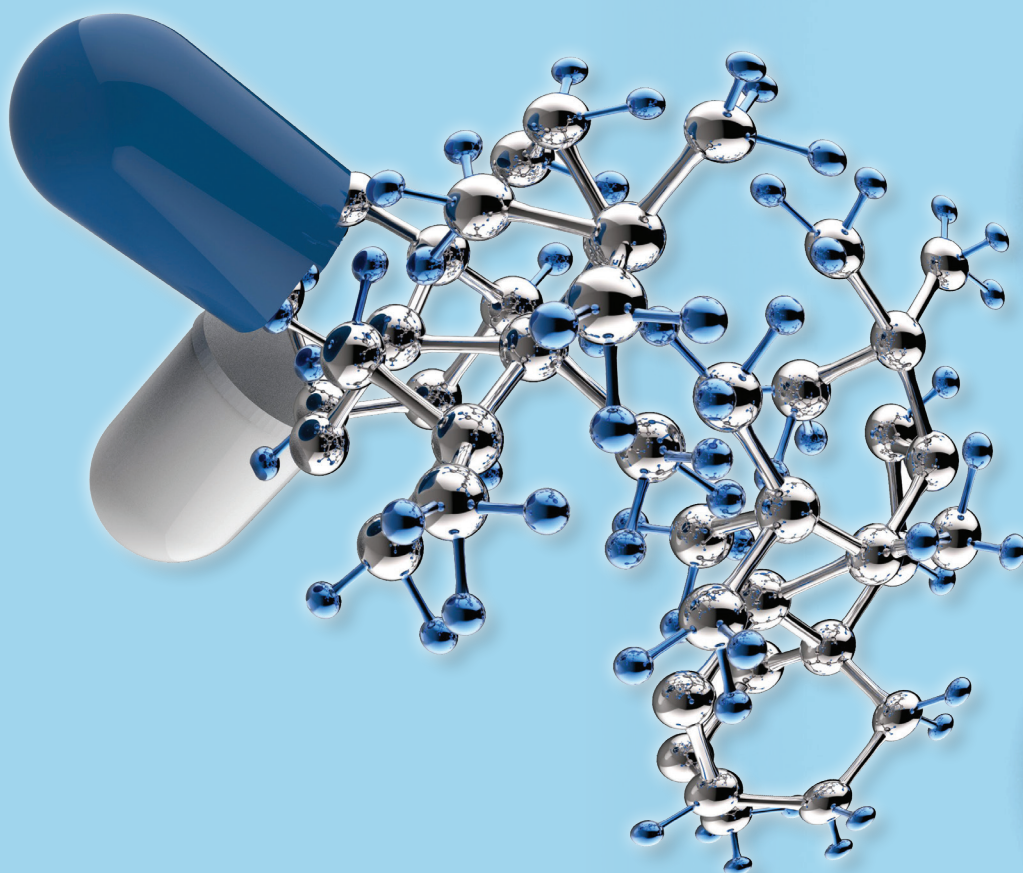




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# ΦΑΡΜΑΚΕΥΤΙΚΗ PHARMAKEFTIKI

ΤΡΙΜΗΝΙΑΙΑ ΕΚΔΟΣΗ ΜΕ ΘΕΜΑΤΑ ΦΑΡΜΑΚΕΥΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ  
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## ΦΑΡΜΑΚΕΥΤΙΚΗ

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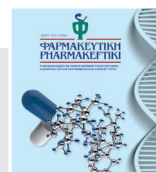
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# Thermodynamic Functions of Chromatographic Retention of Sodium 2-((4-Amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio)acetate and its Impurities

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## KEYWORDS:

**1,2,4-triazoles;  
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## ABSTRACT

The aim of this work was to study the dependence of liquid chromatography retention on temperature and thermodynamic characteristics of the transfer of sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio)acetate, 2-(thiophen-2-yl)acetohydrazide and 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione from the mobile phase to the stationary. HPLC system Agilent 1260 Infinity. Column Zorbax SB-C18; 30 mm x 4.6 mm; 1.8 μm. The mobile phase consisted of water (0,1% HCOOH) and acetonitrile (0,1% HCOOH) (75:25). Flow rate is 0.4 mL/min. The solutions of each substance were chromatographed at different temperatures from 30°C to 70°C with step 5°C. The sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio)acetate and its technological impurities are characterized by negative values of the enthalpy of transfer from the mobile phase to the stationary, so the process of transition of the substances from the mobile phase to the stationary prevails over the reverse process. The entropic term more than enthalpic term for the 2-(thiophene-2-yl)acetohydrazide at the influence on retention.

