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VALIDATION OF ASSAY METHOD OF AMLODIPINE IN TABLETS BY LIQUID CHROMATOGRAPHY

The aim of this study was the validation of methods of quantitative determination of amlodipine in tablets by liquid chromatography. Methods: The chromatographic analysis was performed on amlodipine liquid chromatograph Agilent 1290 Infinity II LC System. Results: A validation of methods of quantitative determination of amlodipine by high performance liquid chromatography tablets has been performed. It was established that the method proves the requirements of the State Pharmacopoeia of Ukraine for the main validation parameters: specificity, accuracy, linearity, robasnist. Conclusion: The results obtained in this study clearly indicate that the developed HPLC method is fast, economical, simple, accurate and suitable for determination of amlodipine in medications.

Key words: amlodipine; quantitative analysis; validation; chromatography; validation parameters

INTRODUCTION

One of the important problems of modern pharmacy is to replenish the range of new drugs and scientific justification of their introduction into clinical medicine. The requirements of the pharmaceutical industry for drug quality control, ensuring their purity and high quality are increasing. Increasing the number of antihypertensive drugs, different chemical structure and physical properties of active pharmaceutical ingredients and the need to identify them while many components are constantly put before standardization of medicines and pharmaceutical chemistry task of improving existing and developing new methods of separation and determination. In this regard, increasing the role of chromatographic methods, and especially – highly efficient options for liquid chromatography (HPLC). Today spectroscopy along with liquid and gas chromatography are the most common instrumental methods of analysis. Over the past decade developed a spectrophotometric methods for determining amlodipine, nifedipine, verapamil, captopril, fosinopril, diltiazem in substance and finished dosage forms, and conducted validation and verification spectrophotometric techniques developed in compliance with SPU and other pharmacopoeias. Along with the unexplored issue is the development of chromatographic methods for analysis of antihypertensive drugs in the standardization of this group of pharmacological agents [5]. HPLC is specific in comparison with spectrophotometry as appropriate to coordinate normalized tested approach to the HPLC method. HPLC in comparison to spectroscopy requires significantly more time for the experiment. So HPLC desirable to use approaches that require minimal time analysis

that can be used by other circuits of the experiment than spectrophotometry [1].

The aim of our study was the validation of methods of quantitative determination of amlodipine in tablets by liquid chromatography.

MATERIALS AND METHODS

The object of the study was Amlodipine tablets ("Astrafarm"). The chromatographic analysis of amlodipine performed on liquid chromatograph Agilent 1290 Infinity II LC System.

Chromatography is performed on liquid chromatograph with spectrophotometric detector under the following conditions [4]:

- ascentis C18 column size 4.6 × 150 mm with a particle size of 5 microns;
- mobile phase: acetonitrile R – 0.1 % solution of trifluoroacetic acid R (40 : 60);
- the rate of mobile phase: 1.0 ml/min;
- column temperature: 30 °C;
- detection wavelength: 237 nm.

Preparation of Test Solution

To sample powder pounded tablets equivalent to 10 mg of amlodipine, add 70 ml of solvent (water R – acetonitrile R (1 : 1)), shake in ultrasonic bath for 15 min. The solution was cooled and adjusted to the volume of solvent 100.0 ml. Filter through a membrane filter with a pore size of 0.45 microns, discarding the first 5 ml of filtrate.

Preparation of SS Solution

27.7 mg of amlodipine besylate USP RS is dissolved in a solvent (water R – acetonitrile R (1 : 1)) and dilute with the same solvent to about 50.0 ml. 5.0 ml of the resulting solution adjusted to 20.0 ml of solvent.

