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DEVELOPMENT OF HPLC-PDA METHOD FOR QUANTIFYING AMLODIPINE BESYLATE IN TABLETS

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Abstract – *Cardiovascular disease is a dangerous disease known to be the leading cause of death globally, and hypertension is considered one of the main risk factors contributing to the disease. Among the high blood pressure medications currently on the market, amlodipine besylate is a commonly used drug to treat high blood pressure and prevent related diseases. This study aims to develop a procedure for the quantification of amlodipine besylate in tablets and drug quality control methods, using high-performance liquid chromatography. The method used in this research was high-performance liquid chromatography (HPLC) combined with a photodiode aray detector (PDA) and then was subsequently evaluated according to the guidelines of the International Conference on Harmonization (ICH). Research results successfully developed a process for quantifying amlodipine besylate in tablets with specificity, linearity with a linear range from 8 to 32 µg/mL, high accuracy, high precision, and a short analysis time (5 minutes) that meets the requirements of the quantification process in drug testing and can be applied to check the quality of amlodipine drugs on the market.*

Keywords: amlodipine besylate tablet, HPLC method, ICH guidelines.

I. INTRODUCTION

Hypertension, the 'silent killer' is one of the leading causes worldwide. According to a WHO report, about 1.28 billion people between the ages of 30 and 79 worldwide have high blood pressure [1]. In Vietnam, the number of deaths due to cardiovascular disease is about 200,000 people each year [2]. The global target for the period 2010–2030 is to reduce the prevalence of hypertension by 33% [1]. To achieve the above goal, in addition to improving the patient's diet and increasing physical activity, using medication to control blood pressure is an easy and inevitable intervention in today's increasingly youthful situation of hypertension.

Commonly used cardiovascular drugs today include diuretics, beta-blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, etc. Among them, amlodipine is one of the calcium channel blockers commonly used currently with indications for hypertension, angina, arrhythmia, Raynaud's syndrome [3], and coronary artery disease (CAD) [4]. However, besides the benefits of treatment, there are still some issues to be aware of when using it, because this is a medicine that must be used daily, even for a lifetime. This requires antihypertensive drugs to be tested for quality in general and drug content in the preparation in particular to ensure quality, safety, and effectiveness for patients when used.

Some current analytical methods for the determination of amlodipine besylate in pharmaceutical dosage forms include liquid chromatography (HPLC) [5, 6], capillary electrophoresis [7], UV-Vis spectroscopy [8, 9], etc. In particular, the HPLC method is a popular method with the outstanding advantages of fast analysis, high accuracy, and the ability to analyze many drugs containing single or multiple ingredients. For these above reasons, this study was carried out to contribute to creating a reference source for testing amlodipine on a laboratory scale as well as contributing to quality management of amlodipine besylate preparations on the market.

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II. LITERATURE REVIEW

Amlodipine besylate is the besylate salt of amlodipine, a calcium channel blocker antihypertensive drug that lowers blood pressure by inhibiting the flow of extracellular calcium ions into vascular and cardiac smooth muscle cells, thereby dilating peripheral arteries, increasing blood flow and reducing total peripheral resistance. In addition to lowering blood pressure, amlodipine is also used to treat angina, arrhythmia, compensated heart failure, etc. [10]. Besides the desired effects of antihypertensive drugs, the use of blood pressure drugs in general and amlodipine in particular also has issues that need attention, including the quality and safety of the drugs. Recently, several research has been conducted to determine the amlodipine content in preparations by using different methods. Specifically, Tran My Thien Thanh et al. [5] successfully developed a process to quantify (S)-amlodipine by HPLC method using dual chiral mobile phase additives with high specificity, accuracy, and repeatability [5]. In 2022, the development of the capillary electrophoresis method for the determination of amlodipine in human plasma was carried out by Nguyen Hong Anh et al. [7]. The study was evaluated for specificity, linearity, accuracy, and repeatability when identifying and quantifying racemic amlodipine and (S)-amlodipine as well as determining limit of detection (LOD) and limit of quantitation (LOQ) when testing (R)-amlodipine [7]. Chitlange et al. [10] also used the HPLC method to simultaneously quantify amlodipine besylate and valsartan in capsules. The results showed that the developed method has a simple sample processing process but high sensitivity that can be applied to the quantification of preparations on the market [10]. Overall, the above procedures for quantifying amlodipine-containing drugs widely use reversed-phase HPLC, using octadecylsilyl-attached silica particles as the stationary phase. Quantification procedures typically use isocratic elution, with a flow rate typically of 1.0 ml/min. The detection wavelength is mainly in the range of 234–238 nm and the analysis time is relatively short (< 10 minutes). These processes often use a mobile phase containing two different solvents, in which a buffer with a pH of 2.5–5.0 is added. However, the disadvantage of using buffer solution is that it reduces the life of the pump seal and the chromatography column. In addition, salt deposits caused by using buffers also affect metal parts in analytical equipment. However, research on the process of quantifying amlodipine besilate without using a buffer solution remains modest. Therefore, based on inheritance of the chromatography technique as well as referring to the sample processing process of previous studies, a reversed-phase liquid chromatography technique combined with a photodiode array detector (HPLC-PDA) is developed. In particular, this quantitative procedure does not use any buffer solution in mobile phase solvent in order to establish a simple, highly reliable procedure for the quantification of amlodipine besilate in tablets with minimal impact on the equipment and that is also obviously different from the previously presented works.

