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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF BEMPEDOIC ACID AND ATORVASTATIN CALCIUM IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A fixed dose combination of Bempedoic acid & Atorvastatin calcium is used in ratio of 180 mg: 80 mg as tablet for treatment of Hypercholesterolemia. A simple, precise, accurate and economical UV spectrophotometric First order derivative spectroscopy and Absorbance ratio methods were developed and validated for Quantitative determination of Bempedoic acid & Atorvastatin calcium in marketed formulation. First order derivative spectrophotometric method two Zero Crossing Points (ZCP) were selected as 257 nm for Bempedoic acid (BMP) and 223 nm for Atorvastatin calcium (ATV). Estimation of BMP was done at 223 nm (ZCP of ATV) and ATV at 257 nm (ZCP of BMP) respectively. Absorbance Ratio Method, two wavelengths 227 nm (λ_{max}) for BMP and 213 nm (Iso-absorptive point) were selected. UV Spectrophotometric method for BMP & ATV was found to be linear over the range of (9-18 µg/ml) and (4-8 µg/ml) respectively for First order derivative spectroscopic method and Absorbance ratio method.

KEYWORDS: Bempedoic acid (BMP), Atorvastatin calcium (ATV), First order derivative spectroscopic method, Absorbance ratio method.

INTRODUCTION

UV-visible spectroscopy is the measurement of the wavelength and intensity of a sample's absorption of visible and near ultraviolet light. When exposed to electromagnetic radiation (EMR), a molecule absorbs a certain amount of that radiation's energy. The scientific term for this phenomenon is absorption, and the technique used to study it is absorption Spectrophotometry. Wavelengths between 200 and 800 nm in the UV-visible range. Through the electronic, vibrational, and rotational transition processes, respectively, molecules can absorb radiation.[1-4] Bempedoic acid (BMP) is chemically known as 8-Hydroxy-2, 2, 14, 14-tetramethylpentadecanedioic acid. It is Very soluble in Methanol and Insoluble in Water. Atorvastatin calcium (ATV) is chemically known as Calcium bis ((3R, 5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate). It is freely soluble in methanol, slightly soluble in ethanol (95%) and very slightly soluble in water. Atorvastatin, a selective, competitive HMG-CoA reductase inhibitor, is used to lower cholesterol and triglycerides in patients with hypercholesterolemia and mixed dyslipidemia and in the treatment of homozygous familial hypercholesterolemia.[5-11]

MATERIALS AND METHODS

Apparatus and Instrument

Electronic Analytical balance (Shimadzu, 0.1 mg), Double beam UV-Visible Spectrophotometer (Shimadzu-1900i, software LabSolution UV-vis ver-2.03) having matched quartz cells of light path 1 cm, Ultrasonicator (Athena Technology), Volumetric flask- 10ml, 100ml

(Borosil), Pipettes - 1, 5, 10 ml (Borosil), Micropipette - 5 µl (Accumax).

Reagents and Materials

Bempedoic acid (Gift sample Exemed Pharmaceuticals, Vapi, Gujarat), Atorvastatin calcium (Gift sample Akums Drugs and Pharmaceuticals, Haridwar), Methanol (AR grade-Thomas Baker, Mumbai).

Spectrophotometric condition

Mode: Scan, Scan speed: Medium, Wavelength range: 200-400 nm, Scale of absorbance: 0.00-2.00 A, Base line correction: Methanol.

Selection of solvent[13-17]

As per solubility study common solvent for both drugs were found to be methanol. Therefore, methanol was selected as solvent for UV methods. In methanol Bempedoic acid and Atorvastatin calcium give linear spectra at their measured wavelength. So, methanol is preferred as a solvent.

PREPARATION OF DERIVATIVE[12]

- **Benzoate/Benzoyl chloride derivative (Schotten Bauman method)**

Weighed 0.5 g compound

↓

Dissolved in 15 ml (5%) NaOH Solution in Iodine flask

↓

Add 1 ml Benzoyl Chloride dropwise

↓

Flask should be well stoppered and shake for 20 minutes

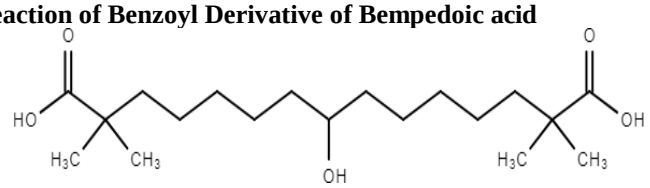
↓

The reaction mixture should be kept cool throughout the addition, finally shaken the reaction mixture vigorously for 10-15 minutes

↓

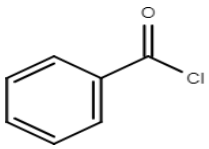
Filter off the product, washed derivative with water and recrystallized from alcohol