## 1. Introduction

Derivatives of 1,2,4-triazole-3-thione exhibit a variety of biological activities, so and they attract a lot of attention from researchers looking for new biologically active substances<sup>1-6</sup>.

Sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio)acetate is an active pharmaceutical ingredient (API) with potential actoprotective action<sup>7-9</sup>.

Thermodynamic characteristics is an important factor in the control of retention in high-performance liquid chromatography (HPLC). The temperature allows controlling the selectivity of the chromatographic separation, and it is also known that with increasing temperature the viscosity of the eluent decreases, which, given the pressure limitations during the operation of the column, is useful and requires detailed study. Due to this factor, water dissolves non-polar compounds better, because the dielectric constant of the medium decreases. The thermostat of the column allows you to avoid fluctuating retention when the room temperature changes<sup>10</sup>. Thus, the study of the thermodynamic parameters of retention of substances, firstly, has a theoretical value, because it helps to clarify the nature of retention, and secondly, it has a direct practical value, because it helps to choose the optimal separation conditions.

Studying the thermodynamics of the retention allows us to better clarify the nature of the chromatographic mechanism as reflected in partitioning, London dispersion bonds, dipole-induced, dipole-dipole, ion-dipole, hydrogen bonds, ionic bonds, etc. Linear dependence of retention factors  $\ln k$  on  $T^{-1}$  as deduced from the Van't-Hoff equation, allows predicting the dependence of chromatographic behavior at different temperatures based on a small number of experiments<sup>11</sup>.

The thermodynamic characteristics obtained by the Van't-Hoff equation also allows describing the properties of the column sorbent and comparing it with other sorbents<sup>11, 12</sup>. Further elucidation of the retention mechanisms will help to more efficiently select and create new stationary phases, as well as change the mobile phase to improve the selectivity of chromatographic systems for the determination of given analytes. Evaluation of thermodynamic characteristics helps to determine whether enthalpic or entropic control prevails in the adsorption of analytes. Many scientists pay attention to the study of thermodynamic parameters of retention<sup>13-25</sup>. Most relevant studies however over the past 20 years have been conducted mainly for enantioselective sorbents on chiral stationary phases<sup>13-22</sup>. Therefore, the study of

classical octadecyl silica gel systems deserves more attention.

The aim of this work was to study the dependence of the retention on temperature, as well as the thermodynamics of mobile to stationary phase transfer of sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)thio) acetate and its impurities-2-(thiophen-2-yl)acetohydrazide and 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione - using a C-18 column.

Derivatives of 1,2,4-triazole-3-thione exhibit a variety of biological activities, so and they attract a lot of attention from researchers looking for new biologically active substances<sup>1-6</sup>.

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Study of thermodynamic parameters of sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate, 2-(thiophen-2-yl)acetohydrazide and 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione retention was held for the first time.

## 2. Materials and methods

### 2.1. Chromatographic equipment.

High-performance liquid chromatographic system Agilent 1260 Infinity consisted of degasser (Agilent Technologies, Japan), binary pump (Agilent Technologies, Germany), autosampler (Agilent Technologies, Germany), thermostat column compartment (Agilent Technologies, Germany), diode array detector (Agilent Technologies, Germany). Software was OpenLAB CDS.

### Chromatographic conditions.

Column was Zorbax SB-C18; 30 mm x 4.6 mm; 1.8  $\mu\text{m}$ . The mobile phase consisted of water and acetonitrile (75:25) in presence of 0,1% HCOOH. The pH of the water:acetonitrile mixture was measured about 2.8. Flow rate was 0.4 mL/min. The wavelengths of the diode array detector were 232, 234, 246 nm. Injection volume 2  $\mu\text{L}$ .

## 2.2. Reagents.

Sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio)acetate (**1**), 2-(thiophene-2-yl)acetohydrazide (**2**), 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**3**) were synthesized at the Department of Natural Sciences for Foreign Students and Toxicological Chemistry of Zaporizhzhia State Medical and Pharmaceutical University. Direct Q 3UV (Millipore, Molsheim, France) was used for the production of high purity water (18 MΩ, 25°C). Acetonitrile HPLC Super Gradient grade (Avantor Performance Materials Poland S.A., Poland), formic acid «For analysis» (98 %) (AppliChem GmbH, Germany).

