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## NEW SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE AND ATORVASTATIN CALCIUM IN TABLET DOSAGE FORMS

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### ABSTRACT

A simple, accurate, precise and reproducible UV spectrophotometric method was developed for simultaneous estimation of Amlodipine besylate (AMD) and Atorvastatin calcium (ATR) in tablet dosage form have been developed. First method is simultaneous equation method; in this method 361nm and 246 nm were selected to measure the absorbance of drugs at both wavelengths. The second method is Q-value analysis based on measurement of absorptivity at 238.8 nm (as an iso-absorptive point) and 246 nm. AMD and ATR at their respective maximum wavelength 361 nm and 246 nm and at isoabsorptive point 238.8 nm shows linearity in a concentration range of 0.5-30 µg/mL. Recovery studies range from >99.82% for AMD and >98.09% for ATR in case of simultaneous equation method and >100% for AMD and >98.45% for ATR in case of Q-analysis method confirming the accuracy of the proposed method. The proposed methods are recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific (no heating and no organic solvent extraction is required).

**KEY WORDS:** Amlodipine, Atorvastatin, Simultaneous equation method, Q-analysis, Absorption correction method.

### 1.INTRODUCTION

Amlodipine (AMD) is a white crystalline powder which is slightly soluble in water, sparingly soluble in ethanol and freely soluble in methanol. It is official in B.P. Chemically Amlodipine, (Fig 1.) is 3-Ethyl-5-methyl ( $\pm$ )-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4- dihydro-6-methyl-3, 5-pyridinedicarboxylatebenzenesulfonat (Budawari,2008). AMD is a dihydropyridine derivative with calcium antagonist activity (Martindale and Sweetman,1999). It is used in the management of hypertension, chronic stable angina pectoris and Prinzmetal variant angina (Oneil,2001). AMD acts by inhibiting the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle and also acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. Atorvastatin (ATR) is a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been used as a lipid lowering agent (Mohammadi,2007). Chemically, Atorvastatin (Fig 2.) is [R-(R\*, R\*)]-2-(4-fluorophenyl)-B, B—dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid (Desager and Hormans,1996). ATR is a competitive inhibitor of HMG-CoA reductase. This enzyme catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme-A to mevalonate, which is the rate-determining step in hepatic cholesterol synthesis. Because cholesterol synthesis decreases, hepatic cells increase the number of LDL receptors on the surface of the cells, which in turn increase the amount of LDL uptake by the hepatic cells, and decrease the amount of LDL in the blood (Malinowski,1998;Burnham,2002).

Literature survey revealed that no UV methods are reported for the simultaneous determination of Amlodipine and Atorvastatin till date. Methods are available for the quantification of Amlodipine individually and with other combinations other than Atorvastatin by HPLC (Bahrami and Mirzaeei,2004;Agrekar and Powar,2000;Vora and Kadav,2008;Kumar;Zarghi,2005), by UV (Priyanka,2009;Topale,2003). Methods are available for the quantification of Atorvastatin individually and with other combinations other than Amlodipine HPLC (Sandeep,2006;Lincy joseph,2008;Bahrami,2005). Present study involves development and validation of two spectrophotometric methods for the simultaneous determination of Amlodipine and Atorvastatin (ATR) in pharmaceutical formulations and in Drug substances.

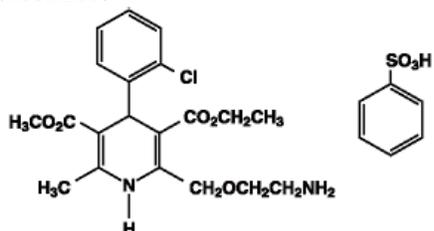


Fig 1.Amlodipine

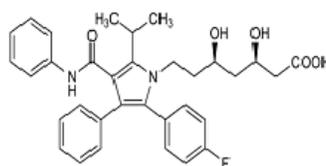


Fig 2.Atorvastatin

## 2. MATERIALS AND METHODS

**2.1. Instrumentation and chemicals:** Spectral runs were made on a Double beam UV-Visible spectrophotometer, model-T80+ was employed with spectral bandwidth of 0.2 nm and automatic wavelength corrections with a pair of 10 mm quartz cells. The software is UVWin5 ver5.1.1, PG instruments. ATR and AMD were purchased from SL drugs & Pharmaceuticals (Hyderabad, India). Purified water was prepared using a Millipore Milli-Q (Nanopure Diamond, Barnstead thermolyne, USA) water purification system. Acetonitrile, Methanol was purchased from Merck Ltd. (Mumbai, India).

