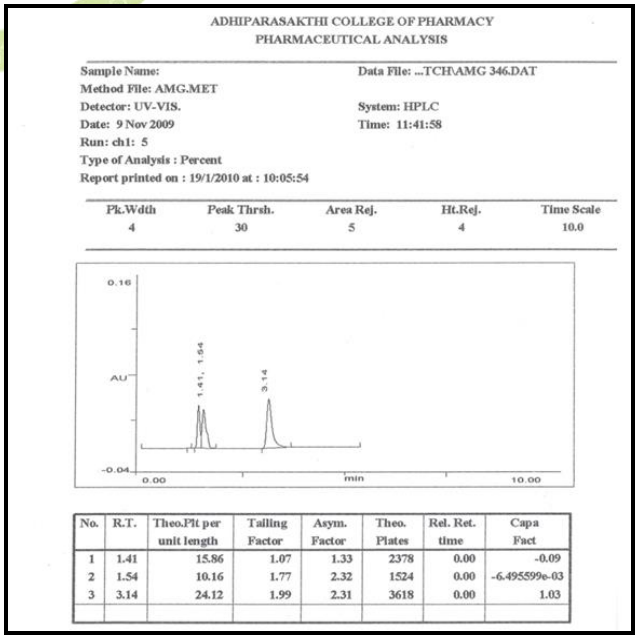
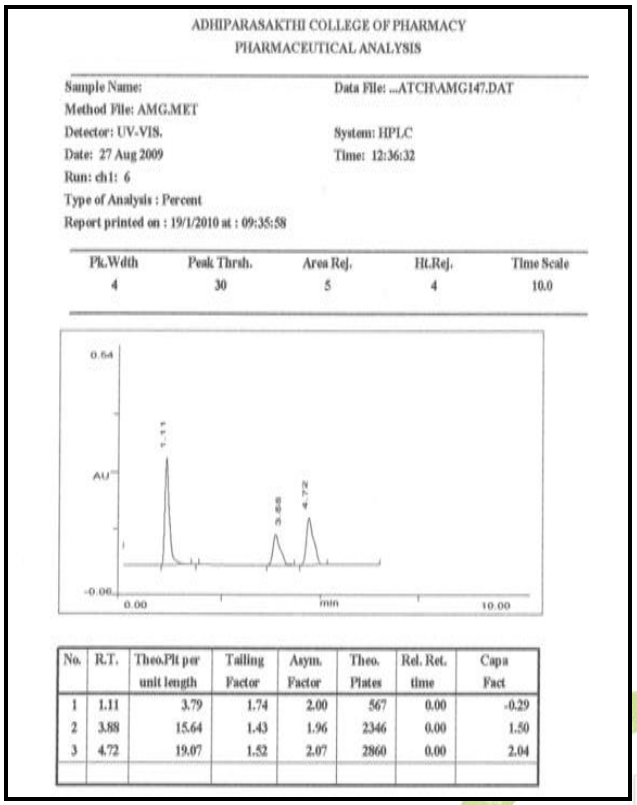


Figure 8: Initial Separation Conditions in Water: Acetonitrile (50:50 % v/v)



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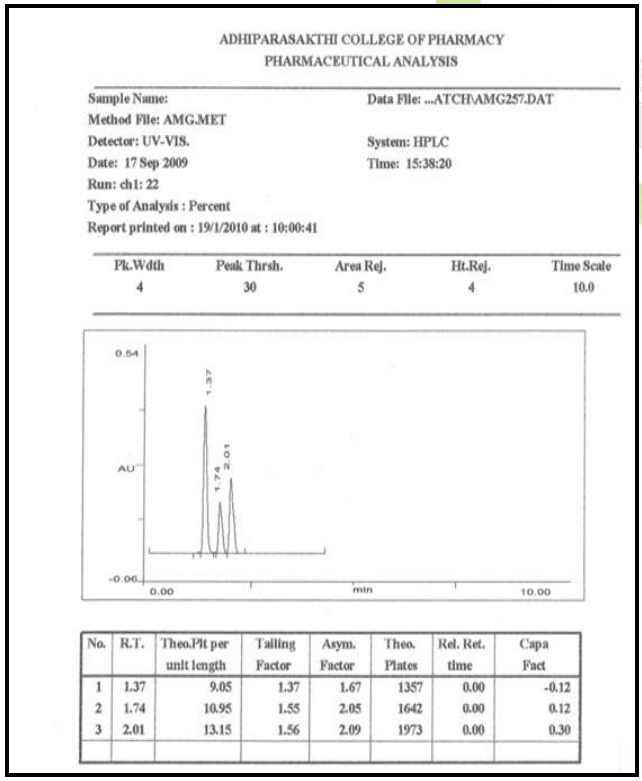
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ...ATCH\AMG147.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 27 Aug 2009 Time: 12:36:32
Run: ch1: 6
Type of Analysis : Percent
Report printed on : 19/1/2010 at : 09:35:58

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0

No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	1.11	3.79	1.74	2.00	567	0.00	-0.29
2	3.88	15.64	1.43	1.96	2346	0.00	1.50
3	4.72	19.07	1.52	2.07	2860	0.00	2.04

Figure 9: Chromatogram for Effect of Ratio of Mobile Phasewater: Acetonitrile (40:60 % v/v)



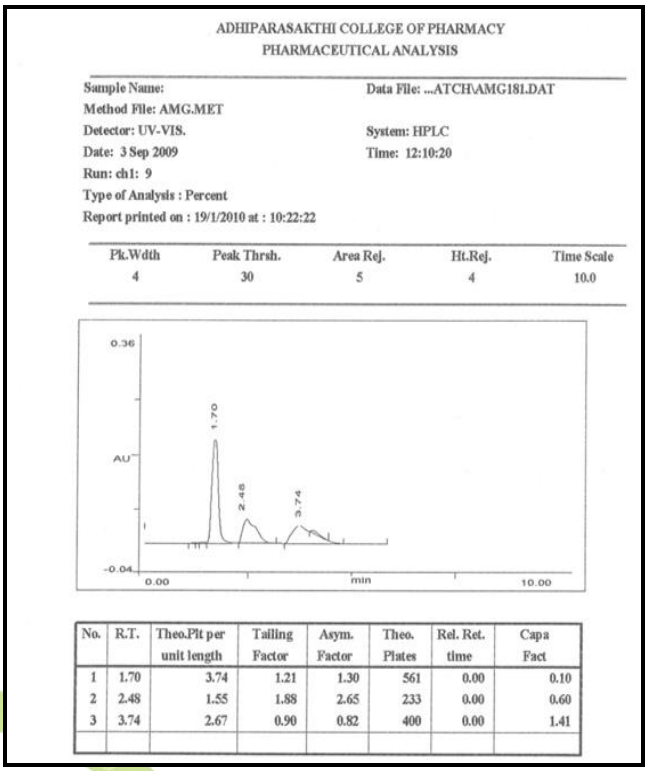
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ...ATCH\AMG257.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 17 Sep 2009 Time: 15:38:20
Run: ch1: 22
Type of Analysis : Percent
Report printed on : 19/1/2010 at : 10:00:41

