ESI-MS fragmentation pathways of some 1,2,4-triazole-3-thiones, the intermediate compounds in the synthesis of active pharmaceutical ingredients

**Aim.** To determine the fragmentation pathways of eight 1,2,4-triazole-3-thiones, which are intermediate products in the synthesis of active pharmaceutical ingredients of potential and registered pharmaceutical formulations.

**Results and discussion.** HPLC-MS analysis of eight 1,2,4-triazole-3-thiones, which are intermediate products in the synthesis of salts of 1,2,4-triazolylthioacetate acids, has been carried out; the mass spectra of the compounds to be analyzed have been registered in ESI-mode with different fragmentor voltage (0, 100, 200 V). The fragmentation pathways and patterns of ion decay for compounds to be analyzed have been proposed.

**Experimental part.** Agilent 1260 Infinity HPLC System with Agilent 6120 mass spectrometer were used. HPLC-MS conditions: column – C18, 4.6 × 30 mm, reverse phase Zorbax SB C18, 1.8 μm, 40°C; mobile phase – 0.1% HCOOH in H2O and 0.1% HCOOH in CH3CN in isocratic mode (50:50, v/v); the flow rate – 0.4 mL/min; ion source – API-ES; positive polarity; drying gas – nitrogen (rate – 10 L/min); the capillary voltage – 4000 V; scanning in the range of m/z 100 – 1000.

For the first time it has been interpreted the mass spectra of 1,2,4-triazole-3-thiones series, the intermediate compounds in the synthesis of active pharmaceutical ingredients of pharmaceutical formulations. The fragmentation pathways and patterns of eight 1,2,4-triazole-3-thiones have been shown.

**Key words:** mass spectrometry; high performance liquid chromatography; 1,2,4-triazole-3-thiones

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Key words: mass spectrometry; high performance liquid chromatography; 1,2,4-triazole-3-thiones

**Conclusions.** For the first time it has been interpreted the mass spectra of 1,2,4-triazole-3-thiones series, the intermediate compounds in the synthesis of active pharmaceutical ingredients of pharmaceutical formulations. The fragmentation pathways and patterns of eight 1,2,4-triazole-3-thiones have been shown.
Heterocyclic systems based on 1,2,4-triazole are the subject of interest for the present-day medicinal chemistry. They have the antioxidative, hepatoprotective and other activities; moreover, some of them have been already registered and used in the present-day veterinary (tryfusol, avesstym) [1, 2], and one of them is on the stage of registration for human use and manufacturing application (thiometrizol) [3].

In this way, the study of the preparation methods and the quality control of all stages of the development and production of the abovementioned compounds and their initial products in the synthesis is the urgent task for the present-day pharmaceutical science and of a scientific interest and practical importance.

One of the main methods, which may be used for the effective and reliable identification and the quantitative determination of the target products of the organic synthesis and impurities is chromatography with mass spectrometric detection. The most appropriate for the analytical goals is a combination of high performance liquid chromatography and mass spectrometry with ionization under atmospheric pressure, in electrospray (ESI), chemical ionization under atmospheric pressure (APCI), photochemical ionization under atmospheric pressure (APPI). The current work is devoted to ionization in electrospray, which is the best choice for the analysis of polar non-volatile compounds, such as the analytes under research.

At the first stage, there was the optimization of the mass spectrometry detection conditions [4], at the second stage the behavior of analytes to be chromatographed was studied [5].

Patterns of hydrazide of definite organic acids and their corresponding hydrazinecarbothioamides mass spectrometric decay were showed [6]. The mass fragmentation patterns of 1,2,4-triazole derivatives were reported in different articles [7–10].

The aim of the present work is to study mass-spectra and offer plausible fragmentation pathways of eight 1,2,4-triazole-3-thiones, the intermediate products in the synthesis of active pharmaceutical ingredients. The elucidation of fragmentation pathways was based on the electrospray ionization single quadrupole mass spectrometry.

