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Hplc Method Development and Validation for the Simultaneous Estimation of Pitavastatin and Telmisartan

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ABSTRACT

Method validation of both the compounds was done on the basis of ICH guidelines. Validation by HPLC method the wavelength was selected at the isobestic point at which the two drugs can be detected using UV detectors. The selected wavelength was 250nm. Optimized liquid chromatographic condition was obtained by performing many trials for the selection of mobile phase and column, for the separation of Pitavastatin & Telmisartan. The method development was conducted with C18, 250 × 4.6 mm, 5μm particle size, Phenomenex with the flow rate of 1.0mL/min. the optimized mobile phase conditions were Acetonitrile and 10mM Ammonium acetate buffer containing 0.1% formic acid in the ratio of 65:35 v/v. Data of Simultaneous shows retention time for Pitavastatin 5.408 & Telmisartan was 7.183. The method found to be linear, accurate, rugged and robust for validated parameters. The linearity range was determined by external standard calibration method in the concentration range of 10μg/ml to 60μg/ml. The amount of recovery was calculated as 98% – 101% and it was observed that all the values are within the limits. Further the precision of the method was confirmed by the repeatable analysis of sample. The results were found to be precise due to low values of the %RSD. It indicated that the method has good precision. Limit of quantification for Pitavastatin & for Telmisartan 1.554μg/ml and 4.709μg/ml respectively. Similarly limit of detection for Pitavastatin & for Telmisartan 0.647μg/ml and 1.959μg/ml respectively. In the robustness study %RSD obtained for change of flow rate and wavelength and ruggedness for change of analyst was found to be below 2, which was within the acceptance criteria. So, simple, sensitive, accurate, precise RP- HPLC methods were developed and validated for the simultaneous estimation of Pitavastatin & Telmisartan.

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Keywords: Pitavastatin, Telmisartan, Acetonitrile HPLC grade, Validation, HPLC method, Simultaneous estimation, Method validation

Introduction

Analytical chemistry is a branch of chemistry which deals with identification of components (qualitative) and determination of quantity of components (quantitative) of substances or samples or mixture. There are two types of analysis, one is qualitative analysis and another one is quantitative analysis. In qualitative analysis, there is identification of components or analyte of mixture or sample is carried out. In quantitative analysis, there is determination of amount of components or analyte of mixture or sample is carried out [1]. Analytical data is required not only in chemistry but also in other sciences like biology, zoology, arts such as painting and sculpture, archaeology, space exploration and clinical diagnosis. Important areas of application of analytical chemistry are quality control in manufacturing industries, monitoring and control of pollutants, clinical and biological studies, geological assays, fundamental and applied research [2]. Analytical chemistry is the study of separation, quantification and chemical components identification of natural and artificial materials constituted with one or more compounds or elements. Analytical chemistry is separated into two main categories, qualitative analysis that is to say the identification with regard to the chemical components exist in the sample, whereas quantitative analysis estimates the amount of certain element or compound in the substance i.e., sample. Pharmaceutical analysis plays a very outstanding role in the examination of pharmaceutical formulations and bulk drugs regarding the quality control and assurance [3-5]. Rapid increase in pharmaceutical industries and production of drug in and around the world bring forward a rise in inevitable demand to seek novel and systematic analytical techniques in the pharmaceutical industries. As a consequence, analytical method development has become the basic activity of analysis. Development in scientific and concrete analytical methods has been resulted from the advancements of analytical instruments [6].

The improvements of the analytical method development and analytical instruments have reduced the time and cost of analysis and enhanced precision and accuracy [7]. Techniques pertaining to analysis are developed and validated for active pharmaceutical ingredients, excipients, related substances, drug products, degradation products and, residual solvents, etc. Resulting which become an integral part of the required necessities for regulatory organization [8].

Analytical Method Development

When there are no authoritative methods are available, new methods are being developed for analysis of novel products. To analyze the existing either pharmacopoeial or non-pharmacopoeial products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits [9].

Analytical methods must be used inside GMP and GLP environments and should be developed by using the given protocols and acceptance criteria in the ICH guidelines Q2 (R1) [10,11].

The requirements for method development are as follows:

- Qualified analysts
- Instruments-qualified and calibrated
- Documented methods
- Reliable reference standards
- Sample selection and integrity
- Change control

Analytical method development is useful for:

- New process and reactions
- New molecule development
- Active ingredients (Macro analysis)
- Residues (Micro analysis)
- Impurity profiling
- Degradation studies
- Herbal products

Materials and Equipments

Table 1: List of Instruments

S. No.	Instruments	Manufacturer
1	UV/VIS Spectrophotometer,	Shimadzu, Japan
2	Digital Weighing balance, (CY220)	Shimadzu, Japan
3	RP-HPLC instrument equipped with PDA detector	Shimadzu, Japan
4	Ultrasonicator	PCi analytics, India
5	Vortex mixer	Remi Scientific Instruments, Mumbai
6	Hot air oven	P. L. Tandon & Co, Delhi
7	Melting Point Apparatus	Remi Scientific Instruments, Mumbai
8	Infrared red spectrophotometer (FTIR)	Bruker Alpha, Berlin, Germany
9	Microcentrifuge	Remi Scientific Instruments, Mumbai
10	Vacuum pump	Suguna single phase, Chennai, India
11.	Nylon 0.22 µm membrane filter	Pall corporation, Mumbai

Table 2: List of Chemicals

S. No	Materials	Source
1	Pitavastatin	Wellona Pharma, Gujara
2	Telmisartan	Dr. Reddys Laboratories, India
3	Methanol	Fisher Scientific India Pvt. Ltd.
4	Ammonium acetate	Merck, Mumbai
5	Acetonitrile HPLC grade	Merck, Mumbai
6	Formic acid	Fisher Scientific India Pvt. Ltd.

