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RESEARCH ARTICLE

Simultaneous Determination of Atorvastatin and Amlodipine in Industrial Tablets by Apparent Content Curve and HPLC Methods

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Introduction: This study proposes the simultaneous determination of atorvastatin and amlodipine in industrial tablets by a quantitative spectrophotometric method, named the apparent content curve method, test method, and by an HPLC method with UV detection as reference method.

Materials and methods: A synthetic mixture and two fixed medicinal combinations containing amlodipine and atorvastatin were investigated by the apparent content curve method, a simple and relatively inexpensive UV-VIS spectrophotometric method based on a mathematical approach derived from the Lambert-Beer law. The results were compared with those obtained by an HPLC method.

Results: A good correlation of the results was obtained, the difference between the pair results was not significant ($p > 0.05$).

Conclusions: The proposed spectrophotometric method is an easier and cheaper alternative for the quantitative determination of amlodipine and atorvastatin in industrial fixed-dose combinations.

Keywords: apparent content curve, UV spectrophotometry, HPLC, atorvastatin, amlodipine

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Introduction

The fixed combination of amlodipine (AML) and atorvastatin (AT) simultaneously reduces both blood pressure and lipid levels, with prevention of cardiovascular diseases [1,2,3]. AML is a dihydropyridine calcium channel blocker and inhibits the extracellular calcium influx into vascular smooth muscle. The consequence is muscle relaxation and vascular dilatation, and for this reason it is used to treat hypertension, angina or coronary heart disease. AT is a competitive inhibitor of HMG-CoA reductase, reducing LDL cholesterol, apolipoprotein B and triglycerides, and increasing HDL cholesterol in the treatment of hyperlipidaemia, when associated with a proper diet and exercise.

Several techniques, including chemometric methods [4,5,6], RP-HPLC [7] and HPLC methods [8] have been employed for the simultaneous determination of AT and AML from fixed combinations.

This study attempts to demonstrate the usefulness and ability of spectrophotometry in the analysis of multicomponent pharmaceutical systems [9]. At first, spectral behavior of AML and AT was studied for their quantitative spectrophotometric determination. Then, the apparent content curve (ACC) method was applied for the simultaneous determination of both substances in industrial tablets [10]. Since the ACC method is considered a secondary method, quantitative determination was also performed by HPLC method, considered as a reference method. The results obtained in these studies are discussed and compared.

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Materials and methods

Chemicals and reagents

The reference substances were AML besylate, working standard provided by Gedeon Richter Romania, AT calcium trihydrate, working standard provided by Synfine. Methanol (Merck) and ultrapure water (Millipore) were used for the ACC method, and acetonitrile, potassium dihydrogen phosphate, methanol (Merck) and ultrapure water (Millipore), for the HPLC method.

Tested pharmaceutical products

Two types of commercial products were tested: product 1 – tablets containing the equivalent of 5 mg AML as AML besylate and the equivalent of 10 mg AT as AT calcium trihydrate; product 2 – tablets containing the equivalent of 10 mg AML as AML besylate and the equivalent of 10 mg AT as AT calcium trihydrate.

Instruments

For spectrophotometric determinations, UV-1601 UV-VIS spectrophotometer (Shimadzu, Japan) provided with 1 cm quartz cells was used. The HPLC analysis was performed with a 1100 HPLC system with UV detection, producer Agilent Technologies (USA), equipped with Luna C18 column (150 × 4.6 mm, 3 µm, Phenomenex).

Procedures

a) The ACC method

The method has been extensively described previously

since it was first published [10] and the same notation has been used in the present study.

Determination of molar absorption coefficient and the selection of the working wavelengths

In order to determine the molar absorption coefficients of AML besylate and AT calcium in methanol, a standard solution of 10 μg/ml AML in methanol and a standard solution of 20 μg/ml AT in methanol were prepared, starting from stock methanol solutions of 100 μg/ml AML and 200 μg/ml AT, respectively. Spectra were recorded on both standard solutions in the 200–400 nm field, using 1 cm quartz cuvettes. By taking into account the mathematical expression of the Lambert-Beer law, the molar absorption coefficients at different wavelengths were obtained.