The analysis considered likely if the requirements of the test "Checking the suitability of the chromatographic
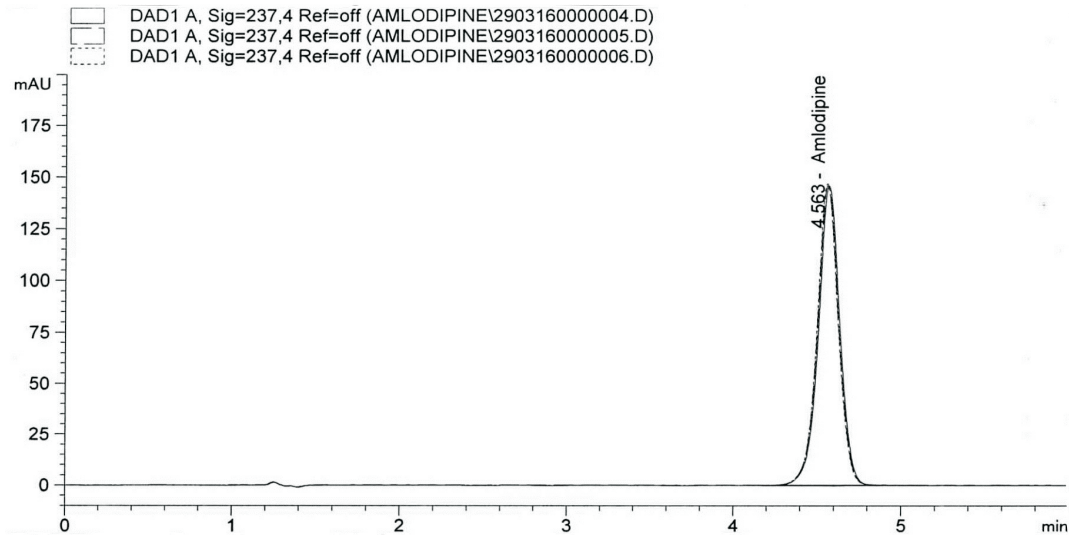
Fig. 1. *HPLC chromatograms of the Standard solution of amlodipine in the terms of identification and quantification amlodipine in medicines*

system". Chromatographic system is considered appropriate if the following conditions are met:

- the effectiveness of the chromatographic column, calculated peak amlodipine, should be not less than 3000 theoretical plates;
- relative standard deviation calculated peak area for amlodipine should be no more than 1.0%.

In accordance with the requirements of the State Pharmacopoeia of Ukraine, methods of quantification of drugs included in the quality control methods should be validated. Set investigated validation characteristics depending on the purpose of analytical methods. Validation of methods of quantitative determination of amlodipine in tablets held by the typical characteristics: specificity, accuracy, linearity, robasnist [2, 3, 6, 7].

RESULTS AND DISCUSSION

For elaboration of the method the chromatograms of the Standard solution of amlodipine (Fig. 1) and the Test solution of amlodipine (Fig. 2), as well as the dependence of the intensity peaks on the retention time were obtained and analysed.

The content of amlodipine (X) in one tablet, in milligrams, calculated by the formula:

$$X = \frac{S_i \cdot m_o \cdot 5 \cdot 0.721 \cdot 100 \cdot b \cdot P}{S_o \cdot 50 \cdot 20 \cdot m_i \cdot 100},$$

where: S_i – average of the peak areas of amlodipine, calculated from the chromatogram of the test solution; S_0 – average of the peak areas of amlodipine, calculated with reference solution chromatogram; m_0 – mass of the sample
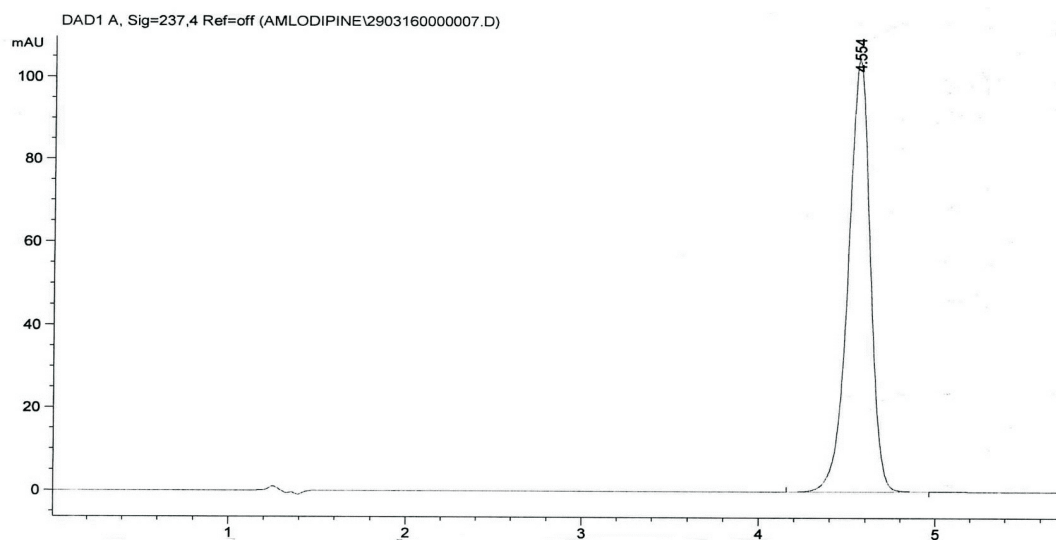