Pre-Formulation Studies

Organoleptic Properties [48,49]

The organoleptic studies like general appearance like nature, color, odor etc. were performed by visual observations.

Color: Small quantity of drug was taken in butter paper and viewed in well illuminated place[12-29].

Odor: Very less quantity of drug was smelled to get the odor [30-35].

Preparation of Calibration Curve [30,35,39,41]

Preparation of Stock Solution of Pitavastatin & Telmisartan in Methanol

Stock solutions (1000 µg/ml) of Pitavastatin & Telmisartan were prepared separately 10mg of each drug in 10 ml methanol was transferred into volumetric flask, then further taken 0.1ml from above each stock solution and put in 10ml volumetric flasks and volume adjusted with methanol up to mark and sonicated for 5 min [39-41]. Absorbance of Pitavastatin & Telmisartan solutions were recorded at 244nm and 296nm against methanol for simultaneous estimation using UV-visible spectrophotometer. The scanning for solution of Pitavastatin & Telmisartan were carried out in the range of 200-400nm against methanol as blank for obtaining the overlain spectra that as used in the analysis [42-51].

Hplc Method [52, 53]

Selection of Detection Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study the Pitavastatin & Telmisartan solution of 10µg/ml was prepared in Methanol and scanned in the UV region of 200-400 nm and a spectrum was recorded.

Selection of Mobile Phase and Optimization of Chromatographic Condition

➢ **Chromatographic conditions**

- **Stationary Phase:** C18, 250 × 4.6 mm, 5µm particle size, Phenomenex
- **Elution mode:** Low pressure gradient mode (65:35 v/v)
- **Mobile phase:** Solvent A was Acetonitrile, Solvent B was 10mM Ammonium acetate buffer containing 0.1% formic acid.
- **Detector:** UV
- **Absorption maxima:** 250 nm
- **Column Temperature:** 30 ^{o}C
- **Flow rate:** 1ml/min.
- **Injection volume:** 10 µl
- **Diluent:** Methanol
- **Run time:** 10 minutes

Standard Stock Solution Preparation (1000 Mg/Ml)

Blank

Diluent was filtered through 0.22 µ millipore membrane filters and injected in HPLC system.

Standard Solution Preparation

An accurately weighed quantity of about 10 mg of Pitavastatinand 10mg of Telmisartan were taken in volumetric flask and dissolved in 10 ml of diluents (as mentioned above) to obtain a solution of 1000 µg/ml as stock and sonicate to dissolve.

Preparation of Calibration Curve of Pitavastatin & Telmisartan

From the standard stock solution, 0.1 ml was pipette out in 10ml volumetric flask and 10ml dilution are made with diluent to obtain working standard solution of concentration ranges from 10µg/ml to 60µg/ml and filtered through 0.22 µ millipore membrane filters and injected in HPLC system. HPLC Chromatogram was recorded of each concentration and the calibration curve was plotted (area vs. concentration). The regression equation and correlation coefficient were obtained

Hplc Chromatogram of Standard

On HPLC analysis of standard, chromatogram was optimized in which Retention time of drugs.

Results and Discussions

Result of Preformulation Study of Drugs

Organoleptic Properties

Organoleptic properties of drug Pitavastatin & Telmisartan found to be as per I.P. monograph. The Organoleptic properties of Pitavastatin & Telmisartan were found to the given table 7.1.

Table 3: Organoleptic Properties of Pitavastatin & Telmisartan

Sr. no.	Properties	Inferences of Pitavastatin	Inferences of Telmisartan
1.	Colour	White to off-white powder,	A white to off-white powder
2.	Odour	Odourless	Odourless
3.	Form	Crystalline	Crystalline

Melting Point [26-28]

Table 4 : Melting Point of Pitavastatin & Telmisartan

Drug	Reference M.P.	Observed M.P.
Pitavastatin	138-139 °C	138-141°C
Telmisartan	261-263 °C	260-262°C

Discussion: The melting point of Pitavastatin & Telmisartan were found to be in range 138-141°C & 260-262°C which is of the pure drug. Hence drug samples were free from any type of impurities.

Ftir of Pitavastatin

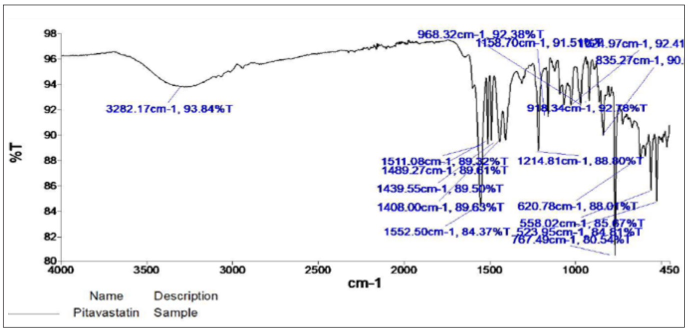


Figure 1: FTIR spectrum of Pitavastatin

Table 5: FTIR interpretation of Pitavastatin

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
O-H stretching	3350.30	3282.17
C=O stretching	1554.46	1599.91
C=C bending	1411.06	1409.82

The FTIR spectra of Pitavastatin were shown in the Figure 1; Table 4. The principal IR absorption peaks of Pitavastatin at 3282.17cm^{-1} (O-H stretching), 1599.91cm^{-1} (C=O stretching), and 1409.82cm^{-1} (C=C bending), were all observed in the spectra of Telmisartan. These observed principal peaks. This observation confirmed the purity and authenticity of the Pitavastatin [57].

