

THE KINETICS OF ALLICIN TRANSFORMATION IN ACETONE AND GC-MS ANALYSIS OF ITS TRANSFORMENTS *

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In this work, the kinetics of alliin transformation was investigated in the conditions of an aprotic solvent, acetone. The kinetics curves, representing the dependence of changes from the alliin concentration in time at the temperatures of 55 and 45 °C, were used to determine the basic kinetic parameters: the reaction order ($n = 0.5$), rate constant ($k_1 = 0.0077 \text{ (mol dm}^{-3}\text{)}^{0.5} \text{ min}^{-1}$, $k_2 = 0.0144 \text{ (mol dm}^{-3}\text{)}^{0.5} \text{ min}^{-1}$), and activation energy ($E_a = 68775 \text{ J mol}^{-1}$). The change of alliin concentration was monitored through the changes in peak area of alliin in HPLC chromatograph and the calibration curve for the concentration range (0 to 3.0 mmol dm⁻³). GC/MS chromatography was used to determine the qualitative and quantitative composition of the reaction mixture. Only 50 % of the isolated components were identified. The most dominant organo-sulphuric molecules in the reaction mixture were 2-vinyl-[4H]-1,3-dithiine, diallyl trisulfide, propyl allyl disulfide and diallyl disulfide.

Key words: alliin, acetone, kinetics of alliin transformation, HPLC, GC-MS.

INTRODUCTION

Although *Allium sativum* (garlic) has been used for its medicinal properties for thousands of years, the investigations of its mode of action are relatively recent. *Allium sativum* has a wide spectrum of activities, the most important among these being:

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antibacterial, antiviral, antifungal, and anti-protozoan. Garlic also has a valuable effect upon the cardiovascular and immune systems [1].

The main carriers of the pharmacological activity of garlic are organic sulfur compounds, and allicin is the most important among them [2-4]. By its chemical composition, allicin is a thioester of the sulfenic acid, or allyl thiosulfinate. Allicin is active against a great number of bacteria, viruses, fungi, and many other parasites [5-7]. Allicin isolation, determination and standardization of allicin based products are made more difficult due to its high instability and volatility. In recent decades its synthesis has become very relevant because pure allicin is hard to obtain commercially. The greatest part of methods of allicin synthesis refers to the oxidation of allyl sulfide by hydrogen peroxide in acid medium [8-12], oxidation of allyl disulfide with *m*-chlorobenzoic acid in chloroform [9], and processing of the dichloromethane solution of diallylsulfide by magnesium monoperoxy hydrate in the presence of ammonium-butyl sulfate [11]. In a previous paper [10], a detailed study of mechanism and kinetics of synthesis of allicin, allyldisulfide and hydrogen peroxide in acidic media was given. S-Allyl cysteine can reduce or prevent nephrotoxicity of gentamicin. Gentamicin is a wide spectrum aminoglycoside antibiotic used to treat infections, particularly caused by Gram-negative bacteria, together with Abramycin and Amikacin [11]. Diallyl disulfide, the compound that gives a characteristic garlic odor, can be used as a convenient compound for the treatment of meningitis. It also shows the activity against HIV-infected host cells, so it can be used for the treatment of these infections [12]. Allyl methyl trisulfide improves the detoxication of undesirable agents and affects the preservation of good health of heart and immunological functions of the human body [12]. Diallyl trisulfide protects thrombocytes from oxidation and aggregation induced by adenosine diphosphate (ADP) [13]. Ajoenes have antibacterial, antifungal, and antiviral activity, anti-diabetic, anticancer, antithrombotic (anticoagulant) properties and are used for the treatment of AML (acute myeloid leukemia), and the regulation of the cardiovascular system (anti-arteriosclerotic and anti-cholesteric activities) [14]. Vinylthiins have high biological activity. They inhibit thrombocyte aggregation, cyclooxygenase, and 5-lipoxygenase, and regulate systolic and diastolic blood pressure [9].

In this paper, the transformation kinetics of allicin in acetone at two different temperatures was investigated. The qualitative and quantitative composition of the reaction mixture, i.e. allicin transformants in acetone at 55 °C, was determined by GC-MS method.

EXPERIMENTAL

Allicin synthesis. Allicin was synthesized from allyldisulfide by the procedure described elsewhere (yield 73 %) [10].

Allicin transformation. The standard allicin transformation was carried out in acetone (10% solution) with reflux at 55 °C and 45 °C. The homogenous reaction blend obtained was dark yellow in color, with a strong odor of garlic.