Fig. 2. *HPLC chromatograms of the Test solution of amlodipine in the terms of identification and quantification amlodipine in medicines*

Table 1

RESULTS OF THE STUDY THE ACCURACY AND PRECISION OF QUANTITATIVE METHODS AMLODIPINE DETERMINATION BY HPLC

Content in model mix, in % of nominal	Found contents to nominal, %	Found contents to input, %
80	79.94	99.93
80	80.10	100.13
80	81.23	101.53
100	100.85	100.85
100	101.38	101.38
100	99.22	99.22
120	120.37	100.31
120	119.50	99.60
120	120.21	100.16
Average value Z	100.35	
The relative standard deviation Sz %	0.78	
The relative confidence interval ΔZ	1.45	
Critical values for results convergence	$\Delta z \% \leq 3.2$	
Systematic error δ%	0.35	
Criterion uncertainty of systematic error	< 0.487	
The general conclusion of the method	correct	

SPhU amlodipine besylate, in milligrams; m_i – mass of the powder pounded tablets, in milligrams; P – content of the main substance in SPhU amlodipine besylate as a percentage; b – average weight tablets in milligrams; 0.721 – amlodipine besylate conversion factor for amlodipine.

The specificity of the methodology adjusted by comparing the chromatogram of the standard, investigated and no-load solutions. Retention time of amlodipine in the chromatogram of test solution meets the retention time of chromatogram peaks corresponding solution. At idle solution chromatogram peaks were found higher than the noise baseline, retention time which coincided with the retention time to the analyzed substances.

To test the accuracy and precision methods of preparing 3 separate mixtures of accurately known content of the substance, which covered a range of application methods (with concentrations of 80, 100, 120 of par). For each model mix was held three parallel tests. According to SPU expected following criteria: systematic error δ% (for accuracy) and the relative confidence interval ΔZ (for precision). The results of the experiments and the calculations in Tab. 1.

Experimental results are characterized by precision spreading relatively acceptable medium and therefore a low standard deviation Sz % (Sz % = 0.78 < 3.2) in the whole concentration range, indicating that the quality of analytics and methodologies applied.

Evaluation of linearity was performed on the entire range of application of the method using standard method. The study of dependence of absorbance on the concentration was conducted using model solutions of the samples. The results obtained were statistically processed by the least squares method according to the requirements of the SPhU. For each of the test solution the average value of the peak area were calculated. The results obtained were processed by the least squares method for line $y = m \times x + b$ and methrological characterictics are shown in Fig. 3.

Requirements for the parameters of a linear relationship in this case performed in the entire range of application methods (80-120 %).

Robasnist – resistance techniques to small changes in experimental conditions tested in the test solution. Terms chromatography varied within ± 10 % of these in the procedure. The research results are presented in Tab. 2.
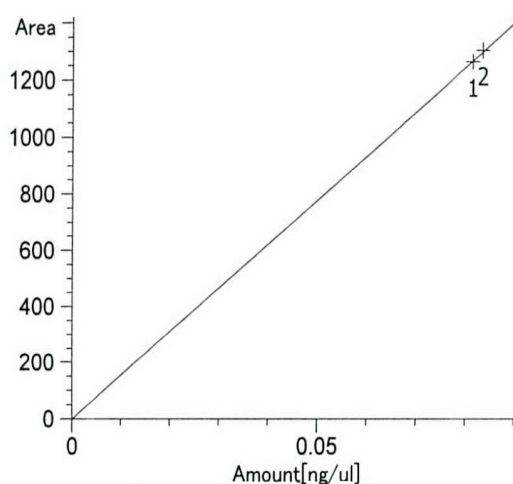

