

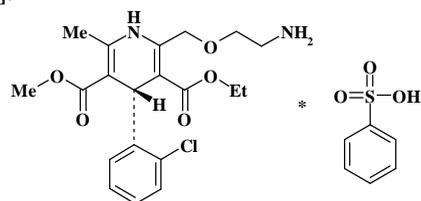
NEW SPECTROPHOTOMETRIC METHOD OF AMLODIPINE
BESYLATE DETERMINATION AND ITS VALIDATIONMarta Sulyma^{1, *}, Svitlana Vasyuk², Yulia Zhuk², Danylo Kaminskyi¹,
Olesya Chupashko¹, Volodymyr Ogurtsov¹<https://doi.org/10.23939/chcht12.04.429>

Abstract. Simple, accurate, and precise spectrophotometric method has been developed and validated for the estimation of amlodipine besylate (AML) in tablets. The method is based on the condensation of AML with sodium 1,2-naphthoquinone-4-sulphonate in an alkaline medium when the reaction mixture was heated at 363 K for 1 min to form an orange-colored product. The spectrophotometric method involved the measurement of the colored product at 459 nm. The reaction conditions were studied and optimized. Beer's law was obeyed in the concentration range of 10–20 $\mu\text{g}\cdot\text{ml}^{-1}$ with RSD of 0.825 and 0.559 % and molar absorption of $2.54\cdot 10^4$, the range of methods application is 67–133 % of the nominal content of amlodipine besylate in the dosage forms.

Keywords: amlodipine besylate; sodium 2-naphthoquinone-4-sulphonate; spectrophotometric assay

1. Introduction

Amlodipine besylate is an important calcium channel blocker belonging to the dihydropyridine family. It is more selective for arterial vascular smooth muscle than for cardiac tissue and is approved for the treatment of hypertension and for variant and stable angina. Amlodipine besylate (AML) is (4R,S)-3-ethyl 5-methyl 2-(2-amino-ethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate monobenzene sulphonate [1, 2].



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The assay procedure listed in European Pharmacopoeia for amlodipine besylate describes the reversed phase high performance liquid chromatographic method [2] for determination of the drug in bulk and pharmaceutical formulations. The literature describes a number of methods for quantifying amlodipine, both in pure form and in dosage forms, among which chromatographic [3-8], electrochemical [9-13] and spectrophotometric [14-19] methods are presented. In spectrophotometric methods for the quantitative determination of amlodipine besylate in the visible region of the spectrum various chemical reactions are used, among which the oxidation-reduction reactions and the reactions of complex formation with charge transfer are predominate. Also, few spectrophotometric methods for amlodipine besylate assay in dosage forms using sodium 1,2-naphthoquinone-4-sulphonate as a color reagent are described [20-21], but both methods are extractive and require more time and reagents.

Therefore, the aim of the presented work was to develop a highly sensitive, easy-to-use and economical method for quantitative determination of amlodipine besylate in the dosage forms based on the reaction with sodium 1,2-naphthoquinone-4-sulphonate, as well as validation of the developed methodology.

2. Experimental

2.1. Materials and Apparatuses

All reagents used were of analytical grade: amlodipine besylate (Fluka Analytical, P500185), sodium 1,2-naphthoquinone-4-sulphonate (SPC "Synbias", batch 53). The solution of sodium 1,2-naphthoquinone-4-sulphonate was freshly prepared. All solvents and other chemicals used throughout this study were of analytical grade. Water was doubly distilled.

Spectrophotometer "SF-56 UV/Vis" (LOMO, OKB Spectr, Saint-Petersburg, Russia), electronic scales ABT-120-5DM, ultrasound bath ELMASONICE60 H were used for all measurements.

2.2. Procedure for the Assay of Amlodipine Besylate

Amlodipine besylate (0.015 g) was dissolved in 40.00 ml of 96 % ethanol and the total volume of the filtrate was made up to 100 ml with distilled water. Aliquots of amlodipine besylate (0.100–0.200 mg) standard solution (1 mg/ml) were pipetted into a series of test tubes. To each test tube 0.50 ml of 1M sodium hydroxide solution and 0.50 ml of 0.5 % sodium 1,2-naphthoquinone-4-sulphonate solution were added, mixed well and heated on a water bath at 363 K for 1 min. After heating, the solutions were cooled at room temperature and transferred to the 10 ml volumetric flask and the volume was made up to the mark with distilled water. The absorbance was measured at 459 nm against a reagent blank treated similarly. The concentration of amlodipine besylate was calculated from calibration curve.