**Results and discussion**

The mass spectra are presented in graphical and tabular form, providing the most intensive peaks starting about 1%. The maximal peak was shown from isotope group peaks. All compounds can exist as a thiol and thione forms. We analyzed the mass spectra and suggested the possible fragmentation pathways of the compounds.

**4-(2-Methoxyphenyl)-5-(pyridin-4-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione.** The ion with m/z 285.0 is observed at 0 V, 100 V, and 200 V of collision voltages (Fig. 1). This ion corresponds to the quasi-molecular ion (protonated molecule) of the current substance. The isotope peaks are also present in the mass spectrum. The ion with m/z 253.1 at 100 V and 200 V is detected (Fig. 2–3, Table 1); it is formed by heterolytic cleavage of bonds between a phenyl carbon and oxygen of the methoxycarbonyl group. It is also possible that this ion corresponds to the structure created as a result of the sulfhydryl group cleavage from the quasimolecular ion.

![Fig. 1. Mass-spectra of 4-(2-methoxyphenyl)-5-(pyridin-4-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione at different fragmentor voltage (0, 100, 200 V)](image)

![Fig. 2. The pathways proposed for the dissociation of 4-(2-methoxyphenyl)-5-(pyridin-4-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 100 V](image)
A pattern for the cation with m/z 275.1 appearing in the mass spectrum at 100 V has been offered (Fig. 2). The methoxy group elimination and the reduction of the triazol cycle are observed. The cation with

Table 1

The values of ions m/z of 4-(2-methoxyphenyl)-5-(pyridin-4-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and the relative abundance at 100 V and 200 V

| No | m/z     | Relative abundance, % |
|----|---------|------------------------|
| 1  | 253.1   | 14.7                   |
| 2  | 275.1   | 0.9                    |
| 3  | 285.0   | 100.0                  |
| 4  | 567.0   | 1.5                    |

| No | m/z     | Relative abundance, % |
|----|---------|------------------------|
| 1  | 169.2   | 1.0                    |
| 2  | 184.0   | 1.2                    |
| 3  | 197.1   | 29.6                   |
| 4  | 212.1   | 6.7                    |
| 5  | 221.0   | 4.8                    |
| 6  | 237.1   | 6.6                    |
| 7  | 253.1   | 16.1                   |
| 8  | 269.9   | 4.8                    |
| 9  | 271.0   | 1.1                    |
| 10 | 285.0   | 100.0                  |
| 11 | 567.1   | 3.4                    |

200 V

| No | m/z     | Relative abundance, % |
|----|---------|------------------------|
| 1  | 105.1   | 27.6                   |
| 2  | 109.1   | 7.9                    |
| 3  | 115.1   | 1.4                    |
| 4  | 118.1   | 5.8                    |
| 5  | 130.0   | 3.1                    |
| 6  | 151.0   | 1.5                    |
| 7  | 157.0   | 7.2                    |
| 8  | 170.1   | 12.1                   |
| 9  | 185.1   | 10.6                   |
| 10 | 212.1   | 2.3                    |
| 11 | 216.0   | 3.0                    |
| 12 | 244.1   | 100.0                  |
| 13 | 265.0   | 1.0                    |
| 14 | 485.0   | 3.6                    |
| 15 | 485.0   | 1.7                    |

Table 2

The values of ions m/z of 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione and the relative abundance at 100 V and 200 V

| No | m/z     | Relative abundance, % |
|----|---------|------------------------|
| 1  | 212.1   | 2.3                    |
| 2  | 244.1   | 100.0                  |
| 3  | 221.0   | 6.7                    |
| 4  | 237.1   | 6.6                    |
| 5  | 253.1   | 16.1                   |
| 6  | 269.9   | 4.8                    |
| 7  | 271.0   | 1.1                    |
| 8  | 285.0   | 100.0                  |
| 9  | 221.0   | 6.7                    |
| 10 | 237.1   | 6.6                    |
| 11 | 253.1   | 16.1                   |
| 12 | 269.9   | 4.8                    |
| 13 | 271.0   | 1.1                    |
| 14 | 285.0   | 100.0                  |
| 15 | 567.1   | 3.4                    |