- **Reaction of Benzoyl Derivative of Bempedoic acid**



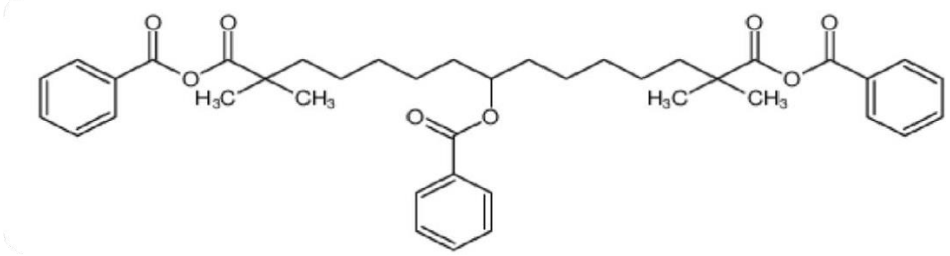
Bempedoic acid

\+



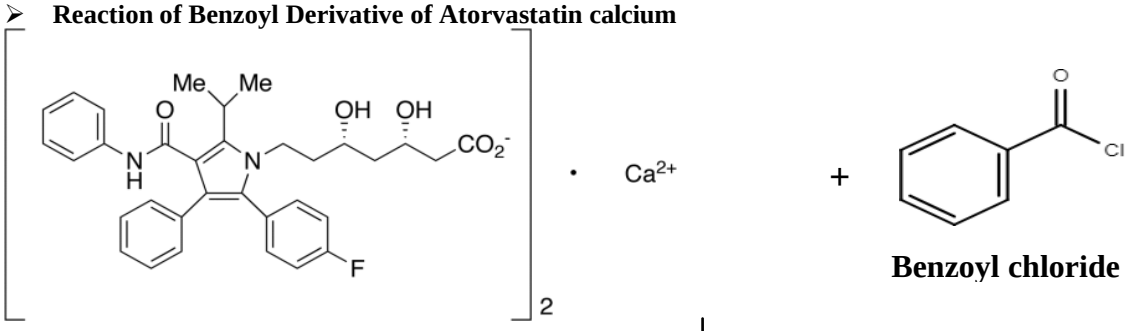
Benzoyl chloride

↓ NaOH



Derivative of Bempedoic acid.

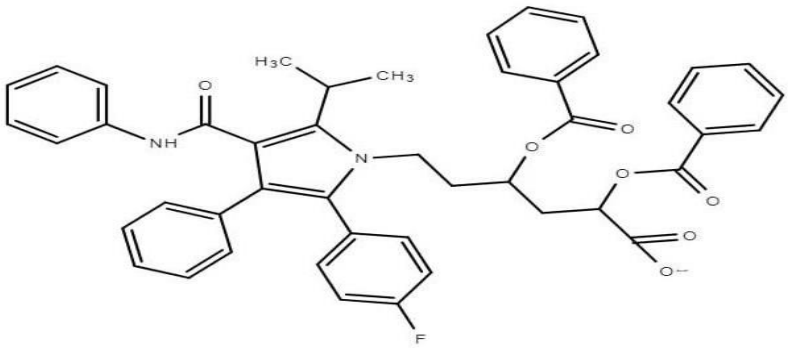
➢ **Reaction of Benzoyl Derivative of Atorvastatin calcium**



Atorvastatin calcium

Benzoyl chloride

NaOH



Derivative of Atorvastatin calcium

PREPARATION OF STANDARD SOLUTION

1. **Preparation of BMP Standard stock solution (1000 µg/ml)**

Accurately weighed 10 mg of Bempedoic acid and take into 10 ml volumetric flask and it was dissolved in methanol and volume was made upto 10 ml with methanol to get stock solution (1000 µg/ml).

2. **Preparation of BMP working stock solution (100 µg/ml)**

Aliquot of 10 ml from above standard stock solution was pipette out into 100 ml of volumetric flask and volume was made upto the mark with methanol to give a solution containing 100 µg/ml.

3. **Preparation of ATV Standard stock solution (1000 µg/ml)**

Accurately weighed 10 mg Atorvastatin calcium and take into 10 ml volumetric flask and it was dissolved in methanol and volume was made upto 10 ml with methanol to get stock solution (1000 µg/ml).

4. **Preparation of ATV working stock solution (100 µg/ml)**

Aliquot of 1 ml from above standard stock solution was pipette out into 10 ml of volumetric flask and volume was made upto mark with methanol to get stock solution (100 µg/ml).

PREPARATION OF CALIBRATION CURVE

1. **Calibration curve for BMP**

Calibration curve for BMP consisted of five different concentrations for standard solution of BMP ranging from 9-18 µg/ml. Each solution was scanned against methanol as blank and corresponding spectra were recorded. The first derivative (dA/dλ) spectra of all these solutions were obtained by transformation of zero order spectra. dA/dλ absorbance at 223 nm (Zero Crossing Point of ATV) was computed and the plot of dA/dλ absorbance vs. concentration was plotted and regression equation was obtained.

2. **Calibration curve for ATV**

Calibration curve for ATV consisted of five different concentrations for standard solution of ATV ranging from 4-8 µg/ml. Each solution was scanned against methanol as blank and corresponding spectra were recorded. The first derivative (dA/dλ) spectra of all these solutions were obtained by transformation of zero order spectra. dA/dλ absorbance at 257 nm (Zero Crossing Point of BMP) was computed and the plot of dA/dλ absorbance vs. concentration was plotted and regression equation was obtained.

VALIDATION OF PROPOSED METHOD

Parameters to be considered for the validation of methods are.

1. **Linearity (n=5)**

For BMP and ATV, the linearity response was determined by examining five different calibration curve levels within the range of 9-18 µg/ml and 4-8 µg/ml,

respectively. Plotting the calibration curve of absorbance versus concentration (dA/dλ) allowed for the calculation of the correlation coefficient and regression line equations for both BMP and ATV.

2. Precision

A. Repeatability (n=6)

Aliquots of 13.5 ml of working stock solution of BMP (100 µg/ml), 0.6 ml of working stock solution of ATV (100 µg/ml) were taken into two separate series of 100 ml and 10 ml volumetric flask and volume was made upto mark using methanol to give a solution containing 13.5 µg/ml of BMP and 6 µg/ml of ATV. Solution was analyzed six times (n=6) and % R.S.D. was calculated.

