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SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND ATENOLOL AS A.P.I. AND IN TABLET DOSAGE FORMS BY VIERODT'S METHOD USING U.V. SPECTROPHOTOMETRY

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ABSTRACT: A new UV-Spectrophotometric method has been described for the simultaneous assay of Amlodipine Besylate and Atenolol in bulk drug and in tablet dosage forms using aqueous medium as the solvent. The method is based on simultaneous equation or Vierodt's method. The λ_{max} values for Amlodipine Besylate and Atenolol in the solvent medium were found to be 238.4 nm and 273.4 nm respectively. The systems obey Beer's law in the range of 4.0 to 32.0 µg/ml and 20.0 to 200.0 µg/ml with correlation coefficient of 0.9984 and 0.9996 for Amlodipine Besylate and Atenolol respectively. Repeatability, Interday and intraday precision were found to be 0.562, 0.474, 0.456 and 0.238, 1.31, 0.337 respectively. No interference was observed from common tablet adjuvants. t –test and F-test have been applied for the recovery studies of the method. The method was successfully applied to the assay of Amlodipine Besylate and Atenolol in tablet formulations.
KEYWORDS: Amlodipine Besylate; Atenolol; UV Spectrophotometry; Vierodt's method

INTRODUCTION

Amlodipine Besylate [1] (AML) chemically 3-Ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate is a long-acting calcium channel blocker used for hypertension and angina pectoris [2-4]. Amlodipine Besylate block the inward movement of calcium by binding to L-Type calcium channels in the heart and in smooth muscle of the coronary and peripheral vasculature relaxing the smooth muscle and dilating arterioles thereby decreasing peripheral resistance. Hence improving blood pressure; in angina it improves blood flow to the myocardium.

Atenolol (ATE) [5] chemically 2-[4-{(2RS)-2-hydroxy-3-[(1-methylethyl) amino] propoxy} phenyl] acetamide is a β-adrenoreceptor blocking agent primarily used for hypertension, angina pectoris & myocardial infarction. It mainly acts by inhibition of renin release and angiotensin -II (AT-II) and aldosterone production.

The official method for estimation of AML includes Non-aqueous titration [6] & HPLC [7] and for ATE is HPLC [8-9]. The methods reported for simultaneous estimation of either drug in combination are RP-HPLC [10], UV- Spectrophotometric [11], HPTLC [12], Derivative Spectrophotometry [13], HPLC [14] and Fluorimetric [15].

In the present method double distilled water is used as solvent for simultaneous estimation of both the drugs by simultaneous equation or Vierodt's method using UV-Spectrophotometry. The present method is relying on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC & HPTLC.

MATERIAL & METHODS

Instrument

ELICO SL 160 Double beam UV-VIS Spectrophotometer with spectral band width of 1.8 nm, wavelength accuracy of ±2 nm and matched quartz cells of 10 mm optical path length was used for all spectral and absorbance measurements.

Reagents and materials

All chemicals used were of analytical reagent grade and double distilled water was used to prepare the solvent medium. Pharmaceutical grade AML and ATE procured from Torrent labs, Hyderabad, India were used as received. A standard solution of AML and ATE were prepared by dissolving 10.0 mg of pure drugs in double distilled water and diluting to 100 ml with double distilled water. These stock solutions (100.0 µg/ml) were diluted with double distilled water to get working concentrations of 4.0 to 32.0 µg/ml for AML and 20.0 to 200.0 µg/ml for ATE.

Method

Aliquots of pure AML and ATE solutions (0.2-1.6 ml) and (1.0-10.0 ml) were transferred into a series of 10 ml calibrated flasks and the total volume was adjusted upto the mark with double distilled water. The absorbance's of the resulting solutions were then measured at 238.4 nm and 273.4 nm respectively in triplicate against double distilled water as blank and calibration curves were plotted between absorbance v/s concentrations (Fig. 1).

Assay of formulations

Twenty tablets each of two brands were weighed and ground into a fine powder. Powder equivalent to 5 mg of AML and 50 mg of ATE was transferred into 100 ml volumetric flask and dissolved in 25 ml of double distilled water. The solution was sonicated for 20 minutes and was filtered through whatmann No. 40 filter paper. The residue was washed with double distilled water and the washings were added to the filtrate. The volume was made upto the mark with double distilled water so as to get a concentration of 50.0 µg/ml of Amlodipine Besylate and 500.0 µg/ml of Atenolol. From this solution, (1.6 ml) was pipetted out into 10 ml volumetric flask and diluted upto the mark with double distilled water so as to get a concentration of 8.0 µg/ml of Amlodipine Besylate and 80.0 µg/ml of Atenolol. The absorbances of these solutions were measured at 238.4 nm and 273.4 nm using double distilled water as blank.