Fig. 3. The pathways proposed for the dissociation of 4-(2-methoxyphenyl)-5-(pyridin-4-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 200 V
m/z 567.1 is the quasimolecular ion (a protonated dimer) of the compound at 0 V, 100 V and 200 V. 200 V of collision voltage cause the appearance of the cation with m/z 237.1. (Fig. 3). It is formed due to partial destruction of the methoxypyrene phenyl cycle. Several structures of the cation with m/z 221.0 have been offered. The first one is splitting off sulfur and the methoxyl group, it leads to formation of the phenylcarbocation. The second one is due to the destruction of the pyridin cycle, the corresponding cation radicals are formed, the third structure is due to the destruction of the metoxypyrene phenyl cycle. The ion with m/z 271.0 appears during cleavage of the methyl group from a quasimolecular ion. The destruction of the triazole cycle is also possible with the formation of the following ions: the radical cation with m/z 212.1 and the cation with m/z 197.1 are formed.

5-(Furan-2-yl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione. At fragmentor voltage of 100 V the quasimolecular (protonated molecule) ion with m/z 244.1 and the dimer cation with m/z 485.0 are formed (Fig. 4, Table 2).

Fig. 4. Mass-spectra of 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione at different fragmentor voltage (100, 200 V)

![Mass-spectra of 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione at different fragmentor voltage (100, 200 V)](image)

Fig. 5. The pathways proposed for the dissociation of 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 200 V
There is also the ion with m/z 212.1, which appears as a result of splitting off sulfur from a quasimolecular ion (Fig. 4). When the fragmentor voltage is increased from 100 to 200 V, more than 10 new ions appear (Table 2, Fig. 5).

The ion with m/z 212.1 is also present at voltage of 200 V. The radical cation with m/z 185.1 appears during cleavage of CO from the ion with m/z 212.1. In the case of additional splitting of the methyl group, the cation with m/z 170.1 appears. The alternative structure of the cation with m/z 170.1 may appear from a quasimolecular ion during the destruction of the furan cycle and elimination of the SH-group. With further cleavage of the methylene group the radical cation with m/z 157.0 appears. During disintegration of the triazole cycle the cation with m/z 151.0 appears at first, then the radical cation with m/z 109.1. A direct elimination of the furan and benzene ring from thiol forms of a quasimolecular ion with the reduction of the triazole cycle; in this case, the appearance of the radical cation with m/z 105.1 is possible. If CO is cleaved from the quasimolecular ion, the cation with m/z 216.1 may be formed. This cation turns into the ion with m/z 130.0 after eliminating the methylene group and benzene ring. In the case of the triazole cycle reduction, the formation of the ion with m/z 118.0 is possible. The radical cation with m/z 115.1 may appear after cleavage of CH₂ from the ion with m/z 130.0.

5-(Pyridin-4-yl)-1,2-dihydro-3H-1,2,4-triazole-3-thione. At voltage of 0, 100, 200, and 300 V the quasimolecular cation of the protonated substance with m/z 179.0 is observed in mass spectra, as well as the dimeric cation with m/z 355.0 and a partially hydrogenized dimeric cation with m/z 357.0 are formed (Fig. 6–7, Table 3).

At 0 V the adduct of the quasimolecular ion of the protonated ion exists with dimethyl sulfoxide with m/z 257.0. The substance studied is solved in dimethyl sulfoxide. The scan range of 160–1000 m/z is used for maximal exclusion of ions, which are the products of transformation of dimethyl sulfoxide in the ion source, it has not entirely obtained yet. The ions of product fragmentation of the compound studied have low intensity (less than 1% of the basic peak intensity); therefore, the interpretation of them is considered to be inappropriate.

5-(Morpholin-4-ylmethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione. At voltage of 100 V the quasimolecular ion MH⁺ with m/z 277.1, as well as the ion with m/z 245.1 are present (Fig. 8, Table 4).