**2.2. Preparation of standard drug solutions:** An accurately weighed 10 mg of each of ATR and AMD was dissolved in 10 ml of methanol to obtain a concentration of 1 mg/mL each. From 1 mg/mL solution 1 ml was taken and made to 10 ml with methanol to obtain a concentration of 100µg/mL each. Daily working standard solutions of AMD and ATR was prepared by suitable dilution of the stock solution with methanol.

**2.3. Determination of maximum wavelength and Iso-absorptive point:** By appropriate dilution of two standard drug solutions with methanol, solutions containing 10 µg /ml of AMD and 10 µg /ml of ATR were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. AMD showed absorbance maxima at 361nm ( $\lambda_1$ ) and 236.5 nm and ATR showed absorbance maxima at 246 nm ( $\lambda_2$ ). The overlain spectra showed  $\lambda$  max of both drugs and also isoabsorptive points at 238.8 nm (Fig 3.).

**2.4. Method I (Simultaneous equation method) (Becket and Stenlake):**

Two wavelengths selected for the method are 361 nm and 246 nm that are absorption maxima of AMD and ATR respectively in methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series of standard solutions of 0.5-30 µg /mL concentrations. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations-

$$Cx = (A_2ay_1 - A_1ay_2) / (ax_2ay_1 - ax_1ay_2), \quad Cy = (A_1ax_2 - A_2ax_1) / (ax_2ay_1 - ax_1ay_2)$$

Where,  $A_1$  and  $A_2$  are absorbances of mixture at 361nm and 246 nm respectively,  $ax_1$  and  $ax_2$  are absorptivities of ATR at  $\lambda_1$  and  $\lambda_2$  respectively and  $ay_1$  and  $ay_2$  are absorptivities of AMD at  $\lambda_1$  and  $\lambda_2$  respectively.  $Cx$  and  $Cy$  are concentrations of ATR and AMD respectively.

**2.5. Method II (Absorption ratio or Q-Analysis method) (Becket and Stenlake):**

From the overlain spectrum of AMD and ATR, two wavelengths were selected one at 238.8 nm which is the isoabsorptive point for both the drugs and the other at 246 nm which is  $\lambda$  max of ATR. The absorbances of the sample solutions are prepared in a similar manner as in the previous method, were measured and the absorbance ratio values for both the drugs at selected wavelengths were also calculated. The method employs Q-values and the concentrations of drugs in sample solution were determined by using the following formula,

$$\text{Conc. of ATR: } C1 = \frac{Q_m - Q_1}{Q_2 - Q_1} x \frac{A}{a}, \quad \text{Conc. Of AMD: } C2 = \frac{Q_m - Q_2}{Q_1 - Q_2} x \frac{A}{a}$$

A = Absorbance of sample at isoabsorptive point, a = Absorptivities of AMD and ATR respectively at isoabsorptive point.  $Q_m$ ,  $Q_1$  and  $Q_2$  are absorbance ratio of mixture, AMD and ATR at Iso-absorptive point to maximum wavelength of one of the component (selected wavelength).

## 3. EXPERIMENTAL

**3.1. Method Validation:** Method was validated accordance to ICH guidelines (FDA,2000;ICH,1995;ICH,1996) for system suitability, linearity, precision, accuracy, limit of detection, limit of quantification and specificity.

**3.2. Linearity:** The linearity of this method was evaluated by Linear Regression Analysis, which was calculated by Least Square method and the drug was linear in the concentration range of 0.5-30 µg/ml for both the drugs. Calibration standards were prepared by spiking required volume of working standard (100µg/mL) solution into different 10 ml volumetric flasks and volume made with methanol to yield concentrations of 0.5, 1, 2, 5, 10, 20 and 30µg/ml. The resultant absorbances of the drugs were measured. Calibration curve was plotted between absorbance of drug against concentration of the drug. These results shown there was an excellent correlation between absorbance and analyte concentration. The linearity graph is presented in [Fig 4.].

