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

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Abstract: The chromatographic behaviour of some ACE inhibitors and their active metabolites was examined under conditions of reversed-phase thin-layer chromatography on RP-18 silica using water–methanol, water–ethanol and water–acetone as binary solvent systems. The relationship between the R_M values and the concentration of organic modifier in the mobile phases was linear. It was found that an increase in the content of the organic modifier in the employed solvent systems led to a decrease of the R_M values, *i.e.*, of the retention. Also, the more hydrophobic compounds had a longer retention. Based on regression analysis of the plots, the lipophilicity parameters R_M^0 and c_0 were calculated. The chromatographically obtained lipophilicity parameters were correlated with the calculated log *P* values.

Keywords: ACE inhibitors, reversed-phase thin-layer chromatography, lipophilicity.

INTRODUCTION

Angiotensin converting enzyme (ACE) inhibitors are known as the most suitable drugs for the prevention and treatment of hypertension and congestive heart failure. These well tolerated drugs are esterified pro-drugs which undergo enzymatic *in vivo* hydrolysis to be converted into pharmacologically active metabolites, *i.e.*, the corresponding di-acid forms characterized by a high affinity for the ACE in arterial, cardiac and renal tissues and a long-lasting and potent ACE inhibitory effect.[1]

A number of methods, such as high performance liquid chromatography (HPLC), capillary zone electrophoresis and gas chromatography have been developed so far for the analysis of ACE inhibitors in biological fluids (urine or serum), as well as in pharmaceutical formulations.[2–5] However, only in a few papers were

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planar chromatography investigations of the above mentioned drugs described.[6,7] There are no data in the available literature referring to systematic examinations of the chromatographic behaviour and separation of a great number of ACE inhibitors and their active metabolites.

In our previous studies of ACE inhibitors in different chromatographic systems,[8–10] salting-out thin-layer chromatography (SOTLC) was found to be a suitable reversed-phase method for the investigation of their hydrophobicity. In continuation of these examinations, in the present work conventional reversed-phase thin-layer chromatography (RP-TLC) was used for the separation and rapid hydrophobicity determination of several ACE inhibitors and their active metabolites.

EXPERIMENTAL

The substances investigated are listed in Table I.

The TLC experiments were performed on commercially available RP-18 silica (Art. 5559, E. Merck, Darmstadt, Germany). The plates were spotted with 1.0 μL aliquots of freshly prepared ethanolic solutions of enalapril, quinapril, fosinopril and cilazapril, aqueous solution of lisinopril and methanolic solutions of enalaprilat, quinaprilat, fosinoprilat and cilazaprilat (about 2 mg/mL), and were developed by the ascending technique.

Water–organic modifier binary systems of widely variable composition were used as the mobile phase: **A**. water –methanol (40 – 80 vol %); **B**. water – ethanol (30 – 70 vol%) and **C.** water – acetone (10 – 50 vol%).

All components of the employed mobile phases were of analytical grade purity.

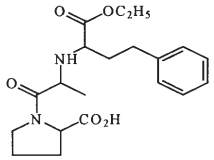
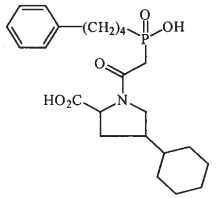
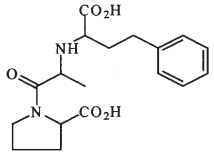
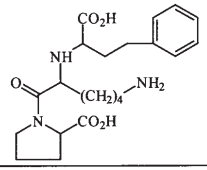
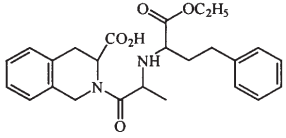
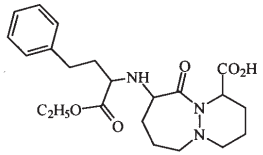
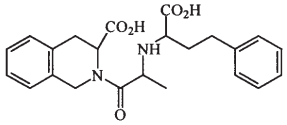
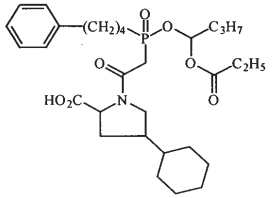
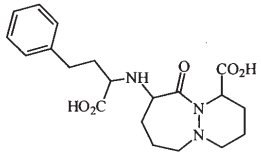
After development, detection was performed by exposing the plates to iodine vapour. All investigations were performed at room temperature (22 ± 2 °C).

RESULTS AND DISCUSSION

The results obtained are listed in Table II. From these data it can be seen that an increase in the concentration of the organic modifier in the mobile phase led to an increase of the hR_F values, *i.e.*, to a decrease of the retention of the investigated substances. More hydrophobic compounds had a stronger retention compared to those with hydrophilic groups in the molecule. Accordingly, the adsorption of the di-acid forms of the ACE inhibitors (metabolites) was lower in comparison with the corresponding pro-drug forms. For the same water content in the mobile phase, the retention was the strongest when methanol was used as the organic modifier. Simultaneously, in that case, the best selectivity in the separation of the metabolites originating from the corresponding pro-drugs was observed (the highest ΔR_F values).

