RESEARCH ARTICLE

Formulation Optimization and Preparation of Amlodipine and Telmisartan Double-layer Tablets (5/40 Mg) using Wet Granulation Method

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ABSTRACT

The two-layer tablet of amlodipine and telmisartan is an effective strategy to manage hypertension disease through a combination of various treatment mechanisms. However, the spray-drying method is currently used to prepare telmisartan tablets due to its extremely low solubility, which requires sophisticated equipment. In this study, wet granulation was applied to fabricate this two-layer tablet. Furthermore, design of experiments were deployed to design and optimize the formulation in order to obtain an *in-vitro* equivalence with the reference drug. As a result, a formulation of the two-layer table using the wet granulation method was successfully developed and optimized. The obtained tablet showed an *in-vitro* equivalence with the reference drug (with f2 superior to 50 for both amlodipine and telmisartan). Furthermore, the obtained tablet also met a set of in-house standards, including appearance, identification, assay, and dissolution rate. This study provided not a novel but effective and simple method of fabrication for amlodipine-telmisartan tablets using wet granulation. Furthermore, the obtained results also highlighted the significant contribution of a well-established design of experiments in the development of a complex tablet formulation.

Keywords: Amlodipine, Telmisartan, Two-layer tablets, *In-vitro* equivalence, Wet granulation, Design experiment.

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INTRODUCTION

Currently, hypertension remains one of the most common diseases for humans, with an increasing risk of serious cardiovascular state. According to the World Health Organization (WHO), around 1.28 billion people worldwide aged 30 to 79 suffered from hypertension in 2021.[1,2] Aiming to increase the effectiveness of hypertension treatment, a combination of drugs from different groups is considered an effective solution. On the market, preparations containing calcium channel blockers (amlodipine), angiotensin receptor blockers (valsartan, telmisartan), and a diuretic (hydrochlorothiazide) is becoming a common regimen. Indeed, amlodipine and telmisartan can provide many advantages to hypertensive patients, such as enhancement of treatment effectiveness, better compliance (especially in a number of taken medications), and minimized side effects.[3] Up to now, several products containing these two active ingredients are accessible in the pharmaceutical market, such as Twynsta by Boehringer Ingelheim Pharmaceuticals Inc., approved by the Food and Drug Administration (FDA) in 2009.[4]

Amlodipine is an antihypertensive agent in the group of calcium channel blockers. One of the important advantages of amlodipine for patients is the effective control of blood pressure for 24 hours. However, it has a slow onset of action.[3,5] telmisartan acts by blocking angiotensin receptors and is often used in the treatment of hypertension alone or in combination with amlodipine to enhance the effectiveness of treating hypertension.[6]

However, the solubility of telmisartan is extremely low (Class II of the Biopharmaceutics Classification System), which requires a special technique to enhance its solubility in industrial production. Currently, spray drying is the only technique to prepare this kind of two-layer tablet.[7] The spray drying method aims to significantly reduce particle size, thereby improving the solubility of the active ingredient. Nevertheless, the application of spray drying is widely considered as a high-cost one, not only in terms of equipment but also in the training of employees. Therefore, in developing countries, there is a high demand of developing of an alternative method that is conventional, cheap, and easy to access but ensures the quality

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of finished products' quality. Amongst different techniques for tablet preparation, the wet granulation method has many significant advantages due to easy formulation methodologies, suitable for upgrading to an industrial scale, which results in cost and time savings. Furthermore, the utilization of design of experiments (DoE, including Design-Expert design and BC PharSoft OPT optimization softwares) in the formulation process is also an effective method, which may help the tablets made by wet granulation to achieve *in-vitro* equivalence with the products fabricated by spray drying. For all the aforementioned reasons, this study was performed to apply the DoE in the formulation and preparation using wet granulation of amlodipine/telmisartan two-layer tablets to achieve an *in-vitro* equivalence with the original drug.

MATERIALS AND METHODS

Materials

Reference samples

Amlodipine besylate, batch number QT145 120122, content 100.3% calculated on anhydrous preparation; Telmisartan, batch number QT217 050122, content 99.4% based on anhydrous preparation provided by Ho Chi Minh City Institute of Drug Testing.

Solvents and chemicals

Acetonitrile (ACN) and methanol meet HPLC standards. Acetic acid and phosphoric acid meet analytical standards.

Amlodipine besylate (India), telmisartan (India), sodium hydroxide, meglumin, avicel PH 112, avicel PH 101, national 78-1551, anhydrous dicalcium phosphate, mannitol, crospovidone XL-10, sodium croscarmellose, aerosil, magnesium stearate, PVP K30, brilliant blue FCF (E133) (India), distilled water and alcohol 99% (Vietnam); reference drug Twynsta®, batch number 104535. Other chemicals and solvents used in analysis meet analytical standards.

Methods

Development and validation of quantification method for amlodipine and telmisartan two-layer tablets

Reversed-phase chromatography technique was applied to assay amlodipine and telmisartan in two-layer tablets, using DAD detection with an acidic mobile phase. Initial chromatography conditions were proposed as follows: column chromatography Zorbax Eclipse Plus C18 (250 x 4.6 mm; 5 µm), column temperature: 40°C, DAD probe with a detection wavelength at 237 nm, mobile phase consisted of acetonitrile and acidic buffer (40:60 v/v) with isocratic mode. Two parameters of chromatography conditions were investigated, including 1) type and ratio in the mobile phase (acid type: H_3PO_4 or CH_3COOH in acetonitrile with different ratios at 35:65, 40:60, 45:55) and 2) column temperature (from 30–40°C). Chromatographic conditions were selected based on the criteria of pure active substance peaks, complete separation (resolution ≥ 1.5), and skewness coefficient (in the range of 0.8–1.5). Once suitable chromatographic conditions were achieved, the validation of the analytical method was conducted according to ICH guidelines, including system suitability, specificity, linearity, accuracy and precision.[8] The validated procedure would be used for subsequent analysis. The analytical method using HPLC is outlined as follows:

Sample preparation

- *Amlodipine standard stock solution*

Prepare a solution with an amlodipine concentration of 100 µg/mL methanol.

- *Telmisartan standard stock solution*

Prepare a solution with a telmisartan concentration of 800 µg/mL methanol.

- *Amlodipine standard solution*

Accurately take 500 µL of amlodipine stock standard solution and transfer it into a 10 mL volumetric flask. Add mobile phase solvent to obtain a solution with a concentration of 5 µg/mL.

- *Telmisartan standard solution*

Accurately take 500 µL of telmisartan stock standard solution and transfer it into a 10 mL volumetric flask. Add mobile phase solvent to obtain a solution with a concentration of 40 µg/mL.

- *Standard mixed solution*

Accurately take 500 µL of amlodipine standard stock solution and telmisartan standard stock solution, and transfer into a 10 mL volumetric flask. Add mobile phase to obtain a solution with a concentration of 5 µg/mL of amlodipine and 40 µg/mL of telmisartan.

- *Sample solution*

Weigh 20 tablets and, calculate the average weight, and crush the tablets into fine powder. Accurately weigh 6.94 mg of amlodipine besylate (equivalent to 5 mg of amlodipine) and transfer it into a 100 mL volumetric flask. Add 60 mL of methanol, shake well and sonicate for 15 minutes. Add a sufficient amount of methanol, shake well, and centrifuge at 4000 rpm for 5 minutes. Accurately take and transfer 500 µL of the obtained solution into a 10 mL volumetric flask, add an adequate amount of mobile phase solvent to obtain a final solution at 5 µg/mL of amlodipine and 40 µg/mL of telmisartan.

- *Placebo solution*

Accurately weigh an appropriate amount of placebo powder, transfer into a 100 mL volumetric flask, add 60 mL of methanol, shake well and sonicate for 15 minutes. Add methanol up to the mark, shake well, and centrifuge at 4000 rpm for 5 minutes. Accurately take 500 µL of the obtained solution and transfer into a 10 mL volumetric flask, add an adequate amount of mobile phase solvent to obtain the final solution of the placebo.

- *Standard stock solution added to placebo solution*

Accurately weigh an adequate amount of placebo powder and transfer into a 100 mL volumetric flask. Add precisely 10 mL of the amlodipine standard stock solution 10 mL telmisartan standard stock solution, add about 40 mL of methanol, shake

Table 1: Criteria for selection of chromatographic conditions

Conditions	*Peak purity*	*Resolution*	*Skewness coefficient*	*Theoretical plate number*
Mobile phase: ACN, Water, MeOH with or without adding pH regulators such as glacial acetic acid, formic acid, triethylamine, phosphoric acid, ...	Passed	> 1.5	0.8-1.5	> 3000
Column temperature (30–40°C)				
Isocratic elution program				
Sample injection volume 10–40 µL				

well and sonicate for 15 minutes. Add methanol up to the mark, shake well, and centrifuge at 4000 rpm for 5 minutes. Accurately take 500 µL of the obtained solution and transfer into a 10 mL volumetric flask, add an adequate amount of mobile phase solvent, and shake well.

- *Blank sample*

Mixture of methanol and mobile phase solvent.

To validate the accuracy of the procedure, prepare placebo solutions with reference active ingredients at three concentrations: 80, 100, and 120% of the quantitative concentration of each analyte. For each concentration level, prepare 3 samples.

All samples were filtered through a 0.45 µm membrane before analysis. Criteria for the selection of suitable chromatographical conditions are presented in Table 1.

