



*J. Serb. Chem. Soc.* 74 (6) 677–688 (2009)  
JSCS–3866

Journal of  
the Serbian  
Chemical Society

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UDC 543.544+66.097.8+577.15:577.121

Original scientific paper

## Normal-phase thin-layer chromatography of some angiotensin converting enzyme (ACE) inhibitors and their metabolites

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(Received 16 October 2008)

**Abstract:** The separation and chromatographic behaviour of five ACE (angiotensin converting enzyme) inhibitors and their four active metabolites were investigated by normal-phase thin-layer chromatography on silica using several mono- and binary non-aqueous solvent systems. The linear relationship between the  $R_M$  values and the composition of employed mobile phase was obtained. The hydrophobicity parameters  $R_M^0$  and  $C_0$  were determined from the regression data of the plots, analogous to reversed-phase chromatography. The chromatographically obtained hydrophobicity parameters were correlated with the calculated  $\log P$  values. The current results were correlated with the lipophilicity of the studied ACE inhibitors and their metabolites, previously estimated by reversed-phase chromatography.

**Keywords:** ACE inhibitors; normal-phase thin-layer chromatography; hydrophobicity.

### INTRODUCTION

Due to the utmost significance of structure/biological activity relationships of pharmaceuticals, interest in this field of research has been continually increasing. The biological activity of a substance depends on the structural, physical and chemical properties of its molecule and the lipophilicity (hydrophobicity), determining to a great extent biological activity, represents a very important feature. Thus, the well-known Lipinski “rule of 5” predicts that poor absorption or permeation of drugs is more likely when there are more than 5 hydrogen-bond donors or 10 hydrogen-bond acceptors, the molecular weight is greater than 500 and the calculated  $\log P$  ( $C \log P$ ) is greater than 5.<sup>1</sup>

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doi: 10.2298/JSC0906677O

As early as 1964, Fujita *et al.*<sup>2</sup> introduced the concept of hydrophobicity and expressed it by the partition (distribution) coefficient as the  $\log P$  value, defining it as the logarithm of the ratio of the concentrations of the examined substance in both phases of a saturated biphasic system consisting of 1-octanol and water:

$$\log P = \log \frac{c_o}{c_w} \quad (1)$$

where  $c_o$  represents the concentration of the substance in 1-octanol and  $c_w$  its concentration in water when the system is at equilibrium.

The so-called “shake flask” method represents a traditional approach for the determination of the lipophilicity of a molecule, *i.e.*, of the  $\log P$  value.<sup>3</sup> However, since this method suffers from several drawbacks, such as poor reproducibility, time consuming, impossibility to be applied for extremely hydrophilic or lipophilic components, the lipophilicity of a biologically active substances is experimentally determined at present by chromatographic methods, primarily by the highly efficient reversed-phase liquid chromatography (RP-HPLC) and reversed-phase thin-layer chromatography (RP-TLC).<sup>3–6</sup> The chromatographic determination of lipophilicity is based on the distribution of the analyte between an expressively non-polar stationary phase (usually RP-18 silica gel) and a polar mobile phase (a binary system water – organic solvent with a relatively high water content). Taking into consideration that under the conditions of normal-phase chromatography, the analyte is distributed during the chromatographic procedure between the two phases significantly differing from each other in polarity, it is to be expected that this chromatographic method might be employed for the determination of relative lipophilicity. There are even several reports in the available literature describing such attempts.<sup>7–9</sup>

The chromatographic behaviour of different organic and inorganic, primarily biologically active substances, under conditions of reversed- and normal-phase planar chromatography has been the subject of our long-range project. Within the scope of these studies, the chromatographic behaviour of a series of ACE inhibitors has been examined by the methods of RP-TLC, applying conventional reversed-phase chromatography on a thin layer of RP-18 silica gel and binary systems water–organic solvent, as well as the salting-out TLC method.<sup>10,11</sup> Based on the obtained results, the parameters of lipophilicity of the examined compounds were calculated and correlated to computer-calculated  $\log P$  values.