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0

No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	1.37	9.05	1.37	1.67	1357	0.00	-0.12
2	1.74	10.95	1.55	2.05	1642	0.00	0.12
3	2.01	13.15	1.56	2.09	1973	0.00	0.30

Figure 10: Chromatogram for Effect of Ratio of Mobile Phase in Water: Acetonitrile (30: 70 % v/v)



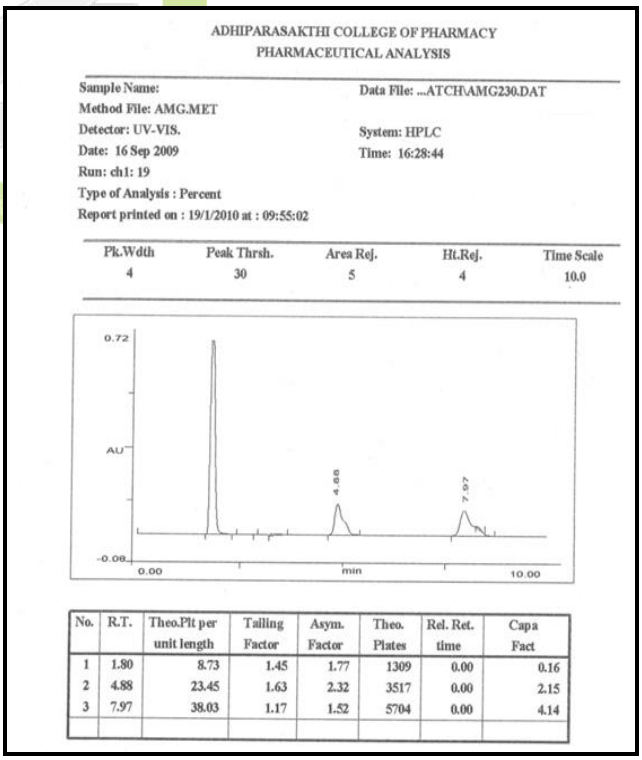
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ...ATCH\AMG181.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 3 Sep 2009 Time: 12:10:20
Run: ch1: 9
Type of Analysis : Percent
Report printed on : 19/1/2010 at : 10:22:22

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0

No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	1.70	3.74	1.21	1.30	561	0.00	0.10
2	2.48	1.55	1.88	2.65	233	0.00	0.60
3	3.74	2.67	0.90	0.82	400	0.00	1.41

Figure 11: Chromatogram for Effect of Ratio of Mobile Phase in 10 mm Phosphate Buffer pH 3: Acetonitrile (30: 70% v/v)



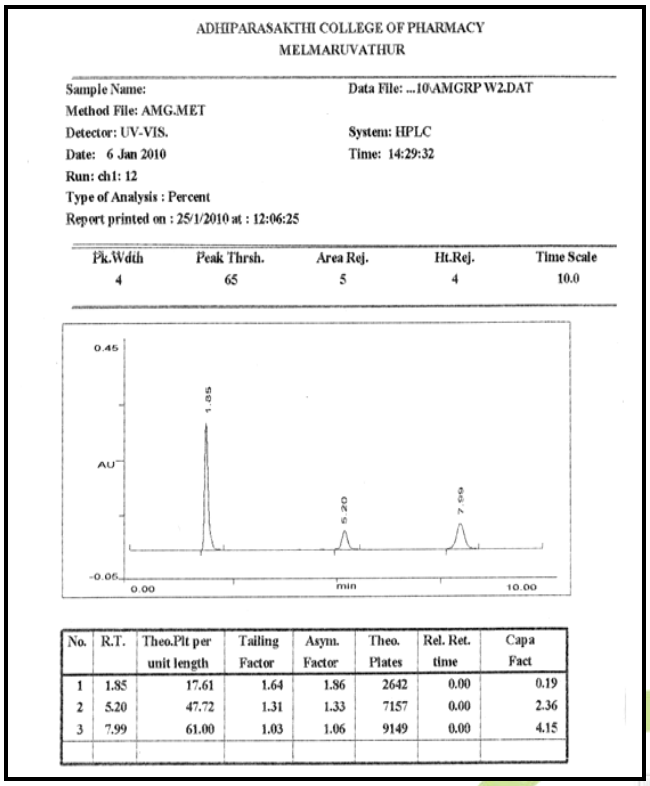
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ...ATCH\AMG230.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 16 Sep 2009 Time: 16:28:44
Run: ch1: 19
Type of Analysis : Percent
Report printed on : 19/1/2010 at : 09:55:02

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0

No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	1.80	8.73	1.45	1.77	1309	0.00	0.16
2	4.88	23.45	1.63	2.32	3517	0.00	2.15
3	7.97	38.03	1.17	1.52	5704	0.00	4.14

Figure 12: Chromatogram for Effect of Ratio of Mobile Phase in 10mm Phosphate Buffer pH 4: Acetonitrile (40: 60 % v/v)



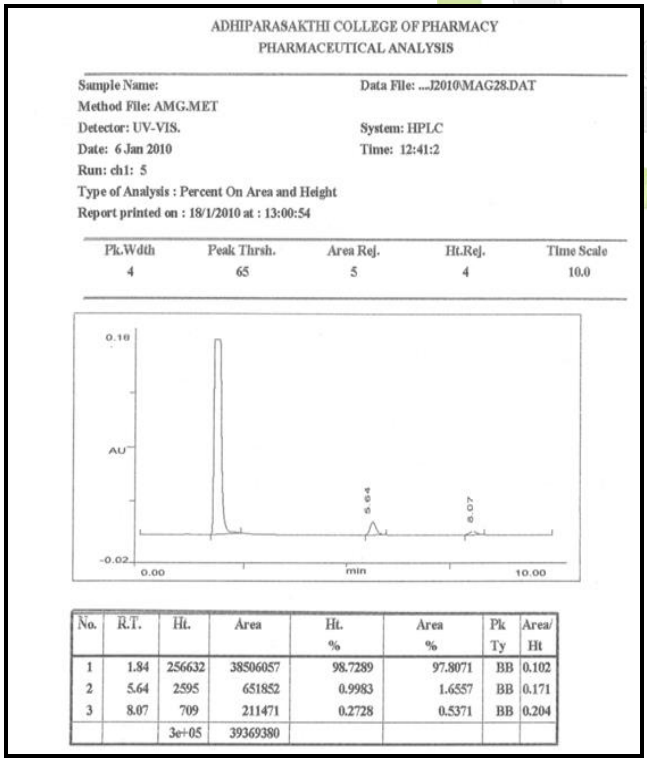
ADHIPARASAKTHI COLLEGE OF PHARMACY
MELMARUVATHUR

Sample Name: Data File: ...10\AMGRP W2.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 14:29:32
Run: ch1: 12
Type of Analysis : Percent
Report printed on : 25/1/2010 at : 12:06:25

