Research Article

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DEVELOPMENT OF THE METHODOLOGY OF THE CHROMATOGRAPHIC DETERMINATION OF AMLODIPINE IN MEDICINES

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ABSTRACT

Analytical method of development and validation is critical to achieving the reliable analytical data you need to support your pharmaceutical development activities. Method of development and validation can be costly and labour intensive. Considerable knowledge, and experience coupled with advanced instrumentation, is critical to developing efficient, accurate, reliable and robust analytical methods. Analysis of amlodipine in substance is described in Pharmacopoeia but the aim of our work was the development of simple, sensitive and accurate analytical methods for the determination of amlodipine besylate in medicines. In developing this technique, column Ascentis C18 was used, which is a classical column, reverse phase and has a high surface area and stability phase. Selected conditions were isocratic elution with binary mobile phase consisting of acetonitrile and 0.1% trifluoroacetic acid solution. The proposed method has the advantage over pharmacopoeial method due to speed and ease of preparation of the mobile phase and reduced chromatography time. In conclusion, we have developed chromatographic method of the quantification of amlodipine in medicines. The proposed method is rapid, economical, simple, accurate, selective, precise and applicable to the analysis of pharmaceutical dosage forms. This method can also give excellent results and can be employed for the routine analysis.

Keywords: amlodipine, high-performance liquid chromatography, validation, linearity, accuracy, range of application.

INTRODUCTION

Traditionally, pharmaceutical analysis is referred to as the chemical analysis of drug molecules. Modern pharmaceutical analysis has evolved beyond this to encompass combination techniques, high-output technologies, chemometrics, micro dosing studies, miniaturization and nanotechnology. These analytical advances are now being employed in all stages of pharmaceutical drug discovery. With new, improved and evolving pharmaceutical technologies (HPLC and UHPLC), as well as new applications for existing technology, the search for new medicines for the prevention and treatment of human diseases continues[1]. The object of the study was chosen amlodipine. Amlodipine besylate, 3-ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulphonate, is a potent dihydropyridine-type calcium channel blocker which is an antihypertensive medicine. Analysis of amlodipine in substance is described in the Pharmacopoeia. Chromatographic conditions to determine Amlodipine besilat tablets have been shown in American Pharmacopoeia monograph that uses chromatographic column categories L1 (with a fixed phase C18) and mobile phase consisting of three components: buffer pH 3.0 consisting of tryethylamine, acetonitrile and methanol[2,3]. Because of creation of the second edition of SPhU and inclusion of articles in the finished products, we have set ourselves the goal to improve to more rapid, simple, selective, more accurate, precise, reliable, less expensive methods of HPLC analysis of amlodipine in medicines and for analysis of their metabolites in next step of the researches.

The aim of our work was the development of simple, sensitive and accurate analytical methods for the determination of amlodipine besylate in medicines.

MATERIALS AND METHODS

The object of study were Amlodipine tablets 10 mg («Farmak») and Amlodipine tablets 5 mg («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:
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 minutes. 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.

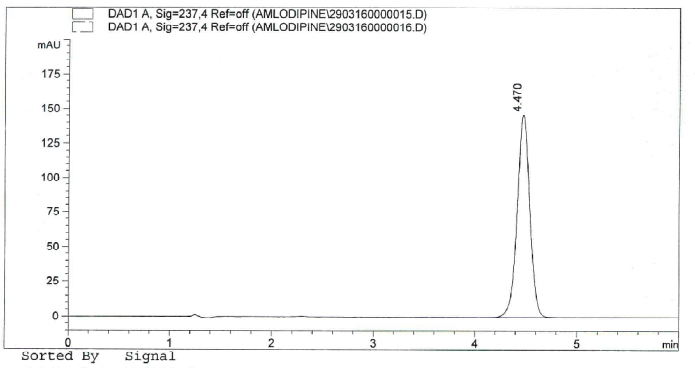
Preparation of SS Solution

27.7 mg of amlodipine besylate sample SPhU 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.

Validation of the method was carried out in accordance with the requirements of the SPhU[4,5].

RESULTS AND DISCUSSION

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



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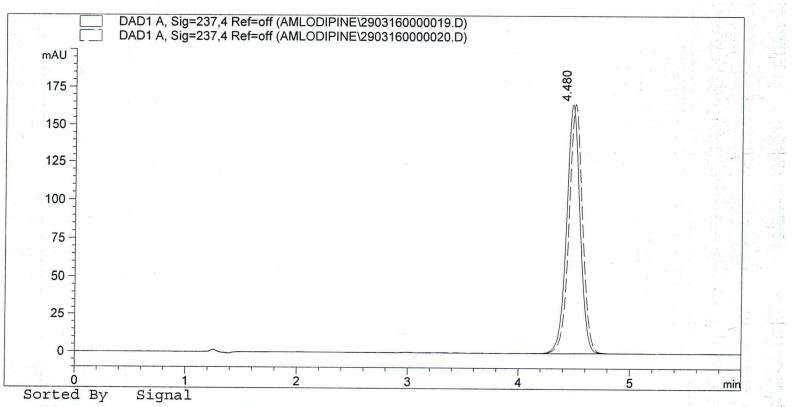
Uncalibrated Peaks : not reported
Signal Description : DAD1 A, Sig=237,4 Ref=off

