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Analytical Method Development And Validation For Simultaneous Estimation Of Atorvastatin And Aspirin In API

And Marketed Formulation By Using U.V-Visible Spectroscopy

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ABSTRACT

A specific, rapid and simple UV-Visible Spectrophotometric method with good sensitivity was developed and validated for the simultaneous estimation of atorvastatin and aspirin in standard solutions and tablets. The method employed solving of simultaneous equations based on the measurement of absorbance of two wavelengths, 295nm and 294nm for Atorvastatin and Aspirin, respectively. The calibration was linear for both the drugs in a concentration range of 10 to 80µg/ml and correlation coefficient provide the desired information on linearity. The results of analysis were validated statistically that included parameters such as Precision, LOD, LOQ, Recovery, Robustness & results were found in the acceptance criteria. It can be concluded from the results that present method for the simultaneous determination of atorvastatin and aspirin in tablets is specific, accurate, rapid and precise.

KEYWORDS: UV-Visible Spectrophotometric method, Atorvastatin, Aspirin, validation, Limit of Detection, Limit of Quantification

INTRODUCTION

Atorvastatin (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxy monocarboxylic acid is a member of the drug class known as statins, used primarily for lowering blood cholesterol and for preventing cardiovascular diseases. It has a role as an environmental contaminant and a xenobiotic. It is an aromatic amide, a member of monofluorobenzenes, a statin (synthetic), a dihydroxy monocarboxylic acid and a member of pyrroles. It is functionally related to a heptanoic acid[1-2].

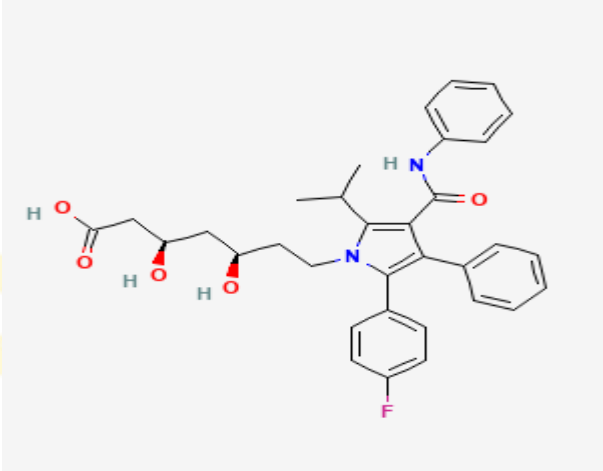

Fig 1: Molecular structure of Atorvaststin

Aspirin (2-(acetoxy) benzoic acid) is the prototypical analgesic used in the treatment of mild to moderate pain. It has anti-inflammatory and antipyretic properties and acts as an inhibitor of cyclooxygenase which results in the inhibition of the biosynthesis of prostaglandins. Acetylsalicylic acid also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis[3-4].

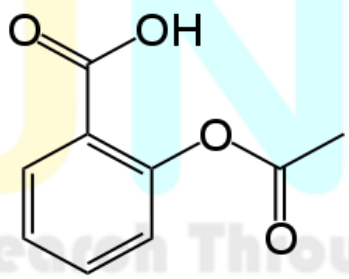

Fig 2: Molecular structure of Aspirin

A detailed literature revealed that several analytical methods have been reported for the determination of Atorvastatin and Aspirin in pharmaceutical dosage form. The aim was to develop a specific, rapid, sensitive, and accurate UV spectroscopy method which can estimate the two compounds simultaneously. The present investigation describes a specific, sensitive and rapid method for simultaneous estimation of Atorvastatin and Aspirin in tablet dosage form. It aims with the developed and validated method for quantification of Atorvastatin and Aspirin in combination of tablet or capsule dosage form and in API(Active Pharmaceutical Ingredient)[5-8].

MATERIALS AND METHODS

MATERIALS:

S.No	Instruments And Glasswares	Model
1	UV- SPECTROSCOPY	Lab india, uv-vis 3000
2	pH meter	Systronics
3	Weighing machine	Shimadzu
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table 1 :- Instruments and glasswares used

CHEMICALS USED:

S.No	Chemical	Brand names
1	Aspirin	Acitophen
2	Atorvastatin	Lipitor
3	Water for uv	Double distilled
4	Methanol for uv	Merck

Table 2:- Chemicals used

METHOD DEVELOPMENT AND OPTIMIZATION

SELECTION OF SOLVENTS:

The solvents for the experiment were selected based on the solubility test and results of both drugs. The solubility tests were performed using common solvents like Acetonitrile, Water, 10% Sodium Hydroxide, Ethylalcohol, Chloroform, Diluted Ammonia and Methanol. From the solubility studies, methanol selected as common solvent for Aspirin and Atorvastatin.