Ftir of Telmisartan

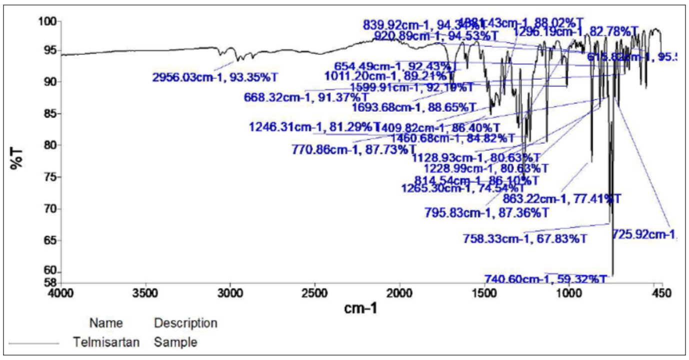


Figure 2: FTIR spectrum of Telmisartan

Table 6: FTIR interpretation of Telmisartan

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
O-H Stretching	3059.10	2956.03
C=Stretching	1693.50	1693.68
C-N Stretching	1278.81	1265.30
CH3 Bending	1342.46	1381.43

The FTIR spectra of Telmisartan were shown in the Figure 2; Table 6 . The principal IR absorption peaks of Telmisartan at 2956.03cm^{-1} (O-H Stretching), 1693.68cm^{-1} (C=Stretching), 1265.30cm^{-1} (C-N Stretching), and 1381.43 cm^{-1} (CH3 Bending), were all observed in the spectra of Telmisartan. These observed principal peaks. This observation confirmed the purity and authenticity of the Telmisartan [58].

FTIR of Pitavastatin & Telmisartan

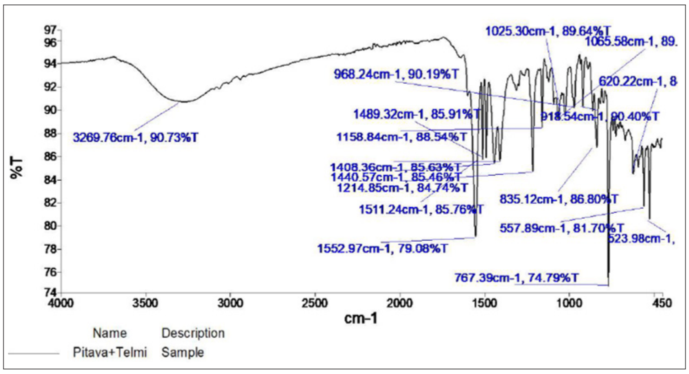


Figure 3: FTIR spectrum of Pitavastatin & Telmisartan

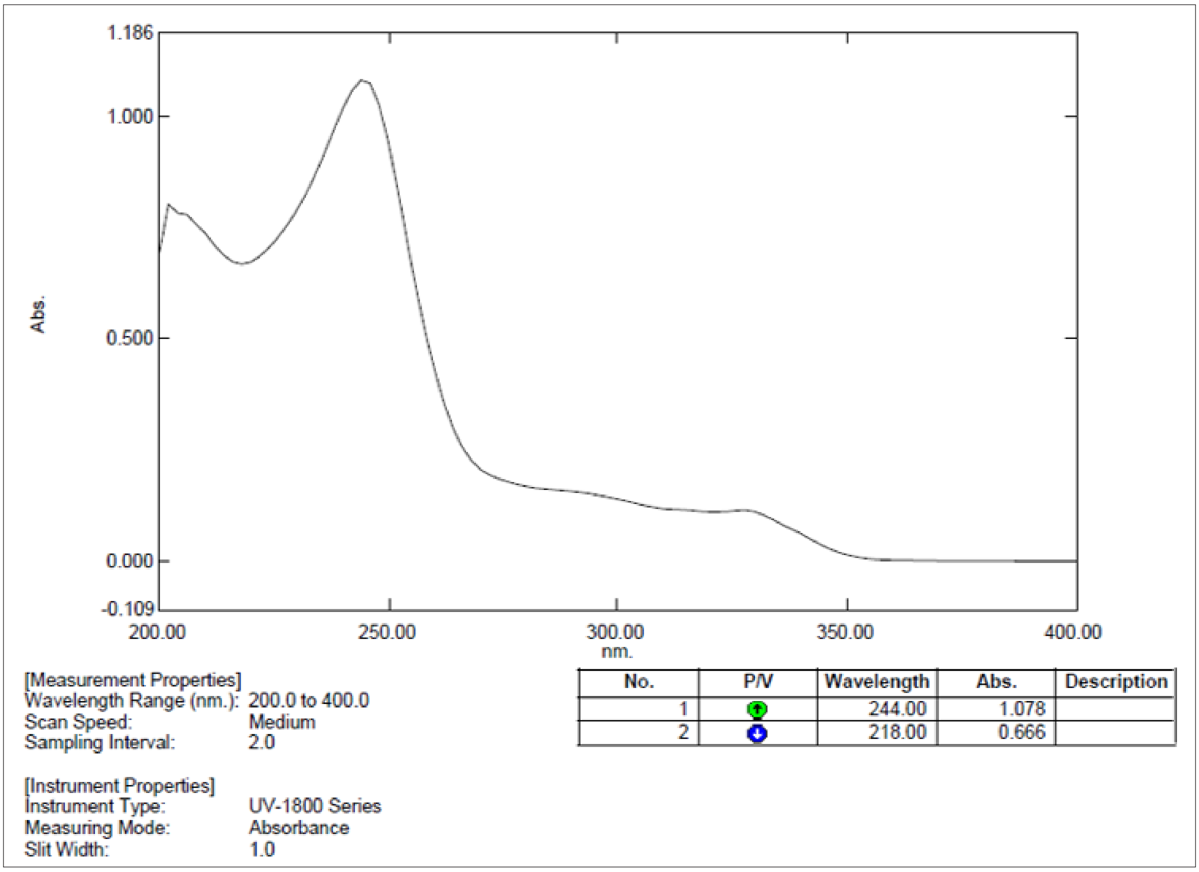
Table 7: FTIR interpretation of Pitavastatin & Telmisartan

