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(RESEARCH ARTICLE)

UV spectrophotometric test for analysis of antihypertensive drug combinations containing amlodipine

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Abstract

The present study provides rapid test for the identity and quantification of Amlodipine besylate as monoproduct and in double and triple antihypertensive drug combinations. Test represents direct analysis - drug mixtures are analyzed in solution without prior separation using UV-VIS spectrophotometry. The spectral characteristics of the complex spectrum are used for authentication, counterfeiting, identification and assay. Basic spectral characteristics are determined and analytical parameters of the validation process are investigated. The method uses 4 types of calculations for normal and derivative spectrophotometry at different analytical zones and fixed wavelengths in the range 190 - 500 nm. For double mixtures Amlodipine / Valsartan, the best results are obtained by determining at the 1st derivative of the function, the 2nd polynomial level, the presence of 3 smoothing points, the analytical range is 190 - 400 nm, and the fixed lengths of the waves - 266 and 360 nm. For triple mixtures containing Amlodipine / Valsartan / Hydrochlorothiazide, the best results are obtained with IVth calculation type for Amlodipine and Valsartan and Ist, IIIrd and IVth for Hydrochlorothiazide. The developed method was compared with available in European Pharmacopoeia and US Pharmacopoeia methods for assay of Amlodipine substance and tablets respectively. The results show comparability and meet the pharmacopoeia criteria.

Keywords: Amlodipine besylate; Antihypertensive drug combination; UV-spectrophotometry; Validation

1. Introduction

Raised blood pressure (BP) is currently the biggest single contributor to global mortality [1], and extensive randomized trial data are consistent in showing that BP reduction substantially reduces cardiovascular morbidity and mortality [2]. However, despite these facts and the widespread availability of effective antihypertensive medications, the vast majority of more than one billion hypertensive patients worldwide remain with uncontrolled BP. Even among hypertensive patients who receive treatment, in most countries at least half of them fail to reach currently recommended BP targets [3].

Recent clinical trials have demonstrated that adequate BP control is possible among the majority of patients if combinations of more than 2 antihypertensive medications are used for treatment [4]. In addition to the potential benefits attributable to possible synergistic pharmacological and physiological actions, this strategy of using a

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combination of 2 or 3 different drugs classes among drug-naive patients may, if provided in a single pill, also improve patient compliance and adherence [5-9].

Among the most widely used combinations are those containing Amlodipine besylate. Amlodipine - (RS)-3-ethyl-5-methyl-2-[[(2-aminoethoxymethyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate belongs to dihydropyridine class from the group of calcium channel blockers. It is prescribed as an antihypertensive agent and for the prevention of angina. It's use leads to constant and effective control of blood pressure and anti-ischemic effect in a single daily dose, is effective in a wide range of patients, has a favorable tolerability profile and proven benefits for reducing cardiovascular mortality and morbidity in randomized clinical trials [10-12]. Amlodipine is given orally as the besylate. It is combined very often in free and fixed dose combinations with other antihypertensive drugs as angiotensin II receptor antagonists (valsartan), thiazide diuretics (hydrochlorothiazide) and others.

Increased use of antihypertensive drugs has also led to quality control issues for double and triple combinations containing drug molecules in various chemical modifications that affect the properties and stability of medicinal products. Also, the widely use of antihypertensive drugs has led to an increase in cases of their falsification and appearance on the market of poor-quality drugs [13-14].

One way to overcome this problem is to introduce fast and reliable identity and quantification tests. A number of analytical techniques for the analysis of amlodipine, valsartan and hydrochlorothiazide - monoproducts and drug combinations - have been described in the literature. [15-22]. Most of the methods, despite the good indicators of the analytical parameters in the process of their validation, are used for very specific purposes, are difficult to apply in drug combinations and are accompanied by a number of limitations. This leads to complications in their application for analytical control, the need to modify the conditions of the tests with subsequent validation or revalidation, incompatibility of the results of different methods and the resulting difficulties in assessing the quality of antihypertensive products. The efforts of specialists and inspection organizations in this direction are to increase the reliability of analytical control tests in order to ensure constant compliance with regulatory standards.