DETERMINATION OF WAVELENGTH

PREPARATION OF STANDARD SOLUTION OF ASPRIN AND ATORVASTATIN:

25mg of Atorvastatin standard was weighed accurately and transferred into 25ml volumetric flask, and the required amount of methanol was added to dissolve. Then the volume was made upto the mark with methanol.

1ml of this solution was transferred into 10ml volumetric flask and the volume is made upto the mark with water. 1ml of this solution was transferred into 10 ml volumetric flask and volume is made upto mark with water. The solution is marketed as working standard solution of atorvastatin.

In the similar way, 25mg of Aspirin was transferred into 25ml volumetric flask and required amount of methanol was added to dissolve. Then volume is made upto the mark with methanol. 1ml of above solution was transferred into 10ml volumetric flask and volume was made upto the mark with water. From this solution 1ml was transferred into 10ml volumetric flask and made upto mark with water. The solution is marked as working standard solution of aspirin.

PREPARATION OF BLANK SOLUTION: Distilled Water is used as blank solution.

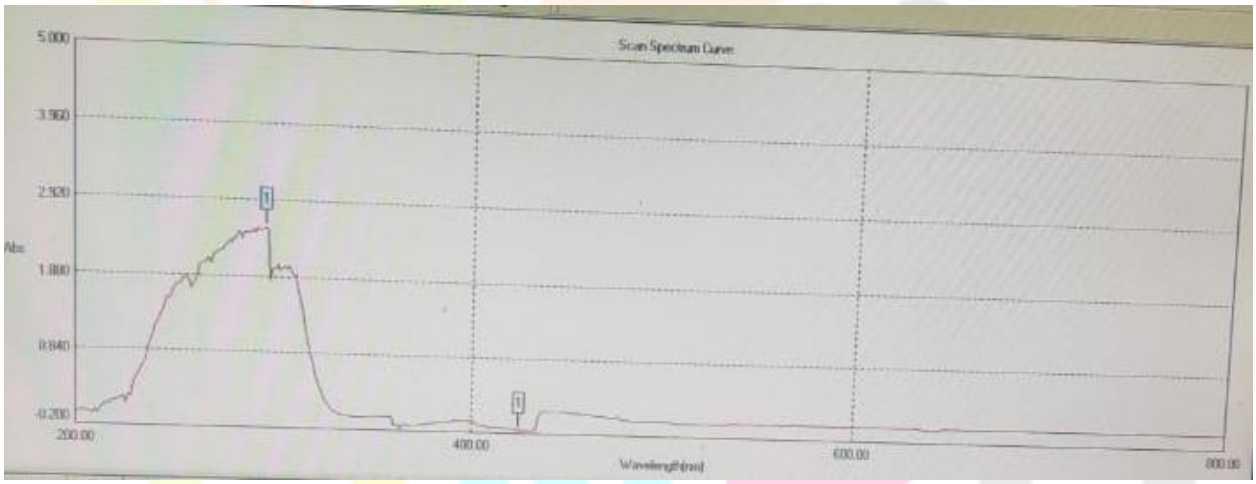

Fig 3 : UV-Visible spectrum of Atorvastatin

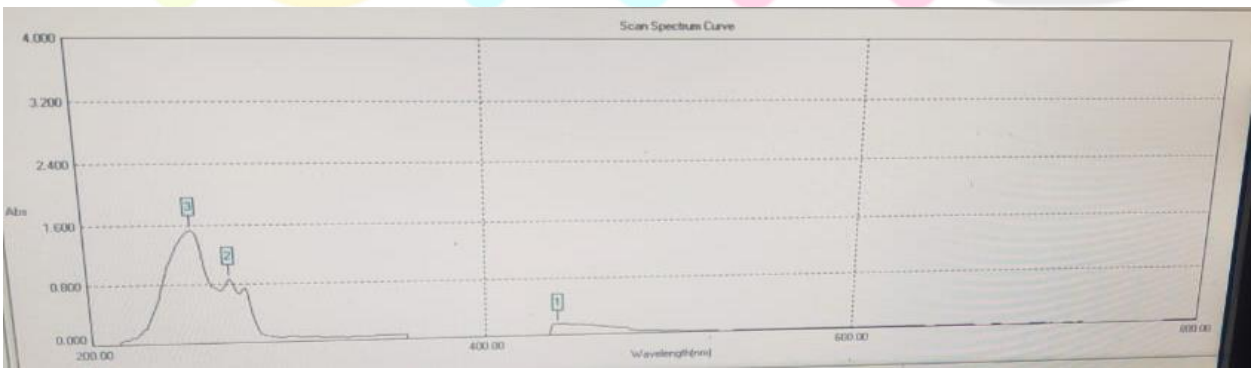

Fig 4 : UV-Visible spectrum of Aspirin

ASSAY:

PREPARATION OF STANDARD SOLUTION: In order to prepare stock solutions, 25 mg of Aspirin and 25 mg of Atorvastatin were accurately weighed into two separate volumetric flasks, and diluted to 25ml with methanol. Standard solution was prepared by further diluting 1ml stock solution with 100ml distilled water to obtain 10 µg/ml concentration of Aspirin and 10 µg/ml of Atrovastatin.

