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THIN-LAYER CHROMATOGRAPHIC STUDIES OF SOME ANGIOTENSIN CONVERTING ENZYME INHIBITORS AND THEIR INCLUSION COMPLEXES WITH β-CYCLODEXTRIN

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ABSTRACT. The aim of this paper is to examine the chromatographic behaviour of some angiotensin converting enzyme (ACE) inhibitors (lisinopril, fosinopril, zofenopril) under conditions of direct phase thin-layer chromatography (Kiselgel 60) and their inclusion complexes with β-cyclodextrin using reversed phase thin-layer chromatography (Kiselgel 60 F254 silaniziert). Binary systems angiotensin converting enzyme inhibitor-β-cyclodextrin were prepared using the kneading method, in 1:1 molar ratio. The thin-layer chromatography reveals that the inclusion complexes show lower hRf values compared to the two components of the inclusion complex, proving that there is an interaction between the active substances and β-cyclodextrin, due to the formation of the inclusion complexes.

Keywords: ACE-inhibitors, β-cyclodextrin, thin-layer chromatography, inclusion complex, kneading method

INTRODUCTION

The angiotensin-converting enzyme (ACE) inhibitors represent a class of substances widely used in the treatment of essential hypertension, congestive heart failure, diabetic nephropathy and after myocardial infarction. Their action is based on the inhibition of ACE, blocking hereby the conversion of angiotensin I in angiotensin II, a vasoconstrictor biomolecule. The inhibition of ACE also induces the increase of the bradikinin, a vasodilatator biomolecule (Ferrar et al., 2003; Remko, 2007).

Lisinopril, (LIS) (1-[N2-[(S)-1-carboxy-3-phenylpropil]-L-lysyl]-L-proline dihydrate), belongs to the class of dicarboxilic-conteining inhibitors and it is not a pro-drug.

Fosinopril, (FOS) (4-cyclohexyl-1-[2-[(2-methyl-1-propanoyloxy-propoxy)-(4-phenylbutyl) phosphoryl] acetyl]-pyrrolidine-2-carboxylic acid, comprises in its molecule the phosphinic group and it has a high lipophilicity (Remko, 2007).

Zofenopril, (ZOF) [1(S),4(S)]-1(3-mercapto-2-metil-1-oxopropil) 4 feniltio-L- prolina-S-benzoilester, is characterized by the presence of sulf in its molecule, which is responsible for the antioxidant properties of this compound. It has high lipophilicity (Evangelista and Manzini, 2005; Subissi et al., 2009).

Fig. 1 presents the chemical structure of the ACE inhibitors used in this study.

Cyclodextrins (CD) are cyclic oligosaccharides consisting of six, seven (β-cyclodextrin), eight glucopyranose units linked by α-(1,4) bonds. The inclusion of various pharmaceutical substances in their cavity leads to the improving of some physico-chemical properties of the included compounds, the enhancing of stability and bioavailability (Loftson et al., 2005, 2007).

MATERIALS AND METHODS

Materials

• Lisinopril dihydrate (Medochemie, Limassol, Cyprus)

• Fosinopril sodium (Terapia–Ranbaxy, Cluj-Napoca, Romania)

• Zofenopril calcium (Berlin-Chemie Menarini, Berlin, Germany)

• β-cyclodextrin (β-CD) (Fluka Chemie GmbH, Germany)

• the substances and the solvents used are of analytical grade requested by the Romanian Pharmacopoeia 10th ed (1993) and by European Pharmacopoeia 5th ed (2004)

• chromatographic plates Kiselgel 60 (Art 5748 DC-Plasstikfolien Kiselgel 60, Merck, Darmstadt, Germany), 20 X 20 cm, with a width of 0.2 mm

• chromatografic plates Kiselgel 60 F254 (Art 5747 DC-Fertigplatten Kiselgel 60 F254 silanisiert Merck, Darmstadt, Germany), 20 X 20 cm, with a width of 0.25 mm.

Preparation of inclusion complexes

Binary systems ACE inhibitors – β-CD were prepared using the kneading method (kneaded products, KP). For this purpose, the amounts corresponding to molar ratio 1:1 guest substance:β-CD were weighed. The obtained mixture was pulverized in a mortar and then kneaded in a quantity of solvent equal to the sum of the amounts of ACE inhibitor – β-CD, until the bulk of solvents evaporated. The solvent used was: water in the case of LIS, ethanol-water (50:50, w/w) in the case of FOS and methanol-water (50:50, w/w) in the case of ZOF. After drying at room temperature, the products were dried in oven at 105˚C. Afterwards, they were pulverized and sieved (100 μm).