High pressure liquid chromatography (HPLC). Kinetics of allicin transforments generation, i.e. kinetics of allicin degradation was monitored by HPLC under the following conditions: apparatus: Agilent 1100; column: Zorbax Eclipse XDB-C18, 4.6 x 250 mm, 5 μ l; eluent: acetonitrile/water = 80:20; mobile phase flow: 1 $\text{cm}^3 \text{min}^{-1}$; injected volume: 20 μ l; detector: DAD Agilent 1200, detection at 205 nm.

The samples were taken from the reaction blend (0.1 cm^3 each) at various time intervals; at the beginning of the synthesis, followed by 15-minute intervals. The samples were topped up with the liquid phase (acetonitrile/water, 80/20) to 10 cm^3 , and then filtered through a membrane filter (0.45 μ) and analyzed to HPLC.

Gas chromatography – Mass spectroscopy (GC-MS). Qualitative and quantitative composition of volatile components in the reaction mixture obtained by the transformation of allicin in acetone was determined by GC-MS method using Hewlett-Packard 6890N gas chromatograph with capillary silica column HP-5MS (30 m x 0.25 mm) coupled with a 5975B Agilent mass selective detector. The carrier gas used was helium with 1.0 $\text{cm}^3 \text{min}^{-1}$ flow. MS spectra were obtained by electronic ionization: the ionization energy was 70 eV, mass range 35-200, scanning time 0.32 s. The components were identified by using Wiley 7NIST 05 and EPA-NBS data library.

RESULTS AND DISCUSSION

In the investigations carried out in scope of this work, the synthesized and purified allicin was the initial precursor subjected to transformations in the conditions of aprotic solvent, acetone, by heating to 55 $^{\circ}\text{C}$ and 45 $^{\circ}\text{C}$. To investigate the kinetics of allicin transformation under these conditions, high pressure liquid chromatography (HPLC) was used. The reaction mixture of allicin and acetone (1:10, v/v) was subjected to heating with reflux at two different temperatures (55 $^{\circ}\text{C}$ and 45 $^{\circ}\text{C}$). At various time intervals (0, 15, 30 and 45 min) the samples were taken and tested by liquid chromatography. The results of these investigations are given in Figure 1 (A, 55 $^{\circ}\text{C}$ and B, 45 $^{\circ}\text{C}$).

From the chromatograms shown it can be seen that the allicin peak for the given chromatography conditions appeared at 2.939 minutes retention time (R_t). In both reaction media, for the given reaction time, the peak area of allicin is decreasing and after 45 minutes almost the total quantity is converted into transforments. Also, the appearance of new peaks can be observed in the chromatograms, and the increase of the area of the existing peaks in the allicin chromatogram (sample in $t = 0$ minutes).