PREPARATION OF SAMPLE SOLUTION: 10 tablets were weighed and powdered, quantity of sample powder containing equivalent to 10 mg of atorvastatin and 75 mg of aspirin was transferred to 100 ml volumetric flask, in which 75 ml of methanol solution was added, and sonicated for 15 minutes. A 10 ml of filtrate was further diluted to 100ml with distilled water to get final concentration of about 47µg/ml of atorvastatin and 35µg/ml of aspirin.

METHOD VALIDATION

VALIDATION PARAMETERS: These include:

- System suitability
- Linearity
- Precision
- Accuracy
- Limit of detection and quantification
- Robustness
- Ruggedness

SYSTEM SUITABIALITY: The system suitability parameters were evaluated by calculating the % RSD (should be <or = 1) of absorbance of Atorvastatin and Aspirin (30µg/ml) at 295nm and 294nm respectively for 6 times.

LINEARITY: Accurately weigh and transfer 25 mg of Aspirin, 25 mg of Atorvastatin working standard into a 25ml of clean dry volumetric flasks add about 10mL of methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

LINEARITY RANGE (1-90 µg/ml)

S.No.	Concentration (µg/ml)	Atorvastatin Abs	Aspirin Abs
1.	10 µg/ml	0.024	0.006
2.	20 µg/ml	0.033	0.009
3.	30 µg/ml	0.045	0.012

4.	40 µg/ml	0.054	0.016
5.	50 µg/ml	0.061	0.021
6.	60 µg/ml	0.066	0.026
7.	70 µg/ml	0.083	0.032
8.	80 µg/ml	0.082	0.036
9.	90 µg/ml	0.109	0.042