Disregarding the structural differences in the metabolites analyzed, a unique retention order of these compounds was established when solvent systems **A** and **B** were employed: R_F(**6**) < R_F(**7**) < R_F(**4**) < R_F(**9**) < R_F(**2**). In the case when the mobile phase **C** was used, an inversion in the order of compounds **7** and **4** was noticed, although the differences in their R_F values were relatively small. In contrast to the metabolites, the pro-drug forms of the examined substances had a regular chromatographic behaviour, *i.e.*, a unique order of the R_F-values in all the examined cases: R_F(**5**) < R_F(**3**) < R_F(**8**) < R_F(**1**).

TABLE I. The investigated substances

No	Structure	Name	No	Structure	Name
1		**Enalapril,** (S)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-*L*-alanyl] -*L*-proline; Krka Research and Development Division	6		**Fosinoprilat,** trans-4-cyclohexyl-1-[[hydroxy (4-phenylbutyl) phosphinyl] acetyl]-*L*- proline; Bristol-Myers Squibb Pharmaceutical Research Institute
2		**Enalaprilat,** (S)-1-[*N*-(1-carboxy-3-phenylpropyl)-*L*-alanyl]-*L*-proline dihydrate; Krka Research and Development Division	7		**Lisinopril,** (S)-1-[N^2-(1-carboxy-3-phenylpropyl)-*L*-lysyl]-*L*-proline dihydrate; Belupo Pharmaceutical&Cosmetic Quality Control Department
3		**Quinapril,** [3S-[2[R*(R*)],3R*]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4 -tetrahydro-3-isoquinolinecarboxylic acid; Hemofarm Pharmaceutical Industry	8		**Cilazapril,** [1S-[1α,9α (R*)]]-9-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino] octahydro-10-oxo-6H-pyridazino[1,2-a] [1,2]diazepine-1-carboxylic acid monohydrate; Roche Pharmaceuticals
4		**Quinaprilat,** [3S-[2[R*(R*)],3R*]]-2-[2-[(1-carboxy-3-phenylpropyl) amino]-1-oxopropyl]-1,2,3,4-tetra hydro-3-isoquinolinecarboxylic acid; Parke-Davis Pharmaceutical Research			
5		**Fosinopril,** [1 [S*(R*)],2α,4β]-4-cyclohexyl-1-[[[2-methyl-1-(1- oxo propoxy)propoxy](4-phenylbutyl) phosphinyl]acetyl]-*L*-proline; Bristol-Myers Squibb Pharmaceutical Research Institute	9		**Cilazaprilat,** [1S-[1α,9α (R*)]]-9-[(1-carboxy-3-phenylpropyl)amino]octahydro-10-oxo-6H-pyridazino [1,2-a] [1,2]diazepine-1-carboxylic acid; Roche Pharmaceuticals

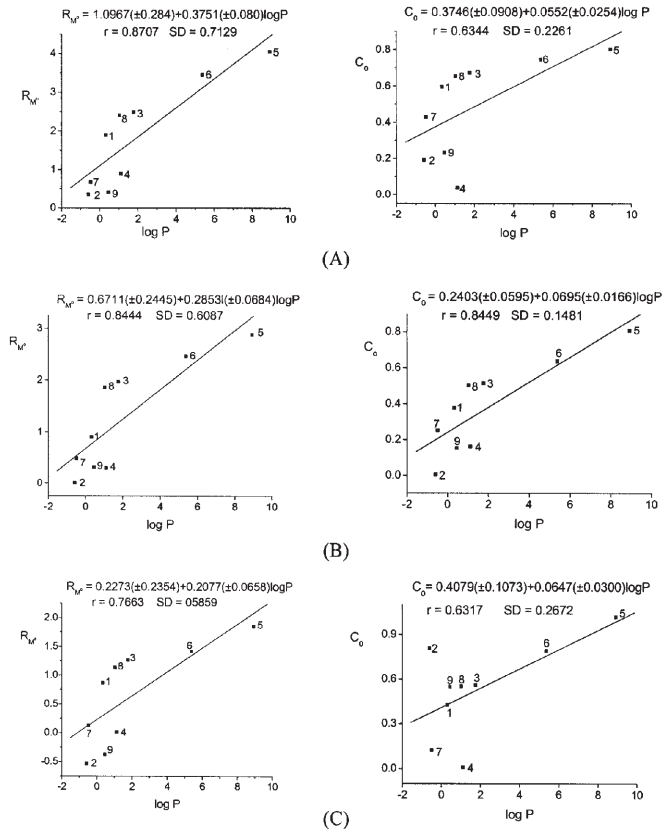


Fig. 1. Correlation between the hydrophobicity parameters R_M^0 and c_0 of the investigated substances and the calculated log *P* values for different mobile phases: water–methanol (**A**), water–ethanol (**B**) and water–acetone (**C**). The numbers on the lines denote the substances from Table I.