**3.3. Intra-day and Inter-day Precision and Accuracy:** Precision and accuracy was studied by quality control samples of standard solutions covering low, medium and high concentrations (3, 15 and 25µg/mL) of linearity range were prepared

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and measured the absorbance of three replicated samples of each concentration. Intra-day precision was studied by six replicate measurements at three concentration levels in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evaluation revealed that relative standard deviation of the drug at different concentration levels for six injections were less than 2. Precision and accuracy data were shown in Table 2 and 3.

**3.4. limits of Detection and Quantification:** The limit of detection of an analytical method may be defined as the concentration, which gives rise to instrument signal that is significantly different from the blank (signal to noise ratio 3) and LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision (signal to noise ratio 10). The LOD and LOQ was calculated based on the Standard Deviation of the Response and the Slope. The values were shown in Table 1.

**3.5. Application of Methods to Tablet Dosage forms:** 20 Tablets (Brand Name: ALNAVAS-A, anla bio) were weighed, and an accurately weighed sample of powdered tablets equivalent to 5mg of AMD and 10mg of ATR [equivalent to one tablet]. For analysis of drug, a standard addition method was used. An accurately weighed 5 mg of pure AMD was added to finely powdered samples to bring the ratio of AMD and ATR to 1:1. Quantity of powder equivalent to 10 mg of AMD and 10 mg of ATR was weighed and dissolved in 60 mL of methanol and sonicated for 10 minutes in a 100ml volumetric flask and this solution was filtered through Whatmann No.1 filter paper. The residue was washed with 10ml methanol three times and volume made upto 100ml with methanol. The solution obtained was diluted with the Methanol so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in five replicates. In Method I, the concentration of both AMD and ATR were determined by measuring the absorbance of the sample at 246 nm and 361 nm. For Method II, the concentration of both AMD and ATR were determined by measuring absorbance of the sample at 238.8 nm and 246 nm and values were substituted in the respective formula to obtain concentrations. Results of tablet analysis are shown in Table 4.

## 4.RESULTS AND DISCUSSION

Drug content in tablet (amount found) was directly found from equations for both the methods. Standard deviations and Coefficient of variation was calculated (Table 2 and 3). The low standard deviation values indicated repeatability, accuracy and reproducibility of the methods. Reproducibility, reliability and interference was also confirmed by recovery studies. Thus, it can be concluded that the methods developed were simple, accurate, sensitive and precise. Statistical analysis and drug recovery data showed that both methods are sensitive, accurate and precise. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully in simultaneous estimation of Atorvastatin calcium and Amlodipine besylate in marketed formulations.

