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

Yulia Kondratova 1, Adebayo Makanjuola Theophilus 2, Logoyda Liliya 3*, Korobko Dmytro 4, Ihor Berdey 5, Tamara Kuchmerovska 6

1Head of Analytical Development Laboratory of R&D Department at Farmak JSC, Ukraine
2I. Horbachevsky Ternopil State Medical University, Ukraine
3Associate Professor of Pharmaceutical Chemistry Department, I. Horbachevsky Ternopil State Medical University, Ukraine
4Dean of Pharmaceutical faculty, Associate Professor of Pharmaceutical Chemistry Department, I. Horbachevsky Ternopil State Medical University, Ukraine
5Associate Professor of Pharmacy Management, Economics and Technology Department, I. Horbachevsky Ternopil State Medical University, Ukraine
6Scientific researcher, Department of Vitamin and Coenzymes Biochemistry, Palladin Institute of Biochemistry, National Academy of Science of Ukraine

Received on: 24/05/16 Revised on: 20/06/16 Accepted on: 25/06/16

*Corresponding author
E-mail: lilya-19@mail.ru

DOI: 10.7897/2277-4343.074128

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, microdosing 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 continues1. The object of the study was chosen amlodipine. Amlodipine besylate, 3-ethyl 5-methyl (4RS)-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 besilate 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 triethylamine, acetonitrile and methanol1-3.

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. 

**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.

---

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

**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 amiodipine, should not be less than 3000 theoretical plates; Relative standard deviation calculated peak area for amiodipine should be no more than 1.0%.

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

\[ X = \frac{S_i \cdot m_0 \cdot 0.721 \cdot 100 \cdot b \cdot P}{S_0 \cdot 50 \cdot 20 \cdot m_i \cdot 100} \]

Where: \( S_i \) - average of the peak areas of amiodipine besylate, calculated from the chromatogram of the test solution; \( S_0 \) - average of the peak areas of amiodipine besylate, calculated with the standard solution chromatogram; \( m_0 \) - mass of the sample SPhU amiodipine besylate, in milligrams; \( m_i \) - mass of the powder pounded tablets, in milligrams; \( P \) - content of the main substance in SPhU amiodipine besylate as a percentage; \( b \) - average weight tablets in milligrams;

0.721 - amiodipine besylate conversion factor for amiodipine.

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 \( y = mx + b \) and metrological characteristics are shown in Figure 3.

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

<table>
<thead>
<tr>
<th>Solution</th>
<th>Put (solution concentration), mg/ml</th>
<th>Found (concentration solution), mg/ml</th>
<th>The relation found to input, Z, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS1</td>
<td>0.0815</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RS2</td>
<td>0.0835</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS 70 %</td>
<td>0.0573</td>
<td>0.0583</td>
<td>101,7</td>
</tr>
<tr>
<td>MS 100 %</td>
<td>0.0810</td>
<td>0.0813</td>
<td>100,4</td>
</tr>
<tr>
<td>MS 130 %</td>
<td>0.1048</td>
<td>0.1062</td>
<td>101,3</td>
</tr>
<tr>
<td>The average</td>
<td></td>
<td>100,8</td>
<td></td>
</tr>
<tr>
<td>The relative standard deviation, ( \Delta ) %</td>
<td></td>
<td>0,4272</td>
<td></td>
</tr>
<tr>
<td>Relative confidence interval, ( \Delta ) %</td>
<td></td>
<td>0,99</td>
<td></td>
</tr>
<tr>
<td>The critical value for convergence results, ( \Delta ) %</td>
<td></td>
<td>Correct</td>
<td></td>
</tr>
<tr>
<td>The criterion of statistical</td>
<td>( \leq 0,33 )</td>
<td>Correct</td>
<td></td>
</tr>
</tbody>
</table>
The obtained result - 101.1%. General conclusion of method – correct (Table 2).

Table 2: Criteria of acceptability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>The obtained result</th>
<th>Criteria</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z, %</td>
<td>Amlodipine 101.1 %</td>
<td>97-103%</td>
<td>Yes □</td>
</tr>
</tbody>
</table>

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 pharmacopeial method due to speed and ease of preparation of the mobile phase and reduced chromatography time.

REFERENCES

6. Logoya L. Validation of chromatographic methods of analysis for the determination of active pharmaceutical ingredients in different medicines. Pharma School association for pharmaceutical development and scientific research 2016; 34.
7. ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and methodology.

Cite this article as:


Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IJRAP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IJRAP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IJRAP editor or editorial board members.