Characteristics Peaks	Reported (cm^{-1})	Observed (cm^{-1})
O-H stretching	3282.17	3269.76
C=O stretching	1599.91	1552.97
C=C bending	1409.82	1408.36
C-N Stretching	1265.30	1214.85
CH3 Bending	1381.43	1408.36

The FTIR spectra of Pitavastatin & Telmisartan were shown in the Figure 3; Table 7 The principal IR absorption peaks of Pitavastatin & Telmisartan at 3269.76cm^{-1} (O-H stretching), 1552.97 cm^{-1} (C=O stretching), 1408.36cm^{-1} (C=C bending), 1214.85cm^{-1} (C-N Stretching), and 1408.36cm^{-1} (C-N Stretching) were all observed in the spectra of Pitavastatin & Telmisartan. These observed principal peaks. This observation confirmed the purity and authenticity of the Pitavastatin & Telmisartan.

UV Spectroscopy

Determination of Absorption Maxima of Pitavastatin & Telmisartan in Methanol

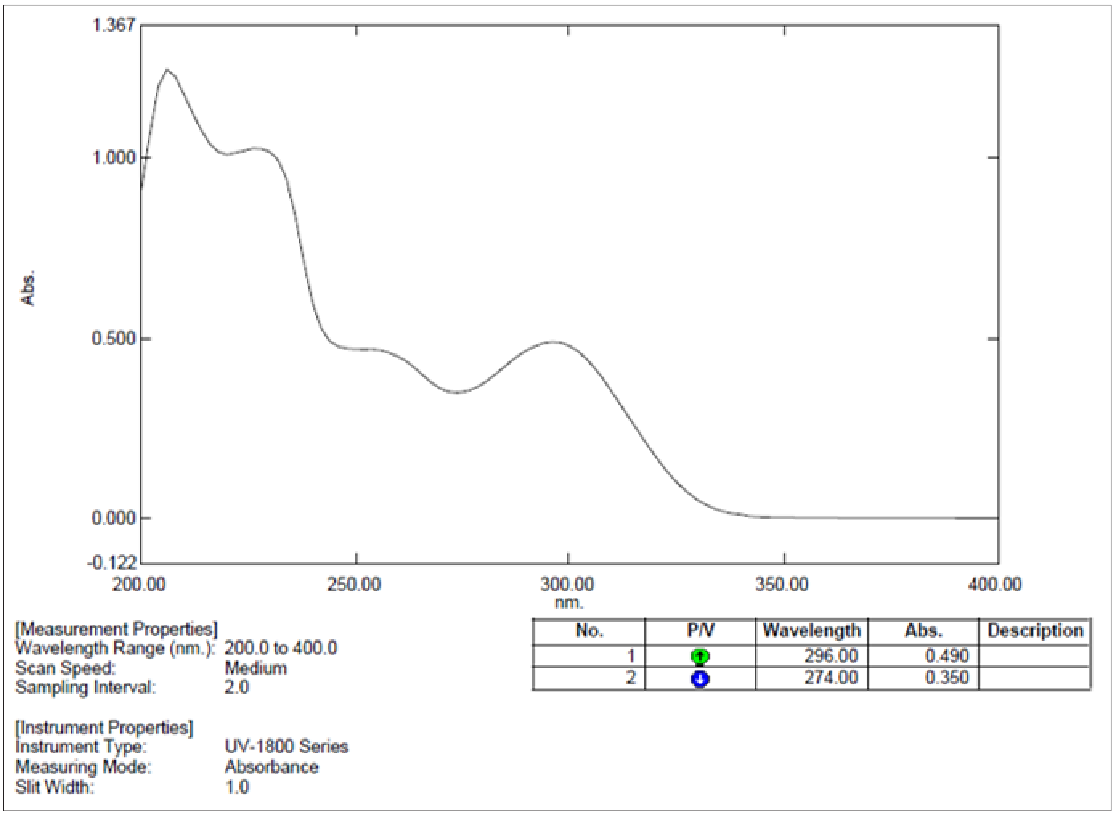


[Measurement Properties]
Wavelength Range (nm.): 200.0 to 400.0
Scan Speed: Medium
Sampling Interval: 2.0

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0

No.	P/V	Wavelength	Abs.	Description
1	↑	244.00	1.078	
2	↓	218.00	0.666	

Figure 4: UV Spectrum of Pitavastatin



[Measurement Properties]
Wavelength Range (nm.): 200.0 to 400.0
Scan Speed: Medium
Sampling Interval: 2.0