## 2.3. Preparation of solutions.

Solutions of sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio)acetate (**1**), 2-(thiophene-2-yl)acetohydrazide (**2**) and 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**3**) were prepared at a concentration of 0,001% in

the mixture of water and acetonitrile (75:25).

**2.4. Determination of temperature dependence.** The final solutions of each substance were chromatographed at different temperatures from 30°C to 70°C with step 5 °C, under conditions described in 2.2.

**2.5. Calculation of octanol water distribution coefficients logD.**

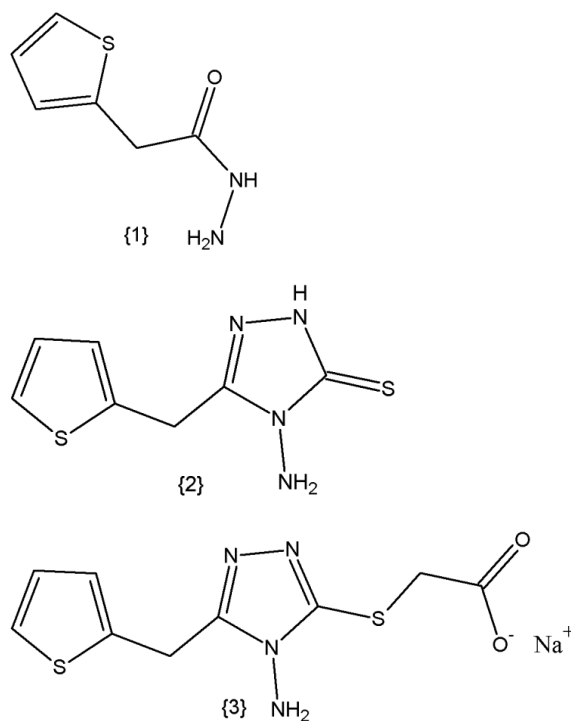
Octanol -water distribution coefficients logD at pH 3 calculated using the on-line service<sup>26</sup>

## 2.6. Statistical Analysis

Microsoft Excel was used for regression analysis and graph construction

## 3. Results and discussions

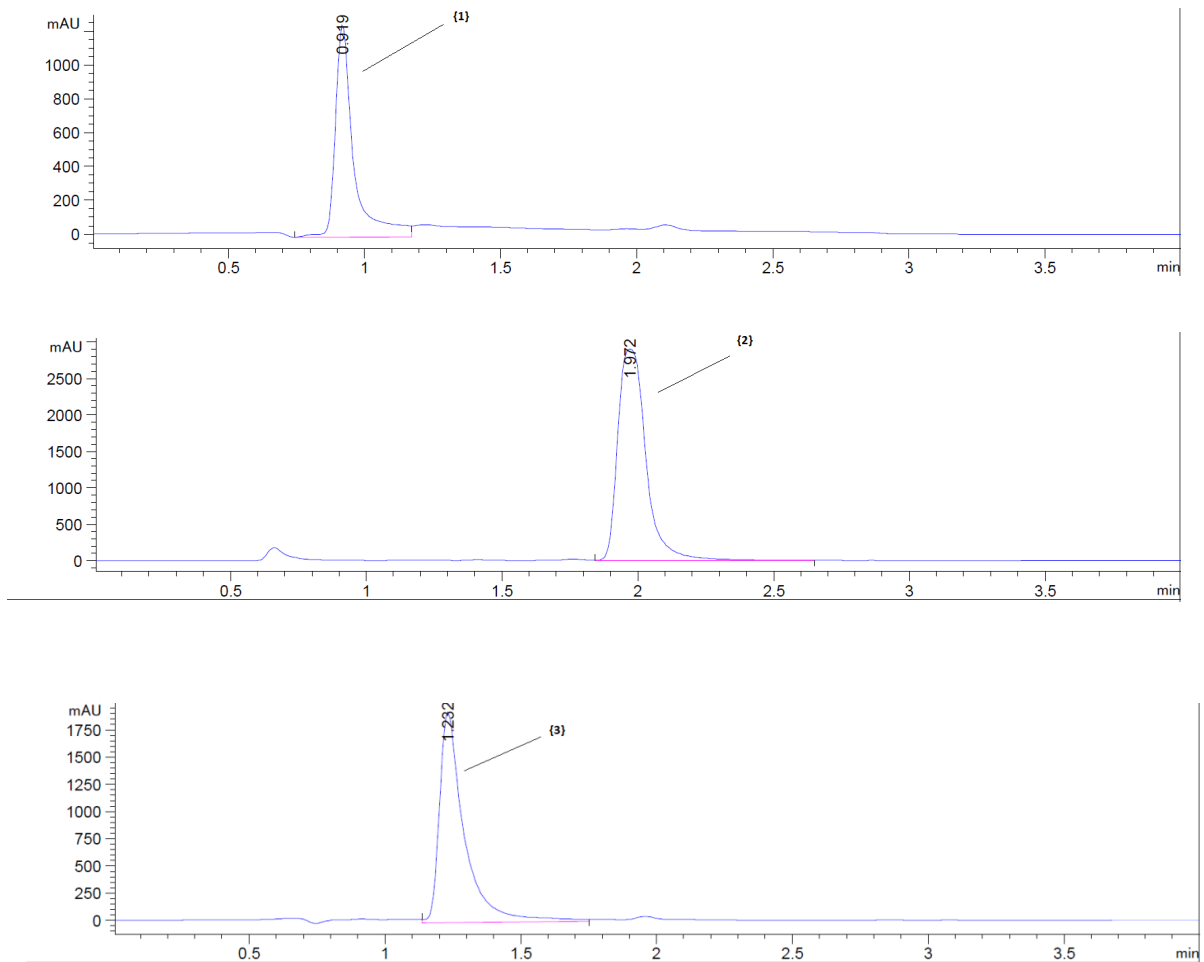
Structures of the sodium 2-(thiophene-2-yl)acetohydrazide (**1**), 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2**) and sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio)acetate are shown at Fig. 1.