III. METHODOLOGY

Time and location of the study

The research was conducted in September 2023 at the Pharmacy Department, Tra Vinh University.

Material

Reference substance: Amlodipine besylate 99.9%; lot number QT145 0124; provided by Institute of Drug Quality Control Ho Chi Minh City.

Solvents: Water, methanol, and acetonitrile meet standards for liquid chromatography; phosphoric acid meets analytical standards.

Equipments: Ultimate 3000 liquid chromatography (UHPLC) system (Thermo Scientific – USA); photodiode array (PDA) detector; Phenomenex C8 and C18 columns (150 x 4.6 mm; 5 μm), analytical balance, ultrasonic bath, and common laboratory glassware have the appropriate precision for each test.

Quantitative method

Based on the physicochemical properties of amlodipine besylate, characteristics of the sample, and reference to several previous studies [5, 6, 10], the HPLC method was chosen to develop a quantitative procedure for amlodipine besylate tablets. After being developed, the process will be evaluated according to ICH guidelines [11], with the criteria of system suitability testing, specificity, accuracy and precision, linearity, and determination range.

Preparation

Blank: Mobile phase solvent.

Stock standard solution: Reference amlodipine besylate is dissolved in acetonitrile to obtain a solution with a concentration of 500 μg/mL. The stock standard solution is then diluted with the mobile phase to obtain standard solutions of appropriate concentration for each test of the validation procedure.

Standard solution: 1 mL of the stock standard solution accurately pipet into a 10 mL volumetric flask. A mobile phase to the mark was added and then shaken well. After shaking, the solution needs to be filtered through a 0.45 μm membrane filter. The resulting solution has a concentration of amlodipine besylate 50 μg/mL.

Sample: 20 tablets were weighed and calculated for the average weight of each tablet. Next, the tablet was grindded into fine powder. A quantity of grinded powder corresponding to 5 mg of amlodipine besylate was accurately weighed into a 10 mL volumetric flask, then 5 mL of acetonitrile was added and sonicated for 10 minutes, and let cool. The solution was made up to volume with acetonitrile, shaken well, and filtered away the first 5 mL of filtrate. After that, exactly 1 mL of the remaining filtrate was taken and transferred into a separate 10 mL volumetric flask. Then a mobile phase was added to the mark. The resulting solution has a concentration of about 50 μg/mL of amlodipine besylate. Finally, this final solution was filtered through a 0.45 μm filter into a vial.

Expected chromatography conditions

Chromatography column: Phenomenex Kinetex C18 column (150 x 4.6 mm; 5 μm); mobile phase: acetonitrile – 1% phosphoric acid water (50: 50); flow rate: 1.0 mL/min; detection wavelength: 237 nm; injection volume: 10 μL.

Investigation of factors affecting separation efficiency

From the expected chromatographic conditions, main factors that affect the separation efficiency of the method such as chromatography column, mobile phase, and mobile phase ratio are evaluated in following section.

IV. RESULTS AND DISCUSSION

A. Results

The survey of chromatographic conditions

- Column

Perform the HPLC method using C8 and C18 columns respectively (100 x 4.6 mm; 5 μm). The results are presented in Table 1; Figure 1 and Figure 2.

