DEVELOPMENT AND VALIDATION OF A HPLC/PDA METHOD FOR SIMULTANEOUS QUANTIFICATION OF AMLODIPINE BESYLATE AND VALSARTAN IN DISSOLUTION MEDIA

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ABSTRACT

Background: *Valsartan, an angiotensin blocker, is often combined with amlodipine (a calcium channel antagonist) in the current treatment of hypertension. There is a generic brand-name drug Exforge® which has been proven to be clinically effective. However, its price is high, making it quite difficult to access, especially in low- and middle-income countries. Therefore, research and development of generic drugs is essential. In addition, research on simultaneous quantification of amlodipine and valsartan in the dissolution medium must be performed to serve as a basis for comparing in vitro equivalence between the two finished products.* ***Objectives:*** *Investigation of the mobile phase for separating amlodipine and valsartan using the isocratic elution method and validation of the simultaneous quantification of amlodipine and valsartan in three different dissolution media with pH of 1.2, 4.5, and 6.8.* ***Materials and methods****: Experimental methods on finished tablets containing amlodipine and valsartan.* ***Results:*** *A procedure for simultaneous quantification of amlodipine and valsartan has been developed and validated in three dissolution media using the HPLC method with DAD detector, ZORBAX Eclipse Plus C18 reversed-phase column (4.6 x 250 mm; 5µm), isocratic elution method, detection wavelength of 237 nm, flow rate of 1 mL/min, an injection volume of 20 µL, and mobile phase composed of acetonitrile-triethylamine 0.7% (adjusted to pH 3.0 with 0.05% of phosphoric acid) with a ratio of 40:60. The quantitative method achieves linearity with a correlation coefficient R2>0.999 and the linearity for amlodipine and valsartan was determined in the range at pH of 1.2 (0.25-10 µg/mL and 2-160 µg/mL), at pH of 4.5 and 6.8 (0.5-10 µg/mL and 8-160 µg/mL). The values of amlodipine and valsartan content in the test sample on the same day and between two different days were less than 2.0%. The recovery rate ranged from 98% to 102% with the relative standard deviation (RSD) not exceeding 2.0%.* **Conclusions:** *The HPLC method with the above chromatographic conditions can be applied for simultaneous quantification of amlodipine and valsartan in three dissolution media with various pH.*

Keywords: *Amlodipine, valsartan, simultaneous quantification, HPLC.*

I. INTRODUCTION

Amlodipine is a calcium channel blocker that helps control blood pressure effectively for 24 hours and has no or very little effect on neurohormonal activation, so it does not cause high blood pressure at the last dose [1], [2]. Valsartan is used in the treatment of hypertension and heart failure through the mechanism of blocking angiotensin receptors and is often combined with amlodipine to increase the effectiveness of treating hypertension [3], [4]. Currently, there are some preparations containing both active ingredients, but the price is expensive and it is difficult for residents in poverty to access them, especially those in developing countries, so research and development of generic drugs is necessary. According to regulations in Circular 07/2022/TT-BYT [5], generic drugs containing amlodipine need evaluating for bioequivalence before being launching on the market, so proving the *in vitro* equivalence of the generic drug with the original brand drug is extremely important before *in vivo* equivalence testing [6], [7].

Developing and validating a procedure for quantifying amlodipine and valsartan in three dissolution environments according to ICH guidelines are urgent to investigate factors affecting tablet quality, the most important of which is the dissolution indicator compared to the control drug Exforge by an index of f_2. However, in the National Pharmacopoeia of Vietnam V, currently, there is no monograph for tablets simultaneously containing the above two active ingredients. In the United States Pharmacopoeia 44, there is a monograph that is not complete for all three dissolution environments with complicated and time-consuming use of gradient mode. That is why this study was conducted with two goals development and validation of a procedure to simultaneously quantify amlodipine and valsartan in three dissolution media with pH of 1.2, 4.5, and 6.8 by isocratic elution mode [8].