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0

No.	P/V	Wavelength	Abs.	Description
1	↑	296.00	0.490	
2	↓	274.00	0.350	

Figure 5: UV Spectrum of Telmisartan

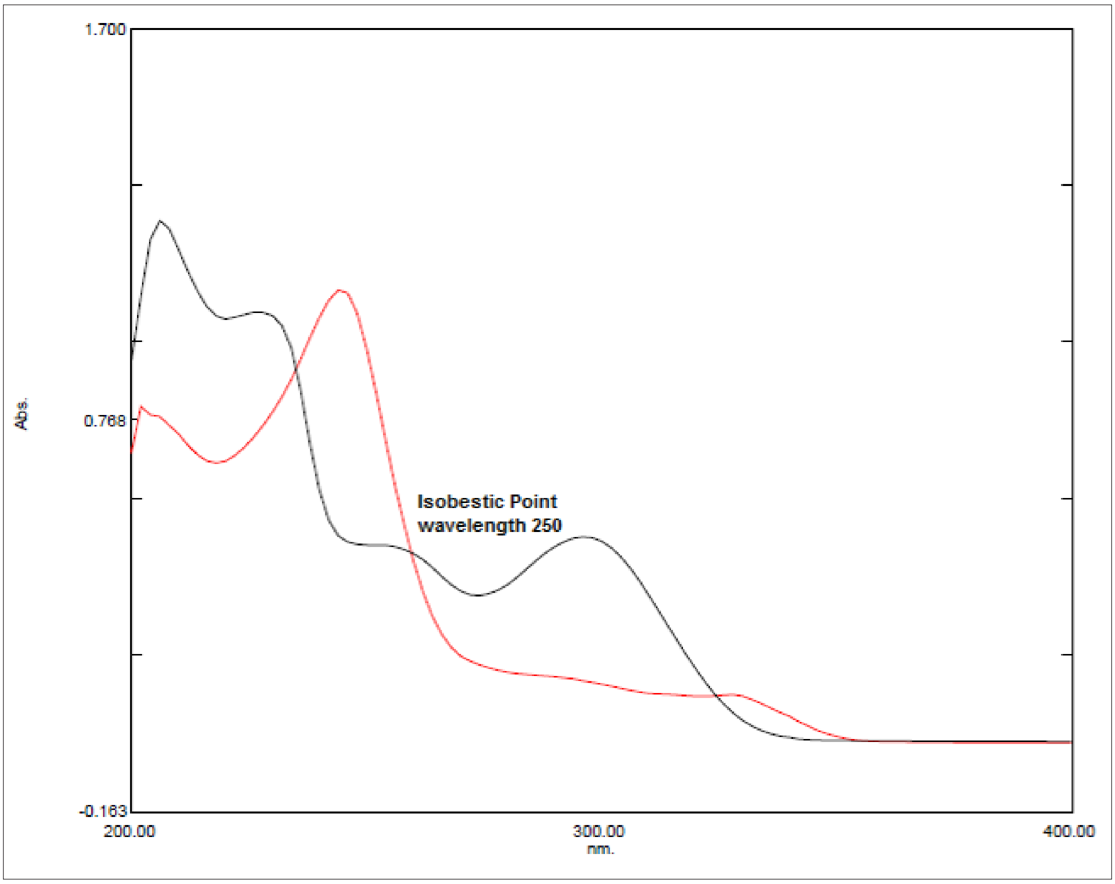


Figure 6: Overlain UV Spectrum of Pitavastatin & Telmisartan in Methanol

A solution of Pitavastatin (10 µg/mL) and Telmisartan (10 µg/mL) were scanned over the UV range from 400 nm to 200 nm and the λmax of Pitavastatin & Telmisartan were found as 244nm and 296nm, respectively. Overlain spectra of Pitavastatin & Telmisartan sodium are showing Isosbestic point at 250nm. Absorption maxima of Pitavastatin & Telmisartan and an overlain spectrum of both the drugs are shown in Figure 4-6.

Hplc Method

Determination of Chromatogram of Blank & Standard (Pitavastatin & Telmisartan)

On HPLC analysis of Blank and standard solution of Pitavastatin & Telmisartan solution of 40µg/ml and 40µg/ml chromatogram was optimized & analyzed as per the proposed method. HPLC analysis of blank and standard chromatogram was shown in Figure 7 & 8.