Table 1: Results of chromatography column survey

	Column	Retention time (minute)	Asymmetry (A_s)	Theoretical plates (N)
Sample	C8	2.510	1.7	4,886
	C18	2.573	1.2	7,576
Standard	C8	2.507	1.8	4,817
	C18	2.570	1.3	7,344

Figures 1 and 2 illustrate the chromatograms of the standard solution and samples with two investigated columns.

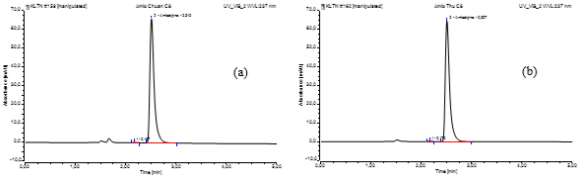

Fig. 1: Chromatograms of standard solution (a) and sample (b) with Phenomenex C8 column (100 x 4.6 mm; 5 μm)

The obtained results presented in Table 1 and Figures 1, 2 showed that with the same solvent system and mobile phase ratio, the C18 column

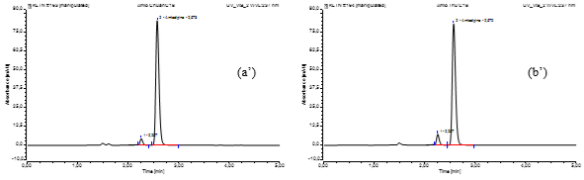

Fig. 2: Chromatograms of standard solution (a') and sample (b') with Phenomenex C18 column (100 x 4.6 mm; 5μm))

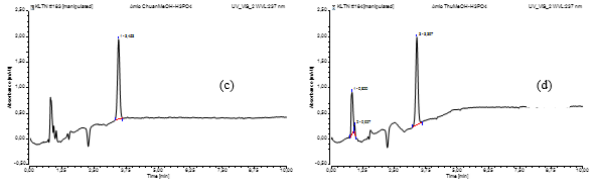

Fig. 3: Chromatograms of standard solution (c), sample (d) with solvent system (1)

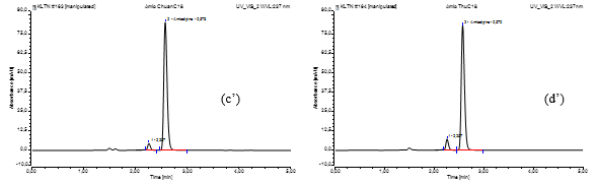

Fig. 4: Chromatograms of standard solution (c'), sample (d') with solvent system (2)

gave a more symmetrical symmetry coefficient of amlodipine (As = 1.2) and the theoretical plate number of amlodipine on the C18 column was higher (N > 7,000) compared to C8 column (As = 1.7 and N = 4,886). These results demonstrated that the C18 column under the evaluated solvent conditions was more suitable for analyzing amlodipine than the C8 column. Therefore, the C18 column was chosen to investigate the factors of solvent and solvent ratio.

- Mobile phase solvent

With choosing the C18 column, the following two solvent systems were selected to study as mobile phase: solvent system (1) includes methanol and 1% phosphoric acid water (40:60); solvent system (2) includes acetonitrile and 1% phosphoric acid water (40:60). The survey results are shown in Table 2, Figures 3 and 4.

Table 2: Results of mobile phase solvent survey

	Solvent system	Retention time (minute)	Asymmetry (A_s)	Theoretical plates (N)
Standard	(1)	-	-	-
	(2)	2.573	1.2	7,576
Sample	(1)	-	-	-
	(2)	2.570	1.3	7,344

Figures 3 and 4 illustrate the chromatograms of the standard solution and samples with the two evaluated solvent systems.

The results presented in Table 2 and Figures 3 showed that during the investigation time of 10 minutes, the amlodipine peak was not eluted by using a solvent system (1). In Figure 4, by using the solvent system (2), the peak of amlodipine eluted at 2.573 minutes with the asymmetry of amlodipine in the range of $0.8 \leq As \leq 1.2$. This indicated that the solvent system (1) was not suitable for analyzing amlodipine. Therefore, solvent system (2) consisting of acetonitrile and 1% phosphoric acid water (40:60) was chosen to continue investigating mobile phase acid water.

- Mobile phase acidic water

The investigation of mobile phase acid water was performed by using two different types of acid water respectively in order to optimize the elution ability of the mobile phase. With the same chromatographic conditions, the investigated solvent systems are solvent system (3) includes acetonitrile-water (40:60); solvent system (4) includes acetonitrile – 1% phosphoric acid water (40:60) and solvent system (5) includes acetonitrile – 1% acetic acid water (40:60). The survey results are presented in Table 3; Figures 5, 6, and 7.

