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Research Article

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The study of thione-thiol tautomerism of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione by HPLC-MS method

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ABSTRACT

HPLC-MS analysis of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione tautomeric derivatives has been performed and composition of the tautomers mixture has been set. The relationship between the structure of synthesized compounds and their UV-spectrums has been studied, the results of atomic charges calculation have been described. Proofs conformity peaks in the chromatogram thione and thiol. Based on these data, we consider that the major component is thione and the minor one is thiol.

Keywords: tautomerism, thione–thiol, 1,2,4-triazole-3-thione

INTRODUCTION

The theory of tautomeric transformations is one of the most important aspects of theoretical organic chemistry. The theory of tautomerism gives an opportunity to understand the diversity of the existence forms of organic compounds. There are many bioactive substances take part in tautomeric transformations. For example pyrazolone derivatives [1].

Traditional methods of studying tautomeric transformations in solutions and in crystalline state (where the influence of molecular interactions are very large) are UV-, IR-spectrophotometry, NMR- spectrometry, X-ray crystallography and quantum chemistry [2-7]. It is known some works, where the presence of thione-thiol equilibrium of 1,2,4-triazole-3-thiones has been proved with the help of UV- spectrophotometry [8,9], however, quantitative assessment have not studied.

Simultaneous using chromatomass- and UV- spectrophotometry in studying tautomerism can identify not only the real "tautomeric" state of monomolecular systems but also give quantitative characterization of two present isomeric forms.

The aim of this work was to study the thione-thiol tautomerism of 1,2,4-triazole derivatives using HPLC-MS method, identify the form of thione and thiol in the chromatogram and set quantitative proportion of thione and thiol in the mixture.

EXPERIMENTAL SECTION

The research of 5-(4-nitrophenyl)-4-amino-3-thio-1,2,4-triazole have been set using the device LC MS: Agilent 1260 Infinity HPLC System (Degasser, Bynary Pump, Autosampler, Thermostat Column Compartment, DAD); Agilent single-quadrupole mass spectrometer 6120 with electrospray ion-source (ESI); OpenLAB CDS Software. Conditions of HPLC-MS studies: 1) Binary gradient. A: H_2O (HCOOH 0.1%), B: CH_3CN (HCOOH 0.1%); 2) Column. Zorbax SB-C18, 30 mm x 4.6 mm, 1.8 um; 3) Column temperature: 25 ^oC; 4) DAD: 210, 254,

280 nm; 5) Ion Source: API-ES; 6) Scan. Mass Range: 160-500; 7) Fragmentor voltage: 10V; 8) Positive polarity. Other reagents were not lower than the qualifications for HPLC.

RESULTS AND DISCUSSION

Tautomerism is a dynamic isomerism as tautomers are readily converted into each other in solution. The study of equilibration processes, in cases when chromophore group undergo isomerization, indicates the migration of double bond. This process is accompanied by significant changes in the electronic spectra. Thus, while thione-thiol tautomerism, the transition of the thione group chromophore to the double bond between nitrogen and carbon connected with thiol group, takes place (Fig.1). It is reported in the literature [2,3] that in condensed phase and in solutions while equilibrium tautomerism thione form usually dominates.

Fig.1.Thione-thiol tautomerism in the molecule of 1,2,4-triazole-3-thione

It is known that in the neutral and the acidic medium in the system thione form prevails, in an alkaline solution, the equilibrium shifts toward the formation of thiol [9]. Thus two versions of substances' sample preparation technique have been used for chromatographic study. In the first one the substances were dissolved in DMSO without adding reagents, in the second one 0.5 mol of sodium hydrogen carbonate was added to 1 mol solution of substances in DMSO.

While chromatography of 5-(4-nitrophenyl)-4-amino-3-thio-1,2,4-triazole (M_r 237) two peaks with the same mass (m/z 235) were recorded in each case, that is why we consider the simultaneous presence of 2 tautomeric forms. In each case, we have found one major and one minor peak. The example of chromatography is shown in Fig. 2, peaks area ratio was 97.27% : 2.73%. In case of adding sodium hydrogen carbonate minor peak increased and the ratio was 94.5% : 5.5% (Fig. 3). Thus we assume that the first peak (a) corresponds to thione form, which with increasing of pH becomes thiol peak (b).

