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Thin-layer chromatography of several antihypertensive drugs from the group of angiotensin converting enzyme inhibitors

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A rapid and simple method for the chromatographic separation of pharmacologically active components contained in some antihypertensive drugs has been developed employing thin-layers of silica gel and polyacrylonitrile sorbent (PANS). The active compounds of *Captopril* – (S)-1-(3-mercapto-2-methyl-1-oxopropyl)-*L*-proline, *Enalapril* – (S)-1- N- 1-(ethoxycarbonyl)-3-phenylpropyl -*L*-alanyl -*L*-proline, *Lisinopril* – (S) -1- N^2-(carboxy-3-phenylpropyl)-*L*-lysyl -*L*-proline, *Quinapril* – 3S- 2 R*(R*) ,3R* , -2- 2- 1(- ethoxycarbonyl)-3-phenylpropyl amino -1-oxopropyl -1,2,3,4-tetrahydro-3-iso-quinoline-carboxylic acid, *Ramipril* – 2S- 1 R*(R*) ,2 3a ,6a -1- 2 1-(ethoxycarbonyl)-3-phenylpropyl amino -1-oxopropyl octahydrocyclopenta b -pyrole-2-carboxylic acid and *Cilazapril* – 1S- 1 ,9 (R*) - 9- 1-(ethoxycarbonyl)-3-phenylpropyl amino octahydro-10-oxo-6H-pyridazino 1,2-a 1,2 diazepine-1-carboxylic acid, were successfully separated by the presented procedures. For their chromatographic separation on silica gel sixteen and on PANS thirteen solvents were used.

Keywords: thin-layer chromatography, silica gel, polyacrylonitrile sorbent, antihypertensive drugs, angiotensin converting enzyme inhibitors.

INTRODUCTION

The compounds examined throughout the present study (Fig. 1) belong to the group of angiotensin converting enzyme inhibitors (ACE inhibitors). Several approaches for their isolation and determination in different biological fluids have been described so far in the available literature, whereby liquid[1–6] and gas crhomatography[7] were usually employed. Only a single paper[8] related to the determination of *Captopril* by thin-layer chromatography (TLC) on silica gel and by liquid, as well as by gas chromatography has been reported. Our previous studies[9–17] were focused on the chromatographic behaviour of different compounds using silica gel, cellulose and PANS thin-layers and the corresponding separation mechanisms were considered. In conti-

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nuation of these studies, the present work was concentrated on the examinations of the chromatographic behaviour of the aforementioned anti-hypertensives on thin layers of silica gel and polyacrylonitrile sorbents which significantly differ in their characteristics. In this way, an attempt was made to establish the optimal conditions for the separation and possible determination of this class of pharmacologically active compounds since their behaviour under conditions of the planar chromatography has not been examined so far.

EXPERIMENTAL

The chromatographic investigations were performed by the method of horizontal TLC on silica gel (Art. 5644 HPTLC Fertigplatten Kieselgel 60 für die Nano-DC, Merck, Germany) and PANS (10 10 cm). For this purpose, a Camag HPTLC Chamber under the Tank Configuration Developing Conditions was used. The PANS preparation and application to plates were performed as previously described.[11] The plates were spotted with small volumes of ethanolic solutions of the samples to be examined with the exception of *Lisinopril* which was dissolved in water.

The compositions of the solvent systems used are listed in Table I. Detection was performed by exposing the plates to iodine vapour.

TABLE I. Solvent systems used

No	Composition	Proportions (v/v)
1	*n*-Propanol	
2	Ethanol	
3	Acetone	
4	*i*-Butanol	
5	*i*-Propanol	
6	Methanol	
7	Methyl-ethyl-ketone	
8	*n*-Butanol	
9	*n*-Bytil-acetate	
10	Methanol : trichloromethane	20 : 80
11	Acetone : benzene	50 : 50
12	*n*-Butyl-acetate : ethanol	50 : 50
13	Ethanol : carbon-tetrachloride	50 : 50
14	Ethanol : acetic acid	95 : 5
15	Benzene : acetone	30 : 70
16	*n*-Butyl-acetate : acetone	50 : 50
17	Methyl-ethyl-ketone : benzene	25 : 50
18	*n*-Butanol : water : ehtanol	75 : 10 : 20
19	*n*-Butanol : water : NH_3	30 : 15 : 60
20	Acetone : *n*-propanol : benzene	50 : 50 : 50
21	*n*-Butyl-acetate : acetone : benzene	75 : 50 : 50
22	Chloroform : methyl-ethyl-ketone : benzene : ethanol	25 : 25 : 50 : 100

v/v Refers to volume in milliliters

RESULTS AND DISCUSSION

TLC on silica gel

As can be seen from Table I, sixteen solvents were used for the chromatographic separation of the examined compounds. The hR_F values obtained in this way are presented in Table II.