Table 3 :- Linearity range

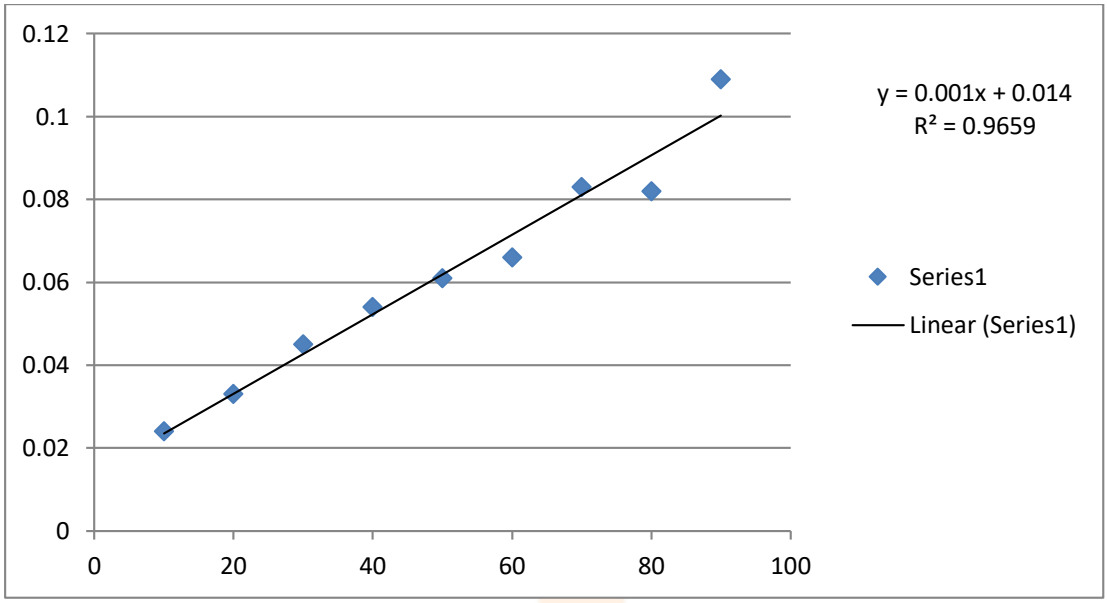

Fig 5 : Linearity Curve of Atorvastatin

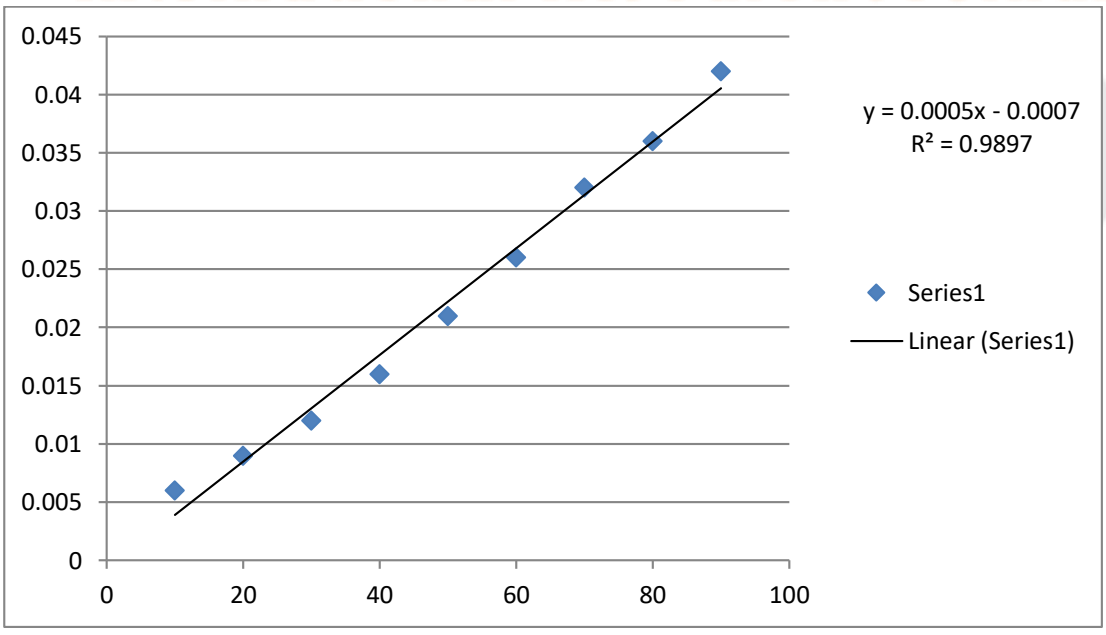

Fig 6 : Linearity Curve of Aspirin

PRECISION: The precision was studied at the levels of intra-day precision and intermediate precision (inter-day) .

INTRA – DAY PRECISION: Mixed standard solution of Atorvastatin and Aspirin (1µg/ml) was prepared and observed the aborbance six times in spectrophotometer within one day (0hrs and 3hrs). Absorbance was recorded and % RSD (<=2) was calculated.

INTERDAY PRECISION : Test solutions of Atorvastatin (10µg/ml) and Aspirin (40µg/ml) were prepared and the absorbance was observed for 6 times.

ACCURACY:

The accuracy of the method was evaluated by determination of recovery of Atorvastatin and Aspirin at three levels of concentrations. It was performed in three different levels for Atorvastatin and Aspirin at 50%, 100%, 150%. Samples analysed at each level in trip + licate. From the results % recovery was calculated.

LIMIT OF DETECTION OF ASPRIN AND ATORVASTATIN:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD=3.3\times\sigma/s$$

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOD = 10\times\sigma/s$$

ROBUSTNESS :

The robustness is the evaluation of the analytical method wherein the results obtained are found to be reliable even when performed in a slightly varied condition. The standard solution containing 10µg/ml were prepared and taken absorbance for six times in spectrophotometer. The robustness was evaluated by calculating the %RSD of absorbance from the six replicate absorbance values (±3).

RUGGEDNESS:

Test results of Atorvastatin (15µg/ml) and Aspirin (15µg/ml) were prepared by different analyst and observed the absorbance for six times, %RSD was calculated. Ruggedness is also considered as the Analyst-Analyst precision.

RESULTS AND DISCUSSIONS

TRIALS

TRAIL1:ATORVASTATIN:ACETONITRILE

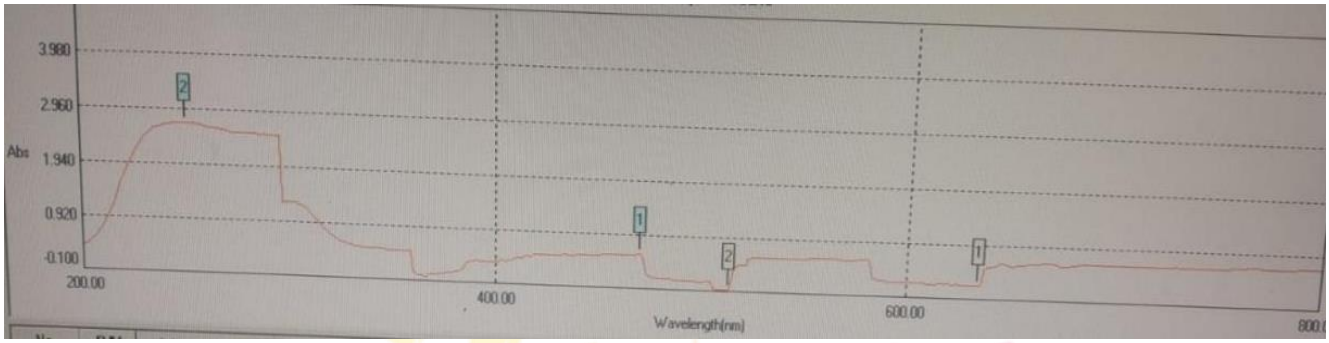

Fig 7 : Trial 1 (Atorva: Aceto)

OBSERVATIONS: In this observation it shows that peak is observed at 251nm wavelength with absorbance 2.732.