Figures 5, 6, and 7 illustrate the chromatograms of the standard solution and samples with the three solvent systems investigated.

The results presented in Table 3 and Figures 5, 6, 7 showed that, with the solvent system (4), the peak of amlodipine eluted at retention time t_R = 2.573 was shorter than with solvent

Table 3: Results of mobile phase acidic water survey

	Solvent system	Retention time (minute)	Asymmetry (A_s)	Theoretical plates (N)
Standard	(3)	26.623	1.8	586
	(4)	2.573	1.2	7,576
	(5)	3.477	2.4	4,106
Sample	(3)	-	-	-
	(4)	2.570	1.3	7,344
	(5)	3.443	2.3	3,969

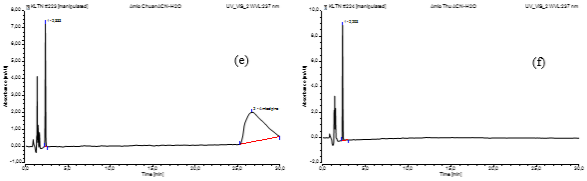

Fig. 5: Chromatograms of standard solution (e) and sample (f) with the solvent system (3)

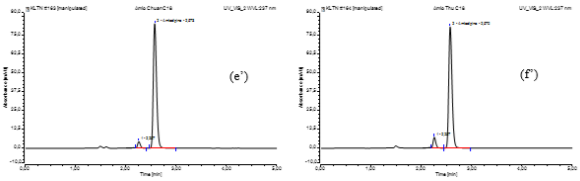

Fig. 6: Chromatograms of standard solution (e') and sample (f') with the solvent system (4)

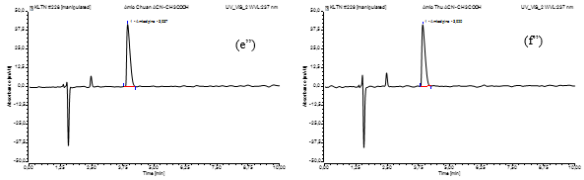

Fig. 7: Chromatograms of standard solution (e") and sample (f") with the solvent system (5)

systems (3) and (5) (t_R = 26.623 and t_R = 3.477, respectively). The asymmetry of amlodipine As = 1.2 with the solvent system (4) was better compared to solvent systems (3) and (5) As $\geq$ 1.7. The solvent system (4) gave the theoretical plates N > 7,000 while solvent systems (3) and (5) gave the theoretical plates N < 4,000. This proved that solvent systems (3) and (5) were not suitable for analyzing the substance to be studied. Therefore, the solvent system (4) consisting of acetonitrile and 1% phosphoric acid water (40:60) was chosen to continue investigating the mobile phase solvent ratio.

- Mobile phase ratio

From the survey results of acid water used in the mobile phase and the previous survey results on solvent ratios, the following mobile phase solvent systems were selected for investigation: solvent system (6) includes acetonitrile and 1% phosphoric acid water (35:65), solvent system (7) includes acetonitrile and 1% phosphoric acid water (40:60), solvent system (8) includes acetonitrile and 1% phosphoric acid water (45:55). The survey results are presented in Table 4; Figures 8, 9 and 10.

Figures 8, 9, and 10 illustrate the chromatograms of standard solution and samples with the three solvent systems investigated.

Table 4 and Figures 8, 9 and 10 presented results of the solvent system survey with the mobile phase solvent ratio acetonitrile – 1% phosphoric acid water being 35:65; 40:60 and 55:45, the results showed that the solvent system (6) eluted the amlodipine peak at t_R = 3.707 was longer than the solvent system (7) and (8) (t_R = 2.573 and t_R = 1.810, respectively). The asymmetry of the solvent system (6) As = 1.1 was better, compared to the solvent system (7) As = 1.2. However, with the solvent system (8), the asymmetry could not be determined due to the amlodipine peak not being separated from the solvent peak. The resolution between the amlodipine peak and the blank peak of the solvent systems (6) and (7) Rs $\geq$ 3.0 was better compared to the solvent system (8) Rs < 1.5. So, the solvent systems (6) and (7) could be used to analyze the active ingredient amlodipine, in particular, the solvent system (6) gave a better asymmetry and better resolution between the amlodipine peak and the solvent peak compared to the solvent (7). Therefore, the solvent system (6) with the ratio (35:65) of acetonitrile and 1% phosphoric acid water was chosen.