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	1.85	17.61	1.64	1.86	2642	0.00	0.19
2	5.20	47.72	1.31	1.33	7157	0.00	2.36
3	7.99	61.00	1.03	1.06	9149	0.00	4.15

Figure 13: Chromatogram for Effect of Ratio of Mobile Phase in 50 mm Phosphate Buffer pH 4.5: Acetonitrile (40: 60% v/v)



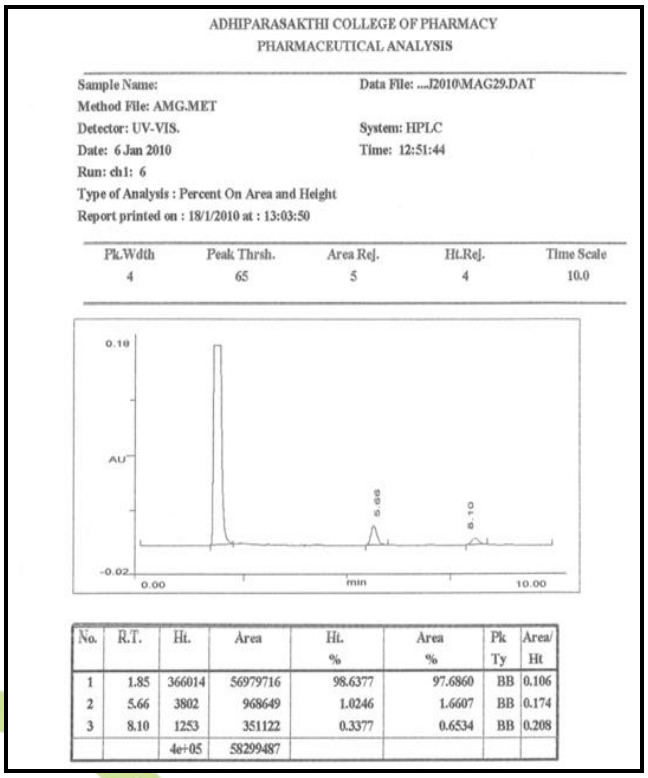
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG28.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 12:41:2
Run: ch1: 5
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:00:54

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.84	256632	38506057	98.7289	97.8071	BB	0.102
2	5.64	2595	651852	0.9983	1.6557	BB	0.171
3	8.07	709	211471	0.2728	0.5371	BB	0.204
		3e+05	39369380				

Figure 15: Linearity Chromatogram of Metformin Hydrochloride, Atorvastatin Calcium and Glimepiride (32, 1.0, 0.2 µg/ml)



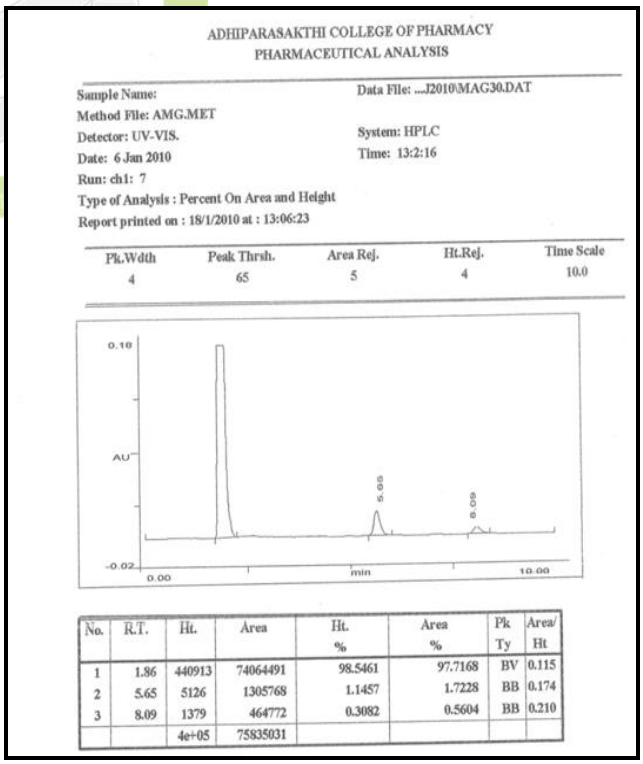
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG29.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 12:51:44
Run: ch1: 6
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:03:50

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.85	366014	56979716	98.6377	97.6860	BB	0.106
2	5.66	3802	968649	1.0246	1.6607	BB	0.174
3	8.10	1253	351122	0.3377	0.6534	BB	0.208
		4e+05	58299487				

Figure 16: Linearity Chromatogram of Metformin Hydrochloride, Atorvastatin Calcium and Glimepiride (48, 1.5, 0.3 µg/ml)



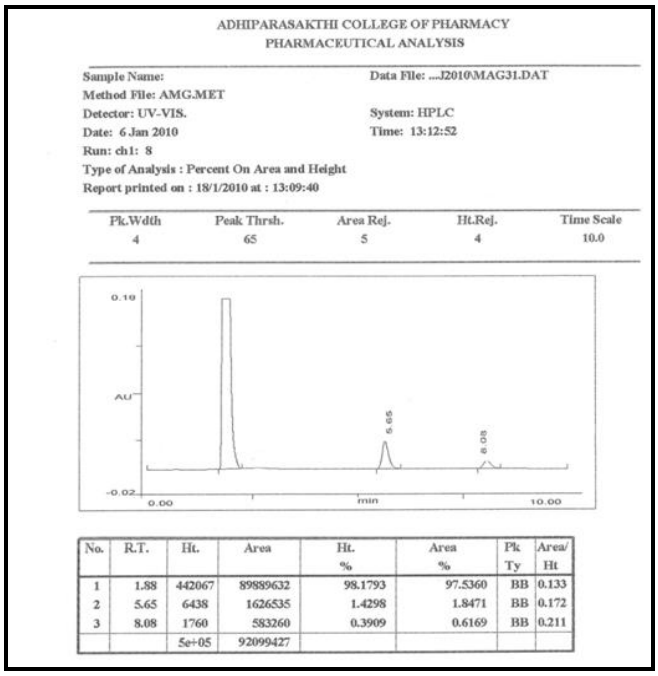
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG30.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 13:2:16
Run: ch1: 7
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:06:23