TRIAL 2: ATORVASTATIN: WATER

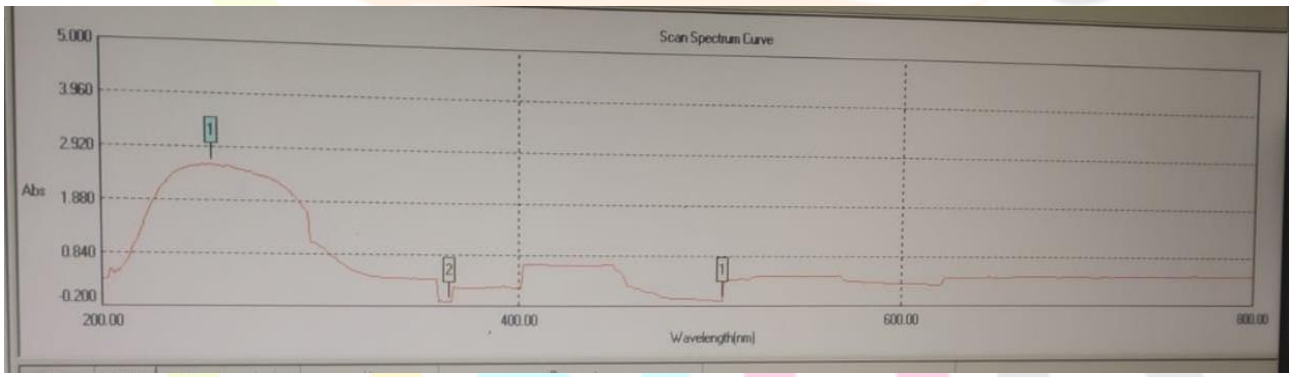

Fig 8 : Trial 2 (Atorva: Water)

OBSERVATION: In this above trail it shows that peak is observed at 251nm wavelength with absorbance 2.569

TRIAL3:ATORVASTATIN:ETHANOL

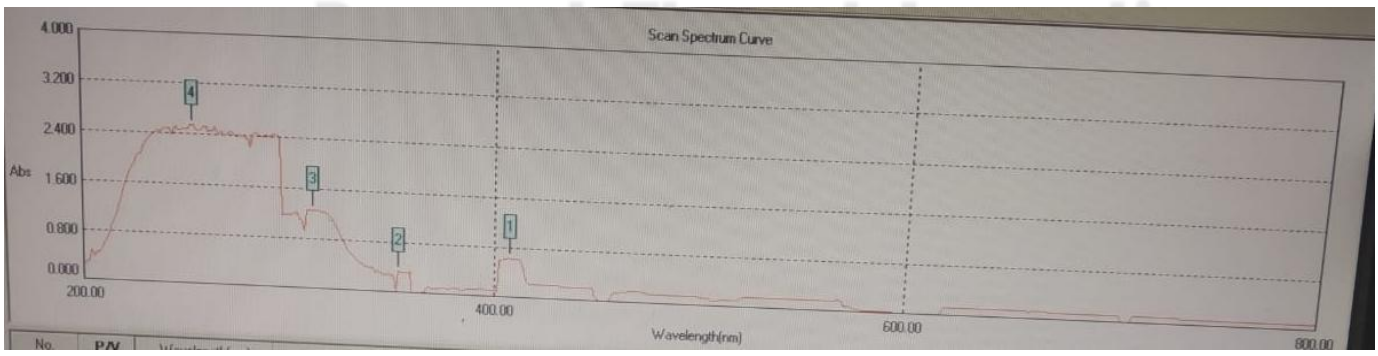

Fig 9 : Trial 3 (Atorva: Ethanol)

OBSERVATON: In this obve trial it shows that peak is observed at 254nm wavelength with absorbance 2.564