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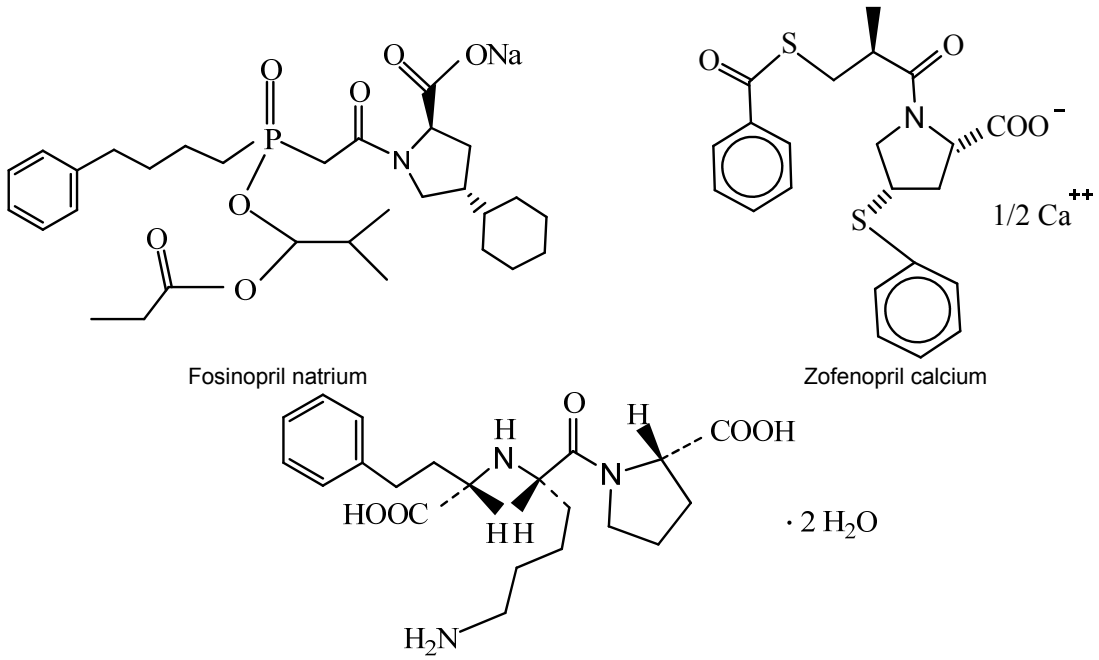

Fig. 1 Chemical structure of lisinopril, fosinopril and zofenopril

Thin-layer chromatography (TLC)

Planar chromatographic method is mentioned in the literature when investigating the chromatographic behaviour of ACE inhibitors (Aleksic et al., 2001; Odovic et al., 2006) as well as the inclusion complexes character between them and cyclodextrins (Şoica et al., 2008; Trandafirescu et al., 2005; Wang et al., 2001).

Chromatographic investigations were performed at room temperature (22 ± 2˚C), by the method of ascendant TLC on silica gel and silica gel silanized. The plates were spotted with 5 µl aliquots of freshly prepared ethanol solution of FOS, water solution of LIS and ethanol:chlorhidric acid 1N 1.98: 0.0459 (v/v) solution of ZOF, (about 2 mg/ml), at a distance of 2 cm between them. The compositions of the mobile phases used are shown in table 1. The hRf values of the examined substances were determined.

For the analysis of inclusion complex character of the active substances with β-cyclodextrin, chromatographic plates were spotted as shown in table 2. The composition of the solvent systems was: n-butanol : acetone : water : glacial acetic acid 80:20:10:0.3 (v/v) for LIS and ethyl acetate : water 80:40 (v/v) for ZOF and FOS.

The migration distance was 10 cm from the start line. The chromatographic plates were dried in air after both development and revelation. Detection was performed by exposing the plates to iodine vapor. TLC analysis conditions are characteristic of the guest substance.

RESULTS AND DISCUSSIONS

According to the dates shown in Table 3, the compounds characterized by high lipophilicity, FOS and ZOF, present a higher mobility compared to LIS. The following order of the hRf was recorded: hRf (FOS) > hRf (ZOF) > hRf (LIS). For mobile phases 6 and 7 an inversion of hRf between FOS and ZOF was observed.

Table 1

The composition of the mobile phases used

No.	Composition	Proportion (v/v)
1	Ethanol	
2	Methanol	
3	Ethanol : water : chlorhidric acid 1N	89 : 10 : 1
4	Metanol : water : chlorhidric acid 1N	97 : 2 : 1
5	Metanol : water : chlorhidric acid 1N	89 : 10 : 1
6	Ethyl acetate : water: chlorhidric acid 1N	95 : 2 :1
7	Ethyl acetate : ethanol : water : chlorhidric acid 1N	80 : 10 : 2 : 1
8	*n*-Butanol : water : chlorhidric acid	95 : 2 : 1
9	*i*-Propanol : water : chlorhidric acid 1N	97 : 2 : 1
10	*n*-Butanol : Acetone	80 : 20

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Table 2

Samples applied on the chromatographic plates