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.86	440913	74064491	98.5461	97.7168	BV	0.115
2	5.65	5126	1305768	1.1457	1.7228	BB	0.174
3	8.09	1379	464772	0.3082	0.5604	BB	0.210
		4e+05	75835031				

Figure 17: Linearity Chromatogram of Metformin Hydrochloride, Atorvastatin Calcium and Glimepiride (64, 2.0, 0.4 µg/ml)



ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG31.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 13:12:52
Run: ch1: 8
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:09:40

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.88	442067	89889632	98.1793	97.5360	BB	0.133
2	5.65	6438	1626535	1.4298	1.8471	BB	0.172
3	8.08	1760	583260	0.3909	0.6169	BB	0.211
		5e+05	92099427				

Figure 18: Linearity Chromatogram of Metformin Hydrochloride, Atorvastatin Calcium and Glimepiride (80, 2.5, 0.5 µg/ml)

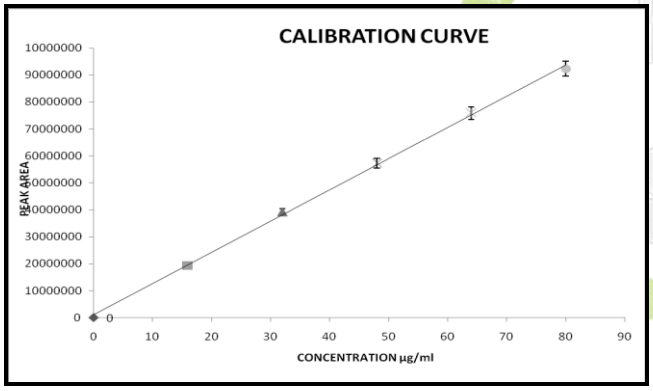


Figure 19: Calibration Curve of Metformin Hydrochloride by RP – HPLC Method

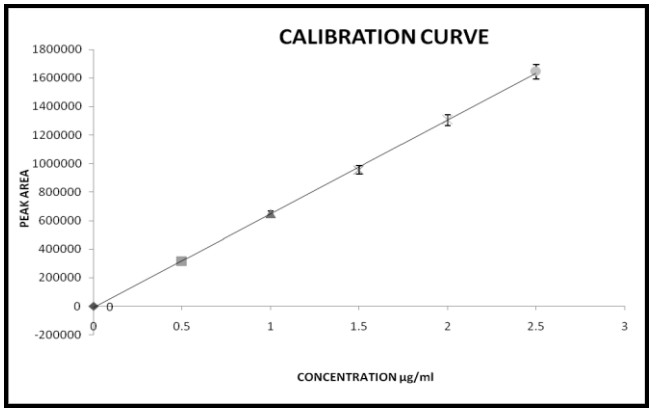


Figure 20: Calibration Curve of Atorvastatin Calcium By RP – HPLC Method

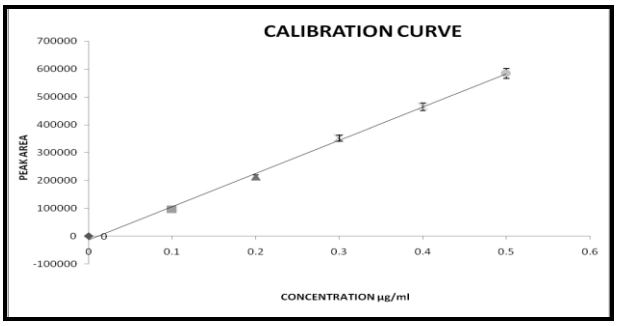
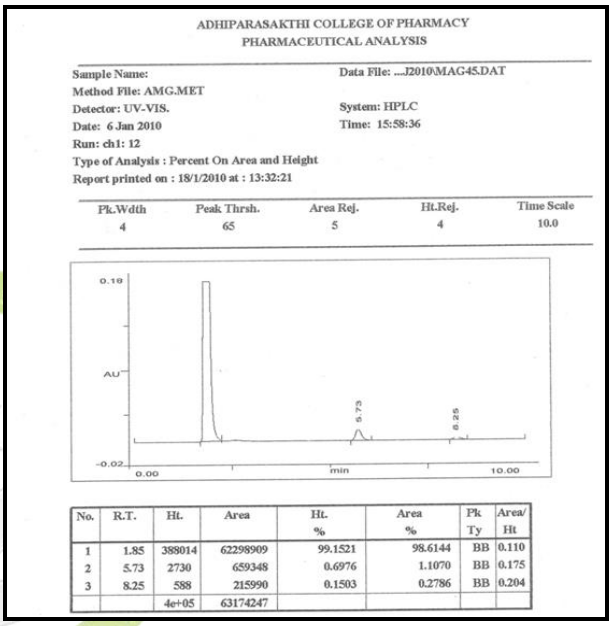


Figure 21: Calibration Curve of Glimepiride by RP – HPLC Method



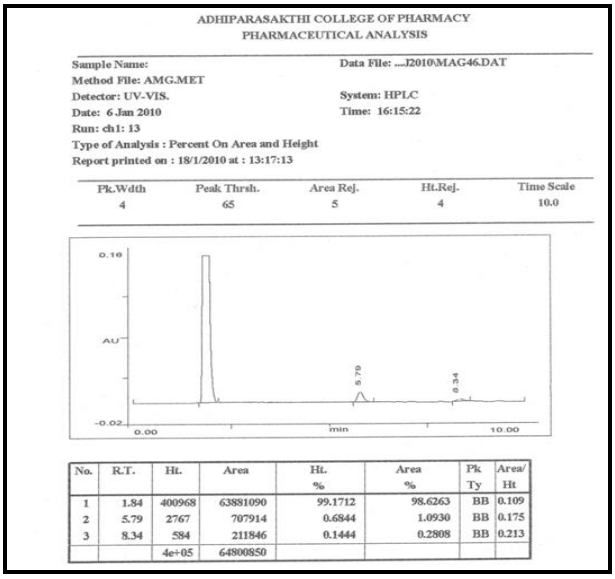
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG45.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 15:58:36
Run: ch1: 12
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:32:21

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.85	388014	62298909	99.1521	98.6144	BB	0.110
2	5.73	2730	659348	0.6976	1.1070	BB	0.175
3	8.25	588	215990	0.1503	0.2786	BB	0.204
		4e+05	63174247				

Figure 22: Chromatogram for Analysis of Formulation – (Cd Pro 2) Repeatability – 1