2.3. Procedure for the Assay of Amlodipine Besylate in Pharmaceutical Preparations

Twenty tablets of “Amlodipine-KV 10 mg” (Kyiv Vitamin Factory, Ukraine) (A) and “Amlodipine-Astrapharm 10 mg” (Astrapharm, Ukraine) (B) were accurately weighed and powdered. The portions of powder (equivalent to 0.6110 g of amlodipine besylate (A) or 0.3052 g (B)) were dissolved in 40 ml of ethanol and diluted to 100 mL with distilled water, mixed in an ultrasonic bath for 2 min, and filtered. 0.50 ml of 1M sodium hydroxide solution and 0.50 ml of 0.5 % sodium 1,2-naphthoquinone-4-sulphonate solution were added to 1.00 ml of filtrate in 10 ml volumetric flask, mixed well and heated on a water bath at 363 K for 1 min. After heating, the solution was cooled at room temperature and the volume was made up to the mark with distilled water. The absorbance was measured at 459 nm against a reagent blank treated similarly.

3. Results and Discussion

3.1. Effect of Sodium Hydroxide Concentration

First, the optimal solvents for used reactants have been chosen: water-ethanol solution containing 40 % ethanol for amlodipine besylate, and water for the sodium 1,2-naphthoquinone-4-sulphonate. To determine the optimal amount of sodium hydroxide, to 1 ml of amlodipine besylate solution, different aliquots (0.1–0.7 ml) of 1M sodium hydroxide solution were added. The results showed that the highest absorbance was obtained with 0.50 ml of sodium hydroxide solution, which remained unaffected with

higher amounts of sodium hydroxide (Fig. 1). Therefore, 0.50 ml of the reagent was added for the determination.

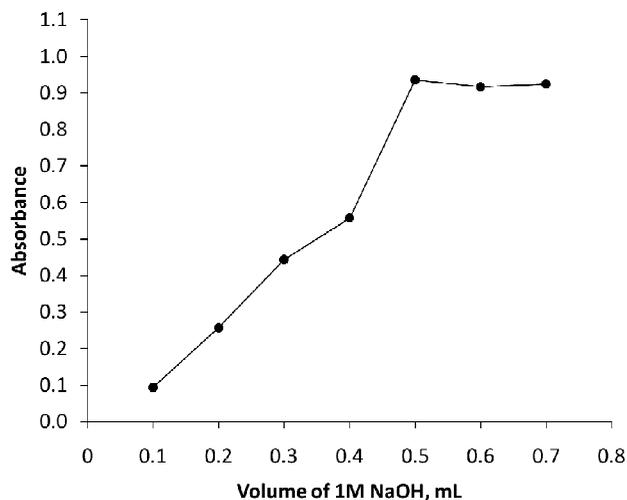


Fig. 1. Effect of 1M sodium hydroxide volume on the absorbance

3.2. Effect of Sodium 1,2-Naphthoquinone-4-Sulphonate Concentration

Aiming to determine the optimal concentration of sodium 1,2-naphthoquinone-4-sulphonate, different volumes (0.4–2.0 ml) of 0.5% sodium 1,2-naphthoquinone-4-sulphonate solution were added to 1 ml of amlodipine besylate solution. The addition of 0.50 ml of 0.5% solution of sodium 1,2-naphthoquinone-4-sulphonate was found to be the optimal one (Fig. 2), since further increase in the concentration of the reagent caused a decrease in the optical density.