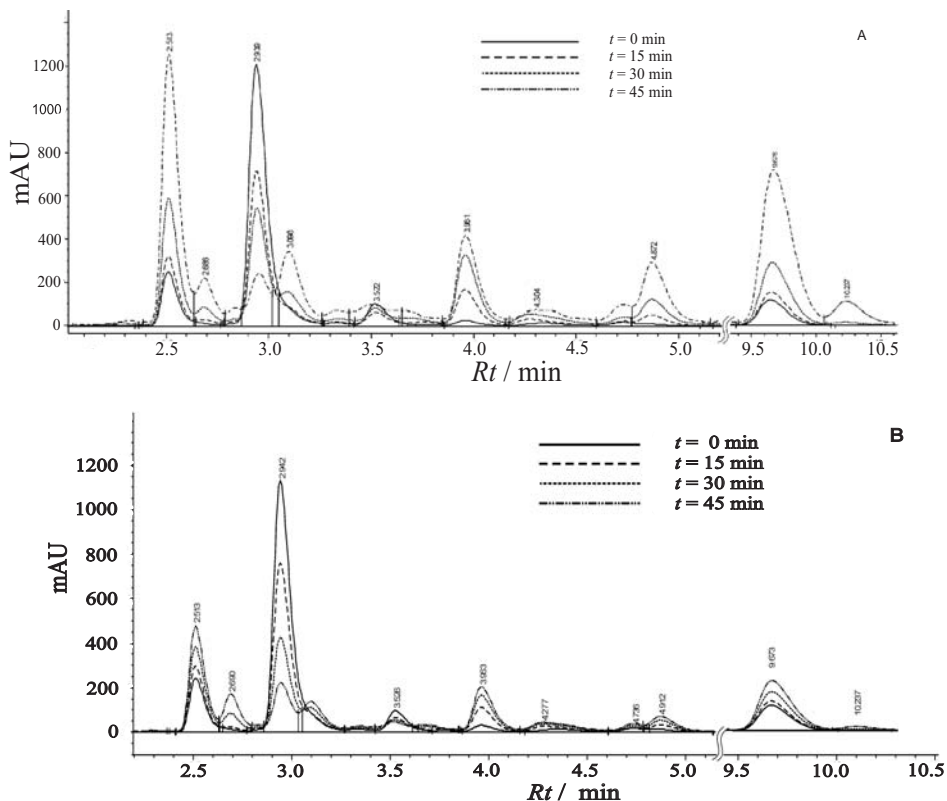


Figure 1. HPLC chromatograms of the reaction mixture of alliin transformation in acetone at 55 °C (A) and 45 °C (B) for various time intervals

These peaks represent the alliin transformts. Since the peak area in HPLC chromatograms is proportional to the concentration of alliin, a calibration curve was constructed for a series of different concentrations of alliin, which shows high linearity and a linear correlation coefficient of 0.998. The line equation which can be represented by the calibration curve is as follows:

$$A_s [\text{mAU}\cdot\text{s}] = 149.2 + 2791.01 c_{M(A)} [\text{mmol dm}^{-3}] \quad (1)$$

where A_s - the peak area, $\text{mAU}\cdot\text{s}$; $c_{M(A)}$ - the concentration of alliin, mmol dm^{-3}

Using equation 1 an unknown concentration of alliin can be calculated in all processes of transformation. Equation (1) can be used to calculate the unknown concentrations of alliin samples by HPLC method, by detection at 205 nm. Equation (1) applies for alliin concentrations range from 0 to 3.0 mmol dm^{-3} . In Figure 2 given is the graph of alliin concentration ($c_{M(A)}$) variations as dependant on the reaction time (t) in the reaction systems at 45 and 55 °C. The curves are exponentially declining and show that the degradation is slightly faster at higher temperatures, i.e. the slope of the curve is steeper.

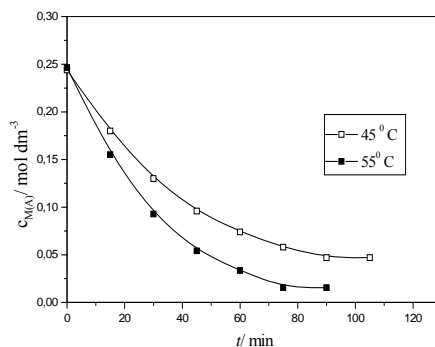


Figure 2. Dependence of alliin concentration on the reaction time at 45 °C and 55 °C (HPLC method)

To determine the kinetic parameters of alliin transformation, the equation for the reaction rate of the n^{th} order is used:

$$-\frac{dc_{M(A)}}{dt} = k \cdot c_{M(A)}^n \quad (2)$$

Logarithm of equation (2) and use of differential method of analysis of the reactants concentration variations in time (t) gives the equation:

$$\ln \left(-\frac{dc_{M(A)}}{dt} \right) = \ln(k) + n \cdot \ln c_{M(A)} \quad (3)$$

Equation (3) represents the equation of the straight line from which kinetic parameters of alliin transformation reaction can be determined, i.e. the chemical reaction rate constant (k), and the order of reaction (n). In Figure 3 given is the linear dependence of function $\ln(-dc_{M(A)}/dt)$ of $\ln c_{M(A)}$, the slope of which determines the order of reaction (n), and the intercept on the ordinate determines the value of the chemical reaction rate constant (k).

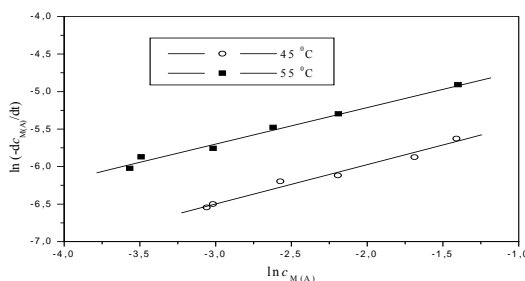


Figure 3. Determination of kinetic parameters of alliin degradation in acetone at 45 and 55 °C (HPLC method)

By using the values of rate constant, k , for the two different temperatures, according to Arrhenius equation [15]:

$$k = A \cdot e^{-\frac{E_a}{RT}} \quad (4)$$

the activation energy and the pre-exponential factor are calculated and the results are given in Table 1.