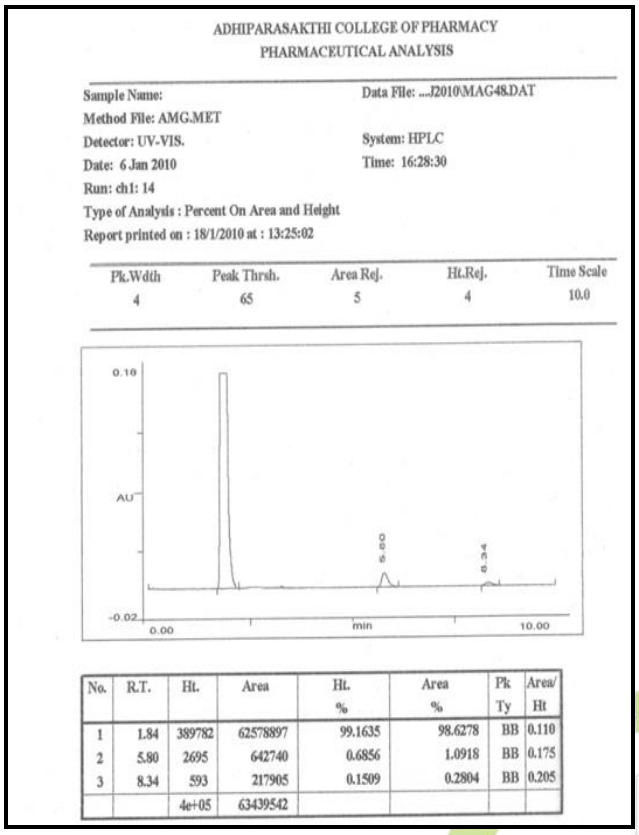
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG46.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 16:15:22
Run: ch1: 13
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:17:13

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.84	400968	63881090	99.1712	98.6263	BB	0.109
2	5.79	2767	707914	0.6844	1.0930	BB	0.175
3	8.34	584	211846	0.1444	0.2808	BB	0.213
		4e+05	64800850				

Figure 23: Chromatogram for Analysis of Formulation – (Cd Pro 2) Repeatability – 2



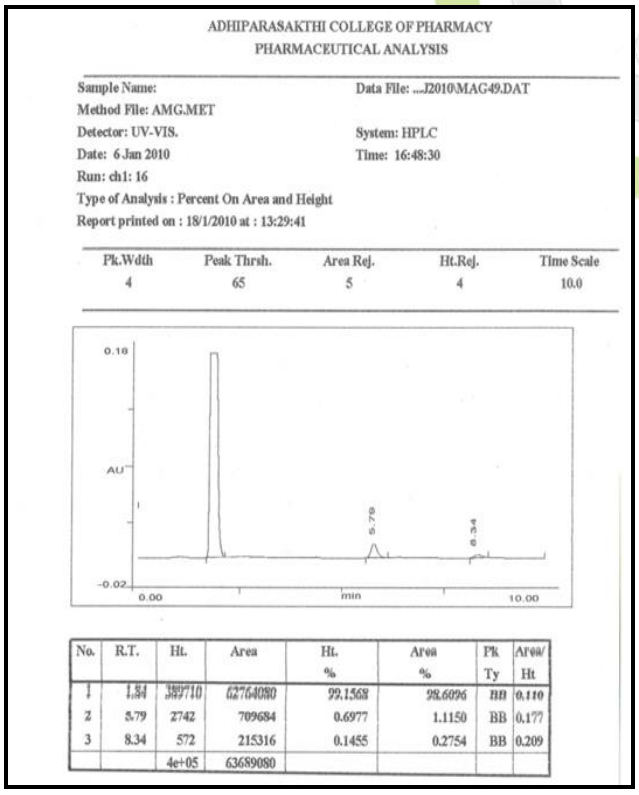
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG48.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 16:28:30
Run: ch1: 14
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:25:02

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.84	389782	62578897	99.1635	98.6278	BB	0.110
2	5.80	2695	642740	0.6856	1.0918	BB	0.175
3	8.34	593	217905	0.1509	0.2804	BB	0.205
		4e+05	63439542				

Figure 24: Chromatogram for Analysis of Formulation – (Cd Pro 2) Repeatability – 3



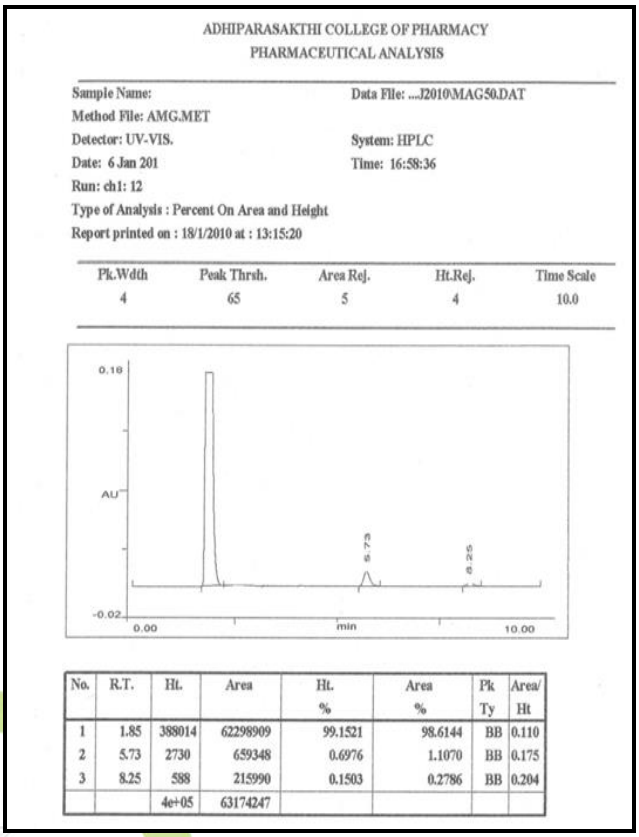
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG49.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 2010 Time: 16:48:30
Run: ch1: 16
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:29:41

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.84	389710	62764080	99.1568	98.6096	BB	0.110
2	5.79	2742	709684	0.6977	1.1150	BB	0.177
3	8.34	572	215316	0.1455	0.2754	BB	0.209
		4e+05	63689080				

Figure 25: Chromatogram for Analysis of Formulation – (Cd Pro 2) Repeatability – 4