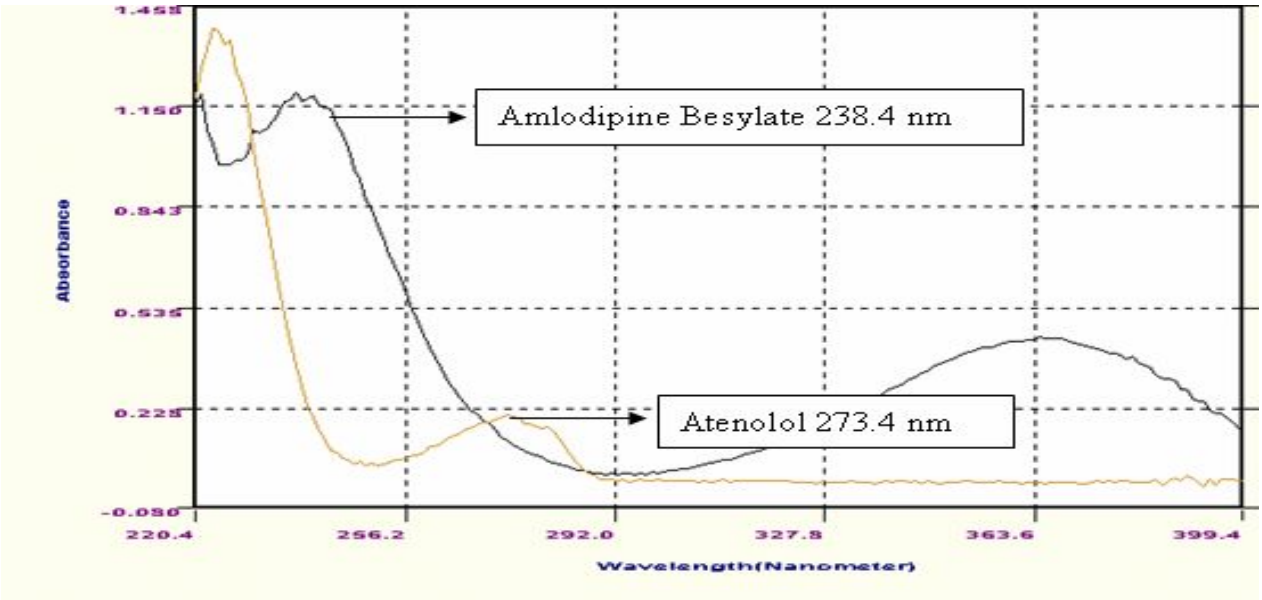

Figure 1: Overlain spectra of synthetic mixture of Amlodipine Besylate and Atenolol between 220-400 nm

RESULTS AND DISCUSSION

Analytical data

A linear correlation was found between absorbances at λ_{max} and concentrations of AML and ATE. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity values are given in Table 1. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values are presented in Table 1. The graph shows negligible intercept as described by the regression equation Y = a + bX where Y is the absorbance and x concentration in μg/ml. The limit of detection and quantification calculated according to ICH guidelines [16] and reveals a very high sensitivity of the methods.

METHOD VALIDATION

Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solutions at three different levels (within the working limits) were analyzed, each determination being repeated three times. The relative standard deviations (%) were less than 1 and indicate the high accuracy and precision for the methods (Table 2). For intra-day and interday precision the relative standard deviation values were in the range of 0.456 -1.31% and represent the best appraisal of the methods in routine use.

Interference study

To investigate the effect of tablet fillers on the measurements involved in the method 0.8, 1.0, 1.2, 1.4 and 1.6 ml of AML and 8.0, 10.0, 12.0, 14.0 and 16.0 ml of ATE were mixed in five 10 ml different volumetric flasks and the volume was made upto mark with double distilled water. The absorbances of the final resulting solutions were measured at 238.4 nm and 273.4 nm. Then a solution containing excipients was added to the above preanalysed solution and filtered the solution using whatmann filter paper no. 40. The ratio of drug: excipients were 1:5 based on ratio available in marketed formulations. The residues were washed with double distilled water and washings were mixed the filtrates. The absorbance's of the resulting solutions were then measured in triplicate and compared to the results of the preanalysed solutions (Fig. 2 & Table 3).