Development and validation of analytical method for amlodipine and telmisartan assay in three dissolution testing environments at pH 1.2, 4.5, and 6.8.

Sample preparation

Proceed similarly to *sample preparation* in section *2.2.1,* standard solution, sample solution, placebo solution, standard stock solution were added to placebo solution and introduce the corresponding dissolution testing environments at pH 1.2, 4.5, and 6.8.

Blank sample

Mixture of methanol, mobile phase solvent and dissolution testing environments.

- *Chromatographic conditions study*

Assays of amlodipine and telmisartan in three dissolution testing environments were conducted using the chromatographic conditions identified in section 2.2.1, and then evaluated according to peak purity, resolution, skewness coefficient, and theoretical plate number. If the parameters in all three environments were satisfied, the validation of the process would be carried out on three dissolution testing environments. Otherwise, simultaneous quantification of amlodipine and telmisartan should be developed in three dissolution testing environments by performing the same procedure as section 2.1.1.

- *Validation of analytical methods in dissolution test*

Validation of the simultaneous quantitative processes for amlodipine and telmisartan in three dissolution testing environments at pH 1.2, 4.5, and 6.8 was conducted according to the conditions described in section *2.2.1.*

Dissolution test of the reference tablets Twynsta® containing 5 mg amlodipine and 40 mg telmisartan

The reference tablet Twynsta® containing 5 mg amlodipine and 40 mg telmisartan was performed using the following conditions: Equipment (Paddle type dissolution apparatus (Type 1)), Environment volume (1000 mL of dissolution testing environments at pH 1.2, 4.5, 6.8.), stirring speed (50 rpm), sampling time (10, 15, 30, 45, 60 minutes), sampling volume (10 mL), sample filtered through 0.45 µm RC membrane before performing chromatography.

Development and optimization of immediate-release two-layer tablet formulation containing amlodipine 5 mg and telmisartan 40 mg

- *Design and optimization of formulation of two-layer tablet containing amlodipine 5 mg and telmisartan 40 mg*

The experimental model was conducted using Design-Expert® software with three independent variables, including x1: the NaOH ratio (%); x2: the ratio of crospovidone XL-10 (%); and x3: tablet hardness.

Independent variables and variation interval selection preliminary studies demonstrated a significant impact of NaOH, crospovidone XL-10 and tablet hardness on the drug release rate. Therefore, these parameters were chosen as independent variables. The degree and range of independent variable variation were presented in Table 2.

The similarity coefficient f_2 of amlodipine and telmisartan in three dissolution testing environments at pH 1.2, 4.5, and 6.8 compared to the reference tablet was chosen as a variable-dependent selection: Where:

y_1, y_2, y_3: similarity coefficient f_2 for amlodipine of the formulation and reference drug at pH 1.2, 4.5, and 6.8.

y_4, y_5, y_6: similarity coefficient f_2 for amlodipine of the formulation and reference drug at pH 1.2, 4.5, and 6.8.

Similarity coefficient (f_2 value)

$$f_2 = 50\log\left\{(1+\frac{1}{n}\sum_{i=1}^{n}(R_i - T_i)^2)^{-0,5}X100\right\} \quad (1)$$

Table 2: Independent variables and its range in design of experiments

Independent variables	*Symbol*	*Degree of variability*		
		Upper level	*Immediate level*	*Lower level*
NaOH ratio (%)	x_1	2	1.5	1
Crospovidone ratio (%)	x_2	10	13	16
Tablet hardness (N)	x_3	50-70	70-90	90-110

n is the number of sampling points, R(t) is the average percentage of the reference drug dissolved at time t since the experiment started, T(t) is the average percentage of the sample drug dissolved at time t since the experiment started.

The design of experiments for optimization of this tablet and its composition are presented in Tables 3 and 4.

The two-layer tablets' preparation process included three main steps, as follows (calculated for 100 tablets)

- *Preparation of the amlodipine layer*

(1) Add amlodipine besylate, avicel PH 112, modified starch national-78-1551, sodium croscarmellose, aerosil, and mix homogeneously. Sieve the mixture through 40-mesh sieve and mix well. (2) Add brilliant blue FCF (E133), sieve through 150-mesh sieve and add to the mixture, mix well. (3) Add magnesium stearate, sieve through a 150-mesh sieve, and fix until homogenously.

Preparation of the telmisartan layer

(1) Add mannitol, dicalcium phosphate, and mix well. Sieve the mixture through 40-mesh sieve and mix well (mixture M). (2) Dissolve sodium hydroxide with a sufficient amount of water, allow it to attain room temperature, and slowly add absolute alcohol (solution A). (3) Add and dissolve sequentially PVP K30, and meglumin in solution A. (4) Add telmisartan and mix thoroughly until telmisartan was completely dissolved (Solution B). (5) Gradually add solution B into mixture M, and granulate through a 16-mesh sieve, in case solution B remains, yet the mixture is sufficiently moist, granulate through 16-mesh sieve, dry at 60°C until obtaining a moisture content < 3.0% and then continue granulating with the remaining solution B. (6) Dry at 60°C until the moisture content < 1,0%. Screen through a 40-mesh sieve. (7) Calculate and add a sufficient amount of crospovidon XL-10, aerosil, sieve through 40-mesh sieve, and mix well. (8) Calculate and add a sufficient amount of magnesium stearate, sieve through a 40-mesh sieve, and mix well to obtain the final granules for compression.

- *Two-layer tablet compression process*

Final compression was performed using a two-layer tablet compression equipment. The first layer of the amlodipine layer was conducted with a mass of 200 mg and a hardness in a range of 15 to 25 N. The second layer of telmisartan was obtained with a total tablet weight of 520 mg, and the final tablet hardness was studied as presented in Tables 3 and 4.

Table 3: Design of experiments for optimization of two-layer tablets

	The formula for one tablet			
	NaOH (%)	*Crospovidone XL-10 (%)*	*Hardness (N)*	*Total weight (mg)*
F1	1	10	50-70	
F2	1	10	70-90	
F3	1	10	90-110	
F4	1	13	50-70	
F5	1	13	70-90	
F6	1	13	90-110	
F7	1	16	50-70	
F8	1	16	70-90	
F9	1	16	90-110	
F10	1.5	10	50-70	
F11	1.5	10	70-90	
F12	1.5	10	90-110	
F13	1.5	13	50-70	
F14	1.5	13	70-90	520
F15	1.5	13	90-110	
F16	1.5	16	50-70	
F17	1.5	16	70-90	
F18	1.5	16	90-110	
F19	2	10	50-70	
F20	2	10	70-90	
F21	2	10	90-110	
F22	2	13	50-70	
F23	2	13	70-90	
F24	2	13	90-110	
F25	2	16	50-70	
F26	2	16	70-90	
F27	2	16	90-110	

Table 4: Bill of materials for amlodipine/telmisartan two-layer tablets' preparation

Bill of materials (100 tablets)		
Item	*Material name*	*Weight (g)*
Amlodipine layer		
1	Amlodipine besylate	0.694
2	National 78–1551	5.00
3	Sodium croscarmellose	0.80
4	Avicel	0.40
5	Brilliant blue FCF (E133)	0.0003
6	Magnesium stearat	0.20
7	Avicel PH 112	QS to 200 g
		Total: 200 mg
Telmisartan layer		
8	Telmisartan	4.00
9	Meglumin	1.20
10	Dicalcium anhydrous phosphate	3.00
11	PVP K30	1.20
12	Aerosil	0.04
13	Magnesium stearat	0.30
14	NaOH	To determine
15	Crospovidon XL-10	To determine
16	Mannitol	QS to 320 g
		Total: 320 mg

Table 5: In-house standards for obtained tablets

Quality attributes	*Testing method*	*Quality standard*
Appearance	Assessment of tablet appearances	Two-layer tablets, with white and blue color, smooth and glossy surface, two layers are not interspersed with each other.
Identification	HPLC as described in section 2.2.1	Two peaks with identical retention times in chromatogram for tested tablets and reference standard.
Hardness	Take randomly 20 tablets and calculate the average hardness	*According to the optimal formula*
Weight uniformity	Weigh randomly 20 tablets and calculate the average weight	There must not be more than two units whose mass is outside the ± 5% difference limit and no unit must have a mass that exceeds ± 10% of the average mass
Dissolution equivalence to reference drug	As described in section 2.2.1	f_2 (of amlodipine and telmisartan in three pH) > 50%
Assay	HPLC as described in section *2.2.1.*	95–105 % (either amlodipine or telmisartan)

To evaluate the quality of obtained tablets, in-house standards were proposed in Table 5.

RESULTS AND DISCUSSION

Development and Validation of Assay Method for Amlodipine and Telmisartan in Two-Layer Tablets

Development of assay method for amlodipine and telmisartan in two-layer tablets

In order to determine appropriate conditions of HPLC for amlodipine and telmisartan assay, type and ratio of mobile phase, and column temperature were investigated.

Firstly, a mobile phase consisting of ACN: phosphoric acid 0.02% at different ratio (35:65, 40:60, 45:55 v/v) was investigated (Table 6, Figure 1).

As showed in the results, though the peaks were completely separated with a satisfactory resolution (Rs > 1.5), the purity and skewness coefficient did not meet the required standard (As < 0.8). Hence, the H_3PO_4 concentration was increased to 0.05% (Table 6, Figure 2).