**Figure 1.** Structures of studied compounds (1-3).

The optimal parameters (maximum separation at maximum retention) are observed at 25% acetonitrile and 40°C of the column thermostat. Retention

of each substance at optimal conditions is shown at chromatograms was acquired at the wavelengths of maximal light absorption (Fig. 2).



**Figure 2.** Chromatograms of compounds (1) at 234 nm, (2) at 246 nm, (3) at 232 nm.

For the study of the thermodynamic parameters of the transfer of the analyte from the mobile phase to the stationary retention factors are determined according to equation (1):

$$k = \frac{(t_r - t_0)}{t_0} = \frac{V_S}{V_M} \quad (1)$$

where  $t_r$  - retention time,

$t_0$  - retention time of an unretained substance determined using  $\text{KNO}_3$  and equal to 0.6 minutes.

$\frac{v_S}{v_M}$  - the ratio of the amount of substance analyte (mole) in the stationary phase to the amount of substance analyte (mole) in the mobile phase.

The ratio of the volume of the mobile phase to the stationary  $\beta$ , also known as the phase ratio, or phase volume ratio is given by equation (2)<sup>11</sup>

$$\beta = \frac{V_M}{V_S} \quad (2)$$

The equilibrium constant  $K$  of the transfer of substance from the mobile phase to the stationary is described by equation (3):

$$K = \frac{[A]_S}{[A]_M} = \frac{v_S}{v_m} \times \frac{V_M}{V_S} = k \times \beta \quad (3)$$

where  $[A]_S$  - concentration of analyte in the stationary phase,

$[A]_M$  - concentration of analyte in the mobile phase.

The well-known Vant-Hoff's equation (4):

$$\ln k = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (4)$$

$$\ln(k \times \beta) = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (5)$$

where  $\beta$  - equation (2)

Based on equation (5), equation (6) is formed:

$$\ln k = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} - \ln \beta \quad (6)$$

Equation (6) is reduced to a linear form, (equation (7)):



$$y = mx + b$$

$$\text{where } y = \ln k, x = T^{-1}, m = -\Delta H^0/R, b = \Delta S^0/R - \ln \beta.$$

The standard enthalpies of transfer of analytes from the mobile phase to the stationary phase may be calculated on the basis of equations (6) and (7):

$$\Delta H^0 = -m \times R \quad (8)$$

So, the dependence of the retention factor on temperature was determined for each substance, and on the basis of the obtained results, the enthalpy of transfer of the substance from the mobile

phase to the stationary one was determined.