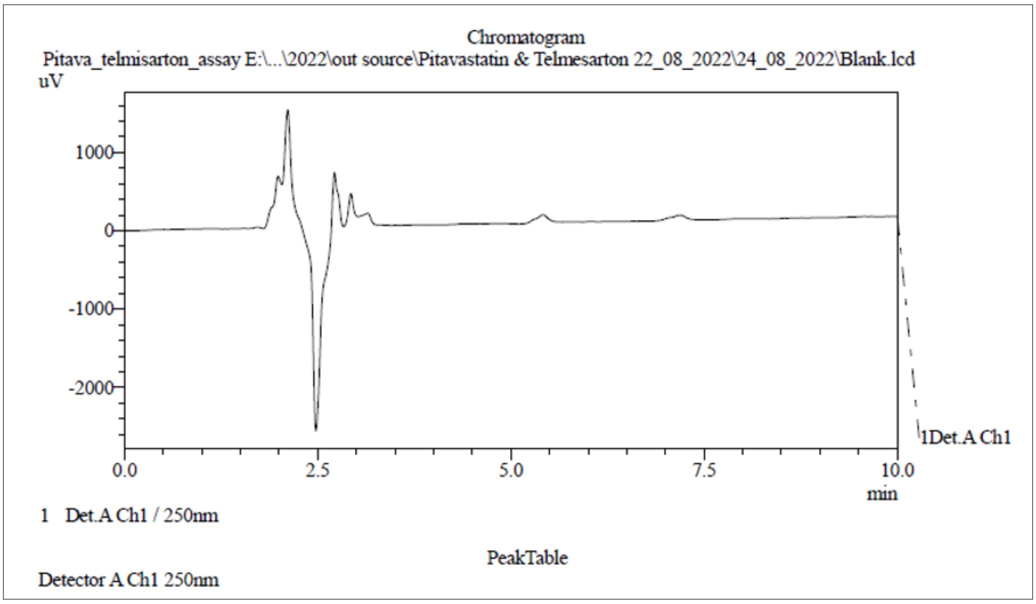


Figure 7: Chromatogram of Blank

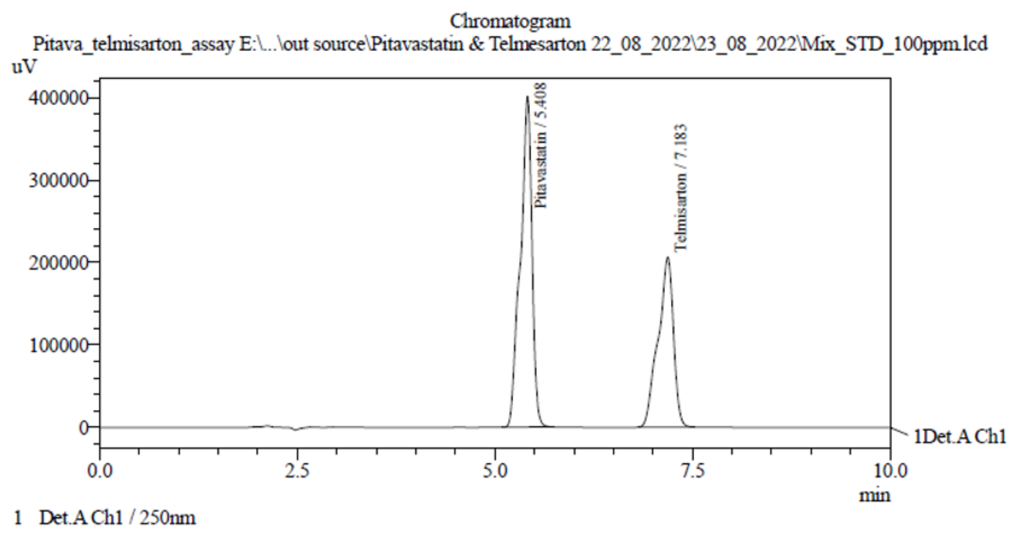


Figure 8: Simultaneous chromatogram of standard Pitavastatin & Telmisartan solution of 40µg/ml and 40µg/ml.

Table 8: Data of Simultaneous Pitavastatin & Telmisartan solution of 40µg/ml and 40µg/ml

S.No.	Compound Name	Retention time	Area
1	Pitavastatin	5.408	4106562
2	Telmisartan	7.183	2777737

Preparation of standard curve of Pitavastatin by RP-HPLC

Table 9 : Calibration curve of Pitavastatin

Sr. No.	Concentration µg/ml	Area
1	10	540745
2	20	1123978
3	30	1632150
4	40	2143217
5	50	2649436
6	60	3229613

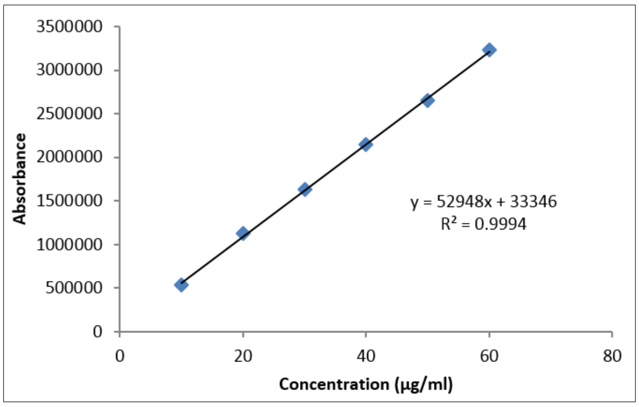


Figure 9: Graph of standard calibration curve of Pitavastatin by RP-HPLC

Table 10: Result of Statistical parameters for estimation of Pitavastatin

Statistical parameters	Results
Regression equation: y=mx+C	Y= 52948x + 33346
Slope (m)	52948
Intercept (C)	33346
Correlation coefficient (r^2)	0.999

Discussion: The calibration curve for Pitavastatin was obtained by using the 10 to 60 µg/ml solution. The area was measured at 250 (Isosbestic point). The calibration curve as shows in graph indicated the regression equation Y = 52948x + 33346 and R^2 value 0.999 which shows good linearity as shown in Figure 9.

Preparation of Standard Curve of Telmisartan by Rp-Hplc

Table 11: Calibration curve of Telmisartan by RP-HPLC

Sr. No.	Concentration µg/ml	Area
1	10	335668
2	20	685539
3	30	982061
4	40	1285405
5	50	1623734
6	60	1953174

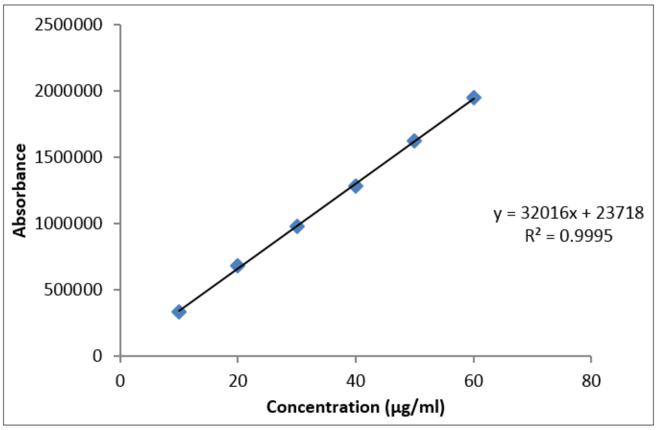


Figure 10: Graph of standard calibration curve of Telmisartan by RP-HPLC

Table 12: Result of Statistical parameters for estimation of Telmisartan

Statistical parameters	Results
Regression equation: y=mx+C	Y= 32016x + 23718
Slope (m)	32016
Intercept (C)	23718
Correlation coefficient (r2)	0.999

Discussion: The calibration curve for Telmisartan was obtained by using the 10 to 60µg/ml solution. The area was measured at 250 (Isosbestic point). The calibration curve as shows in graph indicated the regression equation Y = 32016x + 23718 and R2 value 0.999 which shows good linearity as shown in Figure 10.