When performing chromatography with the mobile phase using acetonitrile:phosphoric acid 0.02% at the ratio 35:65; 40:60; 45:55, the amlodipine peak and telmisartan peak were completely separated with a satisfactory resolution (Rs >1.5). Nevertheless, the purity and skewness coefficient of both peaks did not meet the required standard (As <0.8). Therefore, the phosphoric acid concentration was increased to 0.05% (Table 7, Figure 3).

The results showed that though the peaks were completely separated with a satisfactory resolution (Rs > 1.5), the purity and skewness coefficient did not meet the required standard (As < 0.8). Hence, Another type of buffer solution needs to be investigated to meet the criteria for resolution, purity and skewness coefficient of the peak. Conducting a study of mobile phase using acetonitrile: acetate buffer solution (50 mM ammonium acetate salt adjusted to pH 4.5 using acetic acid) with different ratios Table 8 and Figure 2.

When performing chromatography with the mobile phase using acetonitrile: phosphoric acid 0.05% at similar ratios 35:65, 40:60, 45:55, both peaks did not meet the criteria for

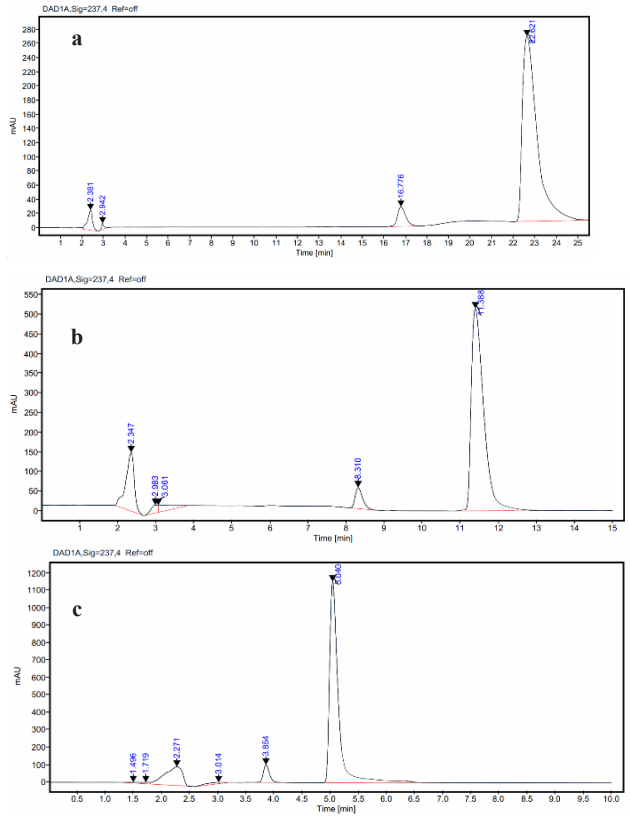

Figure 1: Chromatography of standard mixed solutions with mobile phase using acetonitrile : phosphoric acid 0.02% solution (a. 35:65; b. 40:60; c. 45:55)

skewness coefficient and purity. Another type of buffer solution needs to be investigated to meet the criteria for resolution, purity and skewness coefficient of the peak. Conducting a study of mobile phase using acetonitrile: acetate buffer solution (50 mM ammonium acetate salt adjusted to pH 4.5 using acetic acid) with different ratios.

When performing chromatography with the mobile phase using acetonitrile:acetate buffer solution, the peak purity, resolution and skewness coefficient of amlodipine and telmisartan were satisfactory when chromatography with

Table 6: Chromatographic results using ACN: Phosphoric acid 0.02% as mobile phase

Mobile phase ratio (ACN:H_3PO_4 0.02 %)	*Parameter*	*Amlodipine*	*Telmisartan*
35:65	Skewness coefficient	0.71	0.47
	Resolution	31.285	6.307
	Purity	Failed	Failed
	Theoretical plate number	37709	24218
40:60	Skewness coefficient	0.64	0.47
	Resolution	-	6.55
	Purity	Failed	Failed
	Theoretical plate number	35289	24153
45:55	Skewness coefficient	0.73	0.57
	Resolution	2.197	5.298
	Purity	Failed	Failed
	Theoretical plate number	24976	34163

Table 7: Chromatographic results using ACN: Phosphoric acid 0.05 % as mobile phase

Mobile phase ratio (ACN:H_3PO_4 0.05%)	*Parameters*	*Amlodipine*	*Telmisartan*
35:65	Skewness coefficient	0.50	0.52
	Resolution	17.169	9.754
	Purity	Failed	Failed
	Theoretical plate number	11862	9570
40:60	Skewness coefficient	0.49	0.47
	Resolution	5.175	8.567
	Purity	Failed	Failed
	Theoretical plate number	13136	9246
45:55	Skewness coefficient	0.69	0.43
	Resolution	-	7.440
	Purity	Failed	Failed
	Theoretical plate number	13102	11290

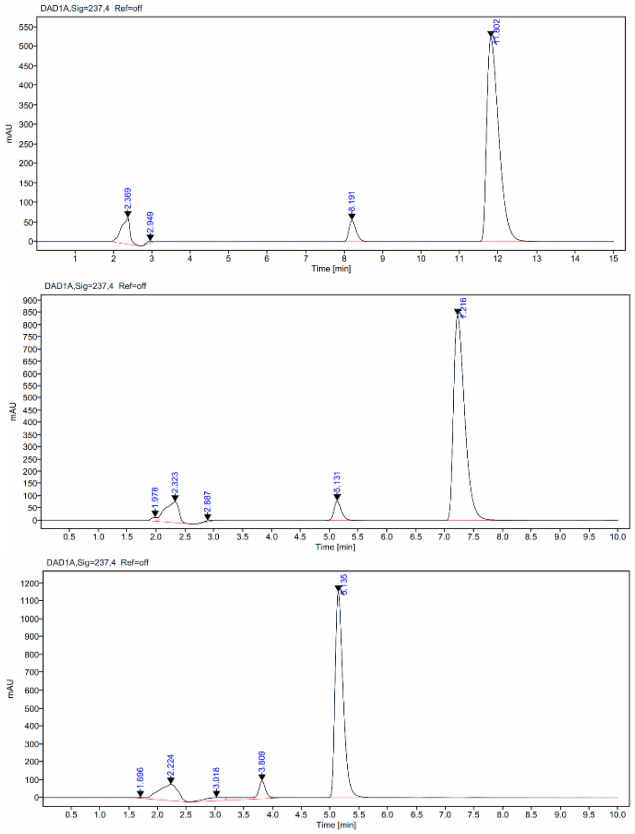

Figure 2: Chromatography of standard mixed solutions with mobile phase using acetonitrile:acetate buffer solution (50 mM ammonium acetate salt adjusted to pH 4.5 by acetic acid) (a. 35:65; b. 40:60; c. 45:55).

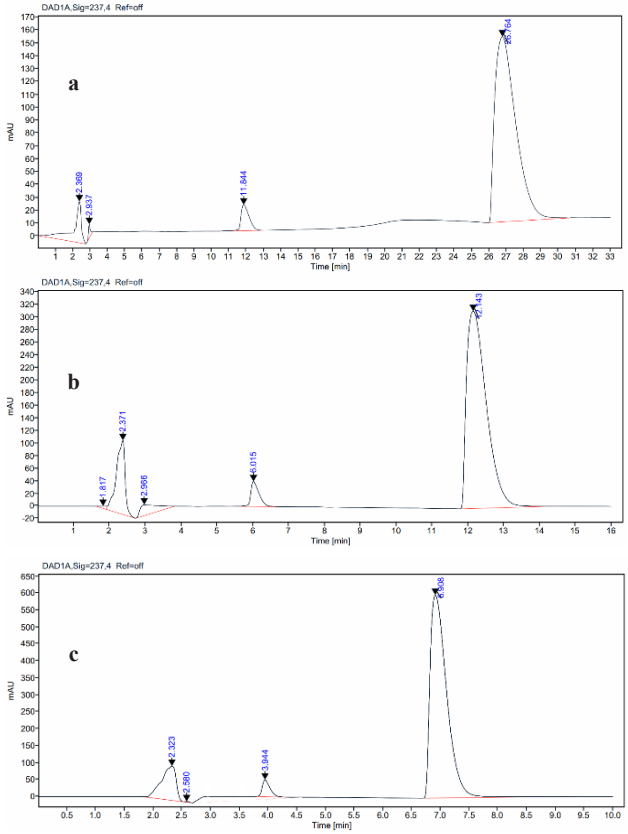

Figure 3: Chromatography of standard mixed solutions with mobile phase using acetonitrile : phosphoric acid 0.05% solution (a. 35:65; b. 40:60; c. 45:55).

mobile phase system at ratios of 35:65, 40:60, 45:55. At the ratio of 40:60, the retention times of amlodipine and telmisartan are 5.1 and 7.2 minutes, respectively, satisfying the criteria of skewness coefficient, resolution, theoretical plate number

Table 8: Chromatographic results using ACN:acetate buffer solution (50 mM ammonium acetate salt adjusted to pH 4.5 by acetic acid)