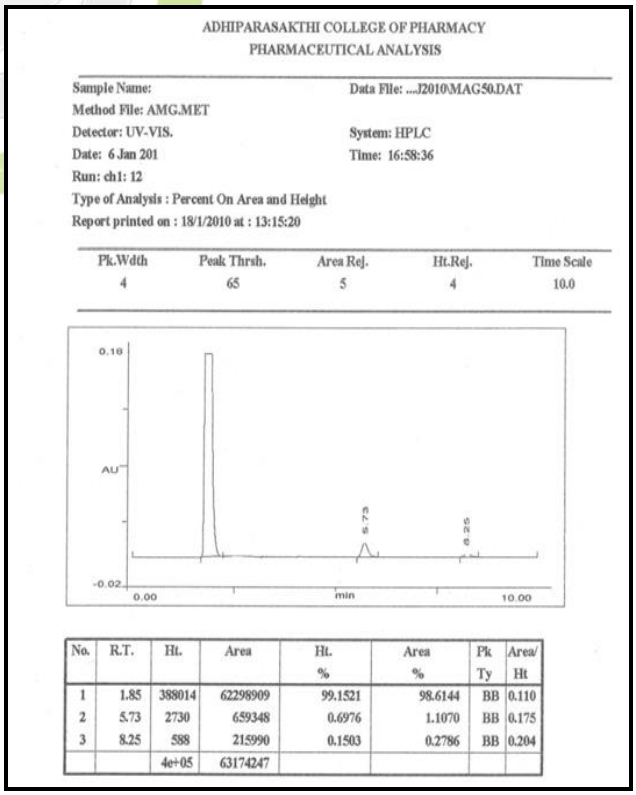
ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG50.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 201 Time: 16:58:36
Run: ch1: 12
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:15:20

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.85	388014	62298909	99.1521	98.6144	BB	0.110
2	5.73	2730	659348	0.6976	1.1070	BB	0.175
3	8.25	588	215990	0.1503	0.2786	BB	0.204
		4e+05	63174247				

Figure 26: Chromatogram for Analysis of Formulation – (Cd Pro 2) Repeatability – 5



ADHIPARASAKTHI COLLEGE OF PHARMACY
PHARMACEUTICAL ANALYSIS

Sample Name: Data File: ....J2010\MAG50.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 6 Jan 201 Time: 16:58:36
Run: ch1: 12
Type of Analysis : Percent On Area and Height
Report printed on : 18/1/2010 at : 13:15:20

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	10.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.85	388014	62298909	99.1521	98.6144	BB	0.110
2	5.73	2730	659348	0.6976	1.1070	BB	0.175
3	8.25	588	215990	0.1503	0.2786	BB	0.204
		4e+05	63174247				

Figure 27: Chromatogram for Analysis of Formulation – (Cd Pro 2) Repeatability-6

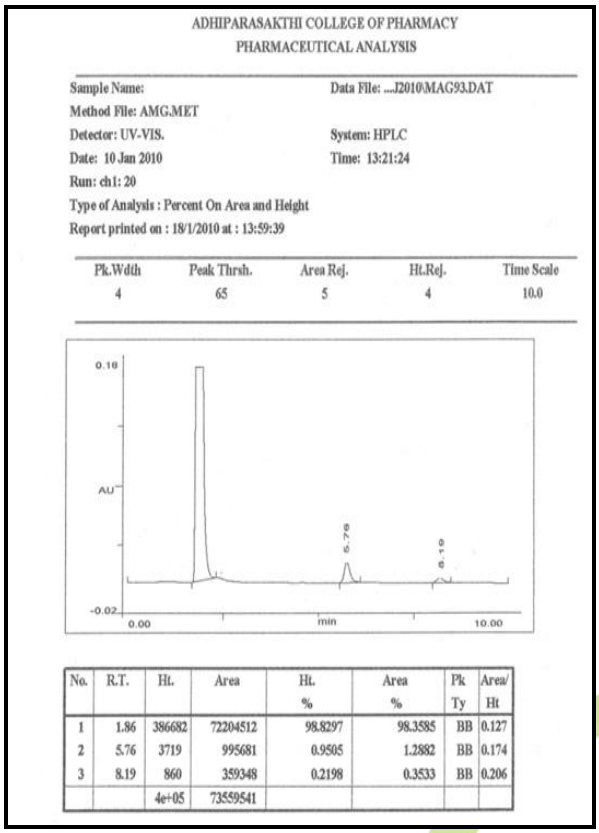


Figure 28: Chromatogram for Recovery of Formulation (Cd Pro 2) – 1

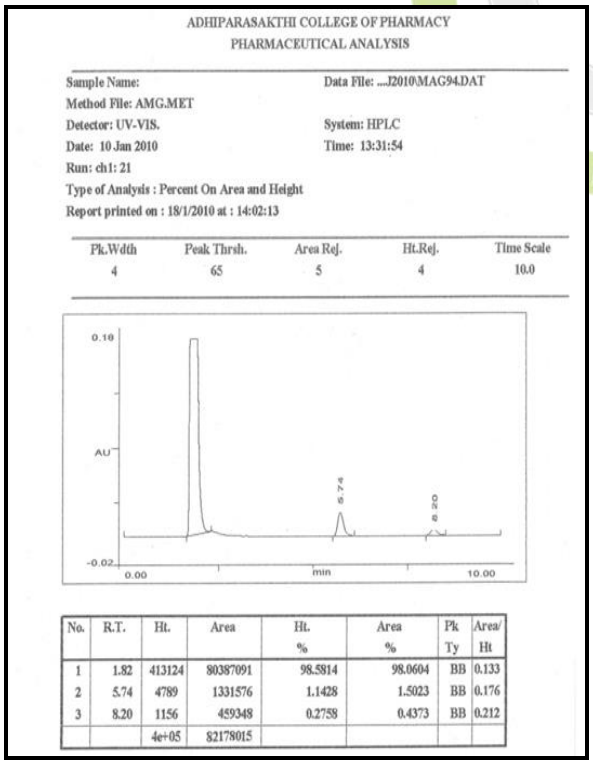


Figure 29: Chromatogram for Recovery of Formulation (Cd Pro 2) – 2

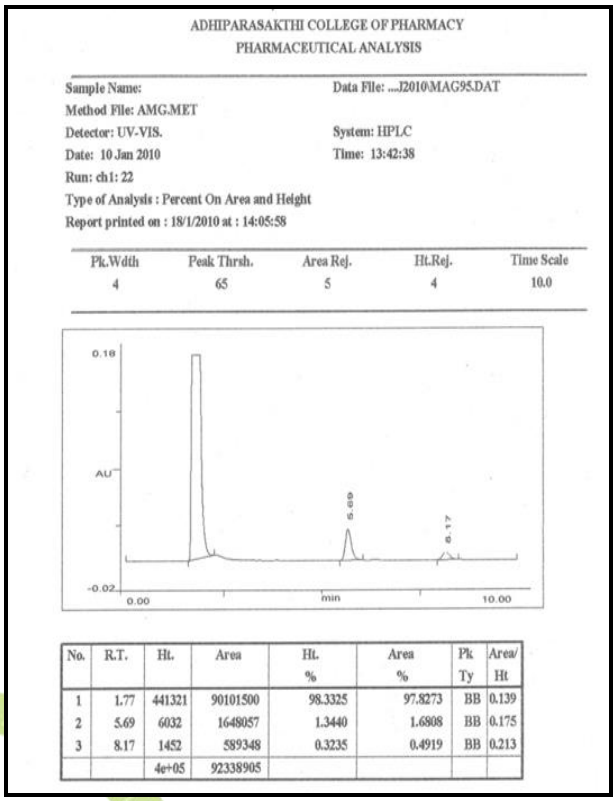


Figure 30: Chromatogram for Recovery of Formulation (Cd Pro 2) – 3

Table 1: Solubility Profile of Metformin Hydrochloride in Polar and Non Polar Solvents