The ion with m/z 245.1 appears after cleavage of sulfur from a quasimolecular ion. The cation with m/z 212.1, which appears as a result of splitting off sulfur from a quasimolecular ion (Fig. 4).

The values of ions m/z of 5-(pyridin-4-yl)-1,2-dihydro-3H-1,2,4-triazole-3-thione and monoisotopic masses of ions

| No | m/z       | Relative abundance, % |
|----|-----------|------------------------|
| 0 V|           |                        |
| 1  | 172.0     | 0.8                    |
| 2  | 179.0     | 100.0                  |
| 3  | 257.0     | 71.9                   |
| 4  | 355.1     | 5.0                    |
| 5  | 357.0     | 10.2                   |
| 100 V|        |                        |
| 1  | 179.0     | 100.0                  |
| 2  | 355.0     | 0.6                    |
| 3  | 357.0     | 0.5                    |
| 200 V|        |                        |
| 1  | 179.0     | 100.0                  |
| 2  | 354.8     | 1.1                    |
m/z 173.1 is formed during forthcoming elimination of the phenyl radical and reduction of the triazole cycle. After cleavage of triazole, cations with m/z 102.1 and 100.1 appear (Fig. 9) [6].

At voltage of 200 V (Table 4, Fig. 10) the quasimolecular ion may be disintegrated in few ways. Firstly, after elimination of sulfur, the triazole cycle reduction and the morpholine fragment separation, the radical cation with m/z 163.0 is formed, and then after cleavage of the methylene radical the cation with m/z 148.0 is observed. The structure of the ion with m/z 148.0 is also possible; it corresponds to the cation, which appears during elimination of sulfur, the morpholine-methylene fragment and partial reduction of the triazole cycle. The cation with m/z 136.0 is formed after cleavage of sulfur, the morpholine-methylene fragment and partial destruction of the triazole cycle.

During elimination of the morpholine fragment from the quasimolecular ion the ion with m/z 190.0 appears. The alternative structure of the radical cation with m/z 163.0 may be obtained by destruction of the triazole cycle. Elimination of sulfur and the morpholine methylene fragment, the triazole cycle destruction lead to the appearance of the radical cation with m/z 105.1. During cleavage of sulfur, the phenyl radical and the triazole cycle destruction the radical cation with m/z 157.1 is formed, it is successively transformed into the cation with m/z 143.0, 131.1 and 100.1 (we suggested two structures; one of them was demonstrated in the previous research paper [6]). After elimination of the phenyl fragment and the morpholine cycle destruction the formation of the alternative

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### Table 4
The values of ions m/z of 5-(morpholin-4-ylmethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione and the relative abundance at 100 V and 200 V

| No | m/z     | Relative abundance, % |
|----|---------|----------------------|
| 1  | 100.15  | 3.3                  |
| 2  | 102.15  | 1.1                  |
| 3  | 173.1   | 1.1                  |
| 4  | 245.05  | 2.2                  |
| 5  | 277.1   | 100.0                |
| 6  | 551.15  | 3.5                  |
|    | **200 V** |
| 1  | 100.1   | 100.0                |
| 2  | 105.1   | 52.8                 |
| 3  | 117.1   | 12.1                 |
| 4  | 131.10  | 89.5                 |
| 5  | 136.0   | 9.5                  |
| 6  | 143.0   | 1.0                  |
| 7  | 148.0   | 4.0                  |
| 8  | 157.1   | 5.5                  |
| 9  | 163.0   | 7.3                  |
| 10 | 190.0   | 83.4                 |
| 11 | 277.1   | 8.1                  |
| 12 | 551.2   | 30.7                 |

### Table 5
The values of ions m/z of 4-methyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and the relative abundance at 100 V and 200 V

| No | m/z     | Relative abundance, % |
|----|---------|----------------------|
| 1  | 100.1   | 100.0                |
| 2  | 128.1   | 15.7                 |
| 3  | 215.1   | 9.5                  |
| 4  | 427.1   | 1.9                  |