Table 4: Results of the survey of mobile phase solvent ratio

Sample	Solvent system	Retention time (minute)	Asymmetry (A_s)	Theoretical plates (N)
Standard	(6)	3.707	1.1	8.9
	(7)	2.573	1.2	3.0
	(8)	1.810	-	<1.5
Sample	(6)	3.700	1.1	8.7
	(7)	2.570	1.3	3.0
	(8)	1.810	-	<1.5

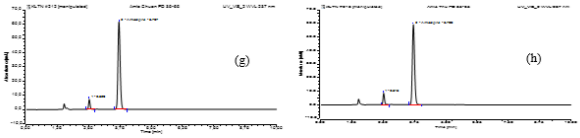

Fig. 8: Chromatograms of standard solution (g) and sample (h) with the solvent system (6)

In short, the appropriate chromatographic conditions for analyzing amlodipine besylate in tablets are: Phenomenex C18 column (100 x 4.6 mm; 5 μm); mobile phase: acetonitril – 1% phosphoric acid water (35:65); flow rate: 1.0 mL/min; detection wavelength: 237 nm; and injection volume: 10 μl.

Results of method validation

- System suitability

Table 5 presented results of the system suitability survey on amlodipine besylate standard solution on C18 column showed that the apparent number of theoretical plates N > 6,000; RSD% value of retention time and peak area of amlodipine besylate was not more than 0.69%; asymmetry 0.8 $\leq$ As $\leq$ 1.2. Thus, the process achieved system suitability.

- Specificity

The results of the specificity survey showed that under the same analytical conditions, the blank chromatogram did not appear as a peak at the retention time corresponding to the standard solution amlodipine besylate. The chromatogram of the sample and the sample with the standard substance added showed a peak with a retention time corresponding to the peak in the chromatogram of the standard. On the chromatograms of the sample and the sample with adding stan-

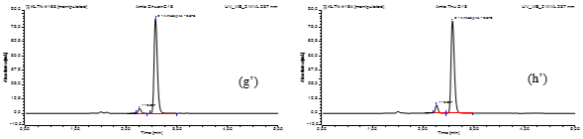

Fig. 9: Chromatograms of standard solution (g') and sample (h') with the solvent system (7)

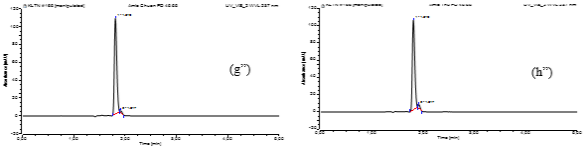

Fig. 10: Chromatograms of standard solution (g") and sample (h") with the solvent system (8)

Table 5: Results of system suitability survey on 50 μg/mL standard solution (n = 6)

Sample	Retention time (minute)	Area (mAU)	Asymmetry (A_s)	Theoretical plates (N)
1	3.693	6.677	1.2	6,252
2	3.690	6.644	1.2	6,268
3	3.683	6.624	1.2	6,209
4	3.687	6.585	1.2	6,381
5	3.687	6.548	1.2	6,289
6	3.687	6.563	1.2	6,263
Average	3.688	6.607	-	6,277
RSD %	0.08	0.69	-	Reached

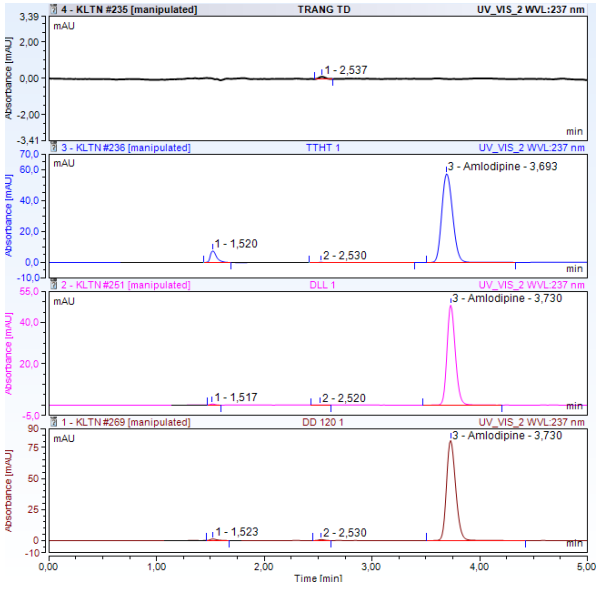

Fig. 11: Specificity chromatograms of blank (i); standard solution (j); sample (k) and sample add standard (l)