Figure 3 shows the effect of temperature on the chromatographic retention of compound (3).

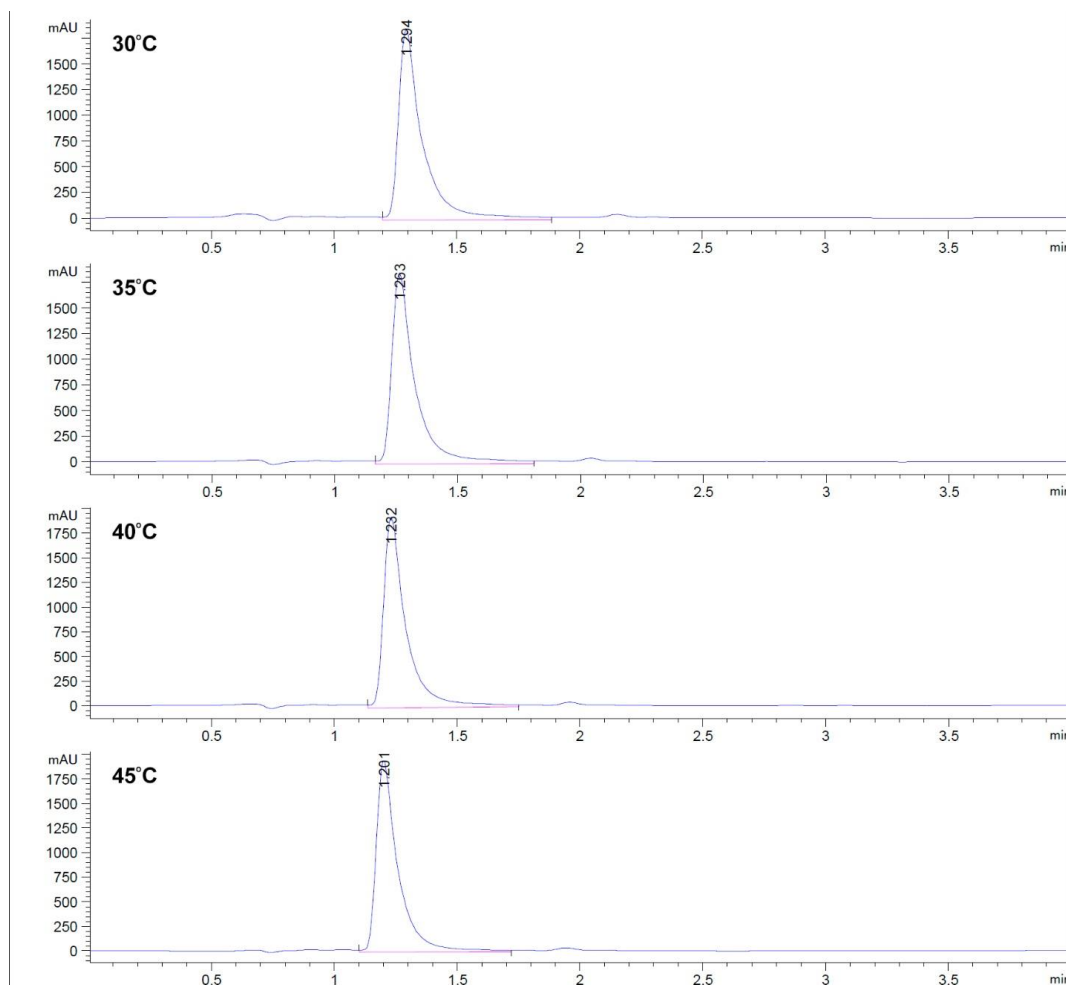
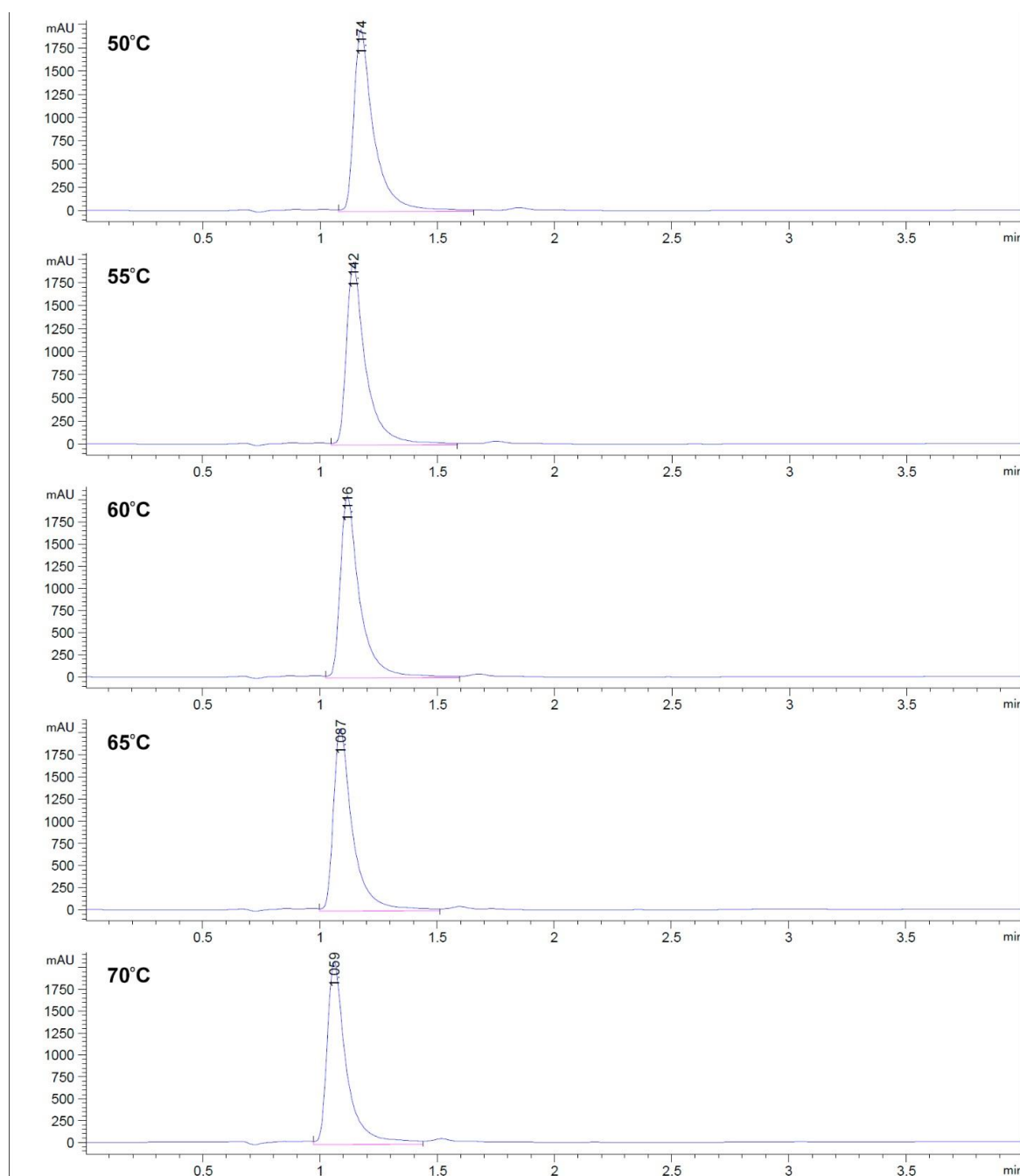