II. MATERIALS AND METHODS

2.1. Materials

Reference substances: Amlodipine besylate (lot number QT145 120122 and content of 100.3% calculated on anhydrous preparation) and Valsartan (lot number QT323 020122 and content of 99.4% calculated on anhydrous preparation provided by Institute of Drug Quality Control Ho Chi Minh city). *Control drug*: Exforge® (Siegfried Barbera Company, SL) was made in Spain with lot number BCXN8 and an expiry date of 02-2025.

Solvents and chemicals: Acetonitrile (ACN) and methanol (MeOH) met liquid chromatography standards. Triethylamine (TEA), phosphoric acid, and solvents used in the analysis met the prescribed analytical standards.

Instruments: Agilent HPLC machine with ZORBAX Eclipse Plus C18 reversed-phase chromatography column (the US), Pharmatest PTWS 120D dissolution tester (Germany), Hanna HI 2550 pH meter (Italy), and KERN AES 220-4 analytical balance (Germany).

2.2. Methods

2.2.1. Sample preparation

Standard solution of amlodipine: The solution has an amlodipine concentration of roughly 100 µg/mL methanol.

Standard solution of valsartan: The solution has a valsartan concentration of approximately 1600 µg/mL methanol.

Standard solution of amlodipine in dissolution media: Accurately 500 µL of a standard solution of amlodipine was drawn into a 10 mL volumetric flask. The dissolution medium was added up to the mark, and then the mixture was shaken well to obtain a solution with a concentration of about 5 µg/mL.

Standard solution of valsartan in dissolution media*:* Accurately 500 µL of standard solution of valsartan was put into a 10 mL volumetric flask. The dissolution medium (as for the standard sample of valsartan in pH 1.2 medium, the mobile phase was added) was added up to the mark, and then the mixture was shaken well to obtain a standard solution with a valsartan concentration of 80 µg/mL.

Mixed standard solution of amlodipine and valsartan in dissolution media: Accurately 500 µL of standard solution of amlodipine and 500 µL of standard solution of valsartan were pipetted out into a 10 mL volumetric flask. The dissolution medium (as for the standard sample of valsartan in pH 1.2 medium, the mobile phase was added) was added until the solution reached 10 mL, and then the mixture was shaken well to obtain a standard solution with amlodipine concentration of 5 µg/mL and valsartan concentration of 80 µg/mL.

Test sample: Tablets simultaneously containing amlodipine and valsartan were tested for dissolution and filtered through a 0.45 µm filter.

Placebo sample: An amount of placebo powder (Crospovidone XL, Aerosil, magnesium stearate, Avicel PH 112) corresponding to the amount of drug powder corresponding to 5 mg of amlodipine was accurately weighed into a 100 mL volumetric flask. About 60 mL of methanol was added to this volumetric flask. Methanol was added until the solution reached 100 mL. Accurately 500 µL of the mixture was pipetted out into a 10 mL volumetric flask. The dissolution medium (as for the placebo sample in pH 1.2 medium, the mobile phase was supplemented) was added up to the mark and then shaken well.

Placebo sample spiked with standard solutions: An amount of placebo powder corresponding to the amount of drug powder containing corresponding to 5 mg of amlodipine was accurately weighed into a 100 mL volumetric flask. Exactly 10 mL of standard solution of amlodipine and 10 mL of valsartan standard solution, and about 40 mL of methanol, shaken well. Accurately 500 µL of the mixture was pipetted out into a 10 mL volumetric flask. The dissolution medium (as for the placebo sample in pH 1.2 medium, the mobile phase was added) was added up to the mark and then shaken thoroughly.

Blank sample: The blank sample included a mixture of methanol, mobile phase solvent, and dissolution medium.