**Fig 3 : Overlain spectra of Amlodipine and Atorvastatin**

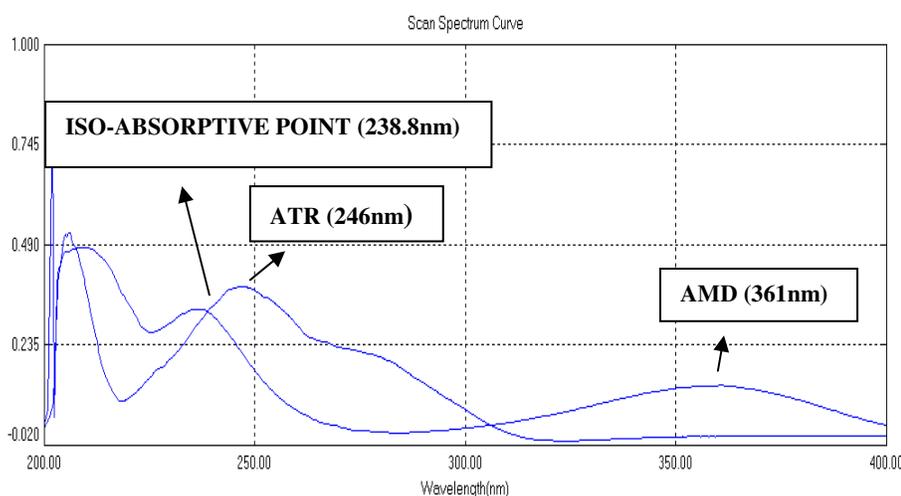


Fig 4: Linearity graph of Amlodipine and Atorvastatin mixture

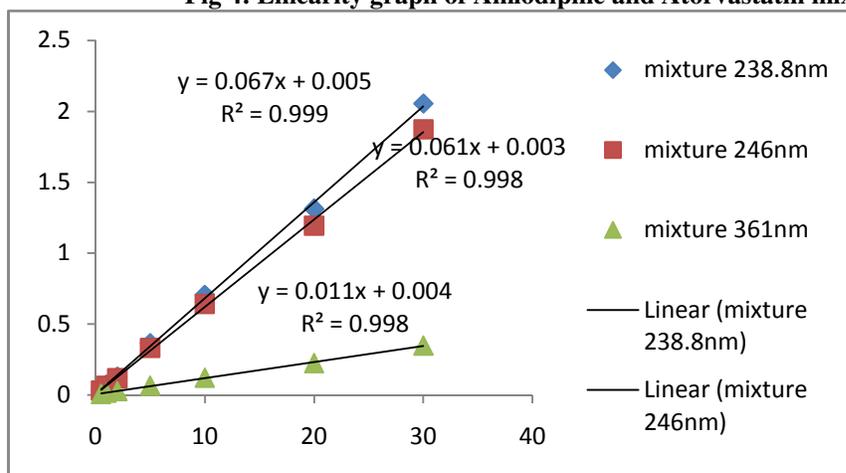


Table 1: Optical characteristics

Parameter	238.8nm		246nm		361nm	
	AMD	ATR	AMD	ATR	AMD	ATR
Beer law limit ( $\mu\text{g/mL}$ )	0.5-30	0.5-30	0.5-30	0.5-30	0.5-30	0.5-30
Molar absorptivity	$1.30 \times 10^4$	$1.78 \times 10^4$	$9.44 \times 10^3$	$2.13 \times 10^4$	$1.35 \times 10^4$	$1.78 \times 10^4$
Regression equation			Y=Mx+c			
Slope (m)	0.067		0.061		0.011	
Intercept (c)	0.005		0.003		0.004	
Correlation coefficient ( $R^2$ )	0.999		0.998		0.998	
Limit of detection ( $\mu\text{g/mL}$ )	0.028		0.054		0.017	
Limit of quantification ( $\mu\text{g/mL}$ )	0.086		0.163		0.052	

Table 2 : Intra-day precision and accuracy of Amlodipine and Atorvastatin

	Conc. ( $\mu\text{g/mL}$ )	Method – I		Method – II	
		Accuracy	%RSD	Accuracy	%RSD
AMD	3	103.77 $\pm$ 1.48	1.429	104.48 $\pm$ 0.30	0.295
	15	101.22 $\pm$ 0.29	0.295	103.24 $\pm$ 0.10	0.103
	25	99.82 $\pm$ 0.17	0.172	104.97 $\pm$ 0.03	0.037
ATR	3	98.09 $\pm$ 0.78	0.797	99.62 $\pm$ 0.29	0.295
	15	105.86 $\pm$ 0.07	0.073	98.43 $\pm$ 0.10	0.103
	25	103.33 $\pm$ 0.56	0.548	100.08 $\pm$ 0.03	0.035

Values expressed Mean $\pm$ SD, (n=6)

Table 3 : Inter-day precision and accuracy of Amlodipine and Atorvastatin

	Conc. ( $\mu\text{g/mL}$ )	Method – I		Method – II	
		Accuracy	%RSD	Accuracy	%RSD
AMD	3	102.88 $\pm$ 1.47	1.430	100.64 $\pm$ 0.46	0.462
	15	103.12 $\pm$ 0.29	1.472	103.24 $\pm$ 0.10	0.103
	25	99.82 $\pm$ 0.17	0.172	104.97 $\pm$ 0.03	0.037
ATR	3	100.08 $\pm$ 0.46	0.462	100.64 $\pm$ 1.06	1.054
	15	104.42 $\pm$ 1.10	1.057	99.31 $\pm$ 1.00	1.103
	25	99.80 $\pm$ 0.17	0.175	100.24 $\pm$ 0.22	0.220

Values expressed Mean $\pm$ SD, (n=9)

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**Table 4: Recovery study from formulation of Amlodipine and Atorvastatin**

Labeled amount (Alnavas-A)	Method – I Accuracy	%RSD	Method – II Accuracy	%RSD
AMD 5mg	101.22±0.69	0.694	103.24±0.10	0.203
ATR 10mg	99.86±0.06	0.063	98.43±0.10	0.503

Values expressed Mean±SD, (n=5)

## REFERENCES

Agrekar AP, Powar SG, Reverse phase High Performance Liquid Chromatographic determination of Ramipril and Amlodipine in tablets, *J.Pharm.Biomed.Anal.*, 21, 2000, 1137-1142.