Figure 3



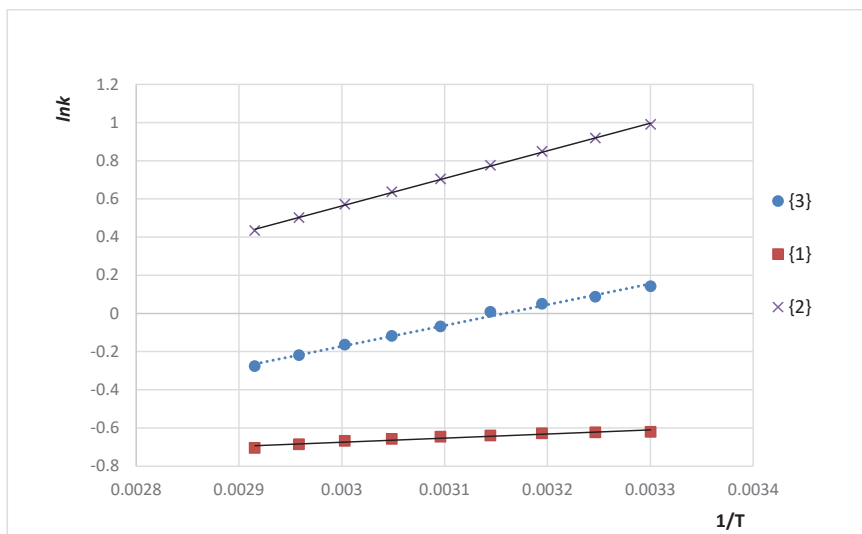
**Figure 3, continued.** Chromatograms of compound (3) at different temperature values (30–70°C).