From two stock solutions of AT calcium and AML besylate with concentrations of 200 μg/ml and 100 μg/ml respectively, a standard mixture of AT and AML with concentrations of 10 μg/ml AT and 5 μg/ml AML in methanol was obtained. The spectra of the obtained standard mixture was recorded and it allowed the building of apparent content curve F = f(λ). The term F is called apparent content being $F_\lambda = (A/\varepsilon_A)_\lambda = c_{apparent}$, where A is the total absorbance of the solution at the wavelength λ and ε_A is the molar absorption coefficient of the substance considered as analyte in the mixture. If there is any interference, the apparent content graphic is a curve line in the spectral interference domain. AT was chosen as analyte and AML as interferent, since in the studied industrial tablets we can find AT in equal or greater concentration than AML. The three working wavelengths λ_1, λ_2 and λ_3 were chosen from apparent content curve around the maximum absorption of the analyte, where the interference is strong. The differences between consecutive wavelengths have to be not more than 5 nm.

Construction of R^i_p resolution calibration curves

An initial standard binary mixture solution of 10 μg/ml AT and 10 μg/ml AML in methanol has been prepared from stock solutions of AML and AT, and a series of solutions were prepared by diluting the initial standard. After measuring the absorbances of these solutions on λ_1, λ_2 and λ_3, the value of qualitative parameter $Q = (F_{\lambda 1}–F_{\lambda 2})/(F_{\lambda 1}–F_{\lambda 3})$ could be calculated and the $R^i_p = f(1/i)$ resolution calibration curve obtained. The term R is called resolution of the binary mixture:

$$R_p = F_{\lambda 1} - F_{\lambda 3} = \left[\frac{A}{\varepsilon_A}\right]_{\lambda 1} - \left[\frac{A}{\varepsilon_A}\right]_{\lambda 3}$$

For a series of standard mixture solutions R_p is a linear function with the degree of dilution 1/i. Of course, the quantitative ratio of the two substances in each standard solution in this series is the same.

The same procedure was applied for both control sample (standard mixture) and tablets samples.

Determination of AT and AML concentration

A. Control sample analysis

A control sample solution with concentration 9/9 μg/ml AML/AT was obtained, and the absorbance was measured at the three selected wavelengths. Values of Q_x and R_x of the control sample were calculated and it was found the degree of dilution 1/i of the initial standard mixture which can produce a new standard mixture with $R^i_p = R_x$ and it has a concentration of interferent C_I equal with the concentration of interferent in the control sample $(C_I)_x$. The control sample solution and the new standard mixture solution were diluted (degree 1/j) in the same way, and the $F_p^j = f(1/j)$ and $F_x^j = f(1/j)$ curves were obtained, at 250 nm. The difference between the slopes of the obtained lines, a and ax, allowed the calculation of AT (analyte) concentration:

$$(C_A)_x = (C_A)_p - (a - a_x)$$

B. Tablets analysis

Extraction from tablets: 10 tablets were weighed accurately and ground into a fine powder. An accurately weighed amount of the powdered tablets equivalent to 5 mg or 10 mg AML and 10 mg AT was transferred into a 100 ml standard flask and shaken with a mixture of methanol and ultrapure water (9:1) for 20 minutes in the ultrasonic bath, then made up to the mark with the solvent and mixed. The obtained solution was centrifuged for 5 minutes at 10,000 rpm at room temperature and then filtered through 0.45 μm PTFE filter. The filtrate was appropriately diluted with the respective solvent to obtain stock solutions of 50 μg/ml AML and 100 μg/ml AT for tablets 5/10, and 100 μg/ml AML and 100 μg/ml AT for tablets 10/10. After that, the sample stock solutions were diluted as follows: in the case of tablets 5/10, a sample solution containing theoretically 6/12 μg/ml AML/AT (sample P1) was analysed, and in the case of tablets 10/10, a sample solution with ratio concentration of 9/9 μg/ml AML/AT (sample P2).

In order to determine the working wavelengths, the ACC was obtained by using samples P1 and P2. After that, the procedure was the same as in the case of control sample.

b) The HPLC method

The optimized HPLC conditions were: column – Luna C18 (Phenomenex), 150 x 4.6 mm, 3 μm; mobile phase – 10 mM potassium dihydrogen phosphate solution (A) and acetonitrile (B); composition gradient – 0–5 min 40% B, 5–6 min 40→60% B, 6–11 min 60% B, 11–12 min 60→40% B, 12–15 min 40% B; mobile phase flow – 1 ml/ min; column temperature – 30 °C; injection volume – 10 μl; detector wavelength – 240 nm.