TABLE II. hR_F values of the investigated substances on silica gel

Substances		Solvent systems[a]															
		1	2	3	4	5	6	7	8	10	11	12	13	14	15	18	19
1	*Captopril*	59	77	58	64	77	63	64	73	79	62	35	35	68	46	56	36
2	*Enalapril*	30	54	9	20	28	57	19	33	70	35	22	32	58	10	22	31
3	*Lisinopril*	0	14	0	0	0	34	0	0	0	0	0	0	0	0	0	0
4	*Quinapril*	56	74	43	49	59	71	42	58	85	65	51	71	78	17	50	44
5	*Ramipril*	39	71	14	42	44	69	33	52	83	53	44	50	75	14	44	41
6	*Cilazapril*	37	63	11	38	33	67	23	39	82	41	37	44	69	12	42	33

[a] The compositions of the solvent systems are given in Table I

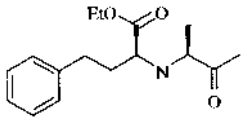
Considernig the complexity of the structures and structural differences among the investigated compounds, no unique retention order could be expected. However, in all cases, a regular chromatographic behaviour of the structurally similar compounds was observed, and the following order of the hR_F values was recorded:

hR_F (2) < hR_F (6) < hR_F (5) < hR_F (4);

hR_F (3) < hR_F (2) < hR_F (1),

where the numbers in parentheses correspond to the ordinal numbers of the compunds listed in Table II. It is known that under the conditions of adsorption chromatography on silica gel, hydrogen bonds formed with the silanol groups of the sorbent, dipole-dipole and other electrostatic interactions determine the retention of the analyzed compounds.[18] Taking into consideration the structure of the compounds examined throughout the present study, it could be supposed that the formation of hydrogen bonds between their electronegative oxygen, nitrogen and sulphur atoms, on the one hand, and the silanol groups of the silica gel, on the other, represents a possible retention mechanism.

As shown in Fig. 1, part of the structure X, *Enalapril*, *Ramipril* and *Quinapril* is identical, where

X = 

Hence, it is clear that differences in the structure of the remaining parts of the molecules determine their chromatographic behaviour. The lower mobility of *Enalapril* compared with the two other comopounds is due to the formation of stronger hydrogen bonds, probably because its five-membered ring is less sterically hindered than in relation to the condensed five- and six-membered rings of *Ramipril* and *Quinapril*, respectively. For the

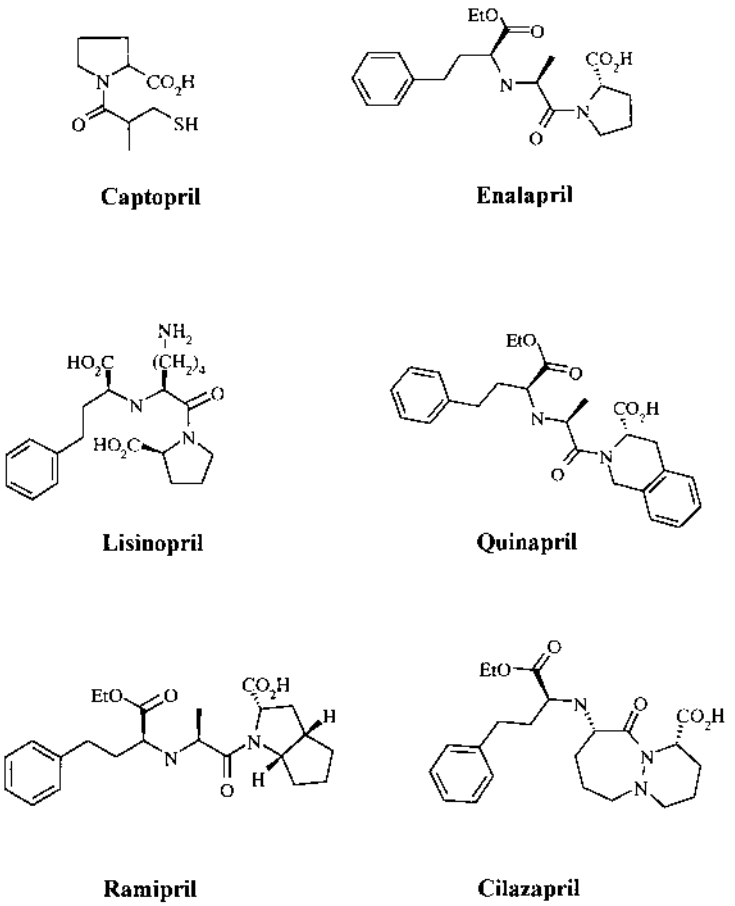


Fig. 1. Structures of investigated ACE inhibitors.

same reason, it is likely that the hR_F values of *Cilazapril*, which is structurally very similar to aforementioned compounds, were higher in all cases than those of *Enalapril*.