Table 1. Values of the kinetic parameters for alliin transformation reaction in acetone obtained by use of HPLC method

Temperature, K	Kinetic parameters	
	$k, (\text{mol dm}^{-3})^{0.5} \text{min}^{-1}$	n
328	0.0077	0.524
318	0.0141	0.486
$Ea, \text{J mol}^{-1}$	68775	

Based on the results from Table 1 it can be seen that the alliin transformation in acetone at two given temperatures has the reaction order of approx. 0.5.

Qualitative and quantitative composition of alliin transforments at 55 °C was determined by GC-MS method. The dominant components in the reaction mixture generated by the alliin degradation in acetone at 55 °C are 2-vinyl-[4H]-1,3-dithiin (Figure 4 A) and diallyl trisulfide (Figure 4 B). Other components that might be significant for the pharmacological activity of the reaction mixture are propyl allyl disulfide, methyl allyl sulfide, diallyl sulfide, diallyl disulfide and 3-vinyl-[4H]-1,2-dithiin (Figures 5-9).

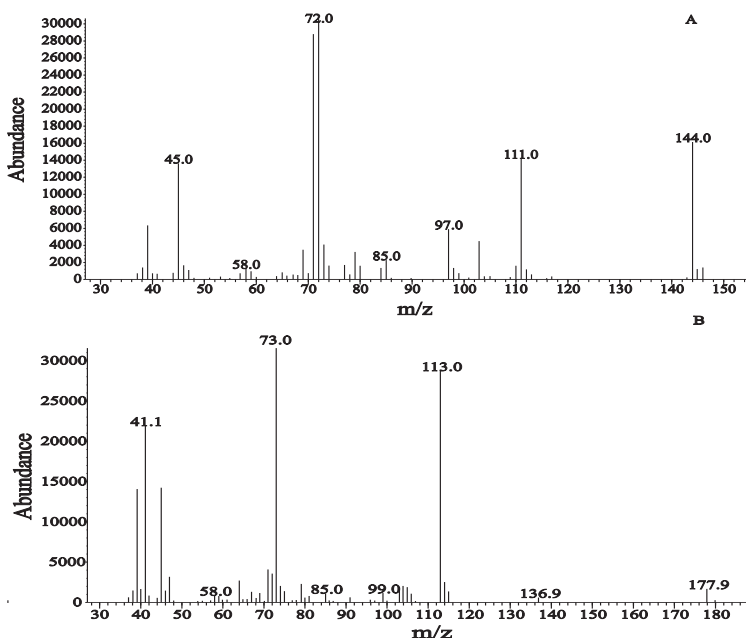


Figure 4. Mass spectrum of 2-vinyl-4H-1,3-dithiin ($R_t = 9.725$ min, A) and diallyl trisulfide ($R_t = 11.880$ min, B)

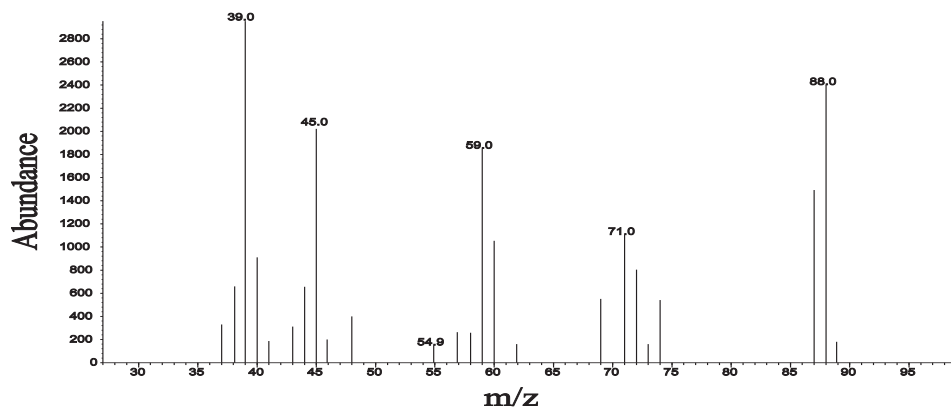


Figure 5. Mass spectrum of methyl allyl sulfide ($R_t = 2.295$ min)