The present study provides rapid UV-spectrophotometry test for the identity and quantification of double and triple combinations containing amlodipine. Test represents direct analysis - drug mixtures are analyzed in solution without prior separation using UV-VIS spectrophotometry. The spectral characteristics of the complex spectrum are used for authentication, counterfeiting, identification and assay. The test includes drug mixtures - free combinations and fixed dose combinations containing amlodipine. Basic spectral characteristics are determined and analytical parameters of the validation process are investigated.

2. Material and methods

2.1. Instrumentation

HP UV visible spectroscopy system (model 8453) with 10 mm quartz cells (Agilent) were used for all absorbance measurements. Balance Mettler Toledo, model AB-304S/M-FACT; LC-P45 was used for weighing the standards.

2.2. Reagents

Methanol, HPLC grade, Fisher Scientific, Valsartan, pharmaceutical secondary standard traceable to US Pharmacopoeia (USP), European Pharmacopoeia (PhEur) and British Pharmacopoeia (BP), LOT LRAA9111, Sigma-Aldrich.

Amlodipine besylate, pharmaceutical secondary standard traceable to USP, PhEur and BP, LOT LRAB1220, Sigma-Aldrich.

Hydrochlorothiazide, pharmaceutical secondary standard traceable to USP, PhEur and BP, LOT LRAA6504, Sigma-Aldrich.

2.3. Experimental Condition

According to the solubility characteristics of drugs, methanol was selected as solvent for analysis.

2.4. Preparation of standard solutions

2.4.1. Amlodipine besylate Stock Solution

69.3 mg Amlodipine besylate was dissolved to 50 ml methanol. (C amlodipine = 1 mg/ml).

2.4.2. Amlodipine besylate reference solution (RS)

2.5 ml Amlodipine besylate Stock Solution was dissolved to 50 ml with methanol (C amlodipine = 0.05 mg/ml).

2.4.3. Hydrochlorothiazide RS

12.5 mg Hydrochlorothiazide standard was dissolved to 100 ml with methanol (C hydrochlorothiazide = 0.125 mg/ml).

2.4.4. Valsartan RS

32 mg Valsartan standard was dissolved to 20 ml with methanol (C valsartan = 1.6 mg/ml).

2.4.5. RS mix

32 mg Valsartan Standard and 1 ml Amlodipine besilate stock solution were dissolved to 20 ml with Hydrochlorothiazide RS (C amlodipine = 0.05 mg/ml, C valsartan = 1.6 mg/ml, C hydrochlorothiazide = 0.125 mg/ml).

2.5. Wavelength selection

The reference solution of amlodipine besylate was scanned at different concentration in the range of 200-400 nm and the λ max was determined. The overlain spectrum of tree drugs and the mix one was also run and presented. It is visible from the sprectrum, that only Amlodipine has absorption at 360 nm – Fig. 1.