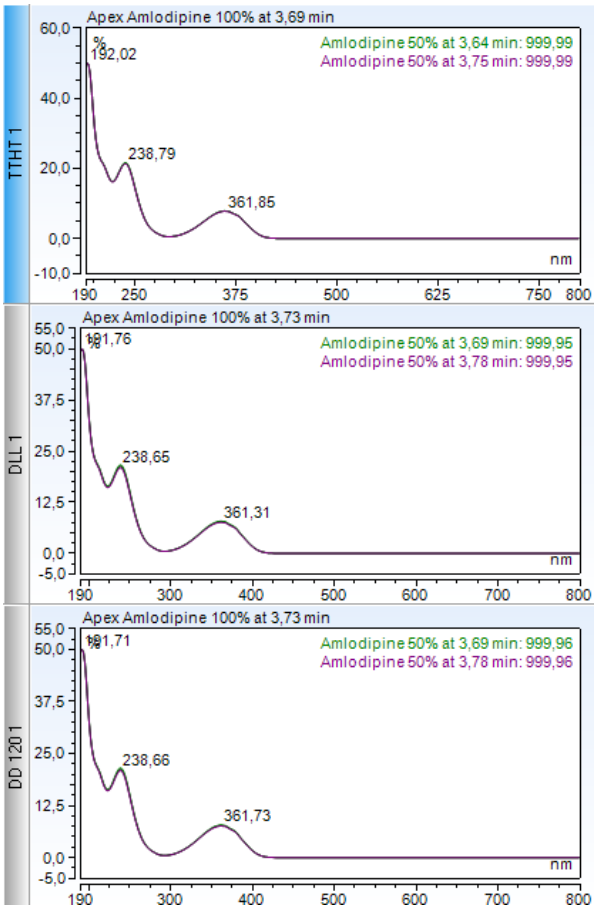

Fig. 12: UV - Vis spectra and purity of standard solution (j'); sample (k') and sample add standard (l')

dard substance, the analytical peak completely separated from the solvent peak with a value of Rs > 8.2. The peaks of the amlodipine besylate in the chromatograms of the sample and the standard were pure. The UV-Vis spectrum of the sample corresponded to the UV-Vis spectrum of the standard solution (Figures 11 and 12). Thus, the method had specificity.

- Linearity, range, accuracy, and precision

The linearity and determination range of amlodipine besylate were investigated on standard solutions, with a concentration range of 8 μg/mL–32 μg/mL. Precision including repeatability and intermediate precision was conducted on samples with a concentration of 20 μg/mL, each factor was conducted with six independent samples. The accuracy of the method was evaluated on samples with standard substance added at three concentration levels of 16 μg/mL; 20 μg/mL and 24 μg/mL, each concentration with three samples.

The analytical method achieved linearity with r2 value > 0.9994. The recovery rate of amlodipine besylate with the tested concentration of 16–24 μg/mL was in the range of 98.4%–101.6% with RSD $\leq$ 1.01%. The accuracy of the method with RSD $\leq$ 1.57%, so the method had high accuracy and precision.

In summary, the method validation of amlodipine besylate in tablets using the HPLC-PDA method meets the requirements for system suitability, with high specificity, accuracy, and precision. The method can be applied to identify and quantify tablets containing the single ingredient amlodipine besylate currently circulating on the market.

B. Discussion

Based on the structure and physicochemical properties of amlodipine, a reversed-phase chromatography technique was selected for the quantification of amlodipine besylate in tablets. The investigation was performed on both Phenomenex C8 and C18 columns. The mobile phase solvents were investigated being polar solvents such as acetonitrile, methanol, and water. In addition, the ratio of mobile phase components and types of acids used in the mobile phase were also evaluated to find appropriate chromatographic conditions. The results of the chromatography column survey showed that both C8 and C18 columns could be used to analyze amlodipine, especially since the C18 column had a better asymmetry than the C8 column. This study result is also consistent with the research of the authors Chitlange et al. [10] who used C18 column, a cheap, popular chromatography column and suitable for most laboratory conditions in their analytical process. However, in their study, the C18 column used was 250 mm long, while in this study the length of the chromatography column