Validation of Hplc Method as Per Ich Guidelines

Linearity

Linearity of Pitavastatin

A calibration curve was plotted over a concentration range of 10 to 60µg/ml for Pitavastatin. Accurately measured working stock solution of Pitavastatin (10, 20, 30, 40, 50 and 60ml) and all the dilutions were filtered through 0.22 µ filter and injected. The area of all solution was taken at their respective wavelength. The Linearity was constructed by plotting concentration against area where each reading.

Table 13 : Linearity of Pitavastatin

Conc. (µg/ml)	Area-1	Area-2	Area-3
10	539594	549269	533373
20	1125276	1124061	1122598
30	1638088	1645828	1612535
40	2132147	2110760	2186745
50	2649254	2647123	2651931
60	3238597	3218174	3232067

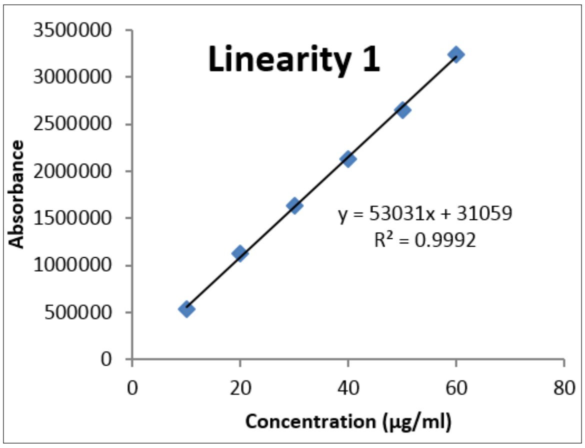


Figure 11: Linearity 1 graph of Pitavastatin

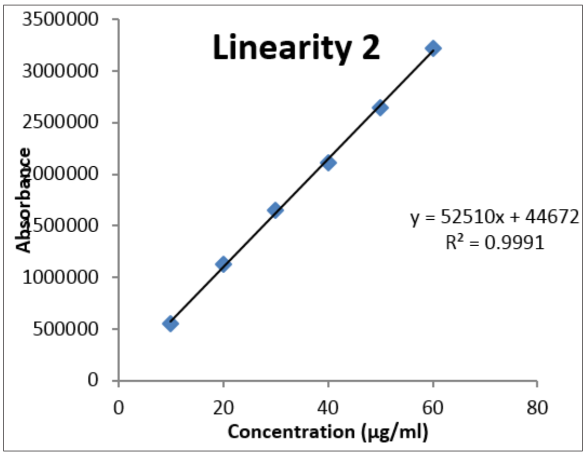


Figure 12: Linearity 2 graph of Pitavastatin

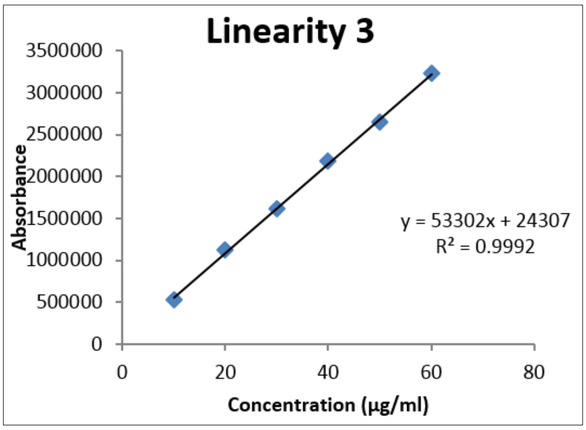


Figure 13: Linearity 3 graph of Pitavastatin

Linearity of Telmisartan

A calibration curve was plotted over a concentration range of 10 to 60µg/ml for Telmisartan. Accurately measured working stock solution of Telmisartan (10, 20, 30, 40, 50 and 60ml) and all the dilutions were filtered through 0.22 µ filter and injected. The area of all solution was taken at their respective wavelength. The Linearity was constructed by plotting concentration against area where each reading.

Table 14 : Linearity of Telmisartan

Conc. (µg/ml)	Area-1	Area-2	Area-3
10	331587	340541	334877
20	684112	689479	683027
30	989812	979853	976517
40	1280884	1283494	1291837
50	1619183	1621107	1630913
60	1974401	1915152	1969968