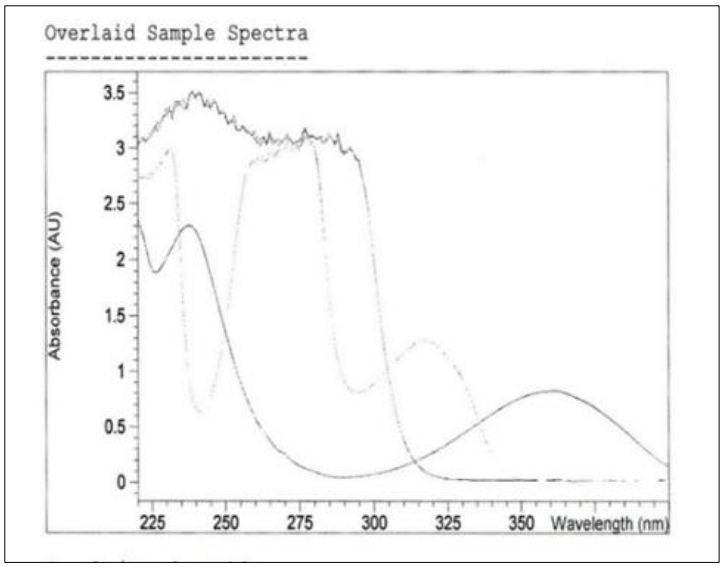

Figure 1 Overlaid spectra of Reference Substances mixture

2.6. Specificity

The specificity of the method was investigated by observing any interference of amlodipine with two other drugs in solution RS mix.

2.7. Calibration Curve Procedure

The calibration curve was prepared by scanning Standard solutions ranging from 15–75 µg/ml at 360 nm. Using weighting method: least squares; calibration curve equation is C=k0+(k1*A). The correlation coefficient (R^2) is 0.99934 (Fig. 2).

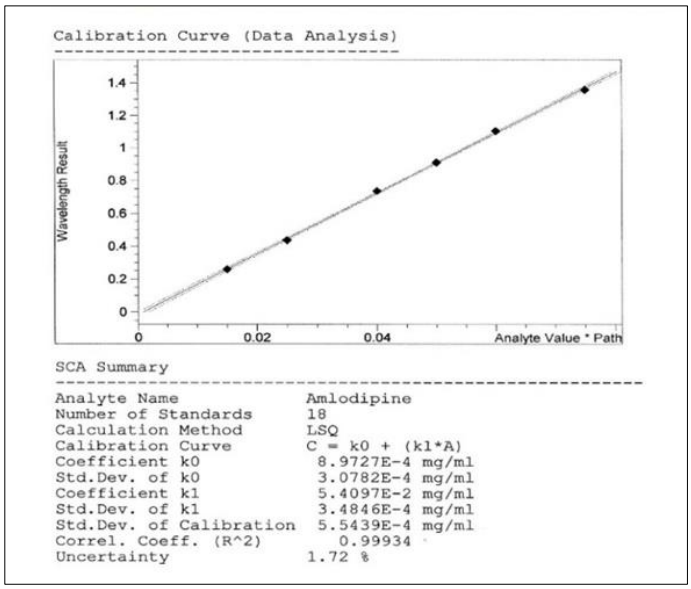

Figure 2 Calibration curve of Amlodipine besylate

2.8. Precision

Precision of the methods was determined by performing interday variation, intraday variation and repeatability studies. In interday variation, the absorbance of standard solutions of Amlodipine (50 µg/ml) were measured on three consecutive days. In intraday variation, the absorbances were measured three times in a day. In repeatability study, six determinations of concentration (50 µg/ml) were analyzed.

2.9. LOD & LOQ Determination

The sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantitation (LOQ). The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level.

2.10. Robustness

Robustness of the method has been evaluated at tree different levels: analyst, days and wavelength (359-364 nm – Fig. 3).