ACE inhibitors belong to a large and very significant family of pharmaceuticals. They are widely applied in clinical practice for the prevention and therapy of hypertension, heart failure and myocardial infarction. These drugs occur in pharmaceutical formulations as esters, which are enzymatically hydrolyzed under *in vivo* conditions to their di-acid forms representing their active metabolites. Lisinopril, already occurring in pharmaceutical formulation in its di-acid form

and captopril, which is not subjected to hydrolysis under *in vivo* conditions but forms disulfides, represent two exceptions among the examined ACE inhibitors.<sup>12,13</sup>

Among the approaches applied for the determination of ACE inhibitors and their metabolites in biological materials and pharmaceutical formulations, several methods, such as HPLC,<sup>14,15</sup> planar chromatography,<sup>15</sup> capillary zone electrophoresis,<sup>16</sup> spectrophotometry,<sup>17</sup> spectrofluorometry<sup>17</sup> and gas chromatography<sup>18</sup> have been employed so far. In addition, the activity and activity/physico-chemical properties relationships of these substances, mainly their lipophilicity,<sup>19–21</sup> were most frequently studied by reversed-phase liquid chromatography procedures.

As a continuation of studies on the chromatographic behaviour of ACE inhibitors, this work was concentrated on the examination of the retention of five ACE inhibitors and their metabolites employing the method of normal-phase thin-layer chromatography (NP-TLC) on silica gel plates. The main objective of this study was to investigate the feasibility of applying the NP-TLC method for the experimental determination of lipophilicity of these compounds.

#### EXPERIMENTAL

The substances investigated throughout the present study are listed in Table I.

The TLC experiments were performed on silica gel 10×10 cm TLC plates (Art. 5644, Merck, Darmstadt, Germany). The plates were spotted with 2 µl aliquots of freshly prepared ethanolic solutions of substances **1**, **3**, **5** and **8**, an aqueous solution of **7** and methanolic solutions of substances **2**, **4**, **6** and **9** (all about 2 mg/ml) and developed by the ascending technique. The solvent systems employed are listed in Tables II and III. All the components contained in the employed mobile phases were of analytical grade purity.

After development, detection was realised by exposing the plates to iodine vapour. All investigations were performed in triplicate at ambient temperature (22±2 °C).

TABLE I. The investigated substances

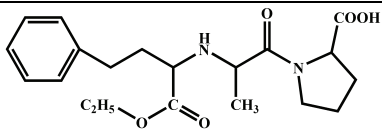
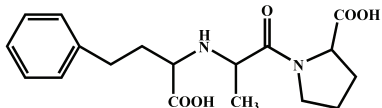
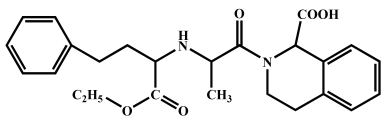
No	Structure	Name
1		Enalapril, (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline Krka Research and Development Division
2		Enalaprilat, (S)-1-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]-L-proline dehydrate Krka Research and Development Division
3		Quinapril, [3S-[2[R*(R*)],3R*]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid Parke-Davis Pharmaceutical Research

TABLE I. Continued

No	Structure	Name
4		Quinaprilat, [3 <i>S</i> -[2[ <i>R</i> *( <i>R</i> *)],3 <i>R</i> *]]-2-[2-[(1-carboxy-3-phenylpropyl)-amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid Parke-Davis Pharmaceutical Research
5		Fosinopril, [1 [5*( <i>R</i> *)],2 <i>α</i> ,4 <i>β</i> ]-4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)-phosphinyl]acetyl]-L-proline Bristol-Myers Squibb Pharmaceutical Research Institute
6		Fosinoprilat, <i>trans</i> -4-cyclohexyl-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline Bristol-Myers Squibb Pharmaceutical Research Institute
7		Lisinopril, ( <i>S</i> )-1-[ <i>N</i> <sup>2</sup> -(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dehydrate Belupo Pharmaceutical & Cosmetic Quality Control Department
8		Cilazapril, [1 <i>S</i> -[1 <i>α</i> ,9 <i>α</i> ( <i>R</i> *)]]-9-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]octahydro-10-oxo-6 <i>H</i> -pyridazino[1,2- <i>a</i> ]-[1,2]diazepine-1-carboxylic acid monohydrate Roche Pharmaceuticals
9		Cilazaprilat, [1 <i>S</i> -[1 <i>α</i> ,9 <i>α</i> ( <i>R</i> *)]]-9-[(1-carboxy-3-phenylpropyl)amino]octahydro-10-oxo-6 <i>H</i> -pyridazino [1,2- <i>a</i> ]- [1,2]diazepine-1-carboxylic acid Roche Pharmaceuticals