Mobile phase ratio (ACN: Buffer acetate)	*Parameters*	*Amlodipine*	*Telmisartan*
35:65	Skewness coefficient	0.67	0.47
	Resolution	17.908	7.925
	Purity	Failed	Failed
	Theoretical plate number	38312	26777
40:60	Skewness coefficient	0.92	0.83
	Resolution	8.631	7.111
	Purity	Passed	Passed
	Theoretical plate number	31161	26832
45:55	Skewness coefficient	1.78	0.60
	Resolution	1.050	5.774
	Purity	Failed	Failed
	Theoretical plate number	21820	26295

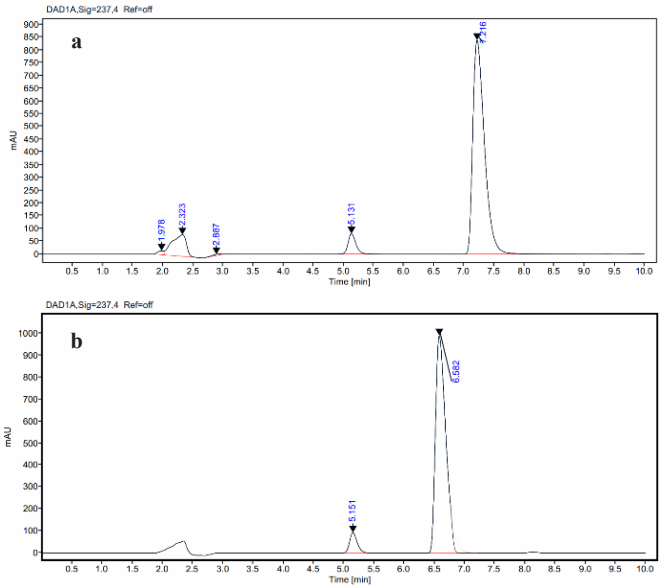

Figure 4: Chromatography of standard mixed solutions with mobile phase using acetonitrile : acetate buffer solution (50 mM ammonium acetate salt adjusted to pH 4.5 by acetic acid) at ratio (40:60) (a. Heating at 30°C; b. Heating at 40°C)

according to proposed in-house standard. The mobile phase containing ACN:acetate buffer solution at a ratio of 40:60 was selected for further investigations.

After having determined the appropriate type and ratio of mobile phase, the column temperature as studied at 30 and 40°C (Table 9, Figure 4).

The chirality coefficient of the analyte has been improved, the retention time of the analyte has been shortened, and

Table 9: Chromatographic parameters of the analyte correspond to the studied chromatography column temperature

Column temperature	*Parameters*	*Amlodipine*	*Telmisartan*
30°C	Skewness coefficient	0.92	0.83
	Resolution	8.631	7.111
	Purity	Passed	Passed
	Theoretical plate number	31161	26832
40°C	Skewness coefficient	1.08	0.99
	Resolution	-	7.234
	Purity	Passed	Passed
	Theoretical plate number	31279	26756

Table 10: Optimal parameters for chromatography conditions to simultaneously quantify the two active ingredients, amlodipine and telmisartan

Parameters	*Descrisption*
Column type	ZORBAX Eclipse Plus C18 (4.6 x 250 mm ; 5 mm)
Detector	DAD
Detection wavelength	237 nm
Flow rate	1.0 mL/min
Sample injection volume	10 mL
Mobile phase	Acetonitrile:acetate buffer solution (50 mM ammonium acetate salt adjusted to pH 4.5 by acetic acid) at ratio (40:60)
Temperature	40°C
Elution program	Isocratic

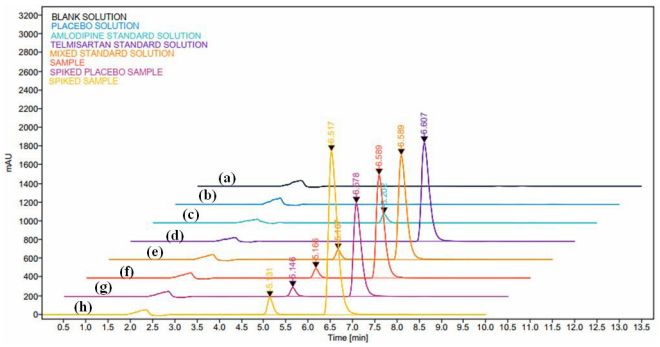

Figure 5: Chromatography of samples when evaluating the specificity of amlodipine and telmisartan (a) blank solution, (b) placebo solution, (c) amlodipine standard solution, (d) telmisartan standard solution, (e) mix amlodipine and telmisartan standard solution, (f) sample, (g) spiked placebo solution, (h) spiked sample

the system pressure has also decreased when increasing the chromatography column temperature from 30°C.

For all aforementioned results, optimal chromatographic conditions for simultaneous quantification of amlodipine and telmisartan by HPLC method are shown in Table 10.

Table 11: Results of system suitability using methanol environment (n = 6)

Active ingredient	*Statistical value*	*Retention time (min)*	*Pic area (mAU x min)*	*Resolution*	*Skewness coefficient*	*Apparent theoretical plate number*
Amlodipine	Mean	5.156	845.1	-	0.838	31420
	RSD (%)	0.13	0.69	-	1.59	0.48
Telmisartan	Mean	6.591	12144.4	5.17	0.825	26734
	RSD (%)	0.07	0.27	0.27	1.67	0.19

Table 12: Results of linearity, value domain, accuracy and precision in methanol

		Amlodipine				*Telmisartan*			
Regression equation		ŷ = 168.33x				ŷ = 290.03x			
Linear interval (µg/mL)		1–10				8–80			
Correlation coefficient (r^2)		0.9998				0.9996			
Accuracy		Repeatability (n=6)		Intermediate accuracy (n=12)		Repeatability (n=6)		Intermediate accuracy (n = 12)	
		%compared to label	RSD (%)	%compared to label	RSD (%)	%compared to label	RSD (%)	%compared to label	RSD (%)
		97.42	1.04	97.32	1.01	95.14	0.62	95.34	0.67
Precision (n = 9)	Concentration level (%)	Recovery Rate (%)		RSD (%)		Recovery Rate (%)		RSD (%)	
	80	99.05		0.63		99.30		0.26	
	100	98.55		0.69		99.33		0.46	
	120	100.01		1.15		99.75		1.05	
Value domain (µg/mL)		4.4–6.6				35.2–52.8			

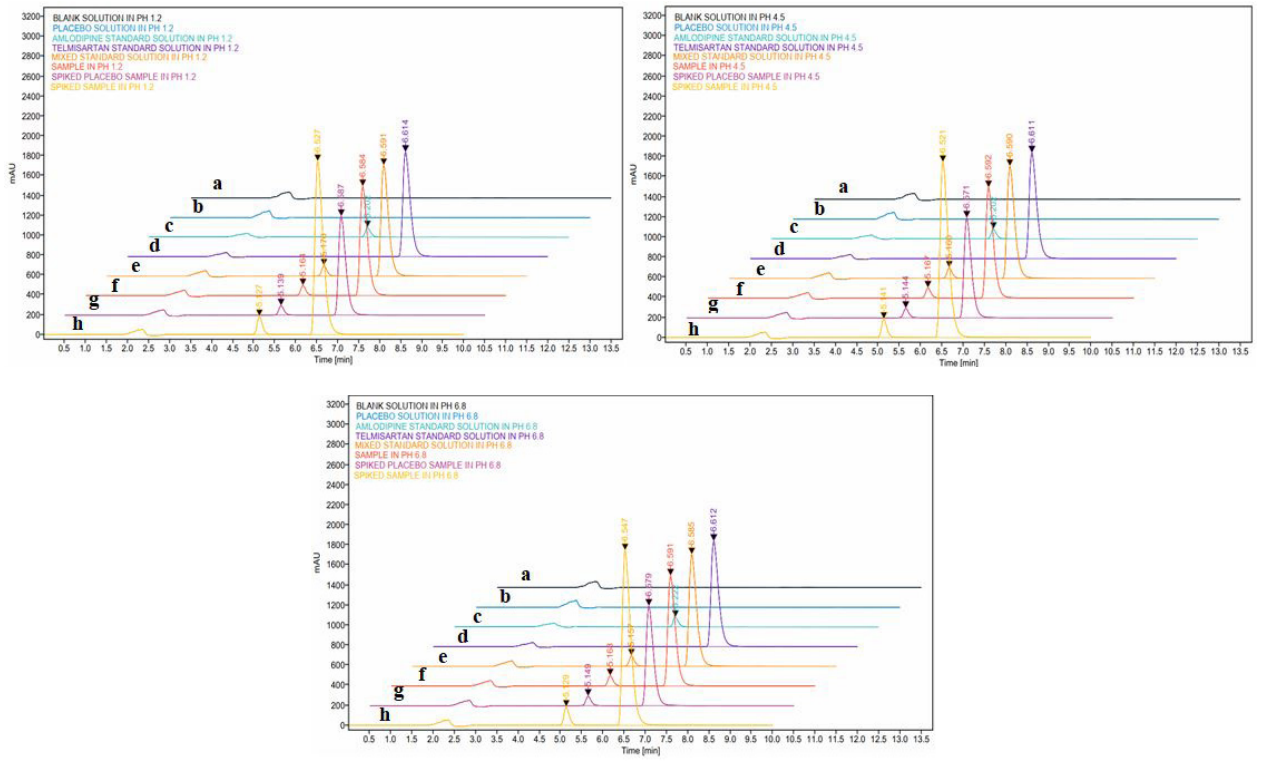

Figure 6: Specificity results in three dissolution testing environments (pH 1.2; b. pH 4.5; c. pH 6.8) (a) blank solution, (b) placebo solution, (c) amlodipine standard solution, (d) telmisartan standard solution, (e) mix amlodipine and telmisartan standard solution, (f) sample, (g) spiked placebo solution, (h) spiked sample

