DEVELOPMENT OF A METHOD TO DETERMINE RELATED IMPURITIES IN BROMIDE 1-(β-PHENYLETHYL)-4-AMINO-1,2,4-TRIAZOLE TABLETS

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Abstract: Aim: to develop a modern method to determine related impurities in bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole tablets by high performance liquid chromatography (HPLC).

Methods: The development of a method to determine related impurities in bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole tablets involved an already developed method for the determination of 4-amino-1,2,4-triazole impurities in tablets by HPLC. The test solution and the comparison solution were prepared and chromatographed alternately according to the developed methods. At least three chromatograms were obtained for each solution for the purpose of the reliability of the study results.

Results: Scientists of the Scientific-Production Association "Farmatron" jointly with the staff of the Pharmaceutical Chemistry Department of Zaporizhzhia State Medical University under the leadership of Professor Mazur I. A. obtained a new original compound – bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole (conditional name "Hypertril"), which exhibits antihypertensive, anti-ischemic and antioxidant properties. It is known that the quality of medicines largely depends on the degree of their purity. Therefore, in accordance with the regulatory documentation and quality control methods of medicines, it is mandatory to carry out tests for impurities. According to the results, the content of impurities of 4-amino-1,2,4-triazole in the tested tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole is in the range from 0.049 % to 0.195 % which meets the requirements of regulatory documentation. No peaks of unidentified impurities were detected on the chromatograms of the test solution.

Conclusion: Therefore, a modern method to determine the related impurity of 4-amino-1,2,4-triazole in tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole has been developed. The methodology is reproducible, accurate and meets the requirements of regulatory documentation.

Keywords: tablets, bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazolium, accompanying impurities, HPLC method, chromatogram, coronary heart disease, arterial hypertension.

1. Introduction

The unfavorable demographic situation in Ukraine continues to deteriorate which is largely due to cardiovascular diseases significantly affecting the main indicators of public health. A constant increase in the total mortality of population and a decrease in life expectancy cause a serious concern and indicate a lack of effectiveness of preventive measures. Although circulatory system diseases are the leading cause of death in economically developed countries, there has been a steady positive trend in health indicators associated with this pathology in recent decades. At the same time, the opposite trend is observed in Ukraine: over the last 30 years, the prevalence of cardiovascular diseases among the population has increased 3.5 times, and the associated mortality rate has grown by 46 % [1–3].

The abovementioned factors prompted the creation of a fundamentally new domestic antianginal and antihypertensive drug. Scientists of the Scientific-Production Association "Farmatron" jointly with the staff of the Pharmaceutical Chemistry Department of Zaporizhzhia State Medical University under the leadership of Professor Mazur I. A. obtained a new original compound – bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole (conditional name "Hypertril"), which exhibits antihypertensive, anti-ischemic and antioxidant properties [4, 5].

It is known that the quality of medicines largely depends on the degree of their purity. Therefore, a very important task of pharmaceutical analysis is to establish the purity of the drug because the presence of impurities affects its physicochemical properties, dosage, pharmacological effect, while contamination with toxic impurities makes the drug dangerous to human life and health. Therefore, the regulatory documentation and methods of drug quality control require tests for impurities.

Therefore, the *aim of work* was to develop a modern method to determine related impurities in tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole.

2. Materials and Methods

We tried to use an already developed method of HPLC to determine impurities in the obtained tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole. The study proved that this method of impurity determination can be used not only for the analysis of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole and injectable solutions, but also for tablets. That is why we have developed a HPLC method for the determination of related impurities in tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole [6, 7].

The related impurities of 4-amino-1,2,4-triazole in tablets of bromide 1-(ß-phenylethyl)-4-amino-1,2,4-triazole were determined by HPLC.

Test solution (a). 1.0 g of powdered tablets is placed in a 25.00 ml volumetric flask and made up to the mark with water. The thoroughly mixed solution is filtered through a filter with a pore diameter of not more than 0.45 μm.

Test solution (b). 5 ml of the filtrate are placed in a 10.00 ml volumetric flask and made up to the mark with eluent.

Comparison solution (a). 25 mg of 4-amino-1,2,4-triazole are dissolved in 10 ml of eluent and made up to 25 ml with eluent.

Comparison solution (b). 20 mg of pharmacopoeial standard sample of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole are placed in a 10.00 ml volumetric flask, dissolved in 5 ml of purified water, 0.1 ml of comparison solution *(a)* is added and made up to the mark with eluent.

Chromatography was carried out under the following conditions:

– HypersilODS (C18) 5 u, 4.6×250 mm, column, particle diameter of 5 μm.

– mobile phase: mixture (10:90 vol. %) of acetonitrile and phosphate buffer pH 3.0 containing ion-vapor reagent: 3.6 g of Na_2HPO_4 and 3.4 g of Bu_4NHSO_4 were dissolved in 900 ml of distilled water, orthophosphoric acid was added to pH 3.0, followed by 100 ml of acetonitrile:

– mobile phase rate: 1 ml/min;

– analytical wavelength of the detector: 220 nm;

– sample volume: 20 μl

3. Results

In order to reach reliable results, the test solution *(b)* and the comparison solution *(b)* (**Fig. 1, 2**) were alternately chromatographed to obtain at least three chromatograms for each solution.