TABLE II.  $R_F \times 100$  values of the investigated substances obtained by mono-component mobile phase

Mobile phase	Substance <sup>a</sup>								
	1	2	3	4	5	6	7	8	9
Methanol	81	54	92	86	94	86	12	64	35
Ethanol	77	22	88	71	92	80	11	44	37
<i>n</i> -Propanol	67	15	63	38	68	42	10	24	4
Isopropanol	60	8	63	22	75	55	0	20	0
Isobutanol	20	2	41	18	43	29	0	16	0
Acetone	18	0	25	12	14	4	0	8	0
Ethyl methyl ketone	30	0	41	17	19	14	0	11	0

<sup>a</sup>The numbers denote the substances, see Table I

TABLE III.  $R_F \times 100$  values of the investigated substances obtained with two-component mobile phases

Substance <sup>a</sup>	$y(\text{ethanol}) / \%$														
	Ethanol–ethyl methyl ketone					Ethanol–carbon tetrachloride					Ethanol–toluene				
	50	40	30	20	10	80	70	60	50	40	80	70	60	50	40
<b>1</b>	59	51	44	34	25	66	59	51	43	35	48	45	42	38	33
<b>2</b>	6	5	4	3	1	19	16	13	10	5	19	15	12	9	5
<b>3</b>	77	73	70	68	66	74	69	66	63	60	79	74	68	64	62
<b>4</b>	67	64	61	57	53	53	47	42	37	29	64	60	57	54	51
<b>5</b>	62	57	53	49	44	70	62	54	47	40	72	68	65	62	58
<b>6</b>	14	11	9	6	4	21	18	16	13	9	37	32	25	18	12
<b>7</b>	5	3	1	0	0	5	3	2	0	0	11	8	6	4	0
<b>8</b>	37	32	28	22	18	50	46	40	34	28	51	48	46	44	41
<b>9</b>	7	5	2	0	0	8	6	4	3	0	16	13	10	7	4

<sup>a</sup>The numbers denote the substances, see Table I

The  $R_M$  values were calculated for each solute in each mobile phase according to the Bate-Smith and Westall equation:<sup>22</sup>

$$R_M = \log (1/R_F - 1) \quad (2)$$

## RESULTS AND DISCUSSION

The chromatographic behaviour of the examined ACE inhibitors and their metabolites and the feasibility of applying the normal-phase TLC method for the determination of their lipophilicity were investigated using thin-layer silica gel plates and several non-aqueous mono- and two-component solvents as the mobile phase. The results are summarized in Tables II and III, respectively.

The results obtained throughout the study of ACE inhibitors and their metabolites employing mono-component solvents (Table II) show a satisfactory accordance to their chromatographic behaviour with the method of normal-phase thin-layer chromatography. Namely, the retention order of the examined substances obtained by alcohols as the mobile phase is in agreement with the elution strength,  $\epsilon^0$ , as well as with the polarity of the applied solvents, *i.e.*, the less polar the solvent, the stronger the retention. Hence, the strongest retention of the examined substances was recorded when isobutanol, as the least polar among the applied alcohols with a  $P'$  value of 3.9, was used and the weakest retention of the ACE inhibitors and their metabolites was observed when the very polar methanol ( $P'$  of 6.6)<sup>23</sup> was employed. However, such a regularity was not observed in case of ketones.

Also, the results obtained during the examinations of the ACE inhibitors and their metabolites applying two-component solvents (Table III) demonstrate a decrease of  $R_F$  values, *i.e.*, increased retention of the examined substances in paral-

lel with increasing concentrations of the less polar component in the mobile phase, which is in accordance with the normal-phase chromatographic mode.

The results summarized in Tables II and III indicate significant differences in the  $R_F$  values, *i.e.*, in the retention of the examined ACE inhibitors and their metabolites. In all instances, it was established that the metabolites exhibit a stronger retention, *i.e.*, they have lower  $R_F$  values compared to the corresponding parent ACE inhibitor. Such a behaviour of the examined substances under conditions of normal-phase TLC is contrary to that observed by reversed-phase chromatography (both the salting-out method and classical reversed-phase TLC chromatography on RP-18 silica) when the retention of the less polar ACE inhibitors was found to be much stronger than that of their metabolites.