S.No.	Solvents	Extent of Solubility	Category
1	Acetone	---	Insoluble
2	Acetonitrile	---	Insoluble
3	Benzene	---	Insoluble
4	Butanol	---	Insoluble
5	Chloroform	---	Insoluble
6	Diethyl amine	---	Insoluble
7	Distilled water	10mg in 30µl	Freely soluble
8	DMF	10mg in 80 µl	Insoluble
9	Ethanol	10mg in 1700 µl	Slightly soluble

10	Ethyl acetate	---	Insoluble
11	0.1M Hydrochloric acid	10mg in 80 µl	Freely soluble
12	Methanol	10mg in 300 µl	soluble
13	Petroleum ether	---	Insoluble
14	2 – Propanol	---	Insoluble
15	0.1M Sodium Hydroxide	10mg in 40 µl	Freely soluble
16	Toluene	---	Insoluble

Table 2: Solubility Profile of Atorvastatin Calcium in Polar and Non Polar Solvents

S.No	Solvents	Extent of Solubility	Category
1	Acetone	---	Insoluble
2	Acetonitrile	10 mg in 0.12 ml	Soluble
3	Benzene	---	Insoluble
4	Butanol	---	Insoluble
5	Carbontetrachloride	---	Insoluble
6	Chloroform	10 mg in 16 ml	Very slightly soluble
7	Diethyl amine	10 mg in 0.07 ml	Freely soluble
8	DMF	10 mg in 3.65 ml	Slightly soluble
9	Distilled water	---	Insoluble
10	Ethanol	---	Insoluble
11	Ethyl acetate	10 mg in 15 ml	Very slightly soluble
12	0.1M Hydrochloric acid	---	Insoluble
13	Methanol	10 mg in 0.12 ml	Soluble
14	Petroleum ether	---	Insoluble
15	2 – Propanol	---	Insoluble
16	0.1M Sodium Hydroxide	---	Insoluble
17	Toluene	---	Insoluble

Table 3: Solubility Profile of Glimepiride in Polar and Non Polar Solvents

S.no	Solvents	Extent of solubility	Category
1	Acetone	---	Insoluble
2	Acetonitrile	---	Insoluble
3	Benzene	---	Insoluble
4	Butanol	---	Insoluble
5	Carbontetrachloride	---	Insoluble
6	Chloroform	10 mg in 0.52 ml	Sparingly soluble
7	Diethyl amine	---	Insoluble
8	DMF	10 mg in 0.4 ml	Sparingly Soluble
9	Distilled water	---	Insoluble
10	Ethanol	---	Insoluble

11	Ethyl acetate	---	Insoluble
12	0.1M Hydrochloric acid	10 mg in 6 ml	Slightly Soluble
13	Methanol	10 mg in 3 ml	Sparingly Soluble
14	Petroleum ether	---	Insoluble
15	2 – Propanol	---	Insoluble
16	0.1M Sodium Hydroxide	10 mg in 8 ml	Slightly Soluble
17	Toluene	---	Insoluble

Table 4: Optical Characteristis of Metformin Hydrochloride by Cramer's Rule Solution Method & Tri-Linear Regression Calibration Method

Parameters	**At 228 nm**	**At 236 nm**	**At 246 nm**
Beers law limit (µg/ml)	3- 15	3- 15	3- 15
Molar absorptivity (L mol^{-1} $cm^{-1)}$	11124.82684	15110.55101	9715.006311
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.014976114	0.011037865	0.017210257
Correlation coefficient (r)	0.9998	0.9999	0.9998
Regression equation (y=mx+c)	0.0664X +0.008093	0.089981 X+0.013185	0.057618X +0.010927
Slope (m)	0.0664	0.089981	0.057618
Intercept (c)	0.008093	0.013185	0.010927
LOD (µg/ml)	0.42782	0.41616	0.38345
LOQ (µg/ml)	1.29642	1.26110	1.12524
Standard Error	0.000896672	0.000918809	0.00028497

Table 5: Optical Characteristcs of Atrovastatin Calcium by Cramer's Rule Solution Method & Tri-Linear Regression Calibration Method

Parameters	**At 228 nm**	**At 236 nm**	**At 246 nm**
Beers law limit (µg/ml)	4- 24	4- 24	4- 24
Molar absorptivity (L mol^{-1} $cm^{-1)}$	37402.67034	40981.60942	45138.2044
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.30095151	0.26877999	0.024323872
Correlation coefficient(r)	0.9994	0.9997	0.9998
Regression equation (y=mx+c)	0.03269x+ 0.008258	0.036904x + (-) 0.00478	0.040834 X+ (-) 0.00764
Slope (m)	0.03269	0.036904	0.040834
Intercept (c)	0.008258	(-)0.00478	(-) 0.00764
LOD (µg/ml)	1.1012	0.83272	0.91802
LOQ (µg/ml)	3.3365	2.52339	2.78188
Standard Error	0.004504707	0.006049735	0.006738828

Table 6: Optical Characteristis of Glimepiride by Cramer's Rule Solution Method & Tri-Linear Regression Calibration Method

Parameters	**At 228 nm**	**At 236 nm**	**At 246 nm**
Beers law limit (µg/ml)	1- 5	1- 5	1- 5
Molar absorptivity (L mol^{-1} $cm^{-1)}$	28307.60586	22291.67014	22588.53418
Sandell's sensitivity(µg/cm^2/0.001 A.U)	0.1741001	0.22108065	0.037175175
Correlation coefficient (r)	0.9991	0.9992	0.9991
Regression equation (y=mx+c)	0.05685x+0.00445	0.044683X+0.00395	0.021783X+0.004183
Slope (m)	0.05685	0.044683	0.021783
Intercept (c)	0.00445	0.00395	0.004183
LOD (µg/ml)	0.33054	0.21173	0.46083
LOQ (µg/ml)	0.80165	0.64163	0.89646
Standard Error	0.000642502	0.000409219	0.00139384