Table 13: Results of systematic suitability in three dissolution testing environments (n = 6)

Environment	*Active ingredients*		t_R *(min)*	*S (mAU x sec)*	N_{bk}	A_s	R_s
pH 1.2	Amlodipine	Mean	5.153	844.7	31546	0.828	-
		RSD	0.07	0.28	0.37	0.49	-
	Telmisartan	Mean	6.585	12161.5	26726	0.818	5.16
		RSD	0.07	0.25	0.25	1.20	0.23
pH 4.5	Amlodipine	Mean	5.156	848.3	31529	0.828	-
		RSD	0.11	0.83	0.30	1.41	-
	Telmisartan	Mean	6.593	12162.6	26652	0.822	5.17
		RSD	0.05	0.31	0.17	1.42	0.29
pH 6.8	Amlodipine	Mean	5.158	841.9	31484	0.825	-
		RSD	0.12	0.57	0.51	1.27	-
	Telmisartan	Mean	6.586	12150.0	26700	0.828	5.17
		RSD	0.11	0.32	0.15	1.41	0.23

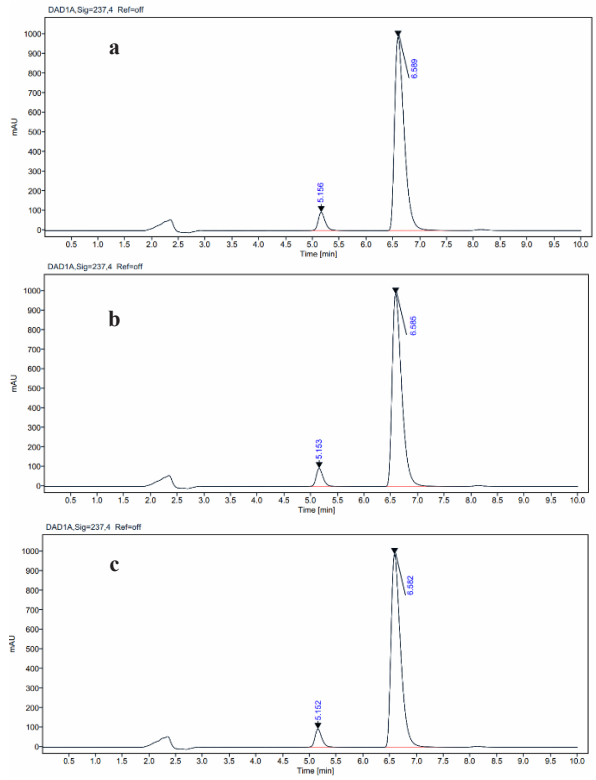

Figure 7: Chromatographical results of standard mixed solutions at three environments (a. pH 1.2; b. pH 4.5; c. pH 6.8)

Validation of Assay Method for Amlodipine and Telmisartan in Two-Layer Tablets

System suitability

The results of the systematic suitability of the procedure using a methanol environment are presented in Table 11.

Results show that the RSD values of retention time, pic area and apparent theoretical plate number of amlodipine and telmisartan were less than 2.0% with a skewness coefficientin range of 0.8 to 1.5. The resolution between the two peaks was greater than 1.5. Therefore, the quantitative procedure achieved the systematic suitability for testing in a methanol environment.

Specificity

The chromatograms of the mixture of standards revealed peaks that coincided with the retention time of the active ingredients, while the chromatograms of the blank sample and the placebo did not. Peaks with retention durations matching those of the in the standard sample emerged on the chromatograms of the test sample and the matrix spike. Consequently, the technique satisfied the specificity requirement (Figures 5 and 6).

Linearity, value domain, accuracy, precision

The results of linearity, value domain, accuracy and precision in methanol environment are summarized in Table 12.

For the studied concentration range of amlodipine and telmisartan, the assay method achieved linearity with a correlation coefficient $R^2 > 0.999$. The difference in amlodipine and telmisartan content in the samples on the same day and between two days were less than 2.0%, indicating that the studied analytical method meets the requirements for repeatability. Furthermore, the accuracy was shown to be satisfactory with our analytical method, as reflected by a conformity in recovery rates of amlodipine and telmisartan at three concentration levels 80, 100, and 120%.

For all these results, the analytical method for amlodipine and telmisartan in methanol using the HPLC method was validated in terms of suitability, specificity, linearity, accuracy and precision. This procedure can be applied to simultaneously quantify amlodipine and telmisartan in subsequent two-layer tablets.

Development and Validation of Analytical Method for Amlodipine and Telmisartan in Three Dissolution Testing Environments at pH 1.2, 4.5, and 6.8

Development of analytical method for amlodipine and telmisartan in three dissolution testing environments at pH 1.2, 4.5, and 6.8

The assay method determined in the previous section was applied in dissolution tests at pH 1.2, 4.5, and 6.8 for amlodipine

Table 14: Linearity, value domain, accuracy, and precision in the pH 1.2 dissolution testing environment

		Amlodipine				*Telmisartan*			
Regression equation		ŷ = 167.76x				ŷ = 306.73x			
Linear interval (µg/mL)		1–10				8–80			
Correlation coefficient (r^2)		0.9998				0.9996			
		Repeatability (n = 6)		Intermediate accuracy (n = 12)		Repeatability (n = 6)		Intermediate accuracy (n = 12)	
Accuracy		%compared to label	RSD (%)	%compared to label	RSD (%)	%compared to label	RSD (%)	%compared to label	RSD (%)
		97.15	1.02	97.23	1.01	95.97	0.71	95.73	0.70
	Concentration level (%)	Recovery Rate (%)		RSD (%)		Recovery Rate (%)		RSD (%)	
Precision (n = 9)	10	98.63		1.12		99.48		0.73	
	80	98.60		0.44		98.66		0.40	
	100	98.13		0.67		98.20		0,78	
	120	97.17		0.63		99.03		1.63	
Value domain (µg/mL)		4.4–6.6				35.2–52.8			

Table 15: Linearity, value domain, accuracy, and precision in the pH 4.5 dissolution testing environment

		Amlodipine				*Telmisartan*			
Regression equation		ŷ = 170.11x				ŷ = 306.65x			
Linear interval (µg/mL)		1–10				8–80			
Correlation coefficient (r^2)		0.9997				0.9996			
		Repeatability (n = 6)		Intermediate accuracy (n = 12)		Repeatability (n = 6)		Intermediate accuracy (n = 12)	
Accuracy		%compared to label	RSD (%)	%compared to label	RSD (%)	%compared to label	RSD (%)	%compared to label	RSD (%)
		97.94	0.80	97.62	1.13	95.90	0.62	95.96	0.58
	Concentration level	Recovery Rate (%)		RSD (%)		Recovery Rate (%)		RSD (%)	
Precision (n = 9)	10	98.13		0.82		101.41		0.77	
	80	98.49		0.45		99.21		0.35	
	100	98.44		0.23		98.42		0.27	
	120	99.24		1.47		98.81		0.23	
Value domain (µg/mL)		4.4 - 6.6				35.2 - 52.8			

and telmisartan (Figure 7). As shown in the results, this method was appropriate, as reflected by no change in peak area, retention time, peak purity, or skewness coefficient.

Validation of Analytical Method for Amlodipine and Telmisartan in Three Dissolution Testing Environments at pH 1.2, 4.5, and 6.8

- *System suitability*

Results of the systematic suitability in three dissolution testing environments at pH 1.2, 4.5, and 6.8 are presented in Table 13.

The results showed that the RSD values of the retention time, peak area, and apparent theoretical plate number of both amlodipine and telmisartane were less than 2.0%. The skewness coefficient of these two active substances was in the range of 0.8 to 1.5. The resolution between the two peaks was greater than 1.5. Thus, the system suitability was achieved for this analytical method at three environments of pH 1.2, 4.5, and 6.8.

Specificity

- *Linearity, value domain, accuracy, precision*

The results of studying linearity, value domain, accuracy, and precision in the pH 1.2, 4.5, and 6.8 dissolution testing environment are summarized in Tables 14-16, Figure 6, respectively.

The validation results showed that the analytical method developed in section 3.1 was suitable for dissolution testing of amlodipine and telmisartan in three environments at pH 1.2, 4.5, and 6.8. Therefore, this method was applied for dissolution testing of further two-layer tablets.