Results and Discussions

As it is shown in Figures 1 and 2, the two substances have a significant spectral interference around 240 nm wavelengths. As working wavelengths were selected the follow-

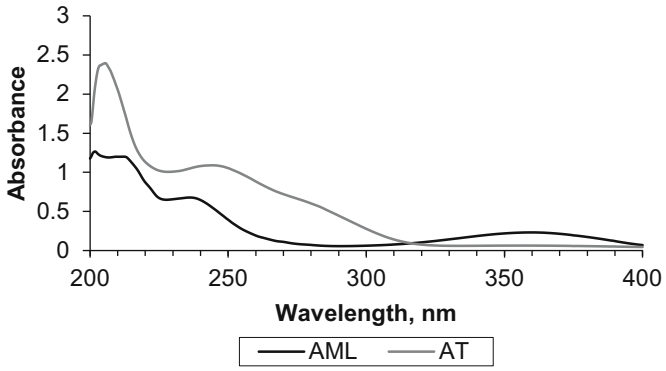

Fig. 1. Overlapped spectra of AML (10 µg/ml) and AT (20 µg/ml) in methanol

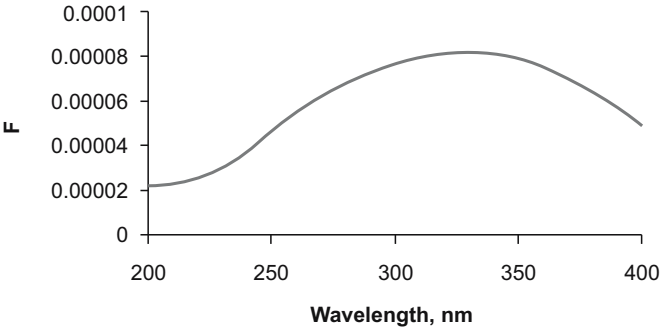

Fig. 2. Apparent content curve of a standard mixture of AML and AT (10 µg/ml AT and 5 µg/ml AML), AT-analyte

Table I. Values of Q and R for standard calibration mixtures used for quantitative determination of control sample (working wavelengths 245, 250 and 255 nm)

	1/i	$(C_A)_p$ [M]	$(C_I)_p$ [M]	Q	R_p^i [M]
	1	$2.70 \cdot 10^{-5}$	$3.68 \cdot 10^{-5}$	0.52	$5.74 \cdot 10^{-6}$
	0.8	$2.16 \cdot 10^{-5}$	$2.94 \cdot 10^{-5}$	0.55	$4.64 \cdot 10^{-6}$
	0.6	$1.62 \cdot 10^{-5}$	$2.21 \cdot 10^{-5}$	0.55	$3.50 \cdot 10^{-6}$
	0.4	$1.08 \cdot 10^{-5}$	$1.47 \cdot 10^{-5}$	0.52	$2.32 \cdot 10^{-6}$
	0.2	$0.54 \cdot 10^{-5}$	$0.73 \cdot 10^{-5}$	0.52	$1.18 \cdot 10^{-6}$
Q_{mean}	$Q_{mean} = 0.53$ (SD 0.01)				
Equation	$R_p^i = 5.72 \cdot 10^{-6}(1/i) + 4.28 \cdot 10^{-8}$, $R^2 = 0.9998$				

Table II. Values of Q and R for standard calibration mixtures used for quantitative determination of tablets samples (working wavelengths 250, 252 and 255 nm)

	1/i	$(C_A)_p$ [M]	$(C_I)_p$ [M]	Q	R_p^i [M]
	1	$2.69 \cdot 10^{-5}$	$3.67 \cdot 10^{-5}$	0.43	$2.07 \cdot 10^{-6}$
	0.8	$2.15 \cdot 10^{-5}$	$2.93 \cdot 10^{-5}$	0.44	$1.75 \cdot 10^{-6}$
	0.6	$1.61 \cdot 10^{-5}$	$2.20 \cdot 10^{-5}$	0.42	$1.18 \cdot 10^{-6}$
	0.4	$1.07 \cdot 10^{-5}$	$1.47 \cdot 10^{-5}$	0.42	$0.81 \cdot 10^{-6}$
	0.2	$0.53 \cdot 10^{-5}$	$0.73 \cdot 10^{-5}$	0.44	$0.45 \cdot 10^{-6}$
Q_{mean}	$Q_{mean} = 0.43$ (SD 0.01)				
Equation	$R_p^i = 2.08 \cdot 10^{-6}(1/i) + 3.17 \cdot 10^{-8}$, $R^2 = 0.993$				