This distinction in the chromatographic behaviour of the ACE inhibitors and their metabolites results from differences in their interaction with silica gel. Namely, due to the presence of two carboxylic groups in the molecule of the metabolites (including substance **7**), their specific interactions with silica gel (hydrogen bonds) are much stronger than those of the corresponding ACE inhibitors, containing only one carboxylic group within their molecule.

Based on the obtained retention parameters of the examined ACE inhibitors and the corresponding metabolites, separation factors ( $\log \alpha$ ) were calculated (Table IV). Comparison of these values and the values of the separation factors calculated for two reversed-phase systems<sup>11</sup> revealed no significant differences in the separation selectivity of the ACE inhibitors and their metabolites between normal- and reversed-phase methods.

The retention behaviour of the examined substances obtained by reversed-phase TLC can be graphically presented as the relationship of the  $R_M$  value and the content of the less polar component of the mobile phase. The obtained linear relationships can be presented by the equation of a straight line  $R_M = R_M^0 + mC$ . The value of the intercept,  $R_M^0$ , represents the lipophilicity of the examined substance, while the value of the slope,  $m$ , corresponds to the specific hydrophobic surface area of this substance and  $C$  represent the content of the more polar component in the mobile phase. Based on the obtained intercept and slope values, another hydrophobic parameter,  $C_0 = -R_M^0/m$ , can be calculated. This hydrophobicity parameter corresponds to the parameter  $\varphi_0$ , previously defined for the HPLC method as the concentration of the organic component in the mobile phase for which the distribution of the analyzed substance between the mobile and stationary phase is equal (1:1).<sup>25</sup>

The same approach was applied in previous attempts to employ normal-phase chromatography for lipophilicity determinations.<sup>7-9</sup> Accordingly, the results obtained throughout the present study by normal-phase TLC are expressed analogously to those obtained by reversed-phase chromatography as the relationship of the  $R_M$  values and the content of ethanol, as the more polar component, in

the mobile phase. As seen from Fig. 1, linear relationships with very high values of the correlation coefficients were recorded for all employed solvent systems. Based on the intercept values and the slope of the plots, the parameter  $C_0$  was calculated. The obtained regression parameters are presented in Table V. The results clearly demonstrate lower  $R_M^0$  values for the more lipophilic compounds, *i.e.* the ACE inhibitors in relation to the corresponding metabolites. This phenomenon was the consequence of the application of normal-phase chromatographic method for the estimation of hydrophobicity of the examined substances and can be solved by presenting linear relationships of the retention and the content of less polar (instead of more polar) component in a binary non-aqueous mobile phase.

TABLE IV. The logarithm of separation factors calculated by relation:  $\log \alpha = |\Delta R_M|^{24}$

Solvent system	$y^a / \%$	Log $\alpha_{1,2}$	Log $\alpha_{3,4}$	Log $\alpha_{5,6}$	Log $\alpha_{8,9}$
Ethanol–ethyl methyl ketone	50 <sup>a</sup>	1.353	0.217	1.001	0.892
	40	1.296	0.182	1.030	0.951
	30	1.174	0.174	1.057	1.280
	20	1.222	0.205	1.178	–
	10	1.519	0.236	1.275	–
Ethanol–carbon tetrachloride	80	0.918	0.402	0.943	1.061
	70	0.878	0.400	0.871	1.125
	60	0.843	0.428	0.790	1.204
	50	0.832	0.462	0.773	1.222
	40	1.010	0.565	0.829	–
Ethanol–toluene	80	0.595	0.326	0.641	0.738
	70	0.666	0.278	0.655	0.791
	60	0.725	0.205	0.746	0.885
	50	0.792	0.180	0.871	1.019
	40	0.971	0.195	1.005	1.222
Water–methanol	40	1.091	1.059	–	1.173
	50	0.863	0.933	–	1.159
	60	0.654	0.701	0.272	0.815
	70	0.540	0.598	0.219	0.577
	80	0.593	0.528	0.340	0.511
Water–acetone	10	1.261	1.201	–	1.431
	20	1.113	0.983	0.505	1.189
	30	1.008	0.844	0.413	1.050
	40	0.806	0.800	0.403	0.957
	50	0.725	0.716	0.429	0.858