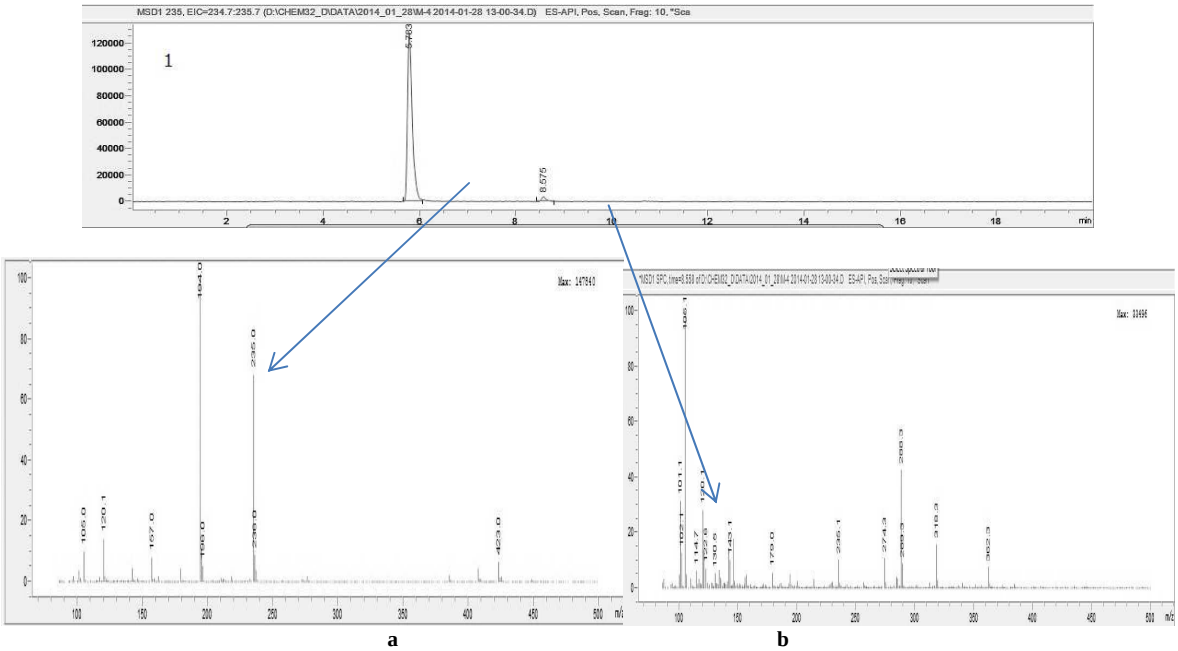

Fig. 2. Chromatogram of 3-thio-4-amino-5-(4-nitrophenyl)-1,2,4-triazole and mass-spectrums of form (a) and (b) (first sample preparation technique)