Table III. The degree of dilution of the initial standard mixtures and the values Q and R for the tested samples

Solution	Q_x	R_x	1/i
Control sample	0.52	$3.51 \cdot 10^{-6}$	0.6
Sample P1	0.44	$0.64 \cdot 10^{-6}$	0.31
Sample P2	0.44	$0.74 \cdot 10^{-6}$	0.35

ing two triples: a) 245, 250 and 255 nm for control sample analysis; b) 250, 252 and 255 nm for tablets analysis. The slight differences between the working wavelenghts for the two type of samples were due to the apparently important interferences from disolved excipients at 200–240 nm.

Following the procedure described before, the results are summarized in Tables I–V.

The final results show that for the control sample we obtained experimental concentrations with relative errors under 1% against theoretical concentrations, which means that the ACC method is suitable for quantitative determination of this pair of substances in mixtures. For the 5/10 tablet sample the found that concentrations are below 3% apart from nominal concentration, which means that the tablets are within acceptance limits of ±7.5% provided by Pharmacopeia [11–13]. In the case of the 10/10 tablet the concentrations were obtained with a relative error of 5.6% for AT and –15.6% for AML from nominal concentrations, the last being above the acceptance limits of ±7.5%.

The same solutions used for the ACC method were analyzed in parallel by the HPLC method. Under the discribed HPLC conditions, the two substances were separated with a good resolution (Figures 3 and 4).

The HPLC method was tested in terms of analytical performance as follows:

Specificity. As shown in the chromatograms, the method is specific for the determination of AT and AML from tablets, without any interference from the existing excipients in the tablets.

Linearity. Calibration curves were linear in the 3–15 µg/ml AML/AT range. The equations of the calibration curves for AML and AT are shown in Table VI.

Accuracy. The method proved to be accurate, the concentration of the standard solutions being recovered with a relative error less than 2%.

Table IV. Parameters of the F_p^j and F_x^j equations

Equation parameters	Control sample		Sample P1		Sample P2	
	F_p^j	F_x^j	F_p^j	F_x^j	F_p^j	F_x^j
Intercept (b)	$-3.23 \cdot 10^{-6}$	$-1.34 \cdot 10^{-6}$	$-5.56 \cdot 10^{-7}$	$-2.82 \cdot 10^{-6}$	$-7.15 \cdot 10^{-7}$	$-4.56 \cdot 10^{-7}$
Slope (a)	$2.586 \cdot 10^{-5}$	$2.572 \cdot 10^{-5}$	$1.393 \cdot 10^{-5}$	$2.230 \cdot 10^{-5}$	$1.447 \cdot 10^{-5}$	$1.686 \cdot 10^{-5}$
R^2	0.9998	0.998	0.9999	0.998	0.9996	0.9998

Table V. Analyte and interferent concentrations in tested samples

Tested sample	Analyte AT			Interferent AML		
	Theoretical concentration, [M]	Found concentration, [M]	Recovery,%	Theoretical concentration, [M]	Found concentration, [M]	Recovery,%
Control sample	$1.623 \cdot 10^{-5}$	$1.622 \cdot 10^{-5}$	99.9	$2.208 \cdot 10^{-5}$	$2.230 \cdot 10^{-5}$	100.8
Sample P1	$1.693 \cdot 10^{-5}$	$1.697 \cdot 10^{-5}$	100.2	$1.154 \cdot 10^{-5}$	$1.130 \cdot 10^{-5}$	97.5
Sample P2	$1.130 \cdot 10^{-5}$	$1.193 \cdot 10^{-5}$	105.6	$1.541 \cdot 10^{-5}$	$1.300 \cdot 10^{-5}$	84.4