In order to check the applicability of normal-phase thin-layer chromatography for the determination of the lipophilicity of the examined substances, the hydrophobic parameters  $R_M^0$  and  $C_0$  were correlated with computer-assisted calculations of the values of  $\log P^{11}$  and with experimentally determined ones.<sup>26</sup> Experimentally determined  $\log P$  values are available for substances **1** (2.45), **3**



(3.72), **5** (6.61) and **7** (−1.22). As it can be seen from Tables VI and VII, relatively satisfactory linear relationships<sup>27</sup> were obtained for all solvent systems employed in this study (in all instances  $r$  was statistically significant at the  $P < 0.05$  level).

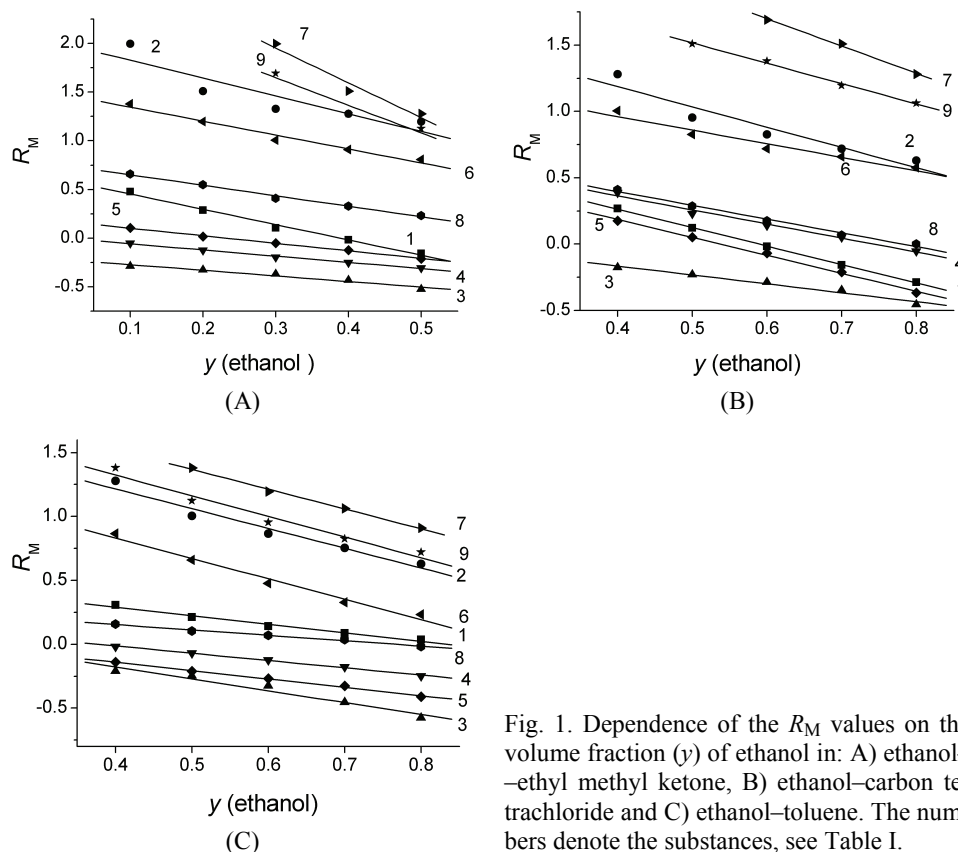


Fig. 1. Dependence of the  $R_M$  values on the volume fraction ( $y$ ) of ethanol in: A) ethanol–ethyl methyl ketone, B) ethanol–carbon tetrachloride and C) ethanol–toluene. The numbers denote the substances, see Table I.

Comparison of these results with previous data obtained by reversed-phase TLC<sup>11</sup> strongly recommends normal-phase thin-layer chromatography as a suitable method for the estimation of the lipophilicity of the examined substances.