TRIAL 4: ATORVASTATIN: METHANOL

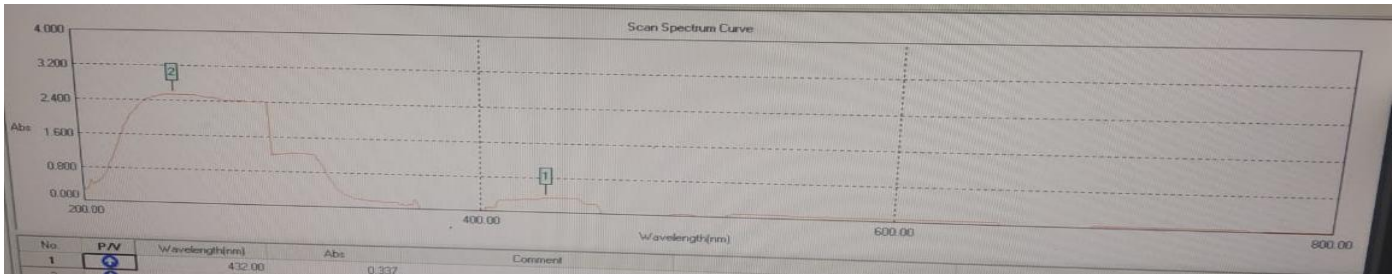

Fig 10 : Trial 4 (Atorva: Methanol)

OBSERVATION: In this above trail it shows that peak is observed at 250nm wavelength with absorbance 2.560.

TRIAL 5: ATORVASTATIN: BUFFER (ACN: KH2PO4)

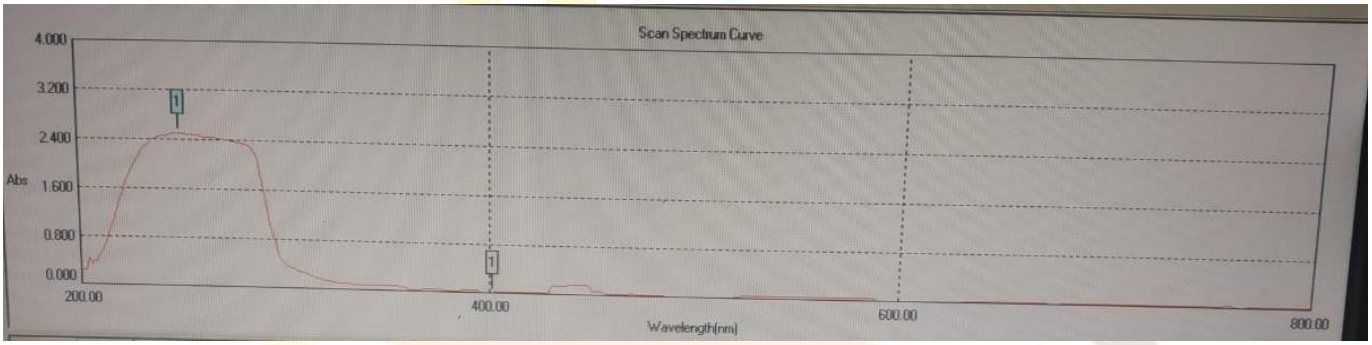

Fig11: Trial 5 (Atorva: Buffer)

OBSERVATION: In this above trial it shows that peak is observed at 250nm wavelength with absorbance 2.517.

TRIAL 6: ASPIRIN: ACETONITRILE

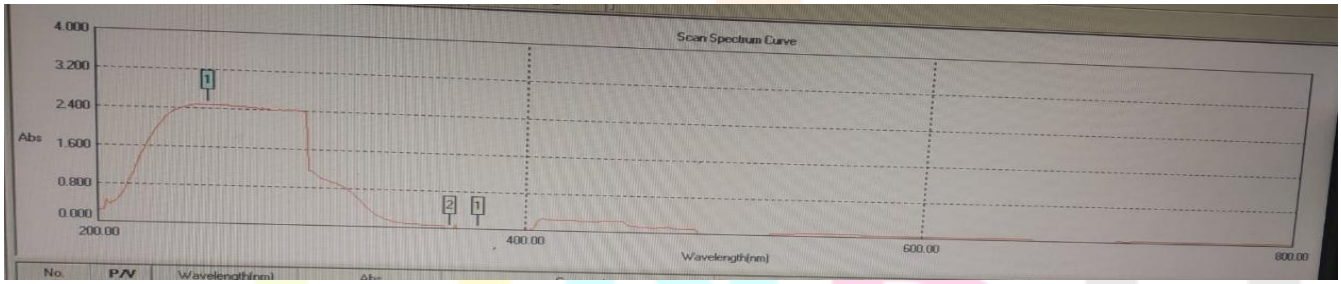

Fig12: Trial 6 (Aspirin: Aceto)

OBSERVATION: In this above trial it shows that peak is observed at 251nm wavelength with absorbance 2.501.