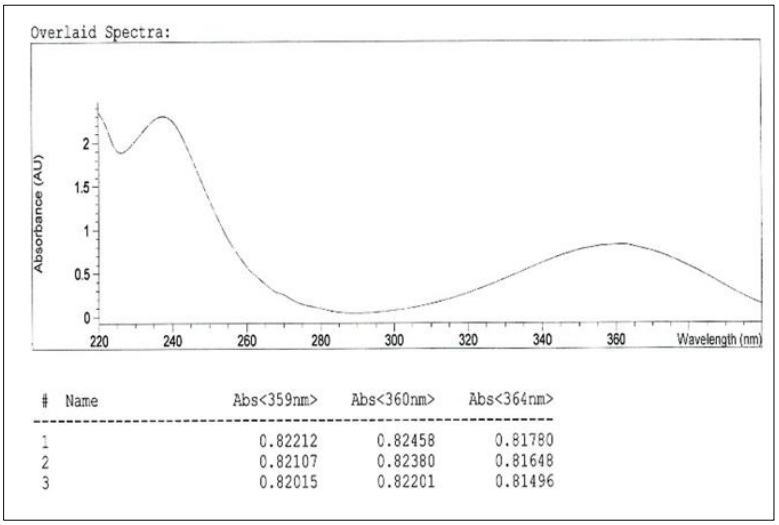

Figure 3 Robustness of Amlodipine at different wavelength

2.11. UV-spectrophotometric study of binary and triple mixtures containing Amlodipine besylate

The UV spectrophotometric study of mixtures was performed in order to optimize the spectral characteristics and for this purpose it was separated in four calculations. Normal spectrophotometry and 1st function derivative were used at polynomial levels 0 and 2nd. Smoothing points were found to be 1 for I and II and 3 for III and IV calculations. Each calculation was performed at a common analytical range (190 - 400, 190 - 500 and 200 - 500 nm) and two additional fixed wavelengths. Table 1 shows the studied parameters for different calculations.

Table 1 Spectral characteristics

Parameter	Calculation I	Calculation IIII	Calculation III	Calculation IVIV
Analysis	Multicomponent analysis	Multicomponent analysis	Multicomponent analysis	Multicomponent analysis
Calibration curve t	Beer's low	Beer's low	Beer's low	Beer's low
Algorithm	Least squares fit	Least squares fit	Least squares fit	Least squares fit
Derivative order	0	0	1	1
Polynomial degree	0	0	2	2
Smoothing points	1	1	3	3
Data Interval	2 nm	2 nm	2 nm	2 nm
Analytical wavelength II	190 – 400 nm	190 – 500 nm	190 – 400 nm	200 – 500 nm
Analytical wavelength IIII	266 nm	250 nm	266 nm	270 nm
Analytical wavelength III	360 nm	360 nm	360 nm	320 nm

3. Results and discussion

A validated UV spectrophotometric method for the quantification of Amlodipine in double and triple mixtures with Valsartan and Hydrochlorothiazide was developed and validated. The method uses 4 types of calculations, normal spectrophotometry, derivative spectrophotometry and determination at different analytical zones and fixed wavelengths in the range 190 - 500 nm. The result evaluation is in terms of analytical parameters and spectral characteristics used for identification. Table 2 shows the parameters of the validation process, the specific absorbance $A_{1\%, 1cm}$ of Amlodipine and the absorption ratio of absorption values at 236 and 360 nm.

Table 2 Analytical and spectral parameters studied in process of validation

Parameter	Amlodipine
Precision (%)	+ 2 /- 3
Specificity	Positive
Accuracy (Recovery %)	-7.6 / + 7.8
Linear range (μg/ml)	15 - 75
Correlation Coefficient (r)	0.99934
LOD (μg/ml)	0.08
LOQ (μg/ml)	1.66
$A_{1\%, 1cm}$ 360 nm	113 - 121
Absorption ratio - A_{236}/A_{360}	1.94

For double mixtures Amlodipine / Valsartan, the best results are obtained by determining at the 1st derivative of the function, the 2nd polynomial level, the presence of 3 smoothing points, the analytical range is 190 - 400 nm, and the fixed lengths of the waves - 266 and 360 nm. The standard deviation is +/- 0.085 for Amlodipine and +/- 0.042 for Valsartan.

Table 3 Results for double mixtures containing Amlodipine besylate

Sample	Putted amount (g/ml)	Calculation	Obtained amount (g/ml)	+/- SD x 10^{-5}	Independence of Stds
Amlodipine	0.000025	I	0.0000192	0.051	3.88
	0.000025	II	0.0000192	0.051	2.96
	0.000025	III	0.0000269	0.085	3.97
	0.000025	IV	0.0000395	0.056	3.97
Valsartan	0.00008	I	0.0000190	0.041	3.88
	0.00008	II	0.0000198	0.074	2.96
	0.00008	III	0.0000869	0.042	3.97
	0.00008	IV	0.0000386	0.043	3.97

For triple mixtures containing Amlodipine / Valsartan / Hydrochlorothiazide, the best results are obtained with IVth calculation type for Amlodipine and Valsartan and Ist, IIIrd and IVth for Hydrochlorothiazide. The differences between the types are in the choice of fixed wavelengths, which for the IVth are 270 and 320 nm.