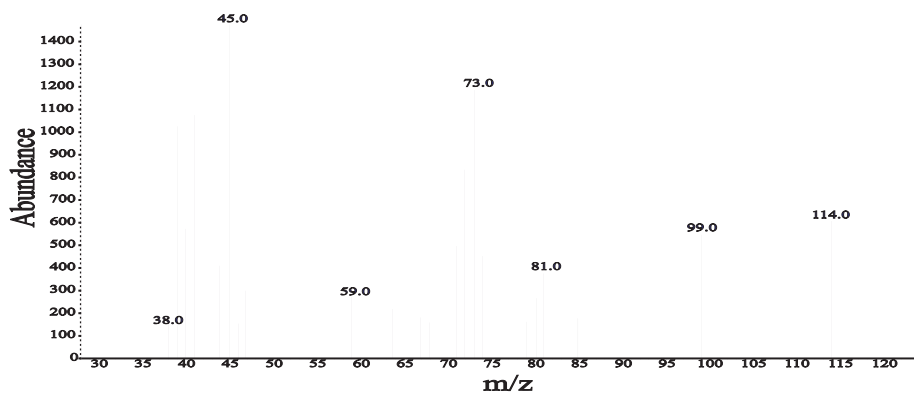


Figure 6. Mass spectrum of diallyl sulfide ($R_t = 3.001$ min)

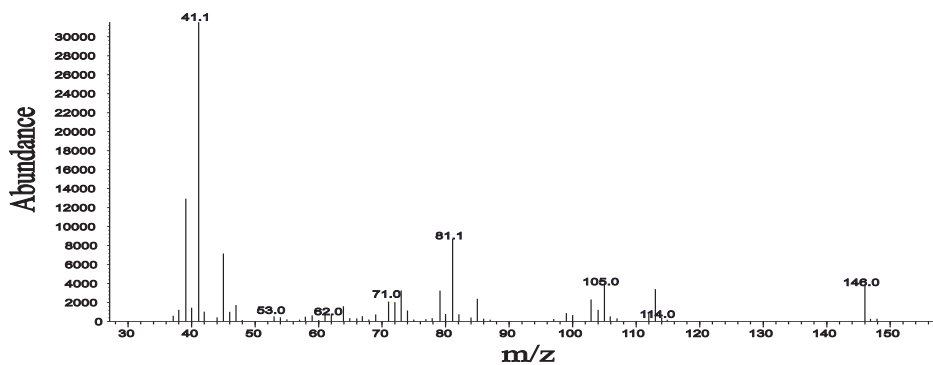


Figure 7. Mass spectrum of diallyl disulfide ($R_t = 6.580$ min)

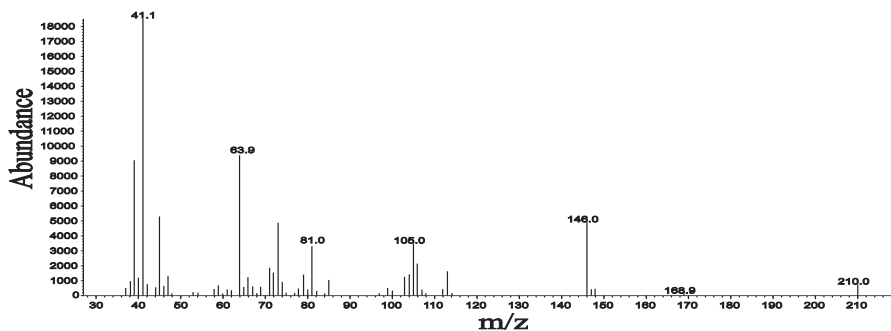


Figure 8. Mass spectrum of propyl allyl disulfide ($R_t = 17.753$ min)

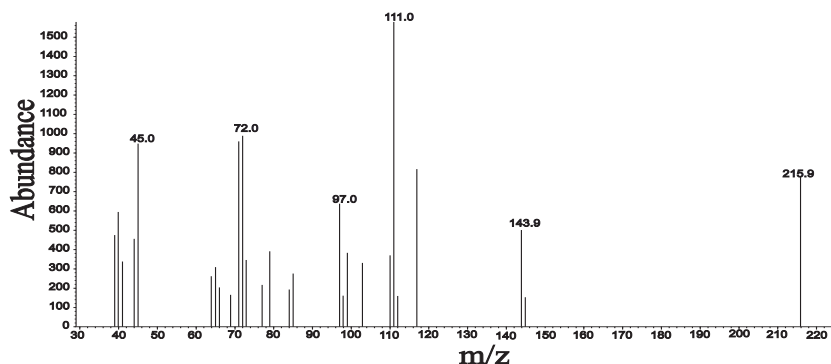


Figure 9. Mass spectrum of 3-vinyl-4H-1,2-dithiin ($R_t = 26.471$ min)

In the above mass spectra present are the peaks from molecule ions generated by removing electrons from the basic molecule and m/z values for these ions correspond to the molecule mass of the respective molecules. Other m/z values in the mass spectra originate from the corresponding fragments generated by basic molecules fragmentation of re-combination of the fragments generated from the basic molecule. In Table 2 shown are the components detected by GC-MS analysis in the reaction mixture by using Wiley 7 Nist 05. L and EPA-NBS data library.