TRIAL 7: ASPIRIN: WATER

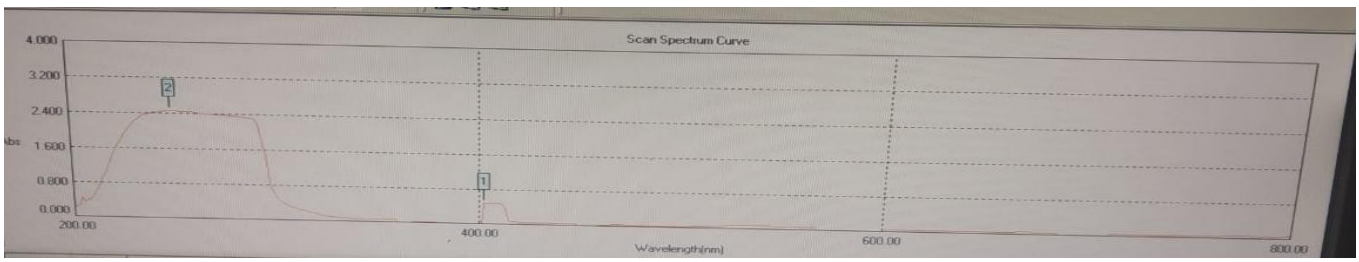

Fig13: Trial 7 (Aspirin: Water)

OBSERVATION: In this above trial it shows that peak is observed at 250nm wavelength with absorbance 2.474.

TRIAL 8: ASPIRIN: ETHANOL

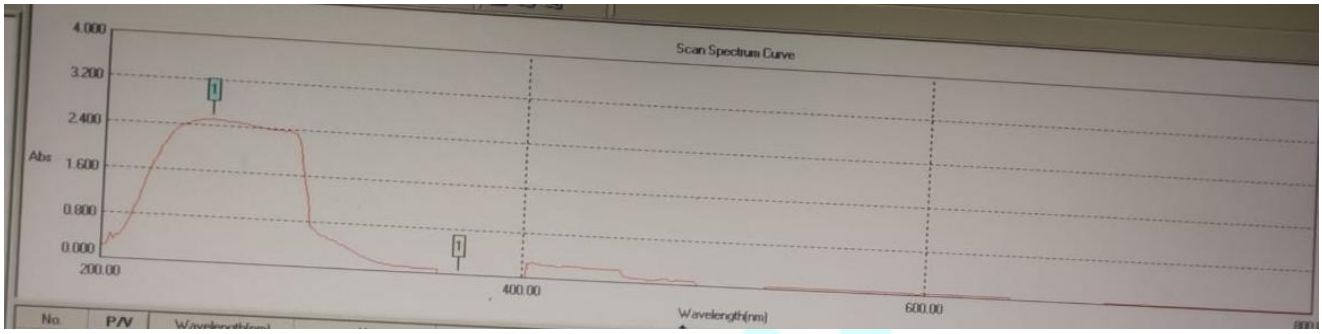

Fig 14: Trial 8 (ASPIRIN: Ethanol)

OBSERVATION: In this above trial it shows that peak is observed at 250nm wavelength with absorbance 2.526

TRIAL9:ASPIRIN:BUFFER

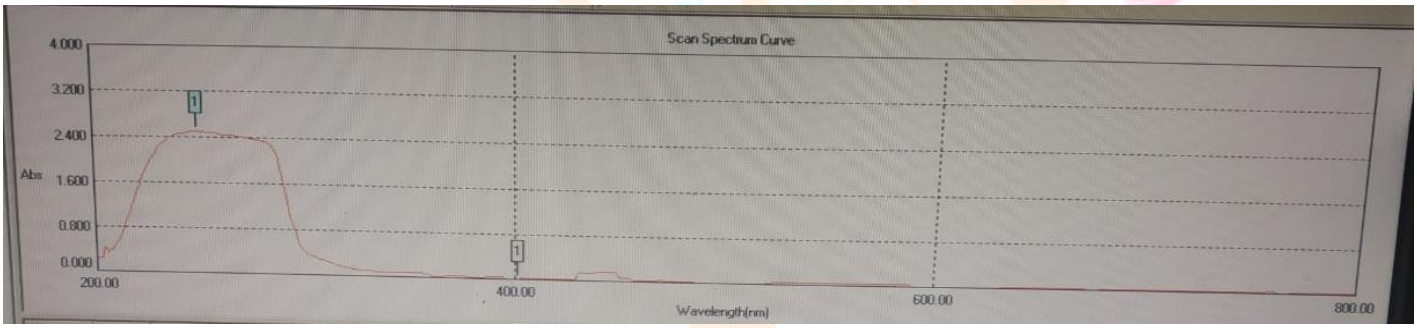

Fig 15: Trial 9 (Aspirin: Buffer)

OBSERVATION : In this above trial it shows that peak is observed at 250nm wavelength with absorbance 2.517.