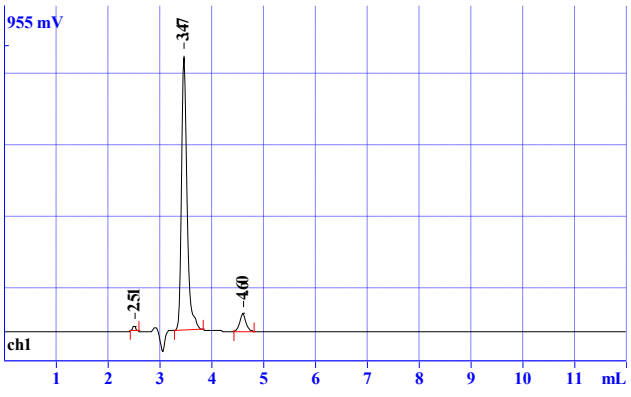

Fig. 1. Test solution chromatogram *(b)*

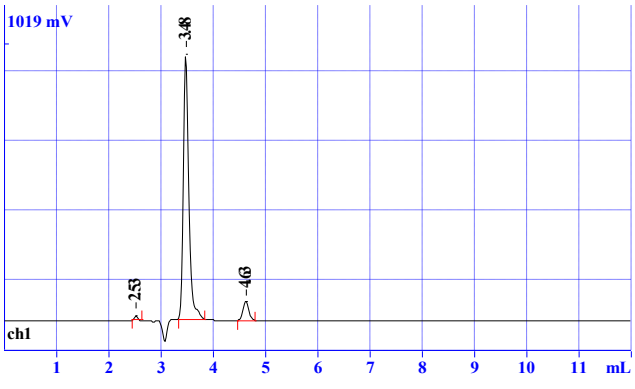

Fig. 2. Comparison solution chromatogram *(b)*

The analysis results are considered significant if the following conditions are met:

– the relative standard deviation of the peak areas of 4-amino-1,2,4-triazole, calculated on the basis of the chromatograms of the comparison solution, must meet the requirements of regulatory documentation;

– the efficiency of the chromatographic system, calculated by the peak of 4-amino-1,2,4-triazole from the chromatograms of the comparison solution, must be at least 1500 theoretical plates;

– the capacity factor calculated from the peak of 4-amino-1,2,4-triazole must be at least 0.5. The wavefront time of the first peak (bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazolium) on the chromatogram of the test solution (retention time is about 2 minutes) is taken as the peak of the substance that is not retained.

The results of the study are presented in **Table 1**. It is seen that the impurity content of 4-amino-1,2,4-triazole in the tested tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole is within from 0.049 % to 0.195 %, that meets the requirements of regulatory documentation. The chromatograms of the test solution showed no peaks of unidentified impurities.

4. Discussion

As a result of previous studies, a new original tablet drug "Hypertril" with the active substance of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole 20 mg was created.

It is known that the purity of medicines is an important aspect in ensuring their quality. That's why, the next step of our research team was to develop a determination method for the related impurities in the created tablets of 1-(β-phenylethyl)-4-amino-1,2,4-triazole bromide. To that end, we chose the method of HPLC, which is widely used for standardization of drugs due to its high sensitivity, specificity and rapidity of analysis.

The experimental studies results indicate that, for the first time, we have developed a rational method for determining the related impurity of 4-amino-1,2,4-triazole, which proved to be reproducible and accurate. Consequently, it can be applied in standardization of the "Hypertril" drug in terms of the "Purity" indicator. Therefore, it provided for the first inclusion of this technique in the draft analytical document – quality control methods for tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole under the conditional name of "Hypertril".

Table 1

Results of determining the content of impurities in bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole tablets

Series No.	4-Amino-1,2,4-triazole peak area, mV·sec	4-Amino-1,2,4-triazole mean peak area, mV·sec	Content of 4-amino-1,2,4-triazole, %
1	6.779	6.782	0.097
	6.628		
	6.939		
2	3.418	3.393	0.049
	3.163		
	3.598		
3	13.149	13.561	0.195
	13.930		
	13.604		
4	5.184	5.085	0.073
	4.972		
	5.099		
5	10.316	10.178	0.146
	10.059		
	10.158		
Comparison solution (*b*)	34.791	34.791	0.500
	34.256		
	35.326		

A set of biological studies permitted to determine the pharmacological characteristics of tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazolium which made it possible to classify the drug as toxicity class IV (low-toxic substances). All our studies prove that the new original tablet drug "Hypertril" is a promising antihypertensive and antianginal drug [8–10], and the developed methods of its standardization are also reliable.

The limitations of the study. The development of the study design involved some difficulties, since the tablets were created on the basis of a new substance – bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole. These tablets are also being prepared for clinical trials, so it is necessary to obtain a trial batch in an industrial setting. A prerequisite for this is the development of a specification that includes the "Purity" parameter. All these problems have been resolved in the course of the research.

The perspectives for further studies. It is planned to use the developed method in order to create specifications for "Hypertril" tablets, as well as to make adaptations for the purpose of quality control of other dosage forms on the basis of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole.

5. Conclusions

In the course of the previously conduced experimental studies, a tablet dosage form based on bromide 1-(β-phenylethyl)-4-amino-1, 2, 4-triazolium has been developed. The drug produces antihypertensive and anti-ischemic action. According to the regulations and quality control methods of medicines, tests for impurities are mandatory. Thus, we have developed a modern method to determine the related impurity of 4-amino-1,2,4-triazole in tablets of bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazole using HPLC. The developed methodology is reproducible, accurate and meets the requirements of regulatory documents.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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