In the medicine and pharmacy industry, numerous developments are being carried out on original multidirectional drugs, among which special attention is drawn to drugs that can be used to treat cardiovascular diseases. The urgency of the search for biologically active compounds for the treatment of cardiovascular diseases is due to the fact that these diseases are the main cause of
mortality, disability, disability of the population all over the world. According to the WHO, Ukraine is the number 1 in Europe and number 2 in the world in the ranking of cardiovascular mortality vascular diseases. Cardiovascular diseases account for 47% of all deaths in European countries [1, 2]. Previous studies have proven the prospect of finding potential biologically active substances in a number of derivatives of 1,3-thiazole [3, 4]. Based on previous studies using modern methods of computer chemistry purposefully designed and synthesized a novel substance [3-Allil-4-(4′-methoxyphenyl)-3H-thiazole-2-ylidene]-3′-trifluoromethylphenyl]amine hydrobromide (Cardiazole) exhibiting cardioprotective, anti-inflammatory, analgesic, hypolipidemic and antioxidant action (Fig. 1) [5, 6].

![Cardiazole Structure](image)

**Fig. 1. Chemical structure Cardiazol [3-Allil-4-(4′-methoxyphenyl)-3H-thiazole-2-ylidene]-3′-trifluoromethylphenyl]amine hydrobromide**

The synthesized compound in the experimental in vitro experiment on isolated rings of the aorta of the laboratory rats was more effective in reducing the contractile response of the smooth muscles to hypoxia compared with L-carnitine and meldonium. The use of a compound in white rats on the model of adrenal infarction has a pronounced and higher cardioprotective effect than the meldonium. The strain is patented, proposed for further preclinical studies under the conditional name Cardiazole [7]. Therefore, the development of an optimal, high-precision, reproducible method of quantitative determination of Cardiazole is very relevant and necessary.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

The introduction of a new medicinal product or substance into medical practice is impossible without the development of methods for its analysis [8, 9]. The development of modern, unified, efficient and simple methods for the analysis of substances is one of the main tasks of pharmaceutical science.

Modern understanding of quality assurance approaches is based on the concept of quality assurance of medicines from the pharmaceutical development and research stage through proper manufacturing, quality control, storage, sales and provision of information to doctors and patients [10, 11].

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

One of the most important stages of the introduction of a new medicinal product or substance into medical practice is the development of quality control methods, in particular, the methods of quantitative determination. Such studies are needed to standardize the substance, to study pharmacokinetics and pharmacodynamics, bioequivalence, pharmaceutical development of dosage forms on its basis.

In previous studies of scientists for quantitative determination of 1,3-thiazole derivatives a method of potentiometric titration in a non-aqueous solvent medium [12], fluorescence spectroscopy [13] was used. The researchers also developed a simple and sensitive spectrophotometric method for the determination of 1,3-thiazole cephalosporins [14]. But the reference in the literature for the use for quantitative determination of 1,3-thiazole derivatives of the method of high-performance liquid chromatography is not numerous, therefore, we consider them to be relevant.

4. The field of research considering the general problem, which is described in the article

According to the recommendations of the SPHU, the quantitative determination of a substance containing atoms of Nitrogen may be carried out by the method of determination of nitrogen after mineralization using sulfuric acid, which is described in the general monograph of SPHU 2.5.9. But the above method is inappropriate for Cardiazole dosage forms, as auxiliary substances may interfere with the determination of the substance. To solve this problem, it is advisable to apply new, more modern methods of analysis; including high-performance liquid chromatography (HPLC), which is one of the most modern high-sensitivity, universal, automated methods of analysis. HPLC can be applied both for identification and for the quantitative determination of drugs, allows results to be obtained quickly, with high accuracy and reliability [15, 16]. Thus, the development of a method for quantifying the substance of Cardiazole using HPLC for further pharmaceutical development is completely necessary and appropriate.

5. Formulating the goals (tasks) of the article

In order to prepare the substance for introduction, we have developed a method for quantitatively determining the substance of Cardiazole using HPLC.

6. Presentation of the main research material (methods and objects) with the justification of the results

Liquid chromatography separation was performed using a Shimadzu Nexera X2 LC-30AD HPLC system (Shimadzu, Japan) composed of a quaternary pump, an on-line degasser, a column temperature controller, the SIL-30AC autosampler (Shimadzu, Japan); the CTO-20AC thermostat (Shimadzu, Japan) as well as the SPD-M20A diode array detector (DAD). Another instruments such as Ultrasonic Cleaner Set for ultra-sonication using (Wise Clean WUC-A06H, Witeg Labortechnik GmbH, Germany), Libra UniBloc AUW120D (Shimadzu Ana-
lytical Scale, Japan); class A analytical vassals that meets requirements of the SPhU (SPhU, 2015) were used in the investigation. HPLC grade acetonitrile (Sigma-Aldrich GmbH, Switzerland), were used in the analysis work. HPLC grade water was obtained from a water purifying system (Millipore, Bedford, MA, USA). Other chemicals and solvents were of analytical grade.

The method of quantitative determination of Cardiazole using HPLC was performed on the column ACE 5 C8 (250 * 4.6 mm, particle size μm). A binary system of mobile phase solvents was used: solvent A (water) and solvent B (acetonitrile). The following profile of the linear gradient elution was used: 55 % A / 45 % B - 0 min, 55 % A / 45 % B - 3 min, 20 % A / 80 % B - 7 min, 20 % A / 80 % B-14 min, 55 % A / 45 % B - 16 minutes and 55 % A / 45 % B - 20 minutes. The flow rate was 1 ml / min, the volume of the injection was 10 μL. The column temperature was constant at 35 °C. Chromatograms were recorded at 300 nm. Under the proposed conditions, the retention time of the investigated component is 13.9 min.