Table 16: Linearity, value domain, accuracy, precision in the pH 6.8 dissolution testing environment

		Amlodipine				*Telmisartan*			
Regression equation		$\hat{y} = 168.92x$				$\hat{y} = 306.21x$			
Linear interval (µg/mL)		1–10				8–80			
Correlation coefficient (r^2)		0.9998				0.9999			
		Repeatability (n = 6)		Intermediate accuracy (n = 12)		Repeatability (n = 6)		Intermediate accuracy (n = 12)	
Accuracy		%compared to label	RSD (%)	%compared to label	RSD (%)	%compared to label	RSD (%)	%compared to label	RSD (%)
		97.10	1.35	97.19	1.14	95.24	0.79	95.56	0.81
	Concentration level (%)	Recovery Rate (%)		RSD (%)		Recovery Rate (%)		RSD (%)	
Precision (n=9)	10	99.14		0.87		99.52		0.26	
	80	99.11		0.40		99.78		0.28	
	100	98.72		0.76		99.16		0.97	
	120	100.63		0.47		99.69		1.06	
Value domain (µg/mL)		4.4–6.6				35.2–52.8			

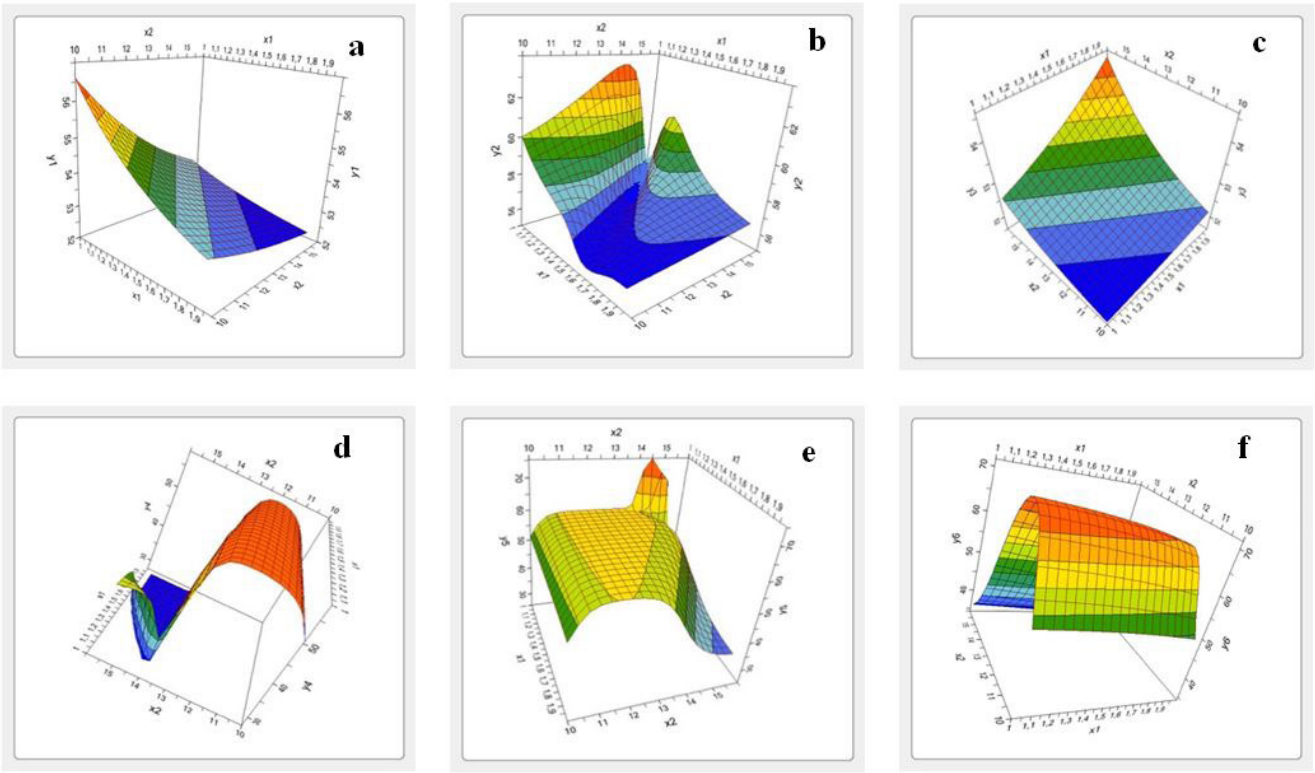

Figure 8: Impact of independent variables x_1 and x_2 on the dependent variable y_1 (a); y_2 (b); y_3 (c); y_4 (d); y_5 (e); y_6 (f)

Development and Optimization of Amlodipine and Telmisartan Two-Layer Tablets

First of all, the dissolution rate of the reference drug was investigated and presented in Table 17.

Afterwards, the DoE was applied to develop and optimize the formulation of two-layer tablets. The results of *in-vitro* equivalence of each formulation compared to the reference drug, as well as predicted optimal conditions, were presented in Tables 18 and 19.

The training results of the program give R^2 training and R^2 testing values in the range of 80 to 100%, indicating a significant correlation between the studied independent variables and the dependent variable. To be specific, the impact of NaOH ratio, crospovidone XL-10 ratio, and hardness on *in-vitro* equivalence

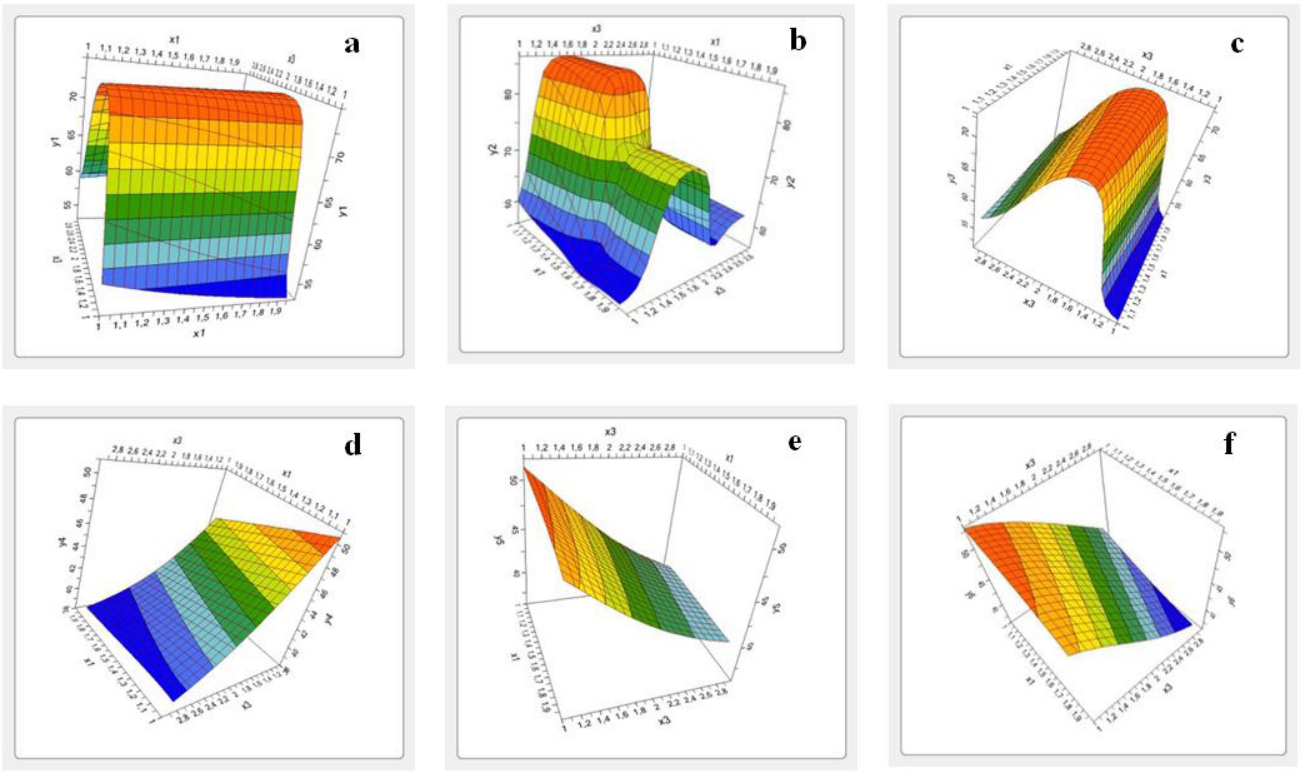

Figure 9: Impact of independent variables x_1 and x_3 on dependent variables y_1 (a); y_2 (b); y_3 (c); y_4 (d); y_5 (e); y_6 (f)