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Amlodipine at exp. RT: 4.556
DAD1 A, Sig=237,4 Ref=off
Correlation:          0.99998
Residual Std. Dev.:    6.69954
Formula: y = mx + b
        m:  15585.77654
        b:     -1.14818e-1
        x: Amount
        y: Area
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Fig. 3. *Calibration curve for HPLC chromatographic determination of amlodipine in tablets and metrological characteristics of linearity*

Table 2

RESULTS OF THE STUDY ROBASNIST METHODS OF AMLODIPINE DETERMINATION BY HPLC

Conditions of analysis	Retention time, min
Standard conditions	4.563
Flow rate 1.1 ml/min (+10 %)	4.712
Flow rate 0.9 ml/min (-10 %)	4.787
Temperature of column 33 °C	4.597
Temperature of column 27 °C	4.589

Most of the results affected by changes in the flow rate of the mobile phase, but they are insignificant. Temperature changes in terms of column chromatography virtually no effect on the analysis.

To confirm the stability of model solutions preparation solution, corresponding to the nominal concentration, made in determining the linearity chromatographed in 24 hours. The results are shown in Tab. 3.

Found value (found / entered) amlodipine in the chromatogram model solutions analyzed 24 hours after cooking is different from 100 % to less than 1.6 %, confirming robasnist this technique.

CONCLUSIONS

A validation of methods of quantitative determination of amlodipine accordance with SPU performed. It was established that the method meets the requirements for SPU specificity, accuracy, precision and robasnist in the range of 80-120% of the nominal content. The results obtained in work clearly indicate that the developed HPLC method is simple, fast, economical and suitable for determination of amlodipine in medicines.

Table 3

CONFIRMATION OF STABILITY OF MODEL SOLUTIONS OF AMLODIPINE

Test solutions	The content of amlodipine, X %	The difference between amlodipine content, %
Freshly prepared	100.0	0.5
After 24 h	100.5	
Requirements: ≤ 1.6 %		Kept

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ВАЛІДАЦІЯ МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ АМЛОДИПІНУ У ТАБЛЕТКАХ МЕТОДОМ РІДИННОЇ ХРОМАТОГРАФІЇ

Метою даного дослідження була валідація методики кількісного визначення амлодипіну в таблетках методом рідинної хроматографії. Методом хроматографічного аналізу проводили дослідження амлодипіну на рідинному хроматографі Agilent 1290 Infinity II LC System. Проведено валідацію методики кількісного визначення амлодипіну методом високоефективної рідинної хроматографії у таблетках. Встановлено, що методика відповідає вимогам Державної фармакопеї України за основними валідаційними характеристиками: специфічність, правильність, лінійність, робасність. Результати, отримані в роботі, чітко зазначають, що розроблений метод ВЕРХ є швидким, економічним, простим, точним і підходить для визначення амлодипіну в лікарських засобах.

Ключові слова: амлодипін; кількісний аналіз; валідація; хроматографія; валідаційні характеристики

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ВАЛИДАЦИЯ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ АМЛОДИПИНА В ТАБЛЕТКАХ МЕТОДОМ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ

Целью данного исследования была валидация методики количественного определения амлодипина в таблетках методом жидкостной хроматографии. Методом хроматографического анализа проводили исследование амлодипина на жидкостном хроматографе Agilent 1290 Infinity II LC System. Проведена валидация методики количественного определения амлодипина методом высокоэффективной жидкостной хроматографии в таблетках. Установлено, что методика соответствует требованиям Государственной фармакопеи Украины по основным валидационным характеристикам: специфичность, правильность, линейность, робасность. Результаты, полученные в работе, свидетельствуют, что разработанный ВЭЖХ метод является быстрым, экономичным, простым, точным и подходит для определения амлодипина в лекарственных средствах.

Ключевые слова: амлодипин; количественный анализ; валидация; хроматография; валидационные характеристики

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Фармацевтична хімія та фармакогнозія