B. Intraday Precision (n=3)

Aliquots of 11.25, 13.5 and 15.75 ml of working solution of BMP (100 µg/ml) were taken into series of 100 ml volumetric flask. Aliquots of 0.5, 0.6, 0.7 ml of working stock solution of ATV (100 µg/ml) were taken into series of 10 ml volumetric flask. Using methanol volume was made upto the mark to give solution containing 11.25, 13.5, 15.75 µg/ml of BMP and 5, 6, 7 µg/ml of ATV. Solution was analyzed for three times (n=3) on the same day within short interval of time and % R.S.D. was calculated.

C. Interday Precision (n=3)

Aliquots of 11.25, 13.5 and 15.75 ml of working solution of BMP (100 µg/ml) were taken into series of 100 ml volumetric flask. Aliquots of 0.5, 0.6, 0.7 ml of working stock solution of ATV (100 µg/ml) were taken into series of 10 ml volumetric flask. Using methanol volume was made upto the mark to give solution containing 11.25, 13.5, 15.75 µg/ml of BMP and 5, 6, 7 µg/ml of ATV. Solution was analyzed for three times (n=3) on three different days and % R.S.D. was calculated.

3. Accuracy

To create a fine powder, twenty tablets were precisely weighed and triturated in a mortar pestle. Then, using the benzoyl derivatization method, the finely ground tablet powder (equivalent to approximately 180 mg of BMP and 80 mg of ATV) is weighed and transferred to a 100 ml volumetric flask. The mixture is sonicated for 5 minutes and volume is then made up to the mark with methanol to obtain a solution containing 1000 µg/ml. The final solution was filtered through Whatman filter paper No. 42. From the stock solution, a 10 ml aliquot was pipetted out and transferred into a 100 ml volumetric flask. The volume was adjusted to the mark with methanol, resulting in a concentration of 100 µg/ml. Then 9 ml was withdrawn from this prepared solution and transferred into another 100 ml volumetric flask. The volume was then adjusted to the mark with methanol to obtain a sample solution containing 9 µg/ml of BMP and 4 µg/ml of ATV, respectively.

4. LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the set of five calibration curves utilized to assess the method's linearity. Calculation of the LOD and LOQ was conducted using the specified formula.

LOD = 3.3 × S.D./Slope

LOQ = 10 × S.D./Slope

Where, S.D. = Standard deviation of the Y- intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves.

➢ RESULT AND DISCUSSION

I. FIRST ORDER DERIVATIVE METHOD:

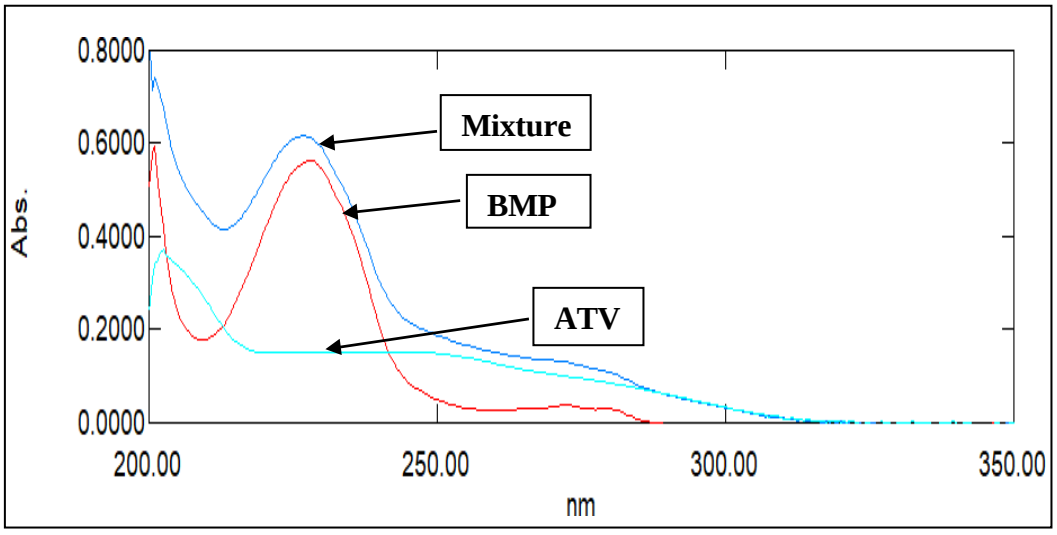


Figure No. 1 Overlain Spectra of BMP (9 µg/ml), ATV (4 µg/ml) and Mixture (9 + 4 µg/ml)

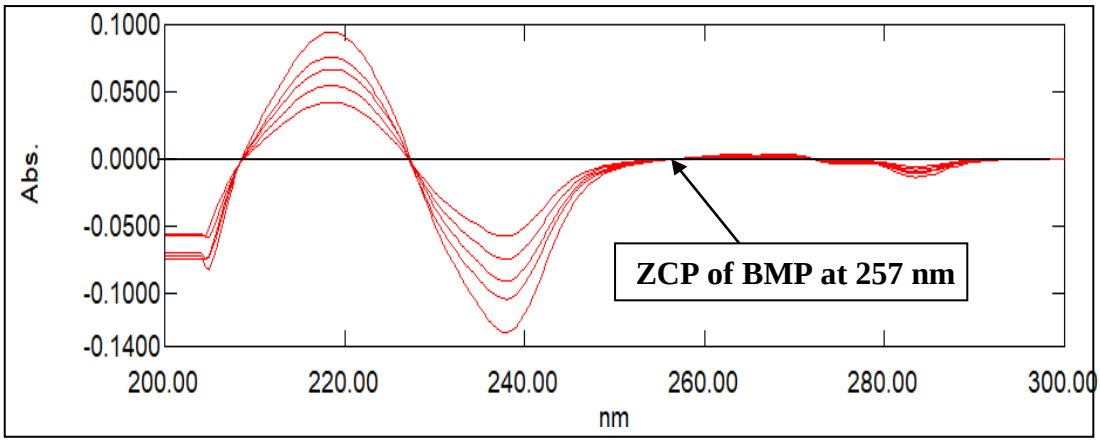


Figure No. 2 ZCP of BMP at 257 nm.