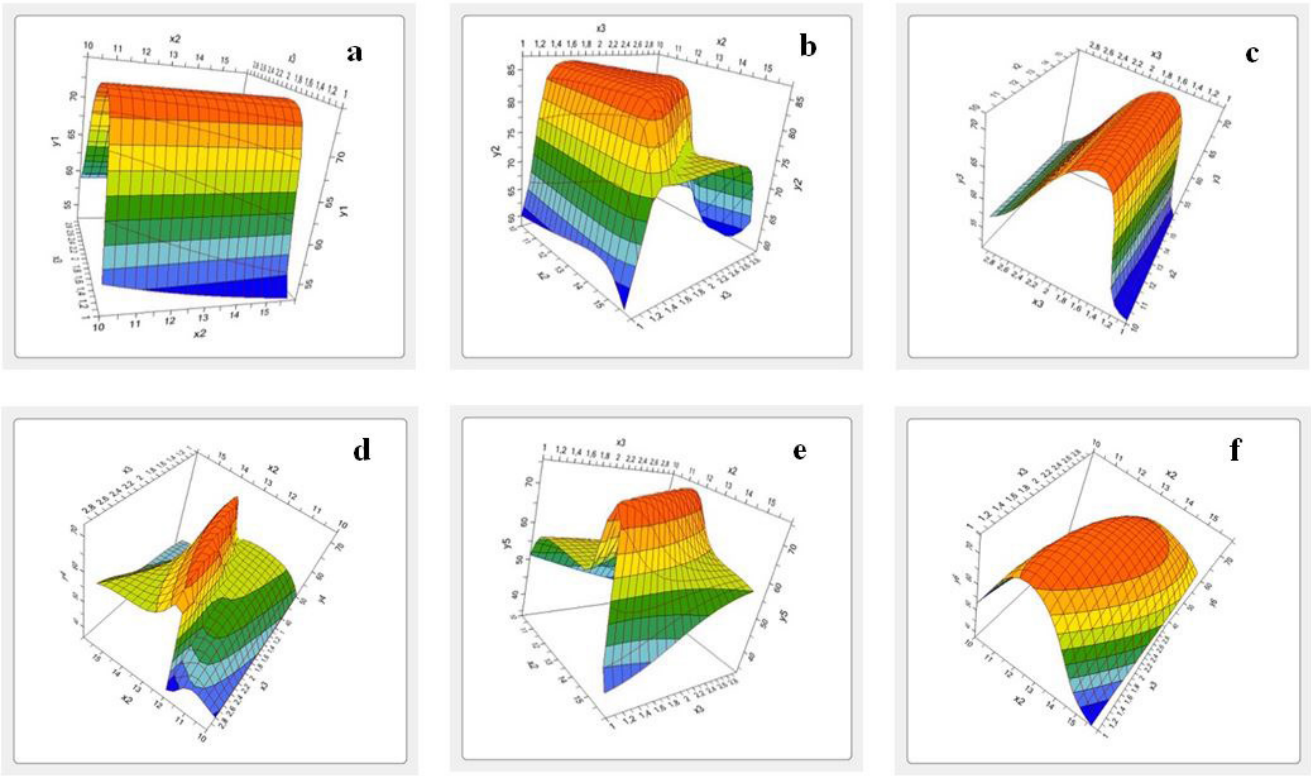

Figure 10: Impact of independent variables x_2 and x_3 on dependent variables y_1 (a); y_2 (b); y_3 (c); y_4 (d); y_5 (e); y_6 (f)

results was also illustrated in Figures 8, 9, and 10.

The optimal results predicted by BCPharSoft OPT software were verified experimentally (Table 20). Three batches of 1000 tablets were prepared with NaOH, crospovidon XL-10 ratio of 1.279 and

Table 17: Average solubility of reference drug

Time (minute)	*Amlodipine*						*Telmisartan*					
	pH 1.2		*pH 4.5*		*pH 6.8*		*pH 1.2*		*pH 4.5*		*pH 6.8*	
	%ROAC	*RSD*	*%ROAC*	*RSD*	*%ROAC*	*RSD*	*%ROAC*	*RSD*	*%ROAC*	*RSD*	*%ROAC*	*RSD*
10	80,17	2,32	79,62	3,05	81,12	1,53	24,85	3,13	20,28	2,65	55,30	2,32
15	81,83	2,04	81,54	2,83	82,76	2,78	35,06	3,47	30,08	3,18	73,78	0,98
30	84,02	1,87	83,18	2,31	84,34	2,29	56,43	2,06	41,94	1,62	79,96	1,75
45	87,39	2,24	86,45	2,68	87,53	3,07	69,67	1,49	47,86	2,04	84,34	1,47
60	90,88	1,92	89,05	1,98	91,82	1,12	78,02	2,86	57,31	1,98	92,85	1,24

*ROAC: release of active compound.

Table 18: Results of design of experiments for the two-layer tablets

No	x_1	x_2	x_3	y_1	y_2	y_3	y_4	y_5	y_6
1	1	10	1	55.87	59.85	51.39	48.63	49.76	51.23
2	1	10	2	78.13	80.02	72.84	44.86	45.92	47.89
3	1	10	3	63.28	68.12	60.43	33.54	35.43	37.71
4	1	13	1	57.32	62.41	55.24	57.09	59.31	61.43
5	1	13	2	80.67	87.05	77.37	73.32	75.87	80.64
6	1	13	3	62.11	63.84	56.91	60.73	62.54	67.17
7	1	16	1	52.86	57.43	51.98	34.54	35.26	40.43
8	1	16	2	72.04	74.17	67.94	47.08	48.19	50.67
9	1	16	3	57.83	58.13	54.41	55.75	56.92	57.98
10	1.5	10	1	55.52	56.86	51.13	49.98	52.19	54.32
11	1.5	10	2	70.14	73.83	68.19	41.32	43.24	45.65
12	1.5	10	3	59.01	61.37	57.29	37.87	38.62	39.97
13	1.5	13	1	50.23	56.12	51.34	56.35	56.98	60.56
14	1.5	13	2	76.67	82.32	74.39	64.51	65.32	69.58
15	1.5	13	3	57.23	62.55	59.96	55.23	56.71	57.13
16	1.5	16	1	52.07	58.63	54.43	36.14	37.48	39.71
17	1.5	16	2	76.17	77.79	73.02	47.18	48.94	49.63
18	1.5	16	3	59.32	63.12	57.84	53.13	54.53	55.67
19	2	10	1	53.18	56.39	50.12	46.14	47.42	48.18
20	2	10	2	73.13	74.43	72.98	38.65	40.98	42.53
21	2	10	3	61,34	60.43	57.19	39.13	40.76	41.86
22	2	13	1	54,94	57.47	53.18	49.92	52.54	54.76
23	2	13	2	72.03	73.16	70.08	58.13	62.05	64.46
24	2	13	3	59.78	60.19	57.54	56.76	57.86	58.76
25	2	16	1	52.76	55.31	54.98	27.42	24.18	25.85
26	2	16	2	74.23	74.04	70.86	34.15	35.76	36.78
27	2	16	3	63.16	65.14	60.08	44.12	46.84	48.64

Table 19: Optimal parameters and predicted results of *in-vitro* equivalence using BCPharSoft OPT software

	y_1	y_2	y_3	y_4	y_5	y_6
R^2 traing	0.9	0.98	0.9	0.99	0.98	0.9
R^2 tesing	0.92	0.82	0.97	0.71	0.86	0.91
R^2	0.92	0.95	0.93	0.92	0.94	0.92

13.675%, respectively, and a hardness in the range of 70–90 N.

The results (Table 21) showed that there is no significant difference between predicted and experimental values ($F = 0.69 < Fcrit = 4.964$), indicating that our mathematical model is reliable and applicable for the development and optimization of this formulation.

Quality tests of optimized two-layer tablets

Tablets in three batches were shown to meet the established in-house standards, as presented in Table 22.

DISCUSSION

Development and Validation of a Procedure for Simultaneous Quantification of Amlodipine and Telmisartan in Two-Layer Tablet Preparations

In this study, three chromatographic conditions were investigated, including solvent type, solvent ratio and column temperature. Initially, in terms of solvent type and ratio, the study began with acetonitrile: 0.02% phosphoric acid in different ratios 35:65, 40:60, 45:55 (v/v), with a flow rate of 1.0 mL/min. However, there is evidence of a peak-dragging phenomenon, as indicated by the peak parameters, including resolution, purity and skewness coefficient, which have not yet been achieved. Continuing the study by changing the ratio of phosphoric acid in the mobile phase, using acetonitrile: acid phosphoric 0.05% with similar ratios 35:65, 40:60, 45:55 (v:v), flow rate 1.0 mL/min. Increasing acid phosphoric ratio to 0.05% has led to some improvement in the peak parameters. However, despite the adjustment, the mentioned parameters still not achived the desired requirements. When compared with other studies, A. Kottai Muthu *et al.* (2010)[9] and Maimoon *et al.* (2017)[10] have developed chromatography conditions for a mobile phase using ACN: phosphoric acid. The difference can be explained by the fact that phosphoric acid is a medium-strength acid and is often used to create a pH 2.0 to 3.0 environment, which can affect the degree of dissociation of telmisartan and increase the hydrophobic attraction to the silica layer,[11] resulting in a greater retention time for telmisartan. On the other hand, acetic acid is a pH control agent at higher values, typically ranging from 3.0 to 5.0. Therefore, the selection of acetic acid to create a pH 4.5 environment contributes to limiting the risks to the column, thereby enhancing the efficiency and longevity of column use during the analysis of various solubility samples. Continuing further investigation of mobile phase including acetonitrile: ammonium acetate salt 50 mM adjusted to pH 4.5 by acetic acid with similar ratios 35:65, 40:60, 45:55 (v/v), flow rate 1.0 mL/min. In which, the mobile phase including ACN: acid acetic in ratio of 40:60, successfully met the requirements for purity, resolution, and skewness coefficient.