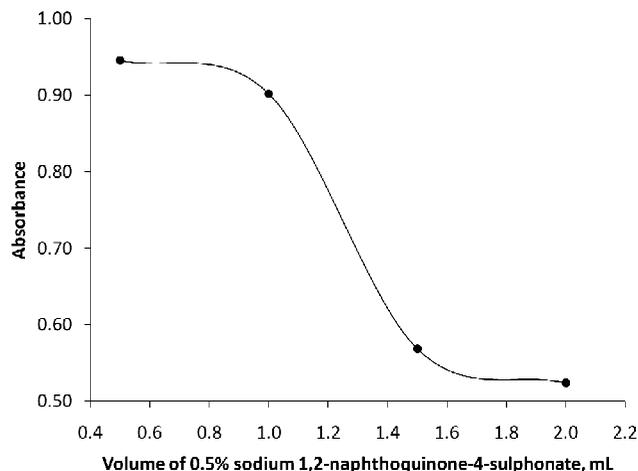


Fig. 2. Effect of sodium 1,2-naphthoquinone-4-sulphonate concentration on the absorbance at λ_{\max} of the coloured product

3.3. Analytical Data

3.3.1. Stoichiometry of the reaction

The stoichiometry of the reactions was studied adopting the limiting logarithmic method [22, 23]. Two straight lines were obtained with slope values of 0.63 and 0.58 (Fig. 3). By dividing the slopes of the two lines, a value of 1.09 was obtained. It was therefore concluded that the reaction proceeds in a molar ratio of 1:1.

The results obtained by the isomolar series confirm this relationship, the maximum values of the optical density of the reaction products are observed under above mentioned proportion (Fig. 4).

3.4. Validation of the Proposed Methods

According to the requirements of the State Pharmacopoeia of Ukraine, methods for quantitative determination of medicinal products should be validated [24].

3.4.1. Overall uncertainty

The predicted total uncertainty of the results of the analysis should not exceed the maximum permissible uncertainty of the results of the analysis $\max \Delta_{As}$. The total predicted relative uncertainty was calculated by using the following equation:

$$\Delta_{As} = \sqrt{\Delta_{SP}^2 + \Delta_{FAO}^2} \quad (1)$$

where Δ_{SP} – uncertainty of sample preparation; Δ_{FAO} – uncertainty of the final analytical operation.

In the case of spectrophotometric analysis, the uncertainty of the final analytical operation is 0.70 % (Table 1).

$$\Delta_{SP} = \sqrt{1.33^2 + 0.12^2 + 0.6^2 + 0.5^2 + 0.0327^2 + 0.12^2 + 0.6^2 + 0.5^2} = 1.74\%$$

Complete uncertainty of the analytical method:

$$\Delta_{As} = \sqrt{\Delta_{SP}^2 + \Delta_{FAO}^2} = \sqrt{1.74^2 + 0.70^2} = 1.87\% \leq \max \Delta_{As} = 3.2\%$$

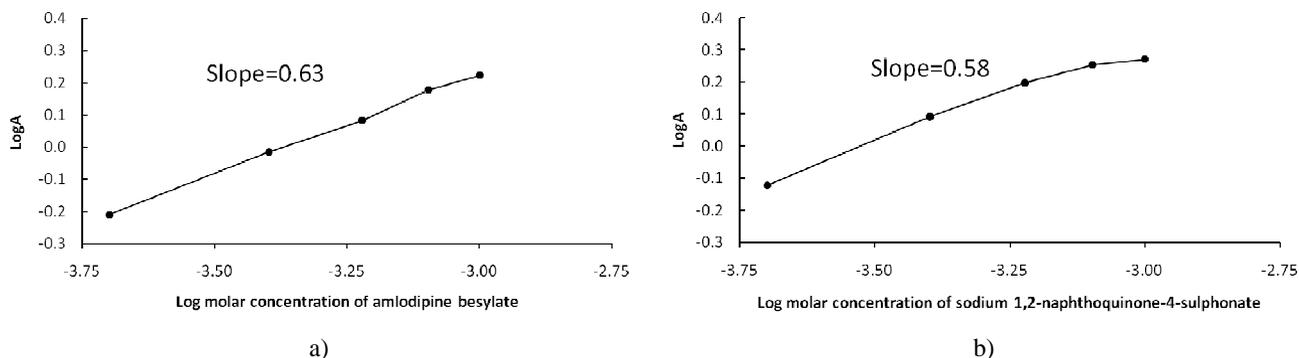


Fig. 3. Stoichiometry of the reaction between amlodipine besylate and sodium

1,2-naphthoquinone-4-sulphonate by adopting the limiting logarithmic method, variable amlodipine besylate concentrations and constant sodium 1,2-naphthoquinone-4-sulphonate concentration (a), variable sodium 1,2-naphthoquinone-4-sulphonate concentrations and constant amlodipine besylate concentration (b)