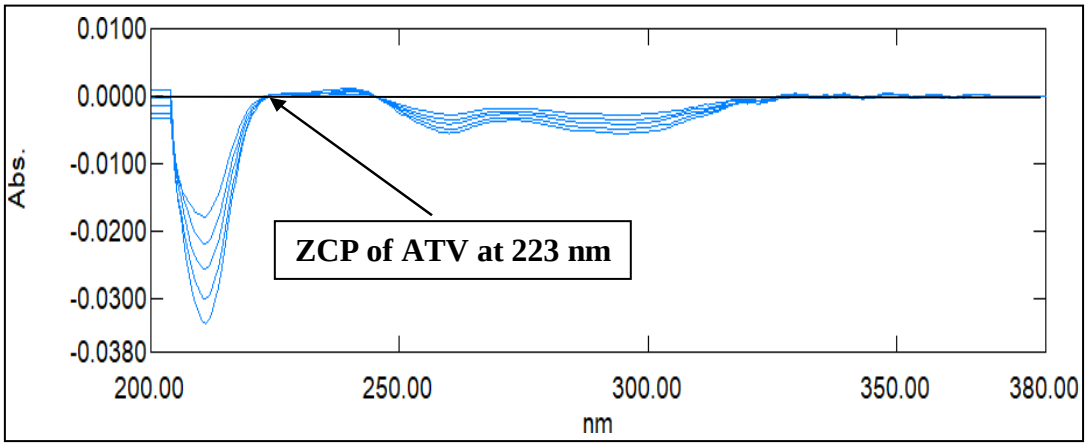


Figure No. 3 ZCP of ATV at 223 nm.

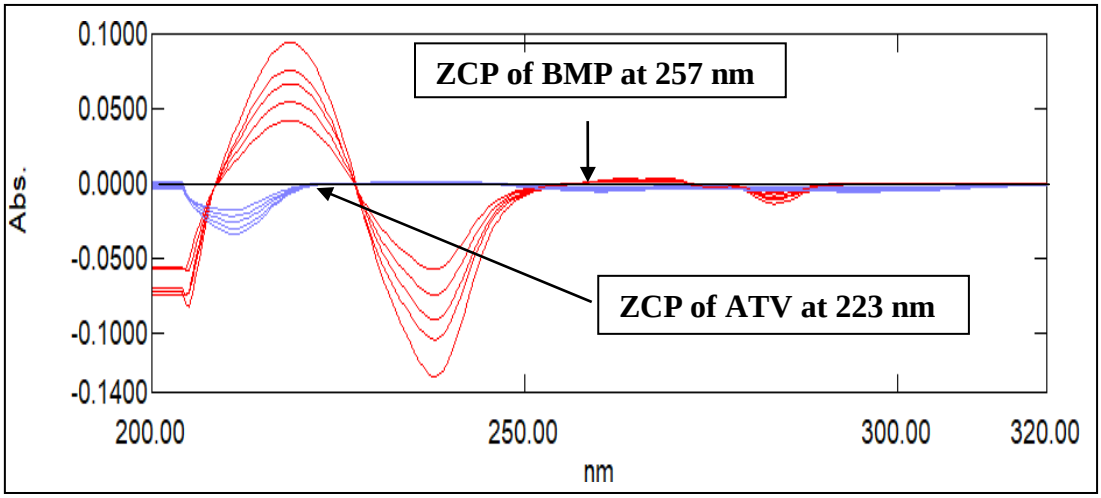


Figure No. 4 Overlain spectra of BMP (9-18 µg/ml) and ATV (4-8 µg/ml).

- **Validation of First Order Derivative Method**

1. Linearity

Table No. 1: Linearity of BMP at 223 nm (ZCP of ATV).

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	% R.S.D.
1.	9	0.0031 ± 0.000024	0.7977
2.	11.25	0.0037 ± 0.000024	0.6589
3.	13.5	0.0043 ± 0.000022	0.5261
4.	15.75	0.0048 ± 0.000022	0.4620
5.	18	0.0053 ± 0.000020	0.3762

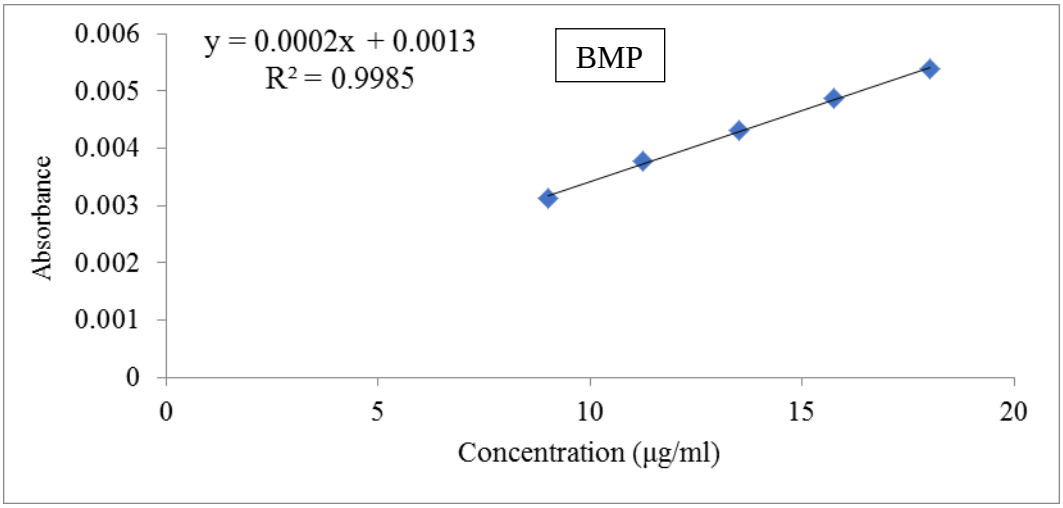


Fig. No. 5: Calibration Curve of BMP at 223 nm (ZCP of ATV).