The chromatogrphic behaviour of the examined substances could be expressed by the R_M values, which were calculated for each solute in each mobile phase according to the Bate–Smith and Westall equation:[11] $R_M = \log (1/R_F–1)$. In all instances, a linear relationship between the R_M values and the concentration of organic modifier in mobile phases was established. This relationship can be expressed by the equation: $R_M = R_M^0 + mc$, where R_M^0 represents the y-axis intercept, m is the slope of the plot and c the concentration of the organic modifier.

From the obtained intercepts (R_M^0) and the slopes (m), the hydrophobicity parameter c_0 was calculated as $c_0 = –R_M^0/m$.[12] The corresponding regression data are listed in Table III. Both chromatographic parameters, R_M^0 and c_0, are generally used for the characterization of hydrophobicity of molecules.[13,14]

From the results listed in Table III, it can be seen that, in most cases, the hydrophobicity parameters R_M^0 decreased when water–methanol as the mobile phase was replaced by the water–acetone system. A lower lipophilicity of the di-acid forms (metabolites) in comparison with those of the corresponding pro-drugs of the ACE inhibitors was clearly observed from the obtained R_M^0 values. Also, a significant difference in the hydrophobicity between the investigated ACE

TABLE II. hR_F values of the investigated substances

Substances[a]	Water–methanol					Water–ethanol					Water–acetone				
	40	50	60	70	80	30	40	50	60	70	10	20	30	40	50[b]
1	18	34	52	70	80	41	52	65	76	86	18	26	34	49	58
2	73	79	83	89	94	79	83	86	91	95	80	82	84	86	88
3	10	16	35	57	75	11	30	51	71	82	8	14	21	30	42
4	56	62	73	84	91	66	72	79	85	91	58	61	65	73	79
5	0	0	8	27	47	0	3	9	20	28	0	3	5	7	10
6	0	8	14	38	66	0	12	20	39	65	5	9	12	16	23
7	51	54	62	72	81	59	64	71	80	89	65	68	71	77	83
8	12	18	38	60	78	13	33	52	73	83	10	17	23	32	44
9	67	76	80	85	92	68	75	82	88	93	75	76	77	81	85

[a]The numbers denote the substances: see Table I; [b]The percentage concentration of organic modifier

TABLE III. Regression hydrophobicity parameters of the investigated compounds

N_o[a]	log*P*	Water/methanol				Water/ethanol				Water/acetone			
		R_M^0	$-m$	$-r$	c_0	R_M^0	$-m$	$-r$	c_0	R_M^0	$-m$	$-r$	c_0
1	0.33	1.895±0.084	3.177±0.136	0.997	0.596	0.902±0.053	2.391±0.106	0.998	0.377	0.866±0.030	2.034±0.090	0.997	0.426
2	–0.59	0.355±0.126	1.859±0.204	0.983	0.191	0.009±0.136	1.773±0.273	0.977	0.005	–0.530±0.01	0.656±0.023	0.998	0.808
3	1.77	2.492±0.113	3.705±0.183	0.996	0.673	1.969±0.170	3.830±0.342	0.992	0.514	1.265±0.022	2.261±0.065	0.999	0.559
4	1.12	0.890±0.134	2.308±0.217	0.987	0.037	0.295±0.076	1.820±0.154	0.993	0.162	0.010±0.046	1.108±0.138	0.978	0.009
5	8.93	4.045±0.506	5.043±0.719	0.990	0.802	2.877±0.281	3.566±0.514	0.990	0.807	1.854±0.043	1.822±0.116	0.996	1.018
6	5.38	3.448±0.296	4.622±0.450	0.991	0.746	2.460±0.234	3.863±0.426	0.994	0.637	1.417±0.044	1.793±0.132	0.992	0.790
7	–0.48	0.671±0.120	1.565±0.194	0.978	0.429	0.484±0.148	1.916±0.297	0.977	0.253	0.129±0.047	1.037±0.142	0.973	0.124
8	1.04	2.401±0.131	3.665±0.213	0.995	0.655	1.858±0.144	3.696±0.290	0.994	0.503	1.138±0.028	2.060±0.084	0.998	0.552
9	0.46	0.411±0.122	1.759±0.197	0.982	0.233	0.310±0.070	2.013±0.141	0.995	0.154	–0.373±0.05	0.682±0.141	0.942	0.547

[a]See Table I.

inhibitors and their metabolites was confirmed by the values of the slopes (m), which are related to the specific hydrophobic surface area of the solutes.[15] Since there are no data in the literature for experimentally determined log P values of the investigated ACE inhibitors, the chromatographically determined hydrophobicity parameters R_M^0 and c_0, were correlated with the calculated log P values[16] (Table III) and the results are depicted in Fig. 1.