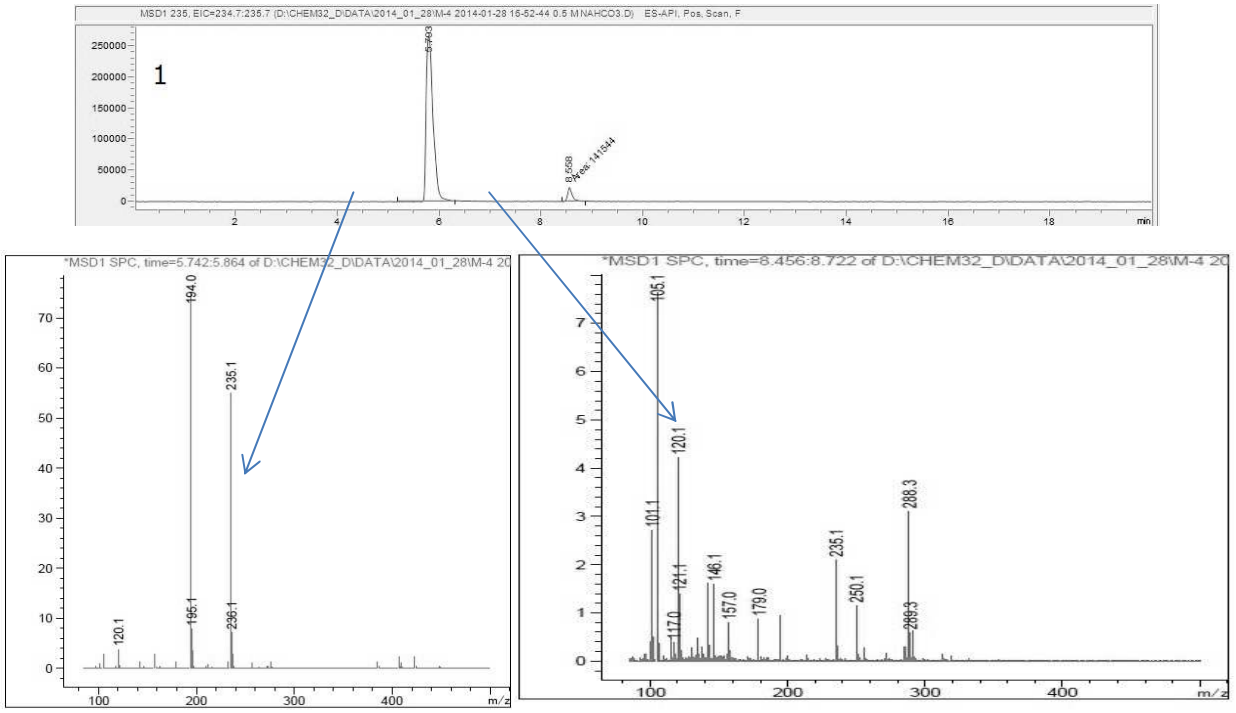

Fig. 3. Chromatogram of 3-thio-4-amino-5-(4-nitrophenyl)-1,2,4-triazole and mass-spectrums of form (a) and (b) (second sample preparation technique)

In order to characterize the main and minor peaks charges on individual atoms of 3-thio-4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole in both thione and thiol forms have been calculated. Calculations have been performed using the Hückel's method. The results are shown in Table. 1 and 2.

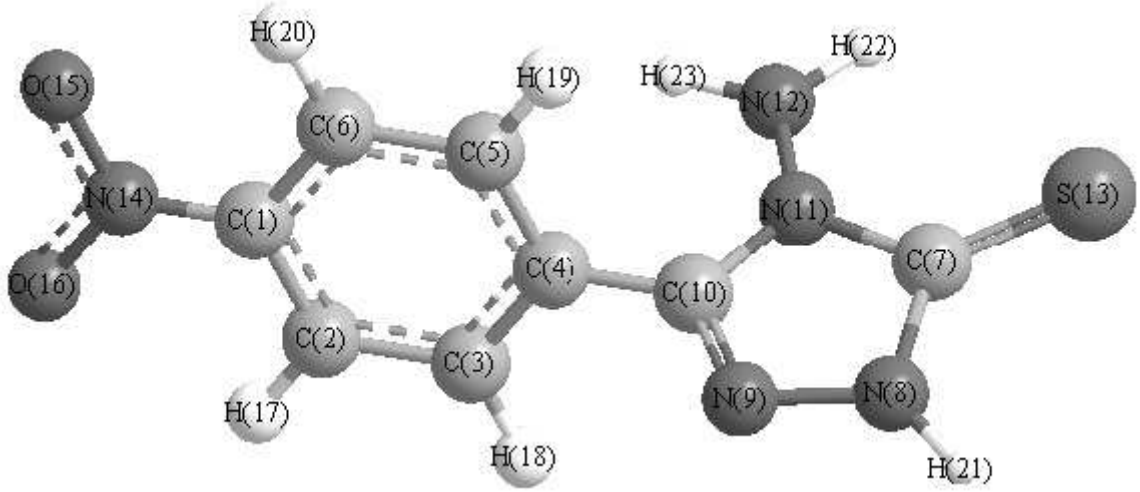

Fig.4. Formula of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H* -1,2,4-triazole-3-thione

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Table 1. Results of the atomic charges calculation and the amount of the molecule fragments charge of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H* -1,2,4-triazole-3-thione

1,2,4-triazole -3-thione		4-nitrophenyl		
C(7)	0.019447	C(1)		-0.114198
N(8)	-0.347656	C(2)		-0.134332
N(9)	-0.052654	C(3)		-0.162678
C(10)	0.083537	C(4)		0.006537
N(11)	-0.277436	C(5)		-0.149941
N(12)	-0.248094	C(6)		-0.134638
S(13)	-0.123958	N(14)		0.519182
H(21)	0.388722	O(15)		-0.345044
H(22)	0.215495	O(16)		-0.34536
H(23)	0.235327	H(17)		0.250434
		H(18)		0.23226
		H(19)		0.234746
		H(20)		0.250302
Sum	-0.10727	Sum		0.10727