Table No. 2: Linearity of ATV at 257 nm (ZCP of BMP).

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	% R.S.D.
1.	4	0.0022 ± 0.000013	0.6318
2.	5	0.0027 ± 0.000015	0.5813
3.	6	0.0033 ± 0.000014	0.4416
4.	7	0.0038 ± 0.000012	0.3190
5.	8	0.0043 ± 0.000016	0.3850

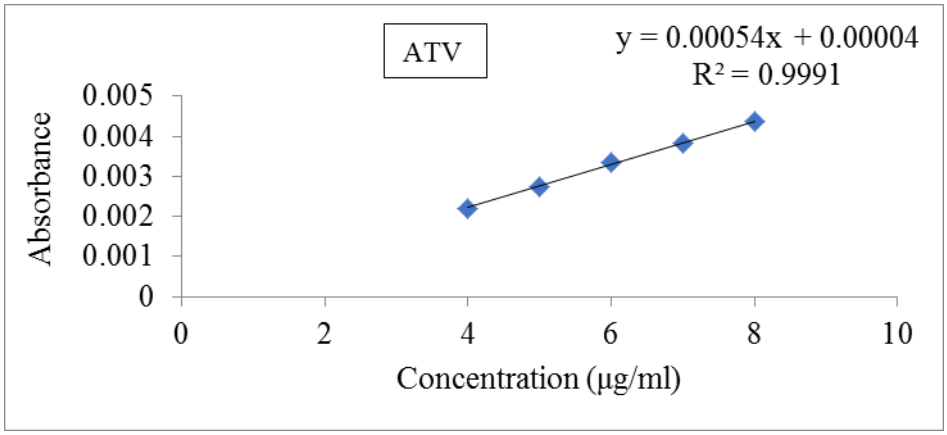


Fig. No. 6: Calibration Curve of ATV at 257 nm (ZCP of BMP).

Table No. 3: Regression line equation, Regression coefficient and correlation coefficient for BMP and ATV.

Sr.No.	Drugs	Regression line Equation	Regression Coefficient (R^2)	Correlation Coefficient (r)
1.	BMP	y = 0.0002x + 0.0013	0.9985	0.9992
2.	ATV	y = 0.00054x + 0.00004	0.9991	0.9995

2. Precision

A. Repeatability (n=6)

Table No. 4: Repeatability data for BMP at 223 nm (ZCP of ATV) and ATV at 257 nm (ZCP of BMP).

Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	% R.S.D.
BMP	13.5	0.0043 ± 0.000026	0.6127
ATV	6	0.0032 ± 0.000016	0.5126

B. Intraday Precision (n=3)

Table No. 5 Intraday Precision data of BMP at 223 nm (ZCP of ATV) and ATV at 257 nm (ZCP of BMP)

Sr.No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	% R.S.D.
1.	BMP	11.25	0.0037 ± 0.000031	0.8426
2.		13.5	0.0043 ± 0.000032	0.7321
3.		15.75	0.0050 ± 0.000026	0.5299
1.	ATV	5	0.0028 ± 0.000019	0.7128
2.		6	0.0034 ± 0.000021	0.6326
3.		7	0.0038 ± 0.000020	0.5219

C. Interday Precision (n=3)

Table No. 6: Interday Precision of BMP at 223 nm (ZCP of ATV) and ATV at 257 nm (ZCP of BMP)

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	% R.S.D.
1.	BMP	11.25	0.0040 ± 0.000035	0.8724
2.		13.5	0.0044 ± 0.000035	0.8021
3.		15.75	0.0050 ± 0.000030	0.6013
1.	ATV	5	0.0029 ± 0.000024	0.8358
2.		6	0.0035 ± 0.000026	0.7651
3.		7	0.0043 ± 0.000027	0.6507

3. Accuracy

Table No. 7: Determination of accuracy of BMP and ATV.

Drugs	Level	Amount of Sample (µg/ml)	Amount of std. spiked (µg/ml)	Total amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
BMP	0%	9	-	9	8.949	99.43
	80%	9	7.2	16.2	16.159	99.74
	100%	9	9	18	17.949	99.71
	120%	9	10.8	19.8	19.788	99.93
ATV	0%	4	-	4	3.970	99.25
	80%	4	3.2	7.2	7.159	99.43
	100%	4	4	8	7.960	99.50
	120%	4	4.8	8.8	8.809	100.10

➢ **Analysis of Marketed Formulation**

Table No. 8 Determination of assay of BMP and ATV.