Table 4 Results for triple mixtures containing Amlodipine besylate

Sample	Putted amount (g/ml)	Calculation type	Obtained amount (g/ml)	+/- SD x 10^{-6}	Independence of Stds
Amlodipine	0.000016	I	0.0000836	5.91	1.26
	0.000016	III	0.0000837	5.91	1.10
	0.000016	IV	0.0000179	0.12	1.09
Valsartan	0.000053	I	0.0000208	7.65	1.26
	0.000053	III	0.0000208	7.65	1.10
	0.000053	IV	0.0000466	0.12	1.09
Hydrochlorothiazide	0.000025	I	0.0000258	1.53	1.26
	0.000025	III	0.0000258	1.53	1.10
	0.000025	IV	0.0000286	0.02	1.09

The developed method was compared with available in European Pharmacopoeia and US Pharmacopoeia methods for assay of Amlodipine substance and tablets respectively. Results are shown on table 5. The results show comparability and meet the pharmacopoeia criteria.

Table 5 Results of pharmacopoeial methods and UV spectrophotometric method for the quantification of Amlodipine besylate

Parameter	Developed UV-spectrophotometric method	European Pharmacopoeia method for substance Amlodipine	US Pharmacopoeia method for tablets containing Amlodipine
Selectivity	positive	positive	positive
Precision (RSD %)	commensurable	commensurable	commensurable
Accuracy (Recovery %)	- 7.6 / + 7.8	- 3 / + 2	+/- 10
LOD (μg/ml)	0.08	-	-
LOQ (μg/ml)	1.66	3.0	3.5
Linearity interval (μg/ml)	15 - 75	-	-

4. Conclusion

Rapid UV-spectrophotometric test has been developed to analyze antihypertensive drug combinations containing amlodipine besylate for identity and assay purposes. The test is based on the study of spectral parameters of the complex spectrum. It is distinguished by increased selectivity and optimal values of analytical parameters comparable to pharmacopoeial tests.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that no conflict of interest existed in this paper.

References

[1] Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. Selected major risk factors and global and regional burden of disease. Lancet. 2002; 360: 1347–1360.

[2] Turnbull F. Blood Pressure Lowering Treatment Trialists Collaboration. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. Lancet. 2003; 362(9395): 1527–1535.

[3] Wolf-Maier K, Cooper RS, Kramer H, Banegas JR, Giampaoli S, Joffres MR, Poulter N, Primatesta P, Stegmayr B, Thamm M. Hypertension Treatment and control in five European countries, Canada, and the United States. Hypertension. 2004; 43: 10–17.

[4] Julius S, Kjeldsen SE, Weber M, Brunner HR, Ekman S, Hansson L, Hua T, Laragh J, McInnes GT, Mitchell L, Plat F, Schork A, Smith B, Zanchetti A. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomised trial. Lancet. 2004; 363: 2022–2031.

[5] Schroeder K, Fahey T, Ebrahim S. How can we improve adherence to blood pressure-lowering medication in ambulatory care? Systematic review of randomized controlled trials. Arch Intern Med. 2004; 164: 722–732.

[6] Wald DS, Law M, Morris JK, Bestwick JP, Wald NJ. Combination therapy versus monotherapy in reducing AH: meta-analysis on 11,000 participants from 42 trials. Am J Med. 2009; 122: 290–300.

[7] Law MR, Wald NJ, Morris JK, Jordan RE. Value of low dose combination treatment with blood pressure lowering drugs: analysis of 354 randomised trials. BMJ. 2003; 326: 1427-1434.

[8] The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 Report. 21 May 2003; 289(19): 2560-71.

[9] Mancia G, De Backer G, Dominiczak A, et al. Guidelines for the management of arterialhypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2007; (6): 1105-87.