In addition, the hydrophobic parameters,  $R_M^0$ , obtained throughout the present study by normal-phase TLC using ethanol–ethyl methyl ketone, were correlated with the  $R_M^0$  parameters obtained by reversed-phase TLC using water–methanol as the mobile phase. Based on the relationship presented in Fig. 2, it can be seen that the examined substances are classified into groups forming two series. The metabolites of the examined ACE inhibitors, being more polar than the corresponding parent molecules, belong to the first series and the second series includes the more lipophilic ACE inhibitors, themselves. The exception is fo-



TABLE V. Regression hydrophobicity parameters of the investigated compounds

No. <sup>a</sup>	Log <i>P</i>	Ethanol-ethyl methyl ketone			Ethanol-carbon tetrachloride			Ethanol-toluene					
		<i>R</i> <sub>M</sub> <sup>0</sup>	- <i>m</i>	- <i>r</i>	<i>C</i> <sub>0</sub>	<i>R</i> <sub>M</sub> <sup>0</sup>	- <i>m</i>	- <i>r</i>	<i>C</i> <sub>0</sub>	<i>R</i> <sub>M</sub> <sup>0</sup>	- <i>m</i>	- <i>r</i>	<i>C</i> <sub>0</sub>
1	0.33	0.612±0.028	1.576±0.084	0.996	0.388	0.82±0.010	1.394±0.016	0.999	0.590	0.559±0.032	0.671±0.053	0.991	0.833
2	-0.59	2.011±0.165	1.832±0.498	0.905	1.097	1.801±0.163	1.532±0.264	0.958	1.175	1.836±0.112	1.549±0.182	0.980	1.185
3	1.77	-0.215±0.022	0.578±0.066	0.981	-0.371	0.104±0.036	0.673±0.059	0.989	0.155	0.194±0.068	0.930±0.110	0.980	0.209
4	1.12	0.006±0.007	0.638±0.022	0.998	0.010	0.789±0.045	1.061±0.072	0.993	0.743	0.216±0.015	0.571±0.025	0.997	0.378
5	8.93	0.179±0.007	0.774±0.022	0.999	0.231	0.727±0.026	1.353±0.042	0.997	0.538	0.121±0.016	0.655±0.026	0.998	0.185
6	5.38	1.489±0.045	1.434±0.134	0.987	1.038	1.372±0.079	1.026±0.128	0.977	1.338	1.472±0.077	1.599±0.124	0.991	0.920
7	-0.48	3.028±0.301	3.584±0.736	0.979	0.845	2.933±0.102	2.057±0.145	0.997	1.426	2.144±0.047	1.551±0.071	0.998	1.382
8	1.04	0.758±0.018	1.077±0.054	0.996	0.704	0.812±0.035	1.039±0.057	0.996	0.782	0.322±0.013	0.421±0.020	0.996	0.766
9	0.46	2.498±0.302	2.834±0.739	0.968	0.881	2.282±0.050	1.532±0.076	0.997	1.490	1.971±0.106	1.618±0.172	0.983	1.218

<sup>a</sup>The numbers denote the substances, see Table I

TABLE VI. Equations and correlation coefficients for  $R_M^0$  and  $C_0$  vs. the calculated  $\log P$  values

Solvent system	Equation	$-r$	$SD$
Ethanol–ethyl methyl ketone	$R_M^0 = 1.874 \pm (0.355) - (1.212 \pm (0.372)) \log P$	0.825	0.786
	$C_0 = 0.775 \pm (0.143) - (0.512 \pm (0.150)) \log P$	0.836	0.317
Ethanol–carbon tetrachloride	$R_M^0 = 1.855 \pm (0.288) - (0.944 \pm (0.302)) \log P$	0.813	0.638
	$C_0 = 1.130 \pm (0.154) - (0.424 \pm (0.162)) \log P$	0.761	0.342
Ethanol–toluene	$R_M^0 = 1.488 \pm (0.247) - (0.868 \pm (0.259)) \log P$	0.832	0.547
	$C_0 = 1.093 \pm (0.097) - (0.460 \pm (0.102)) \log P$	0.897	0.215

TABLE VII. Equations and correlation coefficients for  $R_M^0$  and  $C_0$  vs. the experimentally determined  $\log P$  values