In addition to the common 1-oxo-proline part of the molecule, *Captopril*, *Enalapril* and *Lisinopril* contain chemically different structural parts, but express a regular chromatographic behaviour. *Captopril* has the highest mobility which can be ascribed to the fact that, besides the oxygen of the carboxyl group which is capable of hydrogen bond formation, it contains only the thiol group sulphur which, due to its lower electronegativity, has a weaker interaction with the sorbent. Accordingly, the reason for the lowest mobility of *Lisinopril* should be searched for in its capability of forming four hydrogen bonds.

In conclusion, the application of the solvents employed in the present study makes it possible to establish the optimal solvent system for the separation and determination of compounds belonging to the group of anti-hypertensives employing TLC on silica gel plates.

TLC on PANS

For the separation of the examined compounds on thin-layer of PANS, thirteen solvents were employed (Table I) and the obtained hR_F values are listed in Table III. The following orders of the hR_F values of structurally similar compounds were observed:

hR_F (3) < hR_F (2) < hR_F (1);

hR_F (4) < hR_F (6) < hR_F (2) < hR_F (5).

TABLE III. hR_F values of the investigated substances on polyacrylonitrile sorbent

Substances		Solvent systems[a]												
		1	2	3	6	8	9	11	12	16	17	20	21	22
1	*Captopril*	73	80	93	84	76	76	87	78	90	74	84	94	90
2	*Enalapril*	67	73	83	71	64	29	83	58	86	56	81	92	85
3	*Lisinopril*	9	28	0	55	0	0	0	10	0	0	0	30	23
4	*Quinapril*	61	60	77	65	61	15	50	38	74	9	70	80	57
5	*Ramipril*	70	78	84	74	70	68	87	68	90	63	86	97	94
6	*Cilazapril*	64	63	80	68	62	26	63	52	82	40	72	88	68

[a] The compositions of the solvent systems are given in Table I

The retention order of *Lisinopril*, *Enalapril* and *Captopril* was the same as when they were analyzed by TLC on silica gel. The strongest *Lisinopril* sorption could be the consequence of strong specific interaction of its polar functional groups with the polar parts of the sorbent surface. Namely, based on the studies of the chromatographic behaviour of different organic and inorganic compounds on thin-layer PANS,[11,14–17] it has been established that the sorbent can interact specifically with substances undergoing separation by hydrogen bond formation between the cyano-groups of the sorbent and proton-donor groups of the compounds to be analyzed, or through electrostatic interactions between the methyne hydrogen atoms of the sorbent and electronegative atoms of the compounds being chromatographed, as well as by other electrostatic interactions. The order of the hR_F values of the compounds examined in the present study was the same order both on silica gel and PANS when the same solvent systems were used, but the retention on PANS was weaker than on silica gel. If it is assumed that the formation of hydrogen bonds between the electronegative atoms of the compounds being examined and the hydrogen atoms at the surface of the sorbents is the dominant separation mechanism, the weaker sorption on PANS could be explained in terms the bonds formed with the methyne hydrogen atoms having lower energy than the bonds formed with the hydrogen atoms of the silanol groups.

Examination of the chromatographic behaviour of *Ramipril*, *Enalapril* and *Quinapril* revealed in all cases the following retention order: hR_F (4) < hR_F (2) < hR_F (5). A stronger sorption of *Quinapril* in relation to the other two compounds, as well as in relation to their retention order established on silica gel, is very probably the consequence of the additional aromatic ring within its structure, *i.e.,* it could result from donora–aceptor interactions of this -electron system with the cyano groups of the sorbent.[14,17]

The results presented in this paper demonstrate that quite a number of the examined solvent systems can be successfully applied for the separation of the pharmacologically active components contained in anti-hypertensive drugs from the group of ACE inhibitors.

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И З В О Д

ТАНКОСЛОЈНА ХРОМАТОГРАФИЈА НЕКИХ АНТИХИПЕРТЕНЗИВА ИЗ ГРУПЕ АНГИОТЕНЗИН КОНВЕРТУЈУЋИХ ЕНЗИМ ИНХИБИТОРА

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У раду је у условима хроматографије на танким слојевима силика-гела и полиакрилонитрилног сорбента (ПАНС-а), извршено раздвајање следећих антихипертензива из групе ангиотензин конвертујућих ензим инхибитора (АЦЕ инхибитора): *Каптоприл*-а, *Еналаприл*-а, *Лизиноприл*-а, *Квинаприл*-а, *Рамиприл*-а, *Цилазаприл*-а. За њихово хроматографско раздвајање употребљени су многи растварачи. На основу добијених резултата дискутовани су механизми одвајања. На тај начин дата је једна веома брза и једноставна метода за одвајање поменутих супстанци.

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