2.2.2. Development and validation of a method for simultaneous quantification of amlodipine besylate and valsartan in dissolution media

Based on some documents [8], the investigated chromatographic conditions were chosen as follows: Stationary phase is Agilent ZORBAX Eclipse Plus C18 chromatography column (250 mm x 4.6 mm, 5 µm), isocratic elution mode, sample injection volume of 20 µL, flow rate of 1 mL/min, wavelength of 237 nm, and the temperature of 25^{o}C, mobile phase composed of acetonitrile and triethylamine solution 0.7% (adjusted to pH 3.0 with phosphoric acid 0.05%) with a ratio of 40:60.

Validation of a procedure for the simultaneous determination of amlodipine and valsartan was implemented in three different media with pH of 1.2, 4.5, and 6.8 according to ICH [9] and chromatographic conditions investigated.

III. RESULTS

3.1. System stability

Results of investigating system compatibility of the procedure for the determination of amlodipine and valsartan in the dissolution media with pH of 1.2, 4.5, and 6.8 are presented in Table 1.

Table 1. Results of survey on system suitability (n=6)

Media	Active ingredients		t_R (min)	S (mAU x sec)	N_{bk}	A_s	R_s
pH 1.2	Amlodipine	TB	5.331	168.0	11351	0.875	-
		RSD	0.43	1.60	1.21	1.48	-
	Valsartan	TB	7.747	2367.2	18432	0.869	21.58
		RSD	0.31	1.79	1.83	1.39	1.36
pH 4.5	Amlodipine	TB	5.340	167.9	11420	0.880	-
		RSD	0.51	1.13	1.40	1.84	-
	Valsartan	TB	7.767	2330.5	18952	0.866	21.37
		RSD	0.08	0.91	1.24	1.30	0.98
pH 6.8	Amlodipine	TB	5.338	164.4	11745	0.870	-
		RSD	0.54	1.31	1.33	1.43	-

Media	Active ingredients		t_R (min)	S (mAU x sec)	N_{bk}	A_s	R_s
	Valsartan	TB	7.727	2307.8	18904	0.869	21.60
		RSD	0.06	0.37	0.87	1.46	1.15

Results show that the RSD values of retention time, peak area, and the number of apparent theoretical plates of both amlodipine and valsartan were less than 2.0%. The chiral coefficient of both active ingredients was in the range of 0.8-1.5. The resolution between the two peaks was greater than 1.5. Thus, the quantitative procedure reached the system compatibility in three dissolution media with pH of 1.2, 4.5, and 6.8.