Table 1: Optical and regression parameters of AML and ATE in double distilled water

Parameters	AML		ATE	
λ max (nm)	**238.4 nm**	**273.4 nm**	**238.4 nm**	**273.4 nm**
Beer's law limit (μg/ml)	4-32	20-200	20-140	20-200
Molar absorptivity (l mole^{-1}cm^{-1})	10.8 x 10^3	25.1 x 10^3	19.1 x 10^3	12 x 10^3
Sandell's sensitivity (mg/cm^2/.001absorbance unit)	0.031	0.039	0.052	0.210
Correlation coefficient (r)	0.9992	0.9984	0.9988	0.9998
(R^2)	0.9984	0.9969	0.9976	0.9996
Regression equation (y= a + bc)				
slope (b)	0.0322	0.0047	0.0067	0.0048
intercept (a)	0.0038	0.0243	0.0231	0.0122
S_a** = Standard deviation of slope	0.002	0.001	0.002	0.001
S_b** = Standard deviation of intercept	0.011	0.002	0.014	0.0023

(Y)* = a + bX where Y is the absorbance and x concentration in μg/ml

Table 2- Summary of validation parameters for AML & ATE

S. No.	Parameters	AML	ATE
1.	**Specificity:** -% interference -% agreement	 ≤ 0.5% 100.3-100.4	 ≤ 0.5% 100.3-100.5
2.	**Range (µg/ml):** -Working range -Linearity range -Target range -Test conc. (100%)	 0.87-32.0 4.0-32.0 14.4- 21.6 18.0	 4.67-200.0 20.0-160.0 72.0-108.0 90.0
3.	**Precision: (RSD)** -Repeatability (n =7) -Intraday (n=3) -Interday (3 days)	 1.61 0.586 0.662	 0.371 0.374 0.410
4.	**Accuracy %**	98.25-99.17	98.61-99.95
5.	**Limit of detection, µg mL^{-1}**	0.288	1.51
6.	**Limit of quantification, µg mL^{-1}**	0.872	4.67

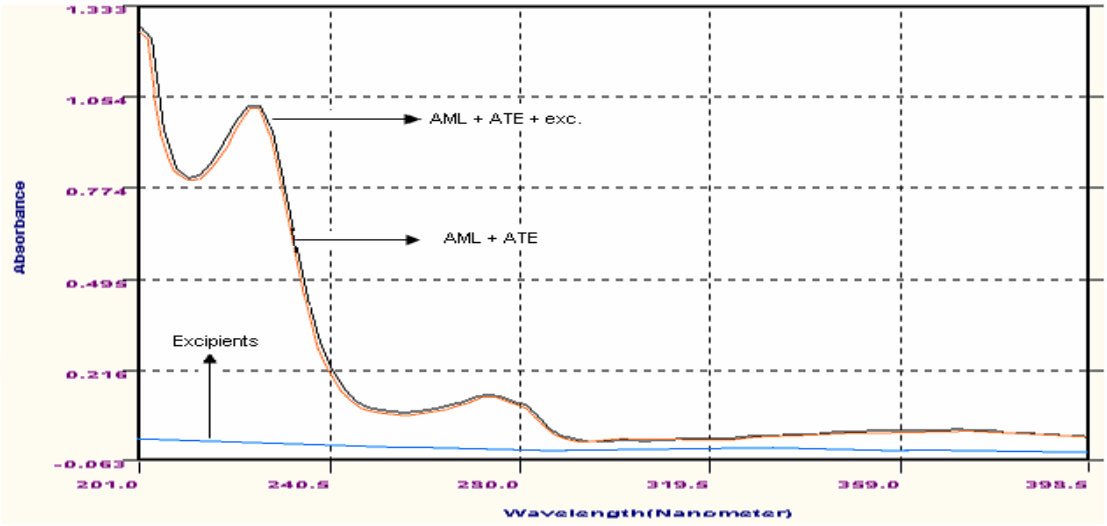

Figure 2: Overlain spectra of AML + ATE, AML + ATE + excipients and excipients at 238.4 & 273.4 nm