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Fig. 8. Mass-spectra of 5-(morpholin-4-ylmethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (100, 200 V)
Fig. 9. The pathways proposed for the dissociation of 5-(morpholin-4-ylmethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 100 V

Fig. 10. The pathways proposed for the dissociation of 5-(morpholin-4-ylmethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 200 V
Fig. 11. Mass-spectra of 4-methyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione at different fragmentor voltage (100, 200 V)

Fig. 12. The pathways proposed for the dissociation of 4-methyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 100 V

Fig. 13. The pathways proposed for the dissociation of 4-methyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 200 V

Fig. 14. Mass-spectra of 4-ethyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione at different fragmentor voltage (100, 200 V)
structure of the cation with m/z 131.1 becomes possible, then it creates the cation with m/z 117.1. At both voltages of 100 V and 200 V the protonated cation of the dimer of the compound with m/z 551.2 mentioned appears (Fig. 9–10).

**4-Methyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione.** At the fragmentor voltage of 100 V the quasimolecular ion with m/z 215.1 of the compound itself can be observed. We can also mark the ion of the dimer of the substance with the reduction of one of the triazole cycle. The cation with m/z 183.1 appears as a result of elimination of sulfur from a quasimolecular ion. The formation of the cation with m/z 100.1 is possible after the destruction of the triazole cycle corresponding to the above-described morpholine methylene derivatives (Fig. 11–12, Table 5).

Voltage of 200 V initiates the formation of the quasimolecular ion with m/z 215.1 and the dimeric ion with m/z 427.1. After elimination of sulfur and partial destruction of the morpholine cycle the radical cation with m/z 128.1 is observed. In the case of destruction of the triazole cycle morpholine methylene the cation with m/z 100.1 appears (Fig. 13, Table 5).

The values of ions m/z of 4-ethyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and the relative abundance at 100 V and 200 V

| No | m/z   | Relative abundance, % |
|----|-------|------------------------|
| 1  | 197.1 | 1.5                   |
| 2  | 229.1 | 100.0                 |
| 3  | 457.2 | 10.6                  |
| 1  | 100.1 | 100.0                 |
| 2  | 114.0 | 3.0                   |
| 3  | 142.0 | 20.9                  |
| 4  | 229.1 | 17.7                  |
| 5  | 455.0 | 1.2                   |
| 6  | 457.1 | 1.4                   |
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4-Ethyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione. At voltage of 100 V we can observe the quasimolecular ion with m/z 229.1 and the dimeric ion m/z 457.1 with partial reduction of one of the triazole cycles. The elimination of sulfur leads to formation of the cation with m/z 197.1 (Fig. 14–15, Table 6).

Voltage of 200 V initiates the appearance of a quasimolecular ion of the substance. The formation of the dimeric cation with m/z 455.0 and the cation with m/z 457.2 is observed. The percentage of the cation with m/z 457.2 decreases in almost eight times comparing with the voltage of 100 V (Fig. 16, Table 6).

After cleavage of sulfur, the partial destruction of the morpholine cycle and the fractional reduction of the triazole cycle the radical cation with m/z 142.0 is formed. This cation after elimination of the methylamine group can be transformed into the cation with m/z 114.0. The appearance of the cation with m/z 100.1 is observed as a result of the triazole cycle destruction as described above.