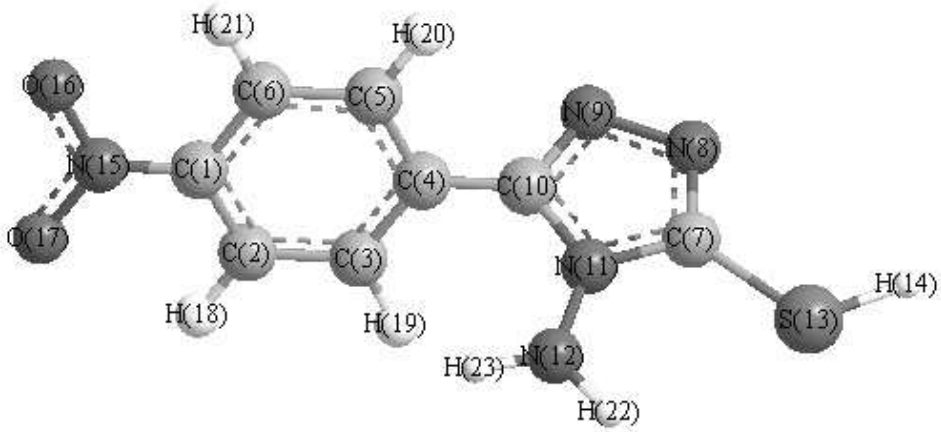

Fig.5. Formula of 4-amino-5-(4-nitrophenyl)-4*H* -1,2,4-triazole-3-thiol

Table 2. Results of the atomic charges calculation and the amount of the molecule fragments charge of 4-amino-5-(4-nitrophenyl)-4*H* -1,2,4-triazole-3-thiol

1,2,4-triazol-3-thiol		4-nitrophenyl	
C(10)	-0.031665	C(1)	-0.121799
N(9)	-0.05002	C(2)	-0.12868
N(8)	-0.092177	C(3)	-0.195815
N(11)	-0.198626	C(4)	0.013515
N(12)	-0.254858	C(5)	-0.160781
S(13)	0.243021	C(6)	-0.130623
H(14)	0.091063	N(15)	0.519813
H(22)	0.223627	O(16)	-0.345194
H(23)	0.22316	O(17)	-0.346623
C(7)	-0.231891	H(18)	0.249454
		H(19)	0.23356
		H(20)	0.240503
		H(21)	0.251034
Sum	-0.078366	Sum	0.078364

The distribution of electrons indicates that thione is more polar form (difference in total charges on atoms for nitrophenyl and triazole fragments is bigger for thione (from 0.10727 to -0.10727), than for thiol (from 0.078364 to -0.078364). As thione is more polar than thiol its retention time on a reversed-phase sorbent should be smaller. So presumably, first peak corresponds to the thione form and the second peak – thiol form.

In addition, we carried out calculations of the molecules of the test compounds with the program ChemSketch, software package ACDLABS 12.00 free version, as a result we have got theoretical proof that the thiol form is a minor component in a mixture of tautomeric forms thione-thiol (Fig. 6).

Fig. 6. Scheme of tautomeric thione-thiol transformations of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H* -1,2,4-triazole-3-thione and 4-amino-5-(4-nitrophenyl)-4*H* -1,2,4-triazole-3-thiol

To conclusively confirm our assumptions and calculations we have analyzed the UV-spectra of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H* -1,2,4-triazole-3-thione and 4-amino-5-(4-nitrophenyl)-4*H* -1,2,4-triazole-3-thiol (Fig. 7. and Fig.8.).