Table 3: Result of specificity study for the synthetic mixture of AML and ATE

S. No.	In absence of excipients		In presence of excipients		% Interference $\frac{(Cp - Ca)*100}{Ca}$	% Agreement Cp/ Ca*100
	Abs.*	Conc. (µg/ml) Ca	Abs.*	Conc. (µg/ml) Cp		
1.	0.255	8.10	0.259	8.13	0.37	100.37
	0.376	80.09	0.378	80.51	0.52	100.52
2.	0.319	10.15	0.324	10.17	0.20	100.20
	0.470	100.11	0.472	100.53	0.42	100.42
3.	0.382	12.09	0.386	12.12	0.25	100.25
	0.563	120.07	0.565	120.34	0.22	100.22
4.	0.446	14.17	0.452	14.19	0.14	100.14
	0.657	140.13	0.659	140.36	0.16	100.16
5.	0.510	16.11	0.515	16.17	0.37	100.37
	0.751	160.08	0.754	160.60	0.32	100.32

Application to analysis of commercial samples

In order to check the validity of the proposed method, AML and ATE were determined in some commercial formulations. Table 4 present the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the labeled claim.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powders were spiked with pure AML and ATE standard solutions at three different levels and the concentration of the sum total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug solution added was quantitative (98.44-99.25 % and 99.84-99.9 % respectively) and revealed that co-formulated substances did not interfere in the determination.

CONCLUSION

The methods for the determination of Amlodipine Besylate & Atenolol have been developed and validated. These are applicable over a range of 4-32 µg/ml for AML and 3.03-27.27 µg/ml for ATE and molar absorptivity of 10.8×10^3 L $mole^{-1}$ cm^{-1} for AML and 12.0×10^3 L $mole^{-1}$ cm^{-1} for ATE. The recoveries of AML and ATE from two brands have been compared by using t- test and F-test for both the methods and results are shown in Table 5. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC, HPTLC. Thus these can be used as alternatives for rapid and routine determination of bulk sample and tablets.

Table 4- Summary of estimation of AML & ATE in different brands

S. No.	Brand	Labeled amount (mg)	Amount found [a] (mg)	% of Labeled amount [a]	RSD
1.	**Amlovas-AT**	5 (AML)	4.98 ± 0.029	99.6 ± 0.588	0.591
		50 (ATE)	49.99 ± 0.172	99.9 ± 0.343	0.344
2.	**Amlopores-AT**	5 (AML)	4.98 ± 0.013	99.5 ± 0.258	0.259
		50 (ATE)	49.96 ± 0.022	99.92 ± 0.04	0.044

a: data represents mean ± SD ; n = 3.

Table 5- Results of t-test and F-test applied for AML & ATE for Amlovas-AT & Amlopores-AT brands

S. No.	Parameters	Amlovas-AT	Amlopores-AT
1	**Mean**	99.44	99.00
2	**Variance**	0.051	0.242
3	**t Stat**	**1.41**	
4	**P (T<=t) two-tail**	0.23	
5	**t Critical two-tail**	**2.78**	
6	**$F_{(2,2)}$ Statistical**	**4.75**	
7	**$F_{(2,2)}$ critical**	**19.0**	

Here, t_{crit} and F_{crit} is greater than t_{stat} and F_{stat}; hence significant difference between the recoveries of Amlodipine Besylate and Atenolol by using the two brands does not exist.

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MATHEMATICAL CALCULATIONS

Amounts of Amlodipine Besylate and Atenolol were determined by solving the simultaneous equations**.** Two simultaneous equations were formed using absorptivity coefficient values.

$$A_1 = 10.8 \times C_1 + 19.1\ C_2 \text{ ---------- (1)}$$

$$A_2 = 25.1 \times C_1 + 12.0\ C_2 \text{ --------- (2)}$$

The concentrations of Amlodipine Besylate and Atenolol were calculated using following two equations.

$$C_1 = -\frac{A_1 \times 4.69 - A_2 \times 7.0}{118.34} \text{ --- (3)}$$

$$C_2 = -\frac{A_2 \times 4.4 - A_2 \times 31.8}{118.34} \text{ --- (4)}$$

Where C_1 and C_2 are concentration of Amlodipine Besylate and Atenolol respectively in gm/liter in the sample solution, A_1 and A_2 are the absorbances of the mixture at 238.4 nm and 273.4 nm respectively.

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