Tablet (Aztor)	Actual Concentration (mg/tablet)		Amount obtained Mean ± S.D. (mg/tablet)		BMP % Purity ± S.D. (n=5)	ATV % Purity± S.D. (n=5)
	BMP	ATV	BMP	ATV		
	180	80	179.8 ± 0.0316	79.74 ± 0.0327	99.88 ± 0.0114	99.67 ± 0.0083

II. ABSORBANCE RATIO METHOD

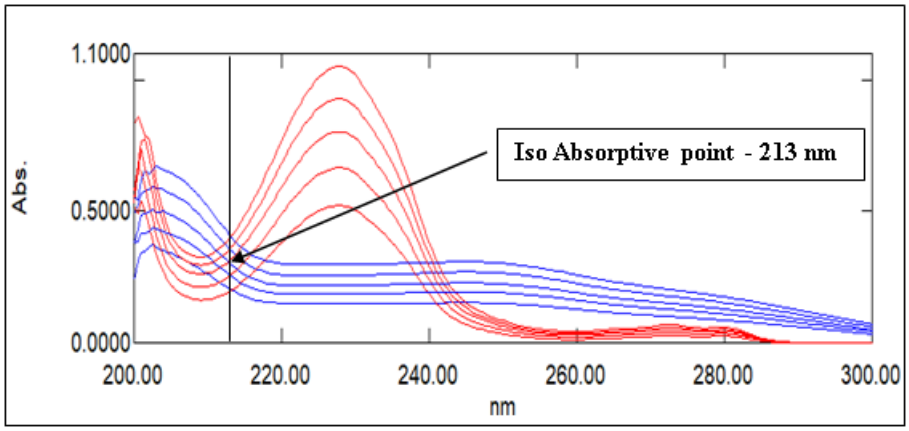


Figure No.7 Overlain spectra of BMP (9-18 µg/ml) and ATV (4-8 µg/ml).

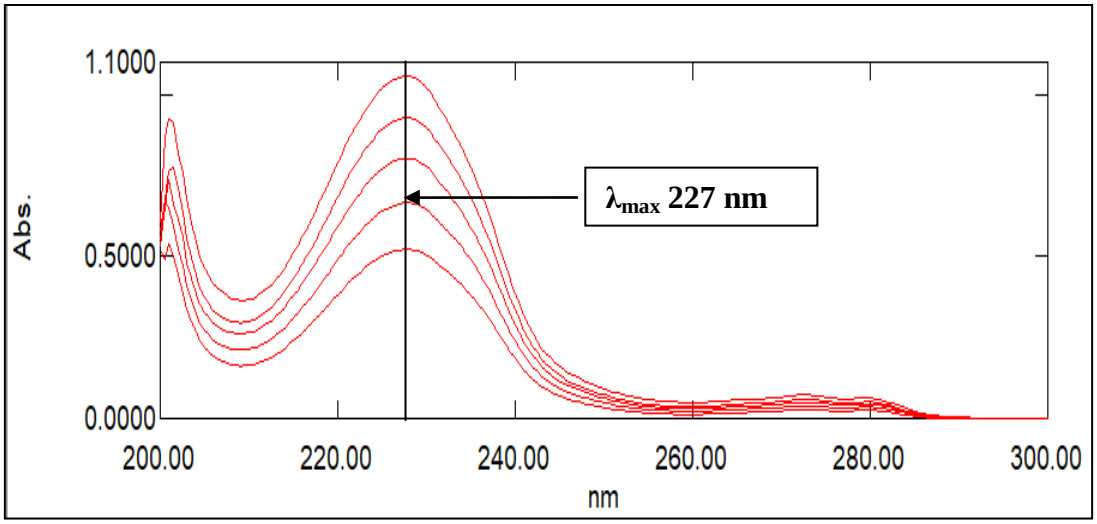


Fig. No. 8: Overlain Spectra of BMP (9-18 µg/ml).

➢ **Validation of Absorbance Ratio Method:**

1. Linearity

Table No. 9: Linearity for BMP at 213 nm.

Sr. No.	Concentration (µg/ml)	Mean ± S.D. (n=5)	% R.S.D.
1.	9	0.2056 ± 0.00093	0.4554
2.	11.25	0.2559 ± 0.00100	0.3912
3.	13.5	0.3022 ± 0.00094	0.3126
4.	15.75	0.3562 ± 0.00104	0.2934
5.	18	0.4083 ± 0.00084	0.2080

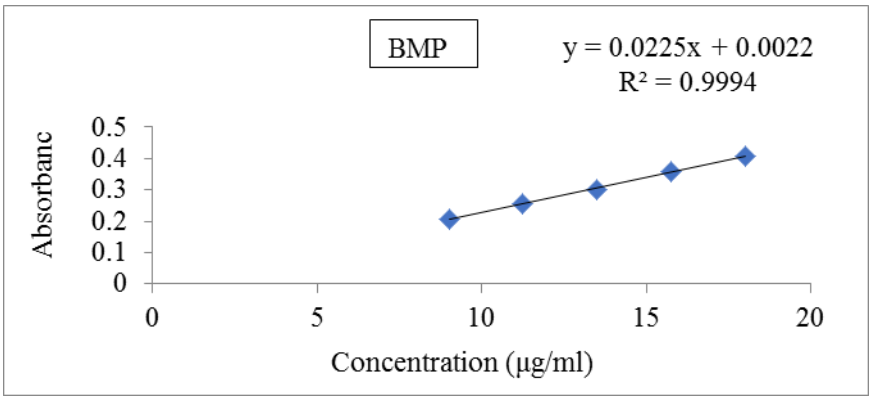


Fig. No. 9 Calibration curve for BMP at 213 nm.

Table No. 10: Linearity for ATV at 213 nm.