On the basis of these results, it can be seen that in both cases a satisfactory correlation was obtained. However an even better correlation was observed for the R_M^0 parameters, especially for the solvent system methanol–water.

The results obtained by studying the pro-drug forms of the ACE inhibitors demonstrate a perfect accordance of all the chromatographically determined hydrophobicity parameters (R_M^0, m and c_0), as well as of the calculated log P values with the established retention order of these compounds.

CONCLUSIONS

The results presented in this paper indicate that reversed-phase thin-layer chromatography is a suitable method for the separation of ACE inhibitors from their metabolites. The separation of the investigated drugs is based on their structural characteristics and their interaction with both the sorbent and the solvents. Although the separation was satisfactory with all solvent systems employed, the best results were obtained with acetone as the organic modifier. The satisfactory correlations of R_M^0, as a measure of hydrophobicity, with the values of the slope (m) of the retention – concentration of organic modifier plots, as well as with the calculated log P values indicate that reversed-phase thin-layer chromatography represents a reliable method for the rapid, simple and inexpensive determination of the hydrophobicity of the investigated compounds.

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ИЗВОД

РЕВЕРСНО-ФАЗНА ТАНКОСЛОЈНА ХРОМАТОГРАФИЈА НЕКИХ ИНХИБИТОРА АНГИОТЕНЗИН-КОНВЕРТУЈУЋИХ ЕНЗИМА (ACE) И ЊИХОВИХ АКТИВНИХ МЕТАБОЛИТА

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Испитивано је хроматографско понашање неких ACE инхибитора и њихових активних метаболита у условима реверсно-фазне танкослојне хроматографије на RP-18

силика-гелу применом следећих бинарних система растварача: вода–метанол, вода–етанол и вода–ацетон. Утврђена је линеарна зависност између R_M–вредности и концентрације органског модификатора у мобилној фази. Нађено је да повећање садржаја органског модификатора у употребљеном растварачу доводи до опадања R_M-вредности, односно до слабљења ретенције. Јача ретенција уочена је за хидрофобније супстанце. На основу одговарајућих регресионих података, израчунати су параметри липофилности R_M^0 и c_0. Хроматографски утврђени параметри липофилности корелисани су са израчунатим log *P* вредностима.

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REFERENCES

1. S. Yamada, I. Muraoka, K. Kato, Y. Hiromi, R. Takasu, H. Seno, H. Kawahara, T. Nabeshima, *Biol. Pharm. Bull*. **26** (2003) 872
2. C. Abbara, G. Aymard, S. Hinh, B. Diquet, *J. Chromatogr. B*. **766** (2002) 199
3. J. A. Prieto, U. Akesolo, R. M. Jimenez, R. M. Alonso, *J. Chromatogr. A* **916** (2001) 279
4. H. H. Maurer, T. Kraemer, J. W. Arlt, *Ther. Drug Monit.* **20** (1998) 706
5. S. Saglik, O. Sagirli, S. Atmaca, *Anal. Chim. Acta* **427** (2001) 253
6. D. Kowalczuk, H. Hopkala, R. Pietras, L. Ersoy, *J. Planar Chromatogr.* **16** (2003) 196
7. V. Čaplar, S. Rendić, F. Kajfez, H. Hofman, J. Kuftinec, N. Blažević, *Jug. Pharmacol. Acta* **32** (1982) 125
8. M. B. Aleksić, D. G. Agbaba, D. M. Milojković-Opsenica, Ž. Lj. Tešić, *Chromatographia* **53** (2001) 442
9. M. B. Aleksić, D. G. Agbaba, R. M. Baošić, D. M. Milojković-Opsenica, Ž. Lj. Tešić, *J. Serb. Chem. Soc.* **66** (2001) 39
10. J. Odović, B. Stojimirović, M. Aleksić, D. Milojković-Opsenica, Ž. Tešić, *Proc. Int. Symp. Planar Separations, Planar Chromatography* 2004, p. 497
11. E. C. Bate-Smith, R. G. Westall, *Biochim. Biophys. Acta* **4** (1950) 427
12. A. Pyka, M. Miszczyk, *Chromatographia* **61** (2005) 37
13. K. Dross, C. Sonntag, R. Mannhold, *J. Chromatogr.* **673** (1994) 113
14. M. L. Bieganowska, A. Doraczynska, A. Petruczynska, *J. Planar Chromatogr.* **8** (1995) 122
15. T. Cserháti, E. Forgács, *J. Chromatogr. A* **771** (1997) 105
16. www.logP.com.