Table 7: Ruggedness Study by Cramer's Rule Solution Method

Drug	**Condition**	**Percentage obtained 1**	**Percentage obtained 2**	**Percentage obtained 3**	**Average %**	**S.D**	**% R.S.D**	**S.E.**
MET	Analyst 1	101.36	101.65	101.62	101.54	0.1595	0.0015	0.0920
	Analyst 2	101.14	101.54	101.73	101.47	0.3011	0.0029	0.1738
ATR	Analyst 1	99.49	100.57	100.62	100.22	0.6384	0.6370	0.3685
	Analyst 2	98.87	99.15	100.59	99.53	0.9228	0.9271	0.5327
GLIM	Analyst 1	100.36	99.48	98.87	99.57	0.7490	0.7523	0.4324
	Analyst 2	99.47	98.58	100.38	99.47	0.9000	0.9047	0.5196

Table 8: Recovery Analysis of Formulation (Cd Pro 2) by Cramer's Rule Solution Method

Drug	Sample No.	Amount present (µg/ml)	Amount added (µg /ml)	Amount estimated* (µg /ml)	Amount recovered (µg/ml)	% Recovery*	S.D	% R.S.D	S.E.
MET	1 2 3	10.23 10.23 10.23	1 2 3	11.22 12.25 13.21	0.99 2.02 2.98	99.00 101.00 99.33	0.8754	0.0087	0.5054
					Mean	99.78			
ATR	1 2 3	9.2374 9.2374 9.2374	1.6 3.2 4.8	10.825 12.450 14.002	1.5876 3.2126 4.7650	99.22 100.39 99.27	0.6615	0.0066	0.3819
					Mean	99.63			
GLIM	1 2 3	2.2389 2.2389 2.2389	0.4 0.8 1.2	2.637 3.045 3.430	0.3981 0.8063 1.1913	99.52 100.78 99.27	0.8093	0.0081	0.4672
					Mean	99.85			

* Mean of Three Observations

Table 9: Quantification of Formulation (Cd Pro 2) By Tri-Linear Regression Calibration Method

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
MET	1 2 3 4 5 6	500 500 500 500 500 500	505.91 506.01 513.31 508.75 510.22 505.44	101.18 101.20 102.66 101.75 104.04 101.08	101.98	1.1676	0.0114	0.4766
ATR	1 2 3 4 5 6	10 10 10 10 10 10	9.82 10.26 10,06 10.03 10.15 9.83	98.20 102.60 100.60 100.30 101.50 98.30	100.25	1.7444	0.0174	0.7121
GLIM	1 2 3 4 5 6	2 2 2 2 2 2	1.91 1.98 2.01 2.01 1.98 1.99	99.48 99.00 100.5 100.5 99.49 99.89	99.81	0.6043	0.0060	0.2467

* Mean of six Observations

Table 10: Intra Day and Inter Day Analysis of Formulation (Cd Pro 2) by Tri-Linear Rgression Method

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
MET	1 2 3	500 500 500	101.19 101.46 101.14	101.22 101.13 101.46	0.1720	0.1940	0.0016	0.0019
Mean			101.26	101.27				
ATR	1 2 3	10 10 10	99.45 100.90 99.90	99.3 101.00 99.85	0.7421	0.8674	0.0074	0.0086
Mean			100.08	100.05				
GLIM	1 2 3	2 2 2	98.50 99.75 98.25	99.75 100.25 99.25	0.8036	0.5	0.0081	0.0050
Mean			98.83	99.75				

* Mean of Three Observations

Table 11: System suitability parameters for the optimized chromatogram by RP – HPLC

Parameters	Metformin Hydrochloride	Atorvastatin Calcium	Glimepiride
Tailing factor	1.64	1.31	1.03
Asymmetrical factor	1.86	1.33	1.06
Theoretical plates	2642	7157	9149
Resolution	Between MET and ATR = 11.16	Between ATR and GLIM = 7.97	

Table 12: Optical characteristics of atorvastatin calcium, metformin hydrochloride and glimepiride by RP – HPLC method

Parameters	Atorvastation	Metformin	Glimipride
λ_{max} (nm)	226	226	226
Beers law limit (µg/ml)	0.5 - 2.5	16 - 80	0.1 - 0.5
Correlation coefficient (r)	0.9997	0.9994	0.9995
Regression equation (y=mx+c)	y= 663830.5x + (-)22621.8	y=1140781 x + 2080939	y= 1229516x + (-)27419.4
Slope (m)	663830.5	1140781	1229516
Intercept (c)	(-)22621.8	2080939	(-)27419.4
LOD (µg/ml)	0.05709	1.01324	0.02159
LOQ (µg/ml)	0.17301	3.0704	0.0654
Standard Error	2623.73415	70850.23662	956.739868

Table 13: Quantification of formulation (cd pro 2) by RP-HPLC method

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
MET	1	500	527.77	105.55	106.33	1.0552	0.0099	0.4307
	2	500	541.88	108.37				
	3	500	530.22	106.04				
	4	500	530.95	106.19				
	5	500	532.09	106.41				
	6	500	527.53	105.50				
ATR	1	10	9.93	99.30	99.18	1.3556	0.0136	0.5534
	2	10	10.04	100.40				
	3	10	9.84	98.40				
	4	10	9.70	97.00				
	5	10	10.07	100.70				
	6	10	9.93	99.30				
GLIM	1	2	1.978	98.93	98.90	0.4016	0.0040	0.0170
	2	2	1.9465	97.33				
	3	2	1.9946	99.73				
	4	2	1.9964	99.82				
	5	2	1.9745	98.72				
	6	2	1.9777	98.88				

*Mean of six observations

Table 14: Recovery analysis of formulation (cd pro 2) by RP – HPLC method

Drug	Sample No.	Amount present (µg/ml)	Amount added (µg/ml)	Amount estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	S.D	% R.S.D	S.E.
MET	1 2 3	53.1675 53.1675 53.1675	8 16 24	61.4697 69.0831 77.2005	8.3022 15.9156 24.033	103.77 99.47 100.13	1.6374	0.0161	0.9453
ATR	1 2 3	1.0463 1.0463 1.0463	0.5 1.0 1.5	1.5344 2.0429 2.5533	0.4881 0.9966 1.507	97.62 99.66 100.46	1.4644	0.0147	0.8454
GLIM	1 2 3	0.1978 0.1978 0.1978	0.1 0.2 0.3	0.2984 0.3960 0.4963	0.1006 0.1982 0.2985	100.6 99.10 99.5	0.7767	0.0078	0.4484

* Mean of Three Observations

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