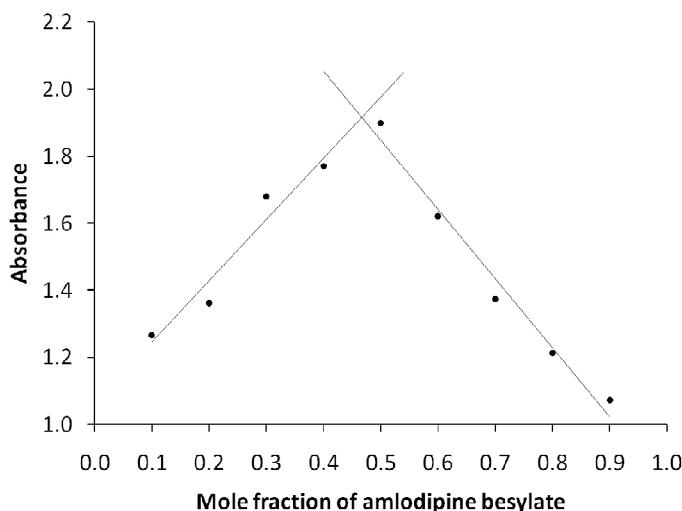


Fig. 4. Dependence of the optical density on the composition of the isomolar solutions

Table 1

Calculation of the uncertainty of sample preparation (tablets “Amlodipine-Astrapharm”)

Phase of the sample preparation	Parameter	Uncertainty, %
Stock standard solution		
1) weighing of exact mass of amlodipine besylate (25 mg)	m_0	$0.2 \text{ mg}/15 \text{ mg} \cdot 100 = 1.33$
2) dilution to 100 ml in a volumetric flask	100	0.12
3) pipetted of aliquot (1.00 ml)	1	0.6
4) dilution to 10 ml in a volumetric flask	10	0.5
Tested solution		
5) weighing of exact mass of tablets powder	m_1	$0.2 \text{ mg}/305.2 \text{ mg} \cdot 100 = 0.0327$
6) dilution to 100 ml in a volumetric flask	100	0.12
7) pipetted of aliquot (1.00 ml)	1	0.6
8) dilution to 10 ml in a volumetric flask	10	0.5

3.4.2. Linearity and sensitivity

The determination of the linearity was carried at the concentrations range according to the main law of light absorption, namely 1–2 mg/100 ml. Standard solution of amlodipine besylate was diluted for obtaining the solutions with known concentration, which were analyzed according to the general procedure. The dependence of the optical density on the concentration of the analyzed solution is described in Fig. 5.

Under the experimental conditions, the optical characteristics and the statistical parameters for the proposed methods are summarized in Table 2.

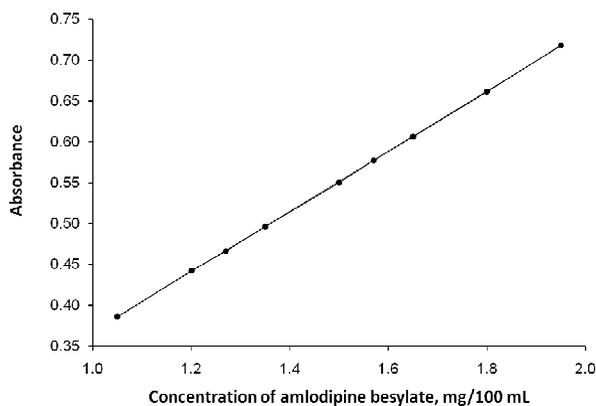


Fig. 5. Calibration curve of amlodipine besylate

The linearity of the method is confirmed throughout the concentration range indicated above (Table 2). Thus, the concentration range of application of the technique is 67–133 % of the nominal content of amlodipine besylate in the drug.