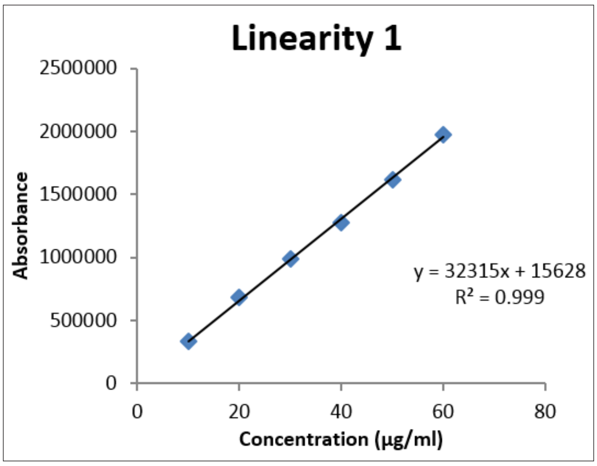


Figure 14: Linearity 1 graph of Telmisartan

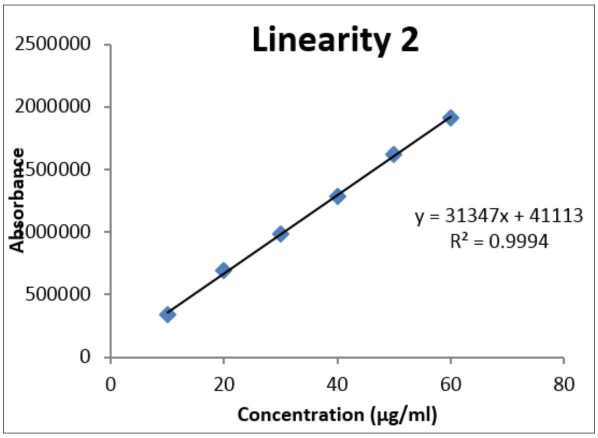


Figure 15: Linearity 2 graph of Telmisartan

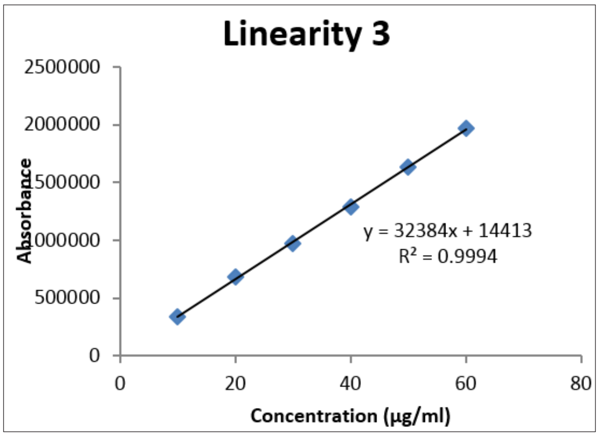


Figure 16: Linearity 3 graph of Telmisartan

Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the 3 concentration levels 80%, 100% and 120% in pre-analyzed sample. In this method the known concentration of standard drug was added to the assay sample.

Table 15: Accuracy Study of Pitavastatin

Level	Amount added of Pitavastatin	Percentage recovery of Pitavastatin	% RSD
80%	20(µg/ml)	100.538	0.488
100%	40(µg/ml)	101.336	1.233
120%	60(µg/ml)	101.893	1.483

Table 16: Accuracy Study of Telmisartan

Level	Amount added of Telmisartan	Percentage recovery of Telmisartan	% RSD
80%	20(µg/ml)	102.87	0.171
100%	40(µg/ml)	98.232	0.898
120%	60(µg/ml)	101.637	0.703

The results indicate that the recoveries are well within the acceptance range of 98% – 101%, indicating a good degree of sensitivity of the method towards detection of analytes in sample. Therefore, method is accurate and it can be used for the estimation of drug.

Precision

Standard solution of Pitavastatin & Telmisartan was prepared and analyzed as per the proposed method.

Table 17: Repeatability and inter-intraday precision study

S.no.	Precision	Percentage recovery of Pitavastatin	% RSD	Percentage recovery of Telmisartan	% RSD
1	Repeatability	99.542	0.426	98.859	0.534
2	Inter Day	97.509	0.829	99.919	0.902
3	Intra Day	100.091	0.592	101.345	0.508

The method was found to be precise due to low values of the %RSD.

Lod and Loq

Table 18: LOD and LOQ data

Drug	LOD (µg/ml)	LOQ (µg/ml)
Pitavastatin	0.647	1.554
Telmisartan	1.959	4.709

The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The results obtained were within the limit.

Conclusion

The work carried out during entire our study includes literature survey of the selected drug, selecting the scheme which had to be followed, the methods and protocols which were to be set as per the scheme, working conditions and analyzing the results and calculations. It was concluded that the proposed new RP-HPLC method & UV spectroscopy developed for the quantitative determination of Pitavastatin & Telmisartan was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. UV Spectrophotometric method and RP-HPLC method was developed for simultaneous estimation of Pitavastatin & Telimisartan. The method was validated according to ICH Guidelines.

Preformulation studies were carried out to characterize the chemical and physical properties of drug substance. Organoleptic properties of drug Pitavastatin & Telmisartan found to be as per I.P. monograph. The melting point of both drugs was determined by capillary method which complies with the melting point given in reference. The melting point of Pitavastatin & Telmisartan were found to be in range 138-141°C & 260-262°C which is of the pure drug. The FT-IR spectrum of drug samples was found to be in concordant with the reference chemical groups present in the structure of the Pitavastatin & Telmisartan. The standard curves of Pitavastatin & Telmisartan in RP-HPLC were prepared and the absorbance data obtained subjected to linear regression. The calibration curve for Sulfamethoxazole was obtained by using the 10 to 60µg/ml solution. The area was measured at 250 (Isosbestic point). The calibration curve as shows in graph indicated the regression equation Y = 52948x + 33346 and R2 value 0.999 which shows good linearity. The calibration curve for Trimethoprim was obtained by using the 10 to 60µg/ml solution. The area was measured at 250 (Isosbestic point). The calibration curve as shows in graph indicated the regression equation Y = 32016x + 23718 and R2 value 0.999 which shows good linearity.

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