#	RT	Type	Width	Area	Height	Area %	Name
1	4.470	VB	0.130	1258.198	146.335	50.023	
2	4.471	BB	0.130	1257.035	146.222	49.977	Amlodipine
		Average		1257.617			
		St.dev.		0.823			

Figure 1: HPLC chromatogram of the Standard solution of amlodipine in the terms of quantification of amlodipine in medicines



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Signal Description : DAD1 A, Sig=237,4 Ref=off

#	RT	Type	Width	Area	Height	Area %	Name
1	4.480	BV	0.129	1394.134	164.763	50.031	
2	4.503	BB	0.128	1392.388	165.029	49.969	Amlodipine
		Average		1393.261			
		St.dev.		1.235			

Figure 2: HPLC chromatogram of the Test solution of amlodipine in the terms of quantification of amlodipine in medicines

The results of the analysis are considered reliable if the requirement of the System Suitability Test are performed. The chromatographic system is considered suitable if the following conditions are performed:

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

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

$$X = \frac{S_i \cdot m_0 \cdot 5 \cdot 0.721 \cdot 100 \cdot b \cdot P}{S_0 \cdot 50 \cdot 20 \cdot m_i \cdot 100},$$

Where: S_i - average of the peak areas of amlodipine besylate, calculated from the chromatogram of the test solution;
S_0 - average of the peak areas of amlodipine besylate, calculated with the standard solution chromatogram;
m_0 - mass of the sample 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.

In developing this technique, column Ascentis C18 was used, which is a classical column, reverse phase and has a high surface area and stability phase. Selected conditions were isocratic elution with binary mobile phase consisting of acetonitrile and 0.1% trifluoroacetic acid solution.

The method has the advantage over pharmacopoeial method due to speed and ease of preparation of the mobile phase and reduced chromatography time.

According to the requirements of the SPhU, methods of quantitative determination of medicines must be validated. We have studied the following validation characteristics: linearity, accuracy and range of application[6,7].

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 9 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 nine test solutions 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 metrological characteristics are shown in Figure 3.

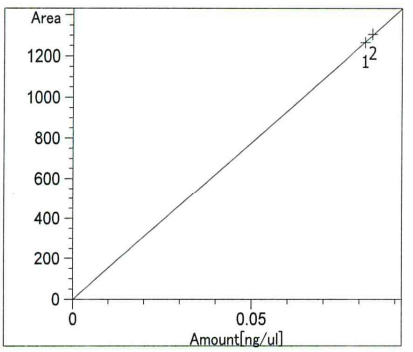

Figure 3: Calibration curve for HPLC chromatographic determination of amlodipine in tablets and metrological characteristics of linearity

Requirements for the parameters of the linear dependence in this case are carried out within the whole range of the method application (70-130 %). Accuracy and convergence were studied by «put-found» on standard solutions of amlodipine. Model solutions were prepared according to the procedure completely repeating the procedure for preparing the test solution. By comparing the two solutions for each analyte built calibration graph (level 1-2, including all parallel injection and specifying the appropriate concentration reference solution), passing through zero. For calibration schedule, each analyte concentration was calculated corresponding model solution (Table 1) .

Table 1: Results of testing the quantitative determination of amlodipine on the accuracy by method HPLC

Solution	Put (solution concentration), mg/ml	Found (concentration solution), mg/ml	The relation found to input, Z, %
RS1	0,0815	-	-
RS2	0,0835	-	-
MS 70 %	0,0573	0,0583	101,7
MS 100 %	0,0810	0,0813	100,4
MS 130 %	0,1048	0,1062	101,3
The average			100,8
The relative standard deviation, Sz%			0,4272
Relative confidence interval, Δz%			0,99
The critical value for convergence results Δz%≤ 3,2			Correct
The criterion of statistical	≤ 0,33	Correct	

insignificance systematic error $\delta\% = \lvert \overline{Z} - 100 \rvert \leq \Delta_z / \sqrt{n}$		
Criterion practical insignificance systematic error $\delta\% = \lvert \overline{Z} - 100 \rvert \leq 0{,}32 \cdot \Delta_{As}$	≤ 2,048	Correct
General conclusion of method		Correct

The obtained result - 101,1 %. General conclusion of method – correct (Table 2).

Table 2: Criteria of acceptability

Parameter	The obtained result	Criteria	Conclusion
Z, %	Amlodipine 101,1 %	97-103%	*Complies* *Yes* ■ *No* □

Using proposed technique were analyzed drugs Amlodipine tablets 10 mg, production «Farmak» and Amlodipine tablets 5 mg, production «Astrafarm», amlodipine contents were 9.98 mg and 5.3 mg respectively.

CONCLUSION

In conclusion, as a result, the study of the literature found amlodipine in medicines require the development of new and improvement of methods used to control their quality. We have developed chromatographic method of assay of amlodipine in tablets. The method has the advantage over pharmacopoeial method due to speed and ease of preparation of the mobile phase and reduced chromatography time.

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