[10] Oliván J, Moreno R, Hoyos M, Ramos E, Rodriguez A, Pizarro JL, Justo E, Perez Cano R. Effect of the treatment with amlodipine on left ventricular hypertrophy in hypertensive patients. An Med Interna. 1996; 13(11): 531-6.

[11] Fak AS, Okucu M, Tezcan H, Bodur G, Oktay A. The Effects of Amlodipine on Left Ventricular Mass and Diastolic Function in Concentric and Eccentric Left Ventricular Hypertrophy. J Cardiovasc Pharmacol Ther. 1996; 1(2): 95-100.

[12] Xi He, Hong-Li Zhang1, Ming Zhao. Amlodipine ameliorates endothelial dysfunction in mesenteric arteries from spontaneously hypertensive rats. Clin Exp Pharm Physiol. 2011; 38(4): 203-284.

[13] Macquart de Terline M, Diop B, Bernard M, Bernard D, Ikama M, N'Gueta R, Balde D, Tchabi Y, Abdallahi A, Toure I, Zabsonre P, Damorou F, Takombe JL, Narayanan K, Fernandez C, Tafflet M, Plouin PF, Empana JP, Marijon E, Antignac M. Substandard drugs among five common antihypertensive generic medications: An analysis from 10 African countries. Journal of Hypertension. 2018; 36(2): 395-401.

[14] Redfern J, Adedoyin RA, Ofori S, Anchala R. Ajay VS, Andrade LD, Zelaya J, Kaur H, Balabanova D, Sani MU. Physicochemical equivalence of generic antihypertensive medicines (EQUIMEDS): protocol for a quality of medicines assessment, BMJ Global Health. 2016; 1(2): e000086.

[15] El-Gizawy SM, Abdelmageed OH, Omar MA, Deryea SM, Abdel-Megied AM. Development and Validation of HPLC Method for Simultaneous Determination of Amlodipine, Valsartan, Hydrochlorothiazide in Dosage Form and Spiked Human Plasma. American Journal of Analytical Chemistry. 2012; 3: 422-430.

[16] Çelebier M, Kaynak SM, Altınöz S, Sahin S. HPLC method development for the simultaneous analysis of amlodipine and valsartan in combined dosage forms and *in vitro* dissolution studies. Brazilian Journal of Pharmaceutical Sciences. 2010; 46(4): 761-768.

[17] Abu-Nameh ESM, Abu-Shandi K, Saket M, Salim M, Othman OMY. Simultaneous Determination of Amlodipine Besylate and Valsartan in Tablets by High Performance Liquid Chromatography with UV Detection. Jordan Journal of Pharmaceutical Sciences. 2011; 4(2): 105-113.

[18] Naveed S, Qamar H, Jawaid W, Bokhari U, Jinnah. Simple UV spectrophotometric assay of Amlodipine University for Women, Karachi, Pakistan. American Journal of Chemistry and Application. 2014; 1(4): 66-69.

[19] Naveed S, Jawaid W, Bokhari U, Qamar H. Degradation study of different brands of amlodipine using UV spectrophotometer. Journal of Scientific and Innovative Research. 2014; 3(4): 414-418.

[20] Erk N. Spectrophotometric analysis of valsartan and hydrochlorothiazide. Analytical Letters. 2001; 35(2): 283–302.

[21] Darwish HW, Hassan SA, Salem MY, El-Zeany BA. Comparative study between derivative spectrophotometry and multivariate calibration as analytical tools applied for the simultaneous quantitation of amlodipine, valsartan and hydrochlorothiazide. Spectrochimica Acta A. 2013; 113: 215–223.

[22] Darwish HW, Hassan SA, Salem MY, El-Zeany BA. Sequential Spectrophotometric Method for the Simultaneous Determination of Amlodipine, Valsartan, and Hydrochlorothiazide in Coformulated Tablets. International Journal of Spectroscopy. 2013; 2013: 8 pages.