**Method**

**Test solution.** 40 mg (precisely weighed amount) of the substance Cardiazole ([3-Allil-4-(41-methoxyphenyl)-3H-thiazole-2-ylidene] - (32-trifluoromethylphenyl) amine hydrobromide) is placed in a 100 ml volumetric flask, dissolved in 30 ml of acetonitrile P and bring the volume of the solution to the mark with the same solvent, mix thoroughly. Filter through a membrane filter with a pore diameter of no more than 0.45 μm. The solution is freshly prepared.

**Comparison solution.** 40 mg (precisely weighed amount) of Cardiazole substance are placed in a 100 ml volumetric flask, dissolved in 30 ml of acetonitrile P, and the volume of the solution is adjusted to the mark with the same solvent, carefully mixed. Filter through a membrane filter with a pore diameter of no more than 0.45 μm. The solution is freshly prepared.

Chromatography was performed on a liquid chromatograph with a spectrophotometric detector under the following conditions:

- a column measuring 250×4.6 mm, filled with octylsilyl silica gel for chromatography P (for example, ACE C8, 150×4.6 mm, YMC company) with a pre-column having a particle size of 5 μm for which the chromatographic system’s suitability conditions are fulfilled;
- speed of the mobile phase: 1.0 ml/min;
- column temperature: 35 °C;
- Detection at wavelength: 300 nm;
- volume of injection: 10 μl;
- mobile phase A: water for chromatography P;
- mobile phase B: acetonitrile P;
- gradient program (shown in Table 1).

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Mobile phase A, %</th>
<th>Mobile phase B, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:00</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>03:00</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>07:00</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>14:00</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>16:00</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>20:00</td>
<td>55</td>
<td>80</td>
</tr>
</tbody>
</table>

Exit time of the main peak is 13.89 min. Fig. 2 shows the chromatogram of the test solution Cardiazole.

The method was validated in accordance with the recommendations of the SPHU [17, 18]. The chromatographic system is considered to be suitable if the following conditions are met for the comparison solution:
the efficiency of the chromatographic column, calculated on the peak of the main substance, must be at least 3000 theoretical plates;

– the coefficient of peak symmetry should be in the range from 0.8 to 1.5;

– the relative standard deviation for peak areas, should not exceed 1.0 %, calculated on the results of 5 injections.

Characteristics of the chromatographic system for the comparison solution of Cardiazole are given in Table 2

<table>
<thead>
<tr>
<th>Substance</th>
<th>Efficiency (number of theoretical plates)</th>
<th>Symmetry factor</th>
<th>Relative standard deviation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiazole</td>
<td>67504</td>
<td>0.99</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Forecast of uncertainty of analysis results

The maximum permissible absolute relative uncertainty of the method of analysis of the substance ΔAS % is related to the symmetric tolerances of the content of the analyzed substance in accordance with the specification (B). That is:

\[
\Delta AS \leq \frac{B_u - B_l}{2} \times 0.32
\]

In the substance of Cardiazole, the quantitative content of its components should be within ±5 %, therefore:

\[
\Delta AS \leq \frac{95 - 105}{2} \times 0.32 = 1.6 %
\]

Calculation of uncertainty of sample preparation is given in Table 3.

<table>
<thead>
<tr>
<th>Operation of sample preparation</th>
<th>Value</th>
<th>Uncertainty, (Δ), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weigh (m)</td>
<td>40 mg</td>
<td>0.5</td>
</tr>
<tr>
<td>Uncertainty of weighing</td>
<td>0.2 mg</td>
<td></td>
</tr>
<tr>
<td>Bringing to volume</td>
<td>100 ml</td>
<td>0.12</td>
</tr>
<tr>
<td>Comparison solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weigh (m)</td>
<td>40 mg</td>
<td>0.25</td>
</tr>
<tr>
<td>Uncertainty of weighing</td>
<td>0.2 mg</td>
<td></td>
</tr>
<tr>
<td>Bringing to volume</td>
<td>100 ml</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Uncertainty of the final analytical operation \(\Delta_{FAO}\) (liquid chromatography)*

| Complete uncertainty of sample preparation \(\Delta sp\)% | 0.73 |
| Uncertainty of the final analytical operation \(\Delta_{FAO}\)% | 1.35 |

Complete uncertainty of the method of analysis \(\Delta_{AS}\)%

\[
\Delta_{AS} = \sqrt{(\Delta sp)^2 + (\Delta_{FAO})^2} = 1.53
\]

Thus, the complete uncertainty of the analysis method \(\Delta_{AS}\)% is calculated to be less than max \(\Delta_{AS}\).
Consequently, the uncertainty of sample preparation and analysis as a whole should provide sufficient accuracy of measurement.

Thus, the developed method of quantitative determination of Cardiazole meets the requirements of “Suitability of the chromatographic system” according to the parameters: the efficiency of the chromatographic column, the coefficient of separation of peaks on the chromatogram, the rate of asymmetry of the peak. The proposed method allows reliable identification of Cardiazole and quantify it.

References

2. The top 10 causes of death // WHO. URL: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

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