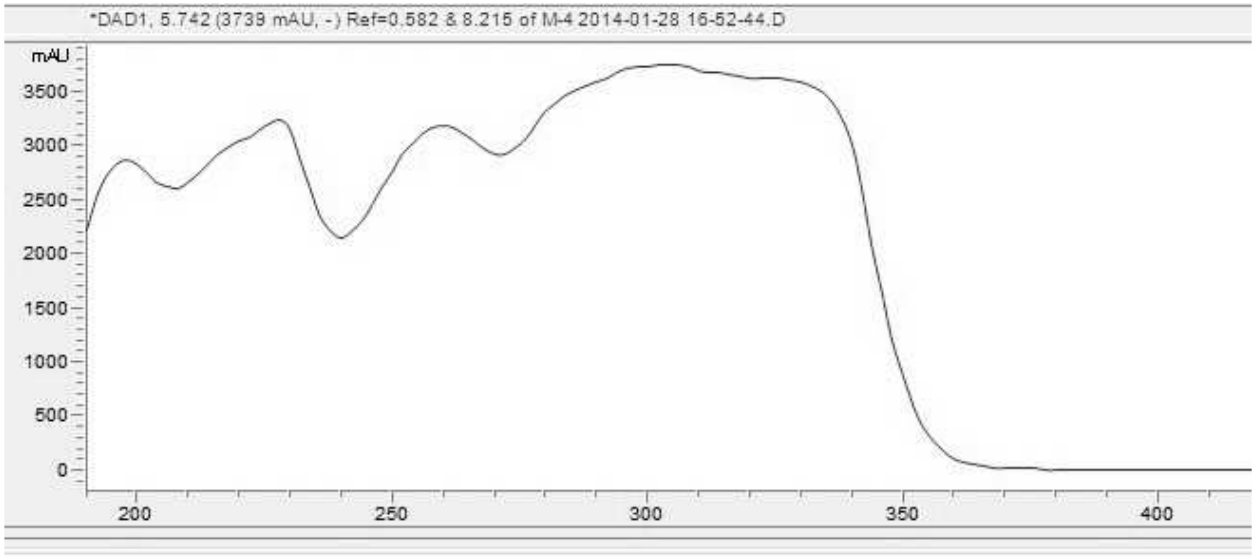

Fig. 7. UV-spectra of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H* -1,2,4-triazole-3-thione (form a)

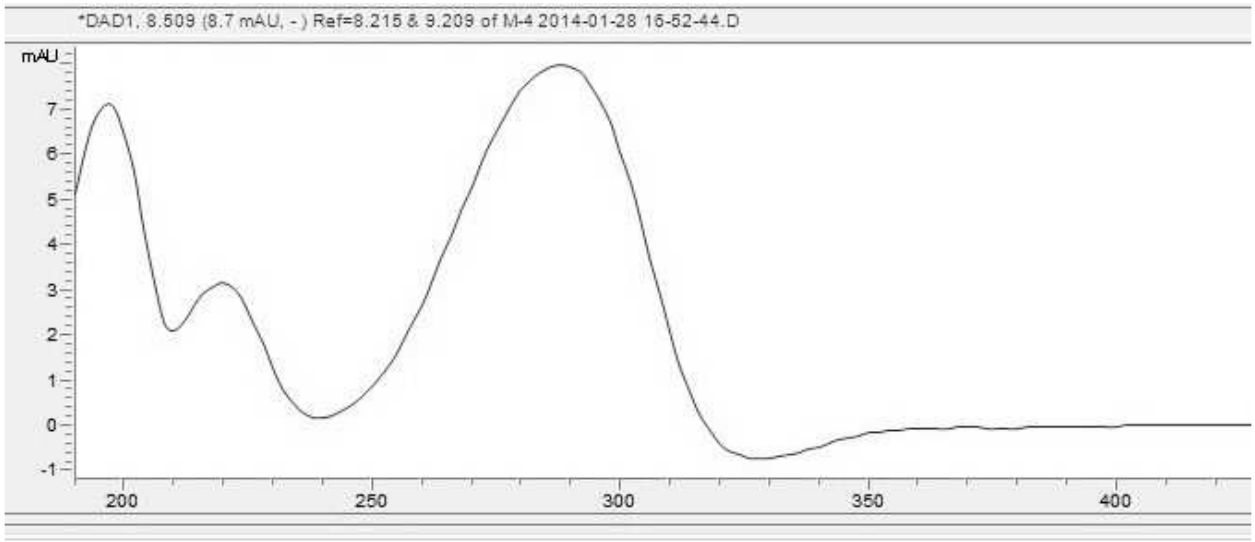

Fig.8. UV-spectra of 4-amino-5-(4-nitrophenyl)-4*H* -1,2,4-triazole-3-thiol (form b)

In the UV-spectrum of major form four absorption bands are traced. The first band is in the range till 200 nm is due to local excitation of the benzene ring electrons. The total optical density of the second absorption band at 210-240 nm is likely due to the overall contribution of $\pi \rightarrow \pi^*$ - transitions in 1,2,4-triazole ring. Third absorption band at 250-300 nm should be considered as a result of p-π - conjugation of the molecule in whole, the fourth band 300-350 is likely due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ chromophore transitions of thione group.