Sr. No.	Concentration (µg/ml)	Mean ± S.D. (n=5)	% R.S.D.
1.	4	0.2056 ± 0.00093	0.4543
2.	5	0.2559 ± 0.00099	0.3900
3.	6	0.3023 ± 0.00093	0.3108
4.	7	0.3562 ± 0.00104	0.2939
5.	8	0.4084 ± 0.00085	0.2096

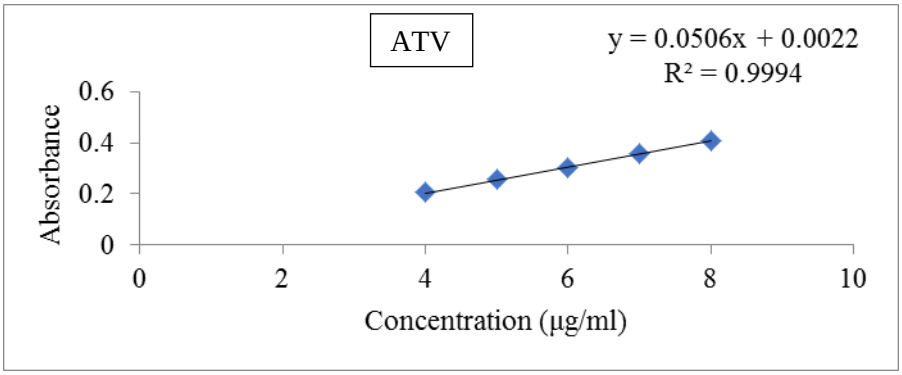


Fig. No. 10 Calibration curve for ATV at 213 nm.

Table No. 11 Linearity for BMP at 227 nm.

Sr. No.	Concentration (µg/ml)	Mean ± S.D. (n=5)	% R.S.D.
1.	9	0.5299 ± 0.0021	0.4052
2.	11.25	0.6680 ± 0.0024	0.3650
3.	13.5	0.8036 ± 0.0023	0.2911
4.	15.75	0.9290 ± 0.0019	0.2137
5.	18	1.0518 ± 0.0018	0.1719

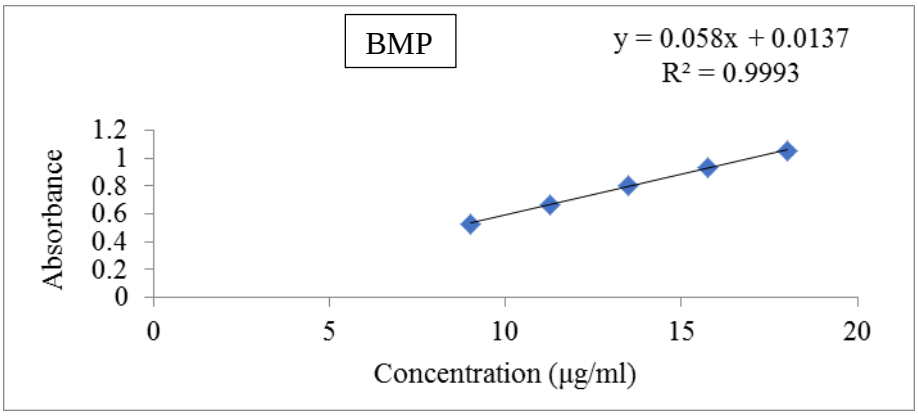


Fig. No. 11 Calibration curve for BMP at 227 nm.

Table No. 12: Linearity for ATV at 227 nm.

Sr. No.	Concentration (µg/ml)	Mean ± S.D. (n=5)	% R.S.D.
1.	4	0.1512 ± 0.00082	0.5481
2.	5	0.1882 ± 0.00090	0.4819
3.	6	0.2217 ± 0.00087	0.3926
4.	7	0.2569 ± 0.00087	0.3385
5.	8	0.3027 ± 0.00084	0.2788

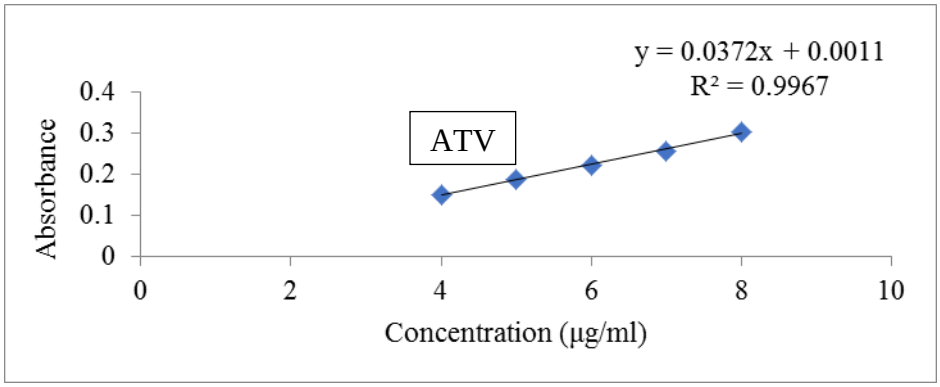


Fig. No. 12 Calibration curve for ATV at 227 nm.

Table No. 13: Correlation coefficient, regression coefficient and regression line equation for BMP and ATV at 213 nm and 227nm.

Sr. No.	Drugs	Regression line equation	Regression coefficient (R^2)	Correlation coefficient (r)
1.	BMP (213 nm)	y = 0.0225x + 0.0022	0.9994	0.9996
2.	ATV (213 nm)	y = 0.0506x + 0.0022	0.9994	0.9996
3.	BMP (227 nm)	y = 0.058x + 0.0137	0.9993	0.9996
4.	ATV (227 nm)	y = 0.0372x + 0.0011	0.9967	0.9983

2. Precision

A. Repeatability (n=6)

Table No. 14: Repeatability of BMP and ATV at 213 nm.