Solvent system	Equation	$-r$	$SD$
Ethanol–ethyl methyl ketone	$R_M^0 = 2.019 \pm (0.650) - (0.387 \pm (0.161)) \log P$	0.861	0.907
	$C_0 = 0.556 \pm (0.341) - (0.098 \pm (0.085)) \log P$	0.632	0.477
Ethanol–carbon tetrachloride	$R_M^0 = 2.036 \pm (0.647) - (0.304 \pm (0.160)) \log P$	0.801	0.902
	$C_0 = 1.042 \pm (0.303) - (0.126 \pm (0.075)) \log P$	0.765	0.422
Ethanol–toluene	$R_M^0 = 1.527 \pm (0.332) - (0.267 \pm (0.082)) \log P$	0.917	0.462
	$C_0 = 1.126 \pm (0.182) - (0.164 \pm (0.045)) \log P$	0.931	0.254

sinoprilat, which practically represents an outlier for the metabolites. However, this deviation is in accordance with the structural diversity of the investigated substances: in contrast to the other studied metabolites that contain two carboxylic groups, fosinoprilat contains one carboxylic and one phosphinyl group. (As is known, a good correlation is only possible in closely related analogue series.<sup>1</sup>)

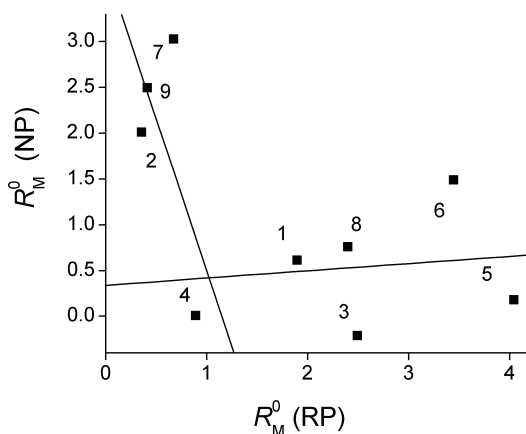


Fig. 2. Correlation between the NP (ethanol–ethyl methyl ketone) and RP (water–methanol) lipophilicity chromatographic data. The numbers denote the substances, see Table I.

#### CONCLUSIONS

The results obtained during the current study on the retention behaviour of several ACE inhibitors and their metabolites applying normal-phase TLC on silica gel plates, *i.e.*, conspicuous differences between the  $R_F$  values of these two

groups of substances, clearly demonstrate that this method is very suitable for their chromatographic separation. Based on the observed correlation of the chromatographically determined hydrophobicity parameters  $R_M^0$  and  $C_0$  and computer-assisted calculated  $\log P$  values, it can be concluded that normal-phase TLC represents a reliable method for an estimation of the lipophilicity of the examined substances. Comparison of the results obtained by normal-phase TLC with those obtained by conventional reversed-phase TLC revealed no significant differences with regard to the estimation of the lipophilicity.

*Acknowledgement.* The authors are grateful to the Ministry of Science of the Republic of Serbia (Project No. 142062) for financial support.

## ИЗВОД

НОРМАЛНО-ФАЗНА ТАНКОСЛОЈНА ХРОМАТОГРАФИЈА НЕКИХ АСЕ  
ИНХИБИТОРА И ЊИХОВИХ МЕТАБОЛИТА

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ДУШАНКА МИЛОЈКОВИЋ-ОПСЕНИЦА<sup>3</sup> и ЖИВОСЛАВ ТЕШИЋ<sup>3</sup>

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Хроматографско раздвајање и понашање пет АСЕ инхибитора (инхибитора ангио-тензин-конвертујућег ензима) и њихова четири активна метаболита испитивано је методом нормално-фазне танкослојне хроматографије на силика-гелу применом неколико једно- и дво-компонентних неводених система растварача. Добијена је линеарна зависност између  $R_M$ -вредности и концентрације етанола у мобилној фази. На основу одговарајућих регресионих података, по аналогији са реверзно-фазном хроматографијом, израчунати су параметри липофилности  $R_M^0$  и  $C_0$ . Хроматографски добијени параметри хидрофобности корелисани су са израчунатим  $\log P$  вредностима. Такође,  $R_M^0$ -вредности добијене у овом раду корелисане су са  $R_M^0$ -вредностима добијеним методом реверзно-фазне танкослојне хроматографије.

(Примљено 16. октобра 2008)

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