In the UV-spectrum of minor form three absorption bands are traced. The first band is in the range till 200 nm is due to local excitation of the benzene ring electrons. The total optical density of the second absorption band at 210-240

nm is likely due to the overall contribution of $\pi \rightarrow \pi^*$ - transitions in 1,2,4-triazole ring. Third absorption band at 250-310 nm should be considered as a result of p-π - conjugation of the molecule in whole.

Wherein significant bathochromic shift associated with the existence of a longer chain of conjugation is observed in form a (Fig. 7) than in the form b (Fig. 8), or because of the appearance of chromophore. In tione both above-mentioned phenomena are observed, and therefore form a corresponds to thione and form b corresponds to thiol (Fig. 9).

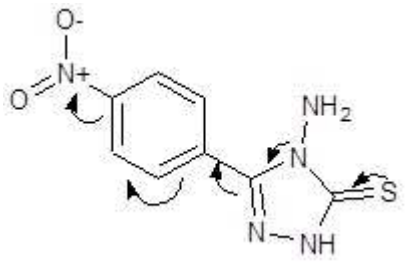

Fig. 9. Electronic effects in molecule of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione

So we got three arguments in favor that the major peak (a) corresponds 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione and a minor peak (b) corresponds to 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-thiol.

As in the first and in the second case 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione had the release time about 5.8 minutes, and 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-thiol had 8.6 minutes. In all mass spectra target ion with m/z 235 has been observed clearly.

Interpretation of the mass spectrum a (Fig. 3) based on chromatographic and mass spectrometric data
Studying the purity of peak, releasing on the 5,791 minute, on the extracted ion chromatograms we observe that m/z = 194, 235, 195 and 236 belong to the same substance (Fig. 10). Thus 195 and 236 refer to isotopic ions. Ion with m/z 120 refers to the background and does not belong to the mass spectrum of the test substance. Thus, major ions observed in the spectrum are ions 194 and 235. And 235 corresponds to the pseudomolecular ion. Ion 194 is a result of fragmentation of the ion. It was confirmed due to disappearing of ion with m/z 235 after increasing of fragmentor voltage from 10 to 100 V. Reconstructed mass spectra is composed only of ions 194, 235, as well as isotopic ions 195 and 236, of low intensity.