Average retention time after six measurements in the same temperature values for substances at temperatures from 30 °C to 70 °C with step 5 °C were used for calculation of retention factor  $k$ .

Equations of linear dependence of  $\ln k$  on  $T^{-1}$  were calculated using the program Microsoft Excel by the method of least squares (Table. 1) and the corresponding graphs were built (Fig.4).

**Table 1. Equation of linear dependence  $\ln k$  on  $T^{-1}$** 

Substance	Equation	$R^2$	S (standard regression error)
1	$\ln k = 214.49 T^{-1} - 1.318$	0.938	0.007767
2	$\ln k = 817.35 T^{-1} - 1.088$	0.995	0.007885
3	$\ln k = 1088.8 T^{-1} - 3.439$	0.994	0,012108

**Figure 4.** Graph of dependence  $\ln k$  on  $T^{-1}$  for compounds 1-3.

The values of  $R^2$  for the compounds (2),(3) higher than 0,99 supports the linear nature of the dependence as a result of homogenous reversed phase retention mechanism. As for substance (1), which is a highly polar molecule, the lower  $R^2$  may indicate the contribution of secondary retention mechanisms most likely ion-exchange due to its interaction with free silanol groups.

The standard enthalpies of transfer of the analyte from the mobile phase to the stationary one were calculated using the equation (8) as follows.

Calculations for 2-(thiophene-2-yl)acetohydrazide (1):  
 $\Delta H^0 = -214.5 \times 8.31 = -1780 \text{ J/mol} = -1.78 \text{ kJ/mol}$

For 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (2):

$\Delta H^0 = -1443.2 \times 8.31 = -11990 \text{ J/mol} = -11.99 \text{ kJ/mol}$

For sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thioacetate (3):

$\Delta H^0 = -1088.8 \times 8.31 = -9050 \text{ J/mol} = -9.05 \text{ kJ/mol}$

Calculated enthalpies are summarized in Table 2 along with the corresponding  $\log D$  values at pH 3.

Octanol-water  $\log D$  as a lipophilicity (hydrophobicity) measure influences the retention mechanism in particular in reversed-phase systems. Indeed higher  $\Delta H^0$  by absolute value corresponds to the higher  $\log D$  values and thereupon and to higher retention factor.

According to equation (6), retention is affected by both the enthalpic term  $-\Delta H^0/RT$  and the entropic term  $\frac{\Delta S^0}{R} - \ln \beta$ . According to the Table 2 enthalpic term increase retention because positive but entropic term decrease retention because negative. Also analysis of the Table 2 show that for compounds (2) and (3) the enthalpic term contributes more than entropic term by absolute value. So these compounds better retained due to more hydrophobic interaction with stationary

**Table 2. Standard enthalpies of transfer of analytes from the mobile phase to stationary and LogD of compounds**

Substance	$-\Delta H^0/RT, \text{J/mol}$	$\frac{\Delta S^0}{R} - \ln\beta, \text{J/mol K}$	$\Delta H^0, \text{kJ/mol}$	$\text{Log}D$
{1}	0.720	-1.32	-1.78	0.06
{2}	4.84	-3.77	-11.99	1.24
{3}	3.65	-3.44	-9.05	0.2

phase and have more ordered state. However for compound (1) prevails the entropic term. Indeed, the retention of compound (1) has the lowest value, denoting that the compound is distributed more in the mobile phase, which corresponds to a more disordered state.

#### 4. Conclusion

Standard enthalpies of transfer of analytes from the mobile phase to the stationary one are determined for all substances. The sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio) acetate and its technological impurities are characterized by negative values of the enthalpy of transfer

from the mobile phase to the stationary, so the process of transition of matter from the mobile phase to the stationary prevails over the reverse process. The entropic term influences more than enthalpic term the more polar compound 2-(thiophene-2-yl)acetohydrazide.

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