3.4.3. Precision

The precision of the method was estimated by measuring nine replicate samples of AML. The assays gave satisfactory results; the one-way confidence interval Δx does not exceed the maximum permissible uncertainty of the analysis, so the method is accurate at the level of convergence (Table 3). This level of precision of the proposed method was adequate for the quality control analysis of AML in its pharmaceutical dosage forms.

3.4.4. Correctness

The correctness of the method was determined by the method of additives. Different amounts of amlodipine besylate solution were added to three equal samples of the dosage form and analyzed three times. The calculated criteria of practical insignificance for both dosage forms do not exceed the maximum permissible uncertainty of the analysis (Table 4).

3.4.5. Robustness

The assessment of robustness was carried out at the stage of development of the methodology. The impact of

Table 2

Spectrophotometric characteristics and statistical data of the regression equations

Parameter	Value
Molar absorptivity, ϵ	25407
Sandell's sensitivity, W_s	0.0223
Limit of detection, C_{\min} , $\mu\text{g}/\text{ml}$	1.12
Linear regression equation	$Y = bX + a$
Slope, $b \pm S_b$	0.3672 ± 0.0006
Intercept, $a \pm S_a$	0.0003 ± 0.0009
Standard error of estimate, $S_{x,y}$	0.00136
Correlation coefficient, r	0.9999

Table 3

Evaluation of the accuracy and precision of the proposed procedure

Formulation names	$\bar{X} (n = 9)$	SD	RSD, %	Δx
"Amlodipine-KV 10 mg"	0.0101	$8.33 \cdot 10^{-5}$	0.825	$1.55 \cdot 10^{-4}$
"Amlodipine-Astrapharm 10 mg"	0.00988	$5.52 \cdot 10^{-5}$	0.559	$1.03 \cdot 10^{-4}$

Table 4

Correctness of the results of quantitative determination of amlodipine besylate

Formulation names	$\bar{Z} (n = 9)$	SD	ΔZ	$\bar{Z} - 100$
"Amlodipine-KV 10 mg"	100.44	0.0711	0.132	0.44
"Amlodipine-Astrapharm 10 mg"	99.61	0.190	0.353	0.39

external factors that may affect the optical density, namely the stability of the analyzed solutions in time, the number of reagents added, and the temperature of the heated solutions of the solutions analyzed, were studied. It has been found that the tablets solutions are stable for at least 40 mins. The influence of other factors is described above. This indicates the reliability of the proposed method during routine procedures.

4. Conclusions

Validated spectrophotometric method for amlodipine besylate assay in tablets has been developed. The method is simple, rapid, accurate, and reliable for the determination of amlodipine besylate in tablets without interference from the common excipients. It was proved by validation characteristics (linearity, precision, correctness, and robustness) that the developed methodology is correct and can be applied in the chemical and pharmaceutical industry.

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РОЗРОБЛЕННЯ НОВОЇ МЕТОДИКИ СПЕКТРОФОТОМЕТРИЧНОГО ВИЗНАЧЕННЯ АМЛОДИПІНУ БЕЗИЛАТУ ТА ЇЇ ВАЛІДАЦІЯ

Анотація. Розроблений та валідований простий, точний і прецизійний спектрофотометричний метод оцінки кількісного вмісту амлодіпін у безилату у таблетках. Метод базується на взаємодії амлодіпін безилату із натрію 1,2-нафтохінон-4-сульфонатом у лужному середовищі, за нагрівання реакційної суміші за 363 K протягом 1 хв., з утворенням продукту помаранчевого кольору. Спектрофотометричний метод включає вимірювання оптичної густини продукту при 459 нм. При розробленні методу вивчено та оптимізовано умови реакції. Підпорядкування закону Бера відбувалось у діапазоні концентрації 10–20 мкг/мл при RSD 0,825 і 0,559 %, а молярний коефіцієнт поглинання складав $2,54 \cdot 10^4$, діапазон застосування методів становить 67–133 % від номінального вмісту амлодіпін безилату у лікарських формах.

Ключові слова: амлодіпін безилат; 2-нафтохінон-4-сульфонат натрію; спектрофотометричний аналіз.