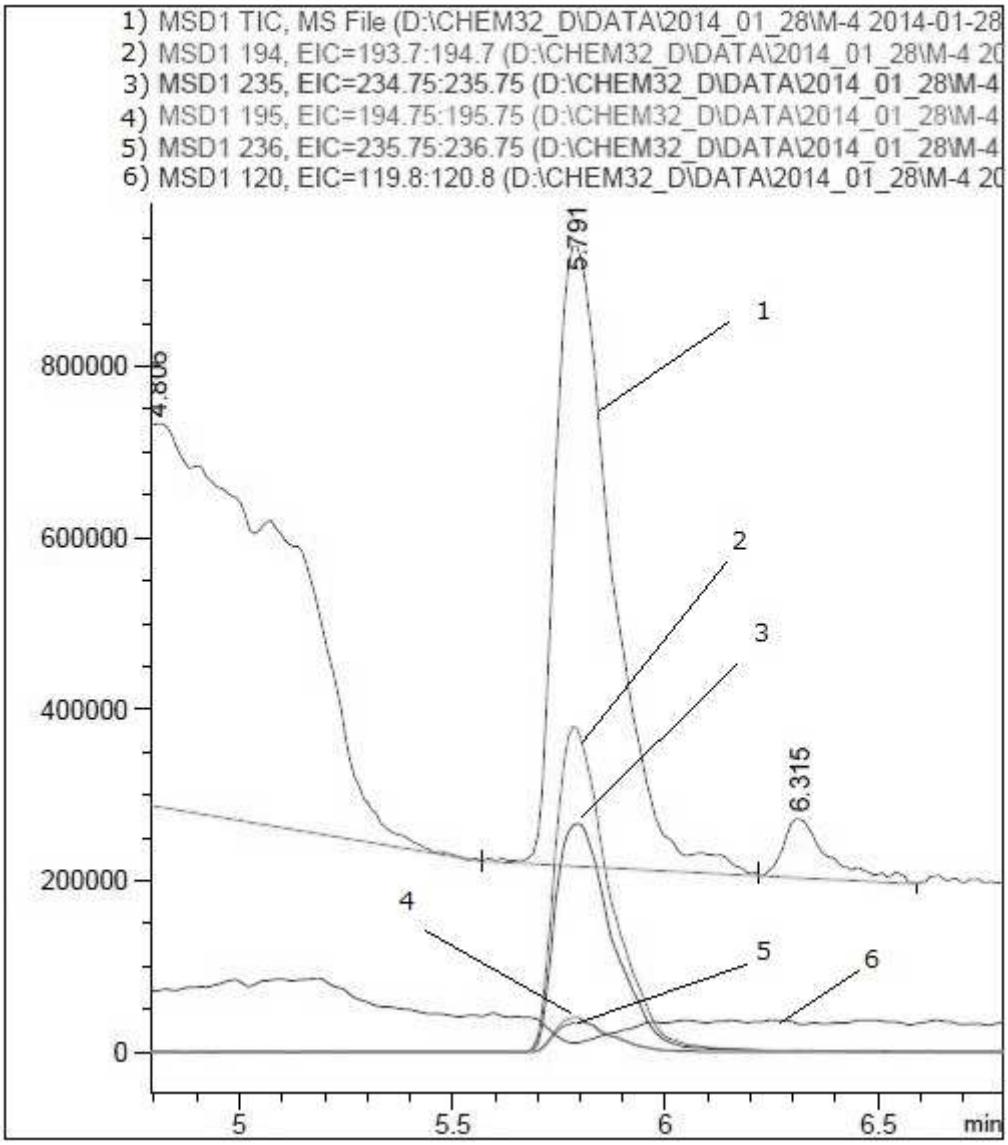

Fig. 10. Extracted chromatograms for ions of peak with retention time 5,791 minute

Interpretation of the mass spectrum b (Fig.3) based on chromatographic and mass spectrometric data

Peak, releasing on the 8,552 minute can be characterized on the basis of analysis of the extracted ions chromatograms (Fig. 10). For minor component with characteristic ion m/z 235 other ions that go along with this peak are not observed. Ions with m/z 105, 120, 101, 142 relate to background ions. Ion peak at m/z 288 is of a different substance, as releases separately from the peak m/z 235. Thus the reconstructed mass spectrum should consist only of the ion with m/z 235 and isotopic ion with m/z 236 (Fig. 11). We do not see selected ion chromatogram of isotope with m/z 236 because of its low intensity.