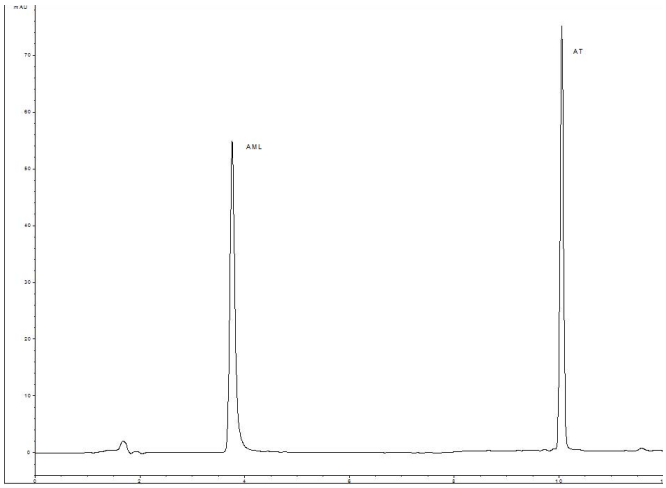

Fig. 3. Typical chromatogram – separation of AML ($t_{R\ AML}$ = 3.79 min) and AT ($t_{R\ AT}$ = 10.08 min) from a standard methanolic solution containing 15 µg/ml of each substances

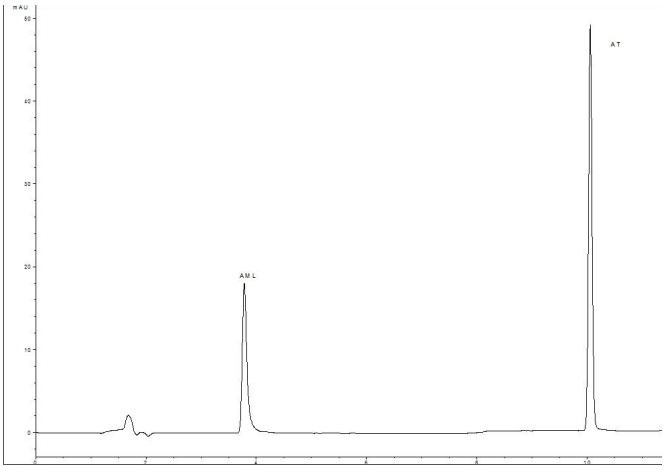

Fig. 4. Chromatogram of 5/10 AML/AT tablet sample

The final results of the determinations by HPLC method are shown in Table VII.

The results obtained from the two methods were compared (Table VIII). The differences, in terms of recovery, were less than 6% and not significant (p = 0.31 for AT and p = 0.44 for AML). The methods are comparable in terms of accuracy. The HPLC method has the advantage of being more sensitive and selective. On the other hand, the ACC method is easier, faster and cheaper.

Conclusions

The study is a new practical application of apparent content curve method. By comparing the results obtained by apparent content curve method with those obtained by reference methods, in this case an HPLC method, we can say that the proposed spectrophotometric method is an easier and cheaper alternative for the quantitative determination of amlodipine and atorvastatin in industrial fixed-dose combinations. In comparison with other methods published regarding the simultaneous determination of the studied substances, the present method is able to provide accurate quantification without analytes separation, it is more understandable in comparison with more abstract chemometric or computational methods, and does not require spectra computation as in derivative spectrophotometry. Nevertheless, the method allows rapid qualitative analysis by a specific parameter.

Table VI. Equation parameters of the calibration curve for HPLC analysis

Compound	Slope (a)	Intercept (b)	R^2
AML	25.36	–2.54	0.9998
AT	22.80	1.84	0.9999

Table VIII. Comparison of the results

Tested sample	Recovery AT %		Recovery AML %	
	ACC method	HPLC method	ACC method	HPLC method
Control sample	99.9	102.0	100.8	101.4
Sample P1	100.2	100.1	97.5	93.0
Sample P2	105.6	99.3	84.4	87.2

Table VII. HPLC results for tested samples

Tested sample	Analyte AT		Interferent AML		Recovery,%	
	Theoretical concentration $C_{theoretical}$, µg/ml	Found concentration C_x, µg/ml	Theoretical concentration $C_{theoretical}$, µg/ml	Found concentration C_x, µg/ml	AML	AT
Control sample	9.00	9.13	9.00	9.18	101.4	102.00
Sample P1	4.72	4.39	9.46	9.47	93.00	100.10
Sample P2	7.13	6.21	7.13	7.08	87.20	99.30

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