Table 20: Optimal parameters of predicted values

Optimal parameters		*Predicted values*			
x_1	1.279%	y_1	73.816	y_4	72.845
x_2	13.675%	y_2	75.163	y_5	58.936
x_3	70–90 N	y_3	70.609	y_6	71.378

Table 21: Experimental results and predicted results

Property	*Experimental results*			*Average*	*Predicted value*
	Batch 1	*Batch 2*	*Batch 3*		
y_1	67.63	71.85	70.03	69.836 ± 2.12	73.816
y_2	72.84	70.07	71.81	71.573 ± 1.96	75.163
y_3	69.92	65.91	70.67	68.833 ± 2.84	70.609
y_4	67.43	71.18	69.13	69.246 ± 2.65	72.845
y_5	58.87	60.34	57.23	58.813 ± 1.04	58.936
y_6	70.17	68.17	69.53	69.291 ± 1.41	71.378

Table 22: Quality tests for three batches of finished products

Evaluation criteria	*Quality standard*			*Result*		
				Batch 1	*Batch 2*	*Batch 3*
Appearance	Two-layer tablets, one layer white, one layer blue, tablets smooth, glossy, two layers are not interspersed with each other			Passed	Passed	Passed
Qualitative	The chromatogram of the testing sample includes two peaks with retention times corresponding to the retention time of the standard sample			True	True	True
Hardness	70–90 N			Passed (77.3 N ± 1.64 %)	Passed (85.8 N ± 1.21%)	Passed (79.7 N ± 1.38%)
Weight uniformity	There must not be more than two units whose mass is outside the ± 5% difference limit and no unit must have a mass that exceeds ± 10% of the average mass			Passed (512.7 mg± 0.53%)	Passed (521.6 mg ± 1.19%)	Passed (510.9 mg ± 0.91%)
Solubility compared to reference drug	f2 (of amlodipine and telmisartan in all 3 environments) > 50 %	Amlodipine	1.2	Passed (67.63 %)	Passed (71.85 %)	Passed (70.03 %)
			4.5	Passed (72.84 %)	Passed (70.07 %)	Passed (71.81 %)
			6.8	Passed (69.92 %)	Passed (65.91 %)	Passed (70.67 %)
		Telmisartan	1.2	Passed (67.43 %)	Passed (71.18 %)	Passed (69.13 %)
			4.5	Passed (58.87 %)	Passed (60.34 %)	Passed (57.23 %)
			6.8	Passed (70.17 %)	Passed (68.17 %)	Passed (69.53 %)
Assay	95–105%	Amlodipine		Passed (101.2 %)	Passed (100.4 %)	Passed (99.5 %)
		Telmisartan		Passed (98.42 %)	Passed (99.42 %)	Passed (101.81%)

Conducting the study at a temperature of 40°C, peak parameters such as resolution, theoretical plate number and skewness coefficient all met the requirements. Furthermore, the retention time was shortened, the peak shape appeared more compact without observing the peak-dragging phenomenon. As the temperature increases, the viscosity of the solution decreases. This can reduce friction between the mobile phase and the analyte, resulting in an increase in the speed of movement of the analyte through the column and thus, a reduced retention time.[12] From there, the chromatography process was chosen to be performed on a Zorbax Eclipse C18 Plus (250 x 4.6 mm; 5 µm). The mobile phase consisted of acetonitrile and acetate buffer in ratio (40:60 v/v) in isocratic mode. The buffer used in the mobile phase contained ammonium acetate salt 50 mM adjusted to pH 4.5 with acetic acid, flow rate 1 .0 mL/min and heated at 40°C. The sample injection volume was 10 µL and measured at 237 nm using a DAD detector. Applied the process to the study of three dissolution testing environments at pH 1.2, 4.5, and 6.8. The results showed that both the area and retention time remained unchanged, suggesting the procedure's suitability for application in three dissolution test environments. The presented results demonstrate that the simultaneous assay of two active ingredients, amlodipine and telmisartan, in tablets was carried out in three dissolution testing environments 1.2; 4.5 and 6.8. The process meets the standards required by ICH in terms of criteria as system suitability, specificity, precision, accuracy, linearity and value domain.

Design and Optimization of Immediate-Release Two-Layer Tablet Formulation Containing Amlodipine 5 mg and Telmisartan 40 mg

In fact, telmisartan is extremely poorly soluble in water, leading to poor bioavailability in the gastrointestinal tract. Thus, improving the solubility of telmisartan is an inevitable issue. Various methods are used to enhance the solubility of telmisartan, such as nanoformulation. However, these methods often require sophisticated equipement and difficult to control procedure parameters. Therefore, the method of salt formation between telmisartan and an inorganic base with strong alkalinity was used in this study. In terms of alkaline agents, many studies showed that NaOH is a suitable base for the salt formation process with telmisartan to better enhance its solubility. Moreover, telmisartan powder has poor flowability, which can affect the control of the weight and hardness of tablets during the tablet compressing process if using the direct compression method. Hence, enhancing the flowability of the powder before tablet compression is a crucial step. To improve the flowability of the powder, the wet granulation method was chosen with Avicel PH 101 as a diluent and also as a binder.[13] The process of producing telmisartan granules includes dissolving NaOH in a minimal quantity of distilled water to minimize water consumption. This aids in reducing the hardness and enhancing the porosity of the resultant granules, thereby facilitating easy disintegration of the tablets and increasing the release rate of the drug. Telmisartan is dissolved in NaOH solution until completely dissolved. This is to ensure that all telmisartan has formed a salt with NaOH to help improve solubility. Crospovidone XL-10, a super disintegrant excipient,, is also selected for the tablet formulation to prevent the formation of excessively hard particles, which can impair tablet disintegration, affecting the release ability of the active ingredient. Adding superdisintegrant excipients at the beginning is optimal since it moderates the rate of tablet disintegration, leading to gradual swelling and capillary action and preventing the excessive release of the active ingredient.[14-16] The binder solution includes telmisartan, NaOH, meglumin, and PVP K30.

The wet granulation method simplifies the preparation process, making it readily accessible and adaptable for tablet formulation compared to the application of the spray drying technique as announced from the patent of reference drug, thereby facilitating the industrial scale upgrading of the formula, significantly reducing production costs of products. Especially, this study was the first study application of wet granulation method to telmisartan in two-layer tablets to improve the solubility of the active ingredient, aiming to evaluate *in-vitro* and *in-vivo* equivalence. Applying the wet granulation method as well as the formula optimization method facilitates the directed progression of the research process, enables rapid identification of the optimal formula, streamlines research steps, and yields cost savings, which enhances the effectiveness of the formulation research process.

When evaluating the influence of the independent variables x_1, x_2 and x_3 on the dependent variable, it shows that for amlodipine, only tablet hardness has a significant impact on the release of the active ingredient in the amlodipine layer, while the ratio of crospovidone XL-10 and NaOH did not affect the amlodipine layer. Specifically, when the hardness of the tablet is too low (50–70 N), the release of the active ingredient of amlodipine tends to increase because the tablet is softer and more prone to disintegration, thereby leading to an increase in the f2 values of the amlodipine layer. However, suppose the hardness is increased too high (90–110 N), causing the tablet to become too compact. In that case, the release of the active ingredient decreases, resulting in a solubility profile that differs from the reference drug, as well as the decrease of f2 value. Therefore, the tablet hardness set at an intermediate level (70-90 N) is suitable for the amlodipine layer. The reason for this is the separate preparation and compression of the two layers of amlodipine and telmisartan, which minimizes the influence of the excipient in the telmisartan layer on the release of amlodipine. Conversely, regarding telmisartan, all three independent variables have an impact on the release level of the active ingredient telmisartan, directly affecting the f2 value. In particular, it is necessary to ensure the balance of all three factors: crospovidone XL-10, NaOH and tablet hardness to ensure that the f2 value of telmisartan reaches > 50% in all three testing environments. Specifically, excessively hard

tablets can result in poor disintegration, thereby reducing the release of the active substance. Tablets with low hardness can lead to soft tablets that are prone to rapid disintegration, resulting in an excessive release of the active ingredient. Both scenarios result in a significant disparity in the solubility of the formulation tablet compared to the reference tablet, leading to a low F2 value. Regarding the impact of crospovidone XL-10, increasing the rate of superdisintegrant excipient too high leads to excessive release of the active ingredient. However, if the percentage is too low may cause a highly rigid table hard to disintegrate, which limits the time telmisartan spends in contact with the solvent, resulting in poor solubility. Both cases reduce the f2 value. Considering the impact of the NaOH ratio, it shows that reducing the NaOH ratio too low can cause the incomplete formation of the salt, reducing the solubility of the tablets. However, increasing NaOH in the formula has the risk of making the tablets become friable and softer because NaOH is quite hygroscopic in nature, which can elevate the release of active ingredients. As a result, there is a significant disparity in the solubility of the formulation tablet compared to the reference tablet, leading to a low F2 value. Hence, it is important to achieve an equilibrium of these parameters to achieve an *in-vitro* equivalence.

After re-evaluating the optimal formulation through research on three batches of 1000 capsules each, it was found that the results of the optimal formulation from BCPharSoft OPT software were highly reliable, resulting in a formulation that met the recommended internal standard and was equivalent *in-vitro* to the reference drug Twynsta®.

CONCLUSION

A two-layer tablet formulation containing simultaneously amlodipine and telmisartan with *in-vitro* equivalence to reference drug was successfully developed using a conventional fabrication method of wet granulation and under important support by design of experiments. This study may be considered a relevant example of the benefit of using DoE in drug formulation regardless of fabrication method. Furthermore, it also highlighted not only technology but also appropriate strategy is essential in the development of drugs. Further studies on the optimization of fabrication processes using DoE will be performed to give a complete example of "quality by design" application in the development of drugs with complex compositions.

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AUTHOR'S CONTRIBUTION

D.T.M.H: Methodology, Conceptualization, Writing-original draft, Project administration, Resources, Supervision. Phuoc-Vinh Nguyen: Methodology, Conceptualization, Writing-original draft Resources, Datacuration, Writing-Review & editing. Other authors: Methodology, Conceptualization, Writing-original draft, Datacuration, Resources.

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