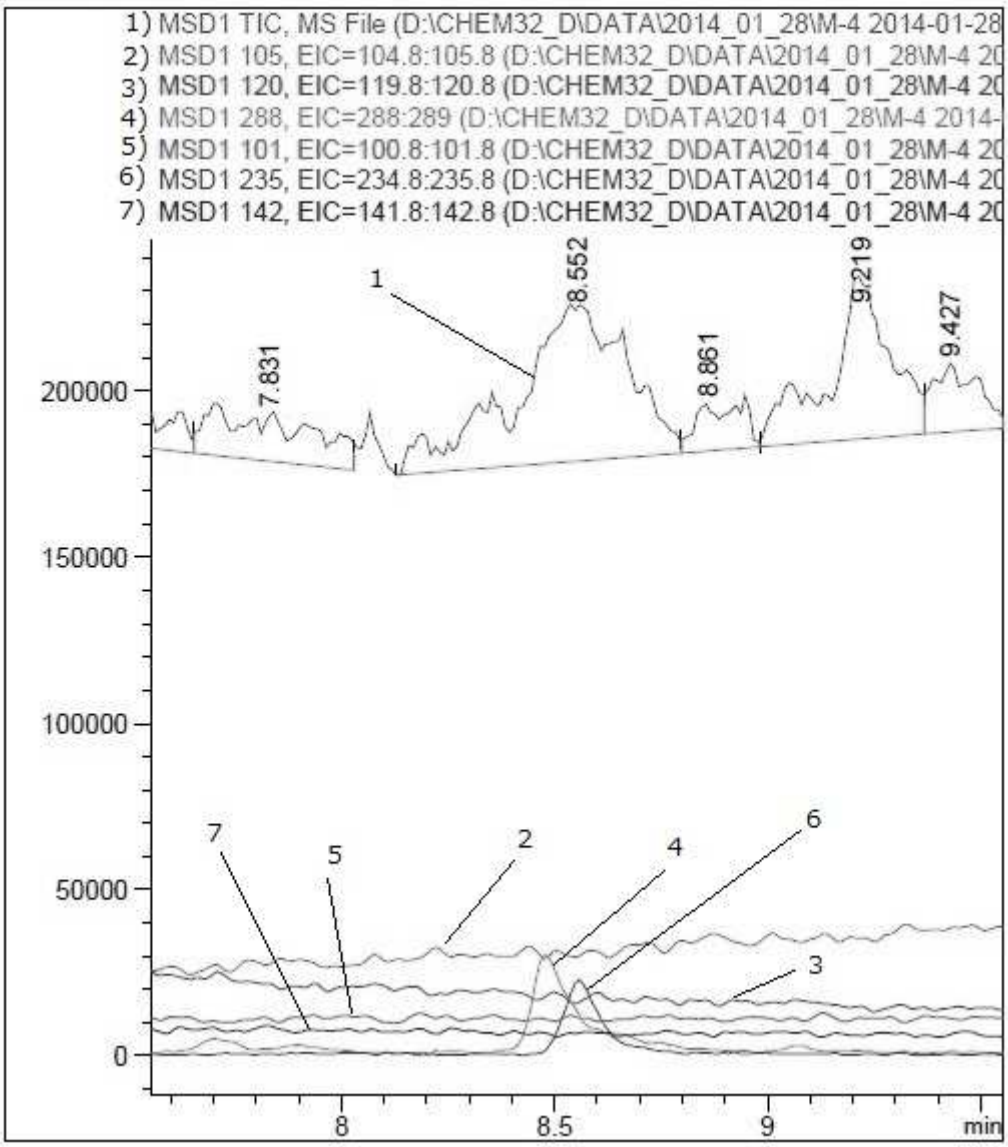

Fig. 11. Extracted chromatograms for ions of peak with retention time 8,552 minute

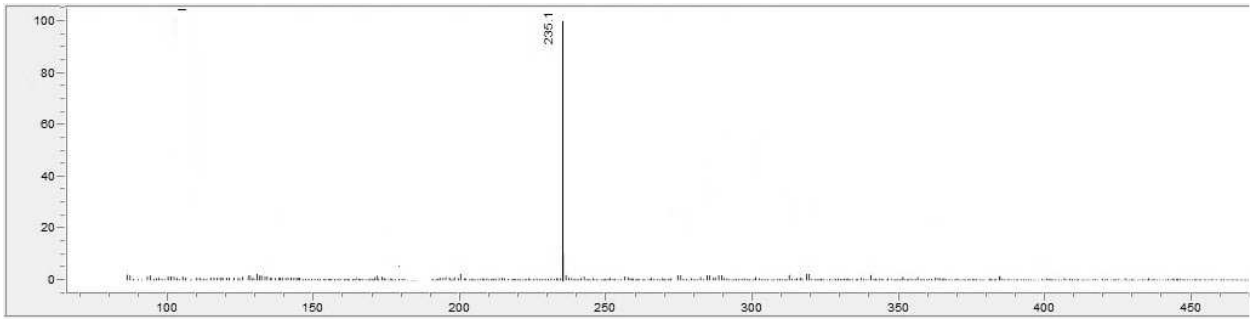

Fig. 12. Mass spectrum of the minor component reconstructed based on chromatographic and mass spectrometrometric data

CONCLUSION

1. HPLC-MS analysis of 4-amino-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione tautomeric derivatives has been performed and composition of the tautomers mixture has been set.
2. The relationship between the structure of synthesized compounds and their UV-spectrums has been studied, the results of atomic charges calculation have been described. Proofs conformity peaks in the chromatogram thione and thiol.
3. Based on these data, we consider that the major component is thione and the minor one is thiol.

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