Table 7
The values of ions m/z of 5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and the relative abundance at 100 V and 200 V

| No | m/z   | Relative abundance, % |
|----|-------|-----------------------|
|    | 100 V |                       |
| 1  | 100.1 | 100.0                 |
| 2  | 114.1 | 4.6                   |
| 3  | 157.1 | 1.0                   |
| 4  | 201.1 | 16.8                  |
| 5  | 284.1 | 6.6                   |
| 6  | 312.1 | 1.0                   |
| 7  | 399.1 | 19.9                  |
|    | 200 V |                       |
| 1  | 100.2 | 2.2                   |
| 2  | 201.0 | 100.0                 |
| 3  | 399.1 | 7.2                   |

Fig. 17. Mass-spectra of 5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione at different fragmentor voltage (100, 200 V)

Fig. 18. The pathways proposed for the dissociation of 5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 100 V

Fig. 19. The pathways proposed for the dissociation of 5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and theoretical monoisotopic masses of ions at 200 V
**Experimental part**

4-(2-Methoxyphenyl)-5-(pyridin-4-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione 1; 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione 2; 5-(pyridin-4-yl)-1,2-dihydro-3H-1,2,4-triazole-3-thione 3; 5-(morpholin-4-ylmethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione 4; 4-methyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione 5; 4-ethyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione 6; 5-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione 7; 5-(2-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione 8 were synthesized in the Zaporizhzhia State Medical University at the Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Physical and Colloid Chemistry Department. The composition of compounds was confirmed by elemental analysis and IR, UV, 1H NMR spectroscopy, chromatography with mass spectrometric detection.

| No | m/z | Relative abundance, % |
|----|-----|-----------------------|
| 100 V |     |                       |
| 1 | 208.0 | 100.0               |
| 2 | 413.0 | 6.2                  |
| 200 V |     |                       |
| 1 | 165.1 | 5.2                  |
| 2 | 176.0 | 1.1                  |
| 3 | 193.0 | 55.6                 |
| 4 | 208.1 | 41.2                 |
| 5 | 413.0 | 23.2                 |
was produced using the Direct Q 3UV Millipore system (Molsheim, France).

**Sample solutions.** Solutions of compounds 2, 4–7 in 50% acetonitrile and compounds 1, 3, 8 in dimethyl sulfoxide were prepared by dissolving to the final concentration of 1 mg/mL.

Agilent 1260 Infinity HPLC System (degasser; binary pump, autosampler) with Agilent 6120 single-quadrupole mass-spectrometer and software OpenLAB CDS were used.

**HPLC-MS conditions.**
1. Column - Ø4.6×30 mm, reversed phase Zorbax SB C18, 1.8 μm; 2. Column temperature - 40°C; 3. Eluent A - 0.1% HCOOH in H₂O; eluent B - 0.1% HCOOH in CH₃CN; isocratic mode (50:50, v/v); 4. Flow rate - 0.4 mL/min; 5. Ion source - API-ES; 6. Positive polarity; 7. Drying gas - nitrogen (rate - 10 L/min); 8. The capillary voltage - 4000 V; 9. Scanning in the range of m/z 100 – 1000 and 160 – 1000 for compounds 2, 4–7 and 1, 3, 8, respectively.

**Conclusions**

1. The ESI mass spectra of eight 1,2,4-triazolethiones at different fragmentor voltage have been shown.
2. For the first time mass spectra of ESI 1,2,4-triazolethiones series, the intermediate materials in the synthesis of active pharmaceutical ingredients of pharmaceutical formulations have been interpreted.
3. The fragmentation pathways and patterns of eight 1,2,4-triazolethiones have been proposed.