Table 2. Results of GC-MS analysis of allicin transformants at 55 °C

	<i>R_t</i> , min	Compound	GC area, %	Retention index
1.	2.295	Methyl allyl sulfide	0.65	956
2.	2.456	Dimethylene methane	0.19	804
3.	2.873	2-Propynyl ether	0.73	846
4.	3.001	Diallyl sulfide	0.41	859
5.	6.580	Diallyl disulphide	6.04	1082
6.	8.281	2-Methylallyl alcohol	6.26	1157
7.	9.131	(2E)-2-Isopropyl-5-methyl-2-hexenal	3.68	1193
8.	9.415	1,3-Dithiolane-2-thione	1.31	1204
9.	9.725	2-vinyl-[4H]-1,3-dithiine	11.81	1217
10.	11.474	1,2-Dithiacyclopentane	0.85	1287
11.	11.880	Diallyl trisulfide	11.04	1303
12.	12.864	2-Ethyltetrahydrothiophene	0.75	1343
13.	13.522	5-methyl-1,2,3,4-tetrathia-cyclohexane	0.44	1369
14.	14.212	Tetraethylene glycol mono-n-dodecyl ether	0.41	1397
15.	17.753	Propyl allyl disulfide	6.77	1531
16.	26.471	3-Vinyl-[4H]-1,2-dithiine	0.72	1954

CONCLUSIONS

Allicin was synthesized by the oxidation method with acid hydrogen peroxide and then subjected to transformation in conditions of aprotic solvent acetone at two different temperatures. By use of HPLC method the calibration curve was constructed for determination of allicin concentration ($R_t = 2.939$ min) in the reaction mixture. HPLC method was used to determine the kinetic parameters of transformation of allicin in acetone for two different temperatures, 45 and 55 °C ($k_1 = 0.0077$ (mol dm⁻³)^{0.5} min⁻¹, $k_2 = 0.0144$ (mol dm⁻³)^{0.5} min⁻¹, $n_1 = 0.524$, $n_2 = 0.486$, respectively, $E_a = 68775$ J mol⁻¹).

The qualitative and quantitative composition of the reaction mixture, i.e. allicin transformant in acetone, was determined by GC-MS method. The dominant components in the reaction mixture generated by the allicin transformation in acetone are 2-vinyl-[4H]-1,3-dithiine and diallyl trisulfide.

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IZVOD

KINETIKA TRANSFORMACIJE ALICINA U ACETONU I GC-MS ANALIZA TRANSFORMENATA

(Originalni naučni rad)

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U radu je ispitana kinetika transformacije alicina u uslovima aprotičnog rastvarača, acetona. Kinetičke krive, koje daju zavisnost promene koncentracije alicina sa vremenom na dve temperature 55 i 45⁰ C iskorišene su za određivanje osnovnih kinetičkih veličina, reda reakcije ($n=0,5$), konstante brzine ($k_1 = 0,0077 \text{ (mol dm}^{-3}\text{)}^{0,5}\text{min}^{-1}$, $k_2 = 0,0144 \text{ (mol dm}^{-3}\text{)}^{0,5}\text{min}^{-1}$) i energije aktivacije ($E_a=68775 \text{ J mol}^{-1}$). Promena koncentracije alicina praćena je preko promena površine pika od alicina u HPLC hromatogramu i na osnovu kalibracione krive za opseg koncentracija (0 do 3,0 mmol dm⁻³). GC/MS hromatografijom određen je kvalitativni i kvantitativni sastav reakcione smeše. Od ukupno razdvojenih komponenata identifikovano je 50 %. Najzastupljeniji organo-sumporni molekuli u reakcionoj smeši su 2-vinil-4H-1,3-ditiin, dialil trisulfid, propil alil disulfid i dialil disulfid.

Ključne reči: Alicin, Aceton, Kinetika transformacije, HPLC, GC-MS.

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