Bahrami GH, Mirzaei Sh, Simple and rapid HPLC method for determination of Amlodipine in human serum with fluorescence detection and its use in pharmacokinetic studies, *Journal of Pharmaceutical and Biomedical Analysis*, 36, 2004, 163–168.

Bahrami Gh, Bahareh Mohammadia, Shahla Mirzaei, Amir Kiani, Determination of Atorvastatin in human serum by RP-HPLC with UV detection, *Journal of Chromatography B*, 826, 2005, 41–45.

Becket AH, Stenlake JB, *Practical Pharmaceutical Chemistry*, 4<sup>th</sup> ed., part-II, CBS publishers and Distributors, 11, Darayaganj, New Deilhi, India.

Budawari S, *The Merck Index*, 23rd ed., Whitehouse Station, New Jersey, 516, 2008, 6235.

Burnham TH, HMG-CoA reductase inhibitors, In: ed. *Drug Facts and Comparisons*, Louis: Facts and Comparisons, 2002, Inc 536-542a.

Desager JP, Hormans Y, *Clinical Pharmacokinetics of 3-hydroxy-3methylglutaryl-coenzyme A reductase inhibitors*, *Clin.Pharmacokinet.*, 31, 1996, 348-371.

FDA., 2000. *Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls*, Federal Register (Notices), 65 (169), 52:776–777.

ICH Q2A, *Guidelines on validation of analytical procedure: Definitions and terminology*, Federal Register, 1995, 60:11260.

ICH Q2B, *Guidelines on validation of analytical procedure: methodology*, Federal Register, 1996, 60:27464.

Kumar R, Sharma A, Mitra M, Chandrashekar T, Stability indicating RP-HPLC method for simultaneous estimation of Amlodipine and Benazepril hydrochloride in capsule dosage form, *Analytical Research*, Ranbaxy Research Laboratories.

Lincy joseph, Mathew George, Venkata ranga rao B, Simultaneous estimation of Atorvastatin and Ramipril by RP-HPLC and spectroscopy, *Pak.J.Pharm.Sci.*, 213, 2008, 282-284.

Lipitor product information, Park-Davis/Pfizer Inc., April 2002.

Malinowski JM, Atorvastatin: A hydroxymethylglutaryl-coenzyme A reductase inhibitors, *J.Health-Syst.Pharm.*, 1998, 2253-2267.

Martindale, Sweetman SC, *The complete drug reference*, 32nd ed., Pharmaceutical Press, 1999, 822, 907.

Mohammadi A, Rezanour N, Ansari M, Dogaheh, Ghorbani F, Bidkorbeh, Hashem M, Walker RB, Stability indicating RP-HPLC assay for simultaneous determination of Atorvastatin and Amlodipine in commercial tablets, *Journal of Chromatography B*, 846, 2007, 215-221.

## Journal of Chemical and Pharmaceutical sciences

Oneil MJ, Smith A, Heckelman PE, Budawari S, The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals, 13rd ed., Merck and Co Inc., White House Station, New jersey, 2001, 488, 865.

Priyanka R Patil, Sachin U Rakesh, Dhabale PN, Burade KB, Simultaneous UV Spectrophotometric Method for Estimation of Losartan potassium and Amlodipine in Tablet Dosage Form, Asian J.Research Chem., 2(1), 2009.

Sandeep SS, Atul AS, Ravindra AF, Sanjay JS, Application of UV-spectrophotometry and RP-HPLC for simultaneous determination of Atorvastatin and ezetimibe in pharmaceutical dosage forms, Eurasian Journal of Analytical Chemistry, 1(1), 2006.

Topale PR, Gaikwad NJ, Tajane MR, Simultaneous UV- spectrophotometric estimation of losartan potassium and Amlodipine in tablet, Indian drugs, 40 (2), 2003, 119-121.

Vora DN, Kadav AA, Development and validation of simultaneous estimation of Amlodipine and Bisoprolol fumarate in tablet formulation, Indian journal of pharmaceutical sciences, 70(4), 2008, 542-546.

Zarghi A, Validated HPLC method for determination of Amlodipine in human plasma and its application to Pharmacokinetic studies, Farmaco, 60, 2005, 789-792.