3.2. Specificity

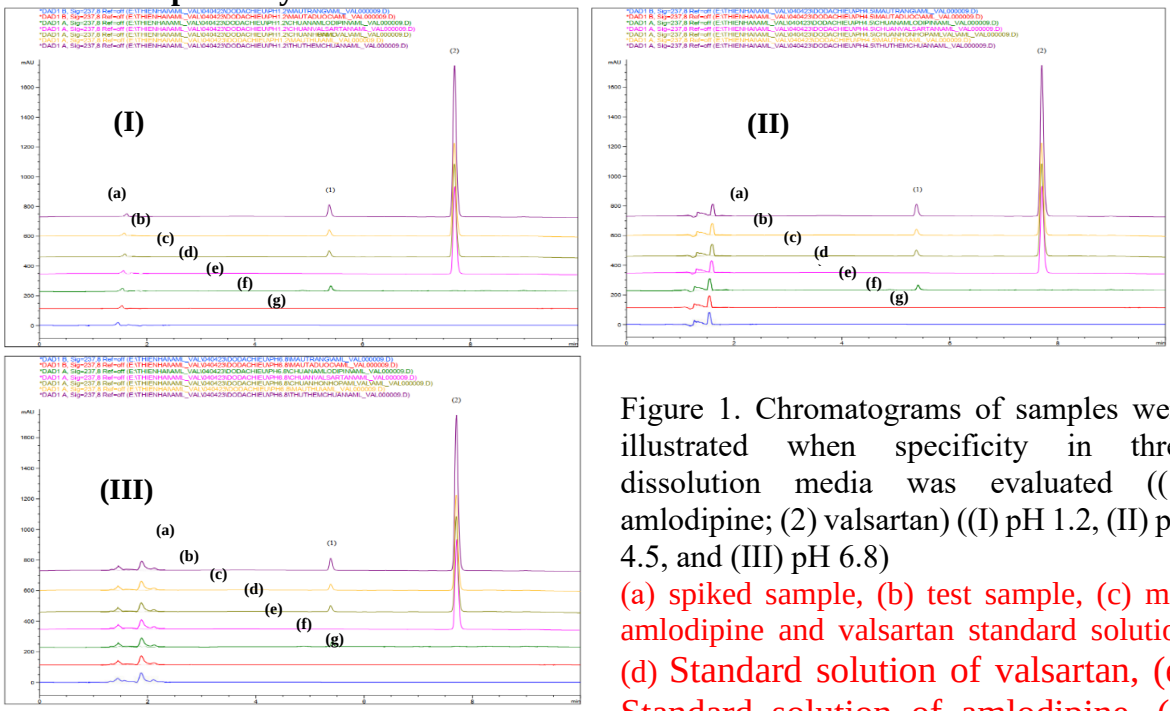

Figure 1. Chromatograms of samples were illustrated when specificity in three dissolution media was evaluated ((1) amlodipine; (2) valsartan) ((I) pH 1.2, (II) pH 4.5, and (III) pH 6.8)
(a) spiked sample, (b) test sample, (c) mix amlodipine and valsartan standard solution (d) Standard solution of valsartan, (e) Standard solution of amlodipine, (f) placebo solution, (g) blank solution

Figure 1. Describes the chromatograms of solutions when evaluating specificity in three dissolution media. The results show peaks for the blank sample, the excipient sample, the standard sample of amlodipine, the standard sample of valsartan, the mixed standard sample, the test sample, and the spiked test sample. In particular, the blank sample and the excipient sample did not have peaks with retention times corresponding to amlodipine and valsartan peaks similar to the standard solution. The chromatogram of the test sample had two peaks corresponding to the retention times of amlodipine and valsartan. The chromatogram of the spiked test sample had a peak height and area higher than the two corresponding peaks in that of the test sample.

3.3. Linearity, range, precision, and accuracy

Table 2. Results of the survey on linearity, range, precision, and accuracy in dissolution media with pH of 1.2, 4.5, and 6.8

			Amlodipine			**Valsartan**		
pH 1.2								
Regression			ŷ = 79.56x			ŷ = 1086.1x		
Linear range (µg/mL)			0.25-10			2-160		
Correlation coefficient (R^2)			0.9994			0.9992		
Precision	Repeatability (n=6)		Intermediate precision (n=12)		Repeatability (n=6)		Intermediate precision (n=12)	
	%, compared to the labeled amount	RSD (%)	%, compared to the labeled amount	RSD (%)	%, compared to the labeled amount	RSD (%)	%, compared to the labeled amount	RSD (%)
	101.84	1.60	100.93	1.42	98.86	1.64	98.04	1.65
Accuracy (n=12)	Concentration level		Recovery rate (%)	RSD (%)		Recovery rate (%)	RSD (%)	
	10%		101.07	0.80		99.21	0.57	
	80%		101.75	0.57		99.25	0.65	
	100%		101.20	1.46		98.80	1.59	
	120%		101.17	0.94		99.17	0.83	
Range (µg/mL)			0.25-10			2-160		
pH 4.5								
Regression			ŷ = 81,785x			ŷ = 1129.1x		
Linear range (µg/mL)			0.5-10			8-160		
Correlation coefficient (R^2)			0.9995			0.9992		
Precision	Repeatability (n=6)		Intermediate precision (n=12)		Repeatability (n=6)		Intermediate precision (n=12)	
	%, compared to the labeled amount	RSD (%)	%, compared to the labeled amount	RSD (%)	%, compared to the labeled amount	RSD (%)	%, compared to the labeled amount	RSD (%)
	101.24	1.01	101.33	1.01	97.12	1.05	96.64	1.05
Accuracy (n=12)	Concentration level		Recovery rate (%)	RSD (%)		Recovery rate (%)	RSD (%)	
	10%		101.67	0.30		98.63	0.38	
	80%		100.75	1.05		99.50	0.81	
	100%		101.60	0.58		98.40	1.22	
	120%		100.83	0.73		99.00	0.73	
Range (µg/mL)			0.5-10			8-160		
pH 6.8								
Regression			ŷ = 82.81x			ŷ = 1069.3x		
Linear range (µg/mL)			0.5-10			8-160		
Correlation coefficient (R^2)			0.9995			0.9997		
Precision	Repeatability (n=6)		Intermediate precision (n=12)		Repeatability (n=6)		Intermediate precision (n=12)	
	%, compared to the	RSD (%)	%, compared to the	RSD (%)	%, compared to the	RSD (%)	%, compared to the	RSD (%)