Table 6: Results of surveying linearity, determination range, accuracy, and precision

Concentration (µg/mL)	Accuracy (n=3)		Determination range (µg/mL)	Precision		Regression	r^2	Concentration range (µg/mL)
	Recovery (%)	*RSD %*		*Recovery (Ave± RSD%)*	*Intermediate precision (Ave ±RSD%)*			
16	100.4–101.6	0.57	16–24	98.2± 1.57	100.2±0.32	y = 0.3319x + 0.0799	0.9994	8–32
20	98.4–99.5	0.55						
24	99.1–101.0	1.01						

used was 150 mm, so the elution time of the amlodipine peak differed slightly but the chromatographic parameters still met the requirements. For investigating the mobile phase solvent, methanol was expected to replace acetonitrile to change the elution force of the mobile phase. Furthermore, methanol has a lower cost than acetonitrile, which helps reduce the costs of the analysis process. However, the results showed that substitution with methanol resulted in the amlodipine peak not eluting within 10 minutes, so methanol was considered unsuitable for the quantification of amlodipine. In contrast, acetonitrile was chosen due to its better elution ability than methanol and this result is also consistent with the results of studies by previous author groups [5, 6, 10] who used acetonitrile in their studies. Additionally, acid water in the mobile phase also significantly affected the separation efficiency of the method. In fact, the research showed that if the mobile phase simply used acetonitrile and water, the separation effect would not be good. Specifically, in this study, if only water and organic solvents were used for elution, the amlodipine's peak would be not yet eluted within 30 minutes. Compared to previous studies [5, 6, 10] that used buffer solutions in the mobile phase to increase elution ability, in this study the author used acidic water to replace buffer solution with the expectation that the buffer solution has the least impact on the longevity of the equipment. In particular, the investigation included two types of 1% phosphoric acid water and 1% acetic acid water. The result was that the mobile phase containing 1% acetic acid had an elution time of 3.443 minutes and an asymmetrical coefficient was $\geq$ 2.3. As a result, 1% phosphoric acid water solution was chosen because the elution time of the peak to be analyzed was 2.573 minutes and As = 1.2. Moreover, the mobile phase ratio of acetonitrile – 1% phosphoric acid water is also an important factor that affects the ability to separate analytes and impurity peaks. Particularly, in the studies of the authors [5, 6, 10], due to their research samples involved 2 or more analytes and these substances had different structures and levels of affinity with the stationary phase. Hence, to completely separate substances and reduce analysis costs, it is necessary to continuously change the mobile phase ratio during chromatography, leading to the gradient elution mode chosen in their studies. In this study, the elution ability affected of the mobile phase ratio was also demonstrated through the separation ability of the analyte peak and the blank solvent peak, however the isocratic elution mode was used instead of the gradient elution as in the above studies. The survey results of mobile phase ratio showed that when increasing the ratio of non-polar solvent, the ability to separate the analyte peak from the solvent peak decreased, particularly when increasing the ratio of acetonitrile from 40% to 45%, the Rs value between the amlodipine's peak and the blank's peak decreased significantly from 3.0 to

< 1.5. On the contrary, when the acetonitrile ratio was reduced to 35%, the Rs value between the amlodipine's peak and the blank's peak increased to 8.9 and the asymmetry of the amlodipine's peak also decreased from 1.2 to 1.1. Thus, the mobile phase ratio of (35:65) acetonitrile – 1% phosphoric acid water was chosen to validate the process.

From the above survey results, a quantitative method for amlodipine besylate by HPLC with a PDA detector was developed. In general, the quantitative method had a short analysis time (< 5 minutes), and the procedure and sample handling process were relatively simple. The method has been successfully validated according to ICH guidelines with a wide linear range, high accuracy, and precision.

V. CONCLUSIONS

The study has successfully developed a quantitative method for amlodipine besylate in tablets using HPLC. The method is determined using the C18 column (As = 1.2, N > 7,000) and mobile phase solvents consisting of acetonitrile and 1% phosphoric acid water (35:65) (t_R = 3.707 minutes, As = 1.1). Moreover, the method validation for quantifying amlodipine besylate in tablets using HPLC meets the system suitability requirements, linearity with a linear range from 8–32 μg/mL, high specificity, accuracy, and precision. From the results of the above investigation and method validation, this procedure can be applied to the quality control of single-component amlodipine besylate tablets on the market.

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