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	% R.S.D.
1.	BMP	13.5	0.3029 ± 0.0010	0.3425
2.	ATV	6	0.3025 ± 0.0010	0.3405

Table No. 15: Repeatability of BMP and ATV at 227 nm.

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	% R.S.D.
3.	BMP	13.5	0.8054 ± 0.0026	0.3236
4.	ATV	6	0.2229 ± 0.0009	0.4128

B. Intraday Precision

Table No. 16: Intraday Precision of BMP and ATV at 213 nm.

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	% R.S.D.
1.	BMP	11.25	0.2558 ± 0.0011	0.4564
2.		13.5	0.3026 ± 0.0012	0.4157
3.		15.75	0.3561 ± 0.0011	0.3238
1.	ATV	5	0.2558 ± 0.0011	0.4529
2.		6	0.3026 ± 0.0012	0.4140
3.		7	0.3560 ± 0.0011	0.3231

Table No. 17: Intraday Precision for BMP and ATV at 227 nm.

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	% R.S.D.
1.	BMP	11.25	0.6685 ± 0.0033	0.4973
2.		13.5	0.8046 ± 0.0033	0.4141
3.		15.75	0.9296 ± 0.0029	0.3173
1.	ATV	5	0.1884 ± 0.0011	0.6121
2.		6	0.2219 ± 0.0011	0.5018
3.		7	0.2570 ± 0.0011	0.4280

C. Interday Precision

Table No. 18: Interday Precision for BMP and ATV at 213 nm.

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	% R.S.D.
1.	BMP	11.25	0.2560 ± 0.0013	0.5156
2.		13.5	0.3026 ± 0.0014	0.4814
3.		15.75	0.3565 ± 0.0014	0.4074
1.	ATV	5	0.2561 ± 0.0013	0.5121
2.		6	0.3027 ± 0.0014	0.4798
3.		7	0.3564 ± 0.0014	0.4068

Table No. 19: Interday Precision for BMP and ATV at 227 nm.

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1.	BMP	11.25	0.6688 ± 0.0037	0.5534
2.		13.5	0.8049 ± 0.0037	0.4662
3.		15.75	0.9309 ± 0.0035	0.3832
1.	ATV	5	0.1887 ± 0.0013	0.7010
2.		6	0.2222 ± 0.0013	0.6187
3.		7	0.2573 ± 0.0014	0.5441

3. Accuracy

Table No. 20: Determination of Accuracy for BMP and ATV.

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
BMP	0%	9	-	9	8.934	99.26
	80%	9	7.2	16.2	16.094	99.34
	100%	9	9	18	17.929	99.60
	120%	9	10.8	19.8	19.859	100.29
ATV	0%	4	-	4	3.973	99.32
	80%	4	3.2	7.2	7.158	99.41
	100%	4	4	8	7.965	99.56
	120%	4	4.8	8.8	8.772	99.68

4. Assay

Analysis of Marketed Formulation (Sample -Tablet).

Table No. 21: Determination of Assay of BMP and ATV.

Tablet (Aztor)	Actual Concentration (mg/tablet)		Amount obtained Mean ± S.D. (mg/tablet)		BMP % Purity ± S.D.	ATV % Purity ± S.D.
	BMP	ATV	BMP	ATV	(n=5)	(n=5)
	180	80	179.27 ± 0.0511	79.65 ± 0.0270	99.59 ± 0.0474	99.56 ± 0.0554

➢ CONCLUSION

1. UV SPECTROPHOTOMETRY

A. FIRST ORDER DERIVATIVE METHOD

Based on the results, obtained from the analysis of BMP and ATV in their tablet dosage form using First Order Derivative Method, it can be concluded that the method has linearity in the range of 9-18 µg/ml for BMP and 4-8 µg/ml for ATV. The regression coefficient (R^2) was found to be 0.9985 and 0.9991 for BMP and ATV and correlation coefficient (r) was found to be 0.9992 and 0.9995 for BMP and ATV at 257 nm (ZCP of BMP) and 223 nm (ZCP of ATV) respectively. Limit of detection for BMP and ATV were found to be 0.1490 µg/ml and 0.3391 µg/ml and Limit of quantification for BMP and ATV were found to be 0.4517 µg/ml and 1.0277 µg/ml respectively. The % assay was found to be 99.88 % w/w and 99.67 % w/w for BMP and ATV respectively. Further % R.S.D. was found to be less than 2% for repeatability, intraday and interday study.

B. ABSORBANCE RATIO METHOD

Based on the results, obtained from the analysis of BMP and ATV in their tablet dosage form using Absorbance Ratio Method, it can be concluded that the method has linearity in the range of 9-18 µg/ml for BMP and 4-8 µg/ml for ATV. The regression coefficient (R^2) was found to be at both wavelength 213 nm and 227 nm 0.9994 and 0.9993 for BMP and 0.994 and 0.9967 for ATV respectively and correlation coefficient (r) was found to be 0.9996 and 0.9996 for BMP and 0.9996 and 0.9983 for ATV at both wavelength 213 nm and 227 nm respectively. Limit of detection for BMP and ATV were found to be at 213 nm 0.1576 µg/ml and 0.0684 µg/ml and at 227 nm 0.1605 µg/ml and 0.07692 µg/ml. Limit of quantification for BMP and ATV were found to be at 213 nm 0.4776 µg/ml and 0.2074 µg/ml and at 227 nm 0.4863 µg/ml and 0.2331 µg/ml respectively. The % assay was found to be 99.59 % w/w and 99.56 %w/w for BMP and ATV respectively. Further % R.S.D. was found to be less than 2% for repeatability, intraday and interday study.

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