			Amlodipine			Valsartan		
	labeled amount		labeled amount		labeled amount		labeled amount	
	99.15	1.14	98.89	1.14	95.27	1.31	96.26	1.29
Accuracy (n=12)	Concentration level	Recovery rate (%)	RSD (%)		Recovery rate (%)	RSD (%)		
	10%	101.40	1.02		98.88	0.79		
	80%	101.00	0.90		99.25	0.64		
	100%	101.60	1.04		98.40	1.39		
	120%	101.00	0.67		99.00	0.83		
Range (µg/mL)		0.5-10			8-160			

Within the investigated concentration range of amlodipine and valsartan, the quantitative method achieved linearity with the correlation coefficient R^2>0.999. The values of amlodipine and valsartan content in the test sample on the same day and between two different days were less than 2.0% and the quantitative results between the two days were not statistically significant. The recovery rates of amlodipine and valsartan at three various concentration levels of 80%, 100%, and 120% of the quantitative concentration were all in the range of 98% to 102% with the RSD of the recovery rate not exceeding 2.0%. The range for amlodipine and valsartan was limited to between 80% and 120% of the quantitative concentration at pH of 1.2 (0.25-10 µg/mL and 2-160 µg/mL), at pH of 4.5 and 6.8 (0.5-10 µg/mL and 8-160 µg/mL).

Validation results show that the procedure for simultaneous quantification of amlodipine and valsartan in different dissolution media with pH of 1.2, 4.5, and 6.8 by HPLC method met the requirements of system compatibility, specificity, wide range, accuracy, and precision. Thus, the procedure can be applied to simultaneously quantify amlodipine and valsartan in dissolution medium.

IV. DISCUSSION

In HPLC, the mobile phase system is the most important factor, the mobile phase system is investigated. The use of mobile phase containing phosphate salt was used to increase the polarity of the mobile phase solvent. With the chemical structure, valsartan is a relatively polar substance due to the presence of the amino group [10]. To convert valsartan to a single neutral form, it was necessary to add more base to the mobile phase system, which is similar to the research of authors Brittain and his colleagues [11]. Triethylamine is a tertiary amine also known as a strong base. In addition, triethylamine is also used to improve the peak shape of the analyte by blocking silanol (silanol-blocking agent) to create a shielding layer on the surface of the stationary phase. The concentration of phosphoric acid 0.05% to adjust triethylamine 0.7% to pH of 3.0 was suitable to achieve separation efficiency and chromatographic parameters while the pH of the mobile phase was still within the allowable limits of the chromatography column (pH from 2.0-9.0).

Overall, a procedure for simultaneous quantification of amlodipine and valsartan in a tablet preparation in three dissolution media met requirements according to the ICH standards regarding system compatibility, specificity, accuracy, precision, linearity, and range. It is proven that the method is suitable for conducting analysis, which is the basis for application in testing finished products.

V. CONCLUSIONS

A procedure for simultaneous quantification of amlodipine and valsartan has been developed and validated in three dissolution media using the HPLC-DAD method with the parameters adhered to the ICH regulations. Simple isocratic mode and short duration help save time and chemical solvents. Meanwhile, the results will be a useful reference for related research.

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