TRIAL 10: ASPIRIN: METHANOL

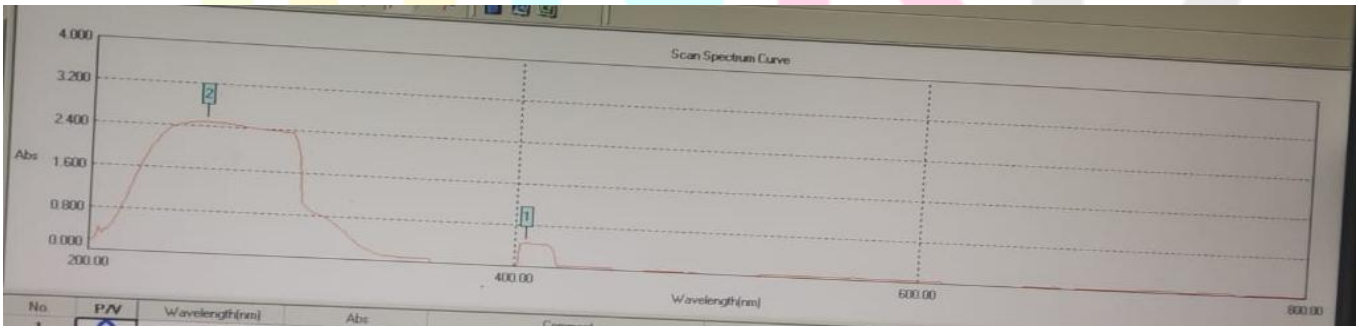

Fig 16: Trial 10 (Aspirin: Methanol)

OBSERVATION: In this above trial it shows that peak is observed at 252nm wavelength with absorbance 2.505.

Table 3: Selection of wavelength

S.No	Drug	λmax (nm)
1.	Atorvastatin	295nm
2.	Aspirin	294nm

Table 4 :- Results of spectrophotometric methods

Parameters	Acceptance criteria	Results obtained
System suitability	% RSD should be < or = 1	Atorva - 0.45 Aspirin - 0.18
Linearity	Correlation coefficient NLT 0.980	Atorva - 0.984 Aspirin - 0.989
Precision: Intraday precision Interday precision	%RSD of Atorva < 2 %RSD of Aspirin < 2	**0 hrs :** Atorva – 1.16 Aspirin – 1.23 **3 hrs :** Atorva – 0.95 Aspirin – 0.96 **Day -1 :** Atorva - 1.1 Aspirin – 1.08 **Day – 2:** Atorva – 0.816 Aspirin – 0.95
Accuracy	Percentage Recovery should be 98 – 100%	Atorva – 99% Aspirin – 99%
LOD		Atorva – 0.83 Aspirin – 0.26

LOQ		Atorva – 0.83 Aspirin - 0.25
Robustness	%RSD should be < 2	**Atorva (±3 nm):** 292 295 298 0.061 1.02 0.35 **Aspirin (±3nm):** 291 294 297 1.09 1.3 1.27
Ruggedness	%RDS should be < 2	**Analyst 1:** Atorva- 0.413 Aspirin – 0.476 **Analyst 2:** Atorva - 0.33 Aspirin - 0.48

The System suitability of Atorvastatin was found to be 0.45 & Aspirin was found to be 0.18. The Linearity of Atorvastatin is 0.984 & Aspirin is 0.989. In Precision the inter-day and intra-day precision has been observed at 0hrs, 3hrs / Day-1& Day-2. In Intra-day precision at 0hrs the atorva is 1.16 & aspirin is 1.23. In 3hrs the atorva is 0.95 & aspirin was found to be 0.96. In intra-day precision at Day 1 of atorva is 1.1 & aspirin is 1.08. In Day 2 the atorva is 0.816 & aspirin is 0.95. The accuracy of both Atorvastatin and Aspirin was found to be 99%. The LOD and LOQ of atorva were found to be at 0.88 & only the difference in aspirin is of LOD is 0.26 & LOQ is 0.25. Ruggedness and Robustness of Atorva & Aspirin were performed and found within the acceptance criteria.

ACKNOWLEDGMENT

I pay reverence to the supreme ubiquitous, omniscient, omnipotent. The almighty for his benevolence and blessing bestowed upon me.

It is really a moment of great pleasure to express my deep sense of gratitude and offer my most sincere and humble regards to my esteemed teachers and guide and **Care College of Pharmacy, Warangal** for their unparalleled and excellent guidance, continuous encouragement and support in completion of my project work successfully Their discipline, principles simplicity and fearless work environment was cherished during the course.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate UV method was developed for the quantitative estimation of Atorvastatin and Aspirin in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Atorvastatin and Aspirin was freely soluble in ethanol, methanol and sparingly soluble in water.

Water was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for UV method was promising.The UV method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Atorvastatin and Aspirin in bulk drug and in Pharmaceutical dosage forms. .

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