**Conflict of interests:** authors have no conflict of interests to declare.

**References**

1. Pruglo, Ye. S.; Pohorlyuk, A. Yu.; Parchenko, V. V.; Panasenko, O. I.; Knysz, Ye. G. Antiviral activity of trifuzol for the broiler at poultry farm. *Zaporozyhe Medical Journal* 2016, 1(94), 77–80. https://doi.org/10.14739/2310-1210.2016.1.64062.
2. Buhusova, I. B.; Berezhkovskiy, A. V.; Kiyva, D. S.; Panasenko, O. I. Assembling a mass spectrometer «Avestom» for investigation of the effectiveness of the enzyme preparation. *SCIENCE¢E®* 2014, 4(1(4)), 94–97. https://doi.org/10.15587/2313-8416.2014.29279.
3. Kaplaushenko, A. G. Determination of the intravenous injection of the parenteral injection of sodium 2-(4-methyl-5-(thiophene-2-yl)-4-aminothiophene-3-thione. *Pharmakov* 2013, 2(3), 115–121.
4. Varynskyi, B. O.; Kaplaushenko, A. G. Optimization of the detection conditions for the series of 1,2,4-triazole-3-thiones for FIA-ESI-MS and HPLC-ESI-MS. *News of Pharmacy* 2016, 1(85), 7–11. https://doi.org/10.24959/nphj.16.2063.
5. Varinska, B. O. Optimization of the detection conditions for the series of 1,2,4-triazole-3-thiones for FIA-ESI-MS and HPLC-ESI-MS. *News of Pharmacy* 2016, 1(85), 7–11. https://doi.org/10.24959/nphj.16.2063.
6. Pruglo, Ye. S.; Pohorlyuk, A. Yu.; Parchenko, V. V.; Panasenko, O. I.; Knysz, Ye. G. Antiviral activity of trifuzol for the broiler at poultry farm. *Zaporozyhe Medical Journal* 2016, 1(94), 77–80. https://doi.org/10.14739/2310-1210.2016.1.64062.
7. Buhusova, I. B.; Berezhkovskiy, A. V.; Kiyva, D. S.; Panasenko, O. I. Assembling a mass spectrometer «Avestom» for investigation of the effectiveness of the enzyme preparation. *SCIENCE¢E®* 2014, 4(1(4)), 94–97. https://doi.org/10.15587/2313-8416.2014.29279.
8. Kaplaushenko, A. G. Determination of the intravenous injection of the parenteral injection of sodium 2-(4-methyl-5-(thiophene-2-yl)-4-aminothiophene-3-thione. *Pharmakov* 2013, 2(3), 115–121.
9. Varynskyi, B. O.; Kaplaushenko, A. G. Optimization of the detection conditions for the series of 1,2,4-triazole-3-thiones for FIA-ESI-MS and HPLC-ESI-MS. *News of Pharmacy* 2016, 1(85), 7–11. https://doi.org/10.24959/nphj.16.2063.
10. Varinska, B. O. Optimization of the detection conditions for the series of 1,2,4-triazole-3-thiones for FIA-ESI-MS and HPLC-ESI-MS. *News of Pharmacy* 2016, 1(85), 7–11. https://doi.org/10.24959/nphj.16.2063.
11. Varinska, B. O. Optimization of the detection conditions for the series of 1,2,4-triazole-3-thiones for FIA-ESI-MS and HPLC-ESI-MS. *News of Pharmacy* 2016, 1(85), 7–11. https://doi.org/10.24959/nphj.16.2063.
12. Pruglo, Ye. S.; Pohorlyuk, A. Yu.; Parchenko, V. V.; Panasenko, O. I.; Knysz, Ye. G. Antiviral activity of trifuzol for the broiler at poultry farm. *Zaporozyhe Medical Journal* 2016, 1(94), 77–80. https://doi.org/10.14739/2310-1210.2016.1.64062.
13. Buhusova, I. B.; Berezhkovskiy, A. V.; Kiyva, D. S.; Panasenko, O. I. Assembling a mass spectrometer «Avestom» for investigation of the effectiveness of the enzyme preparation. *SCIENCE¢E®* 2014, 4(1(4)), 94–97. https://doi.org/10.15587/2313-8416.2014.29279.
14. Kaplaushenko, A. G. Determination of the intravenous injection of the parenteral injection of sodium 2-(4-methyl-5-(thiophene-2-yl)-4-aminothiophene-3-thione. *Pharmakov* 2013, 2(3), 115–121.
15. Varynskyi, B. O.; Kaplaushenko, A. G. Optimization of the detection conditions for the series of 1,2,4-triazole-3-thiones for FIA-ESI-MS and HPLC-ESI-MS. *News of Pharmacy* 2016, 1(85), 7–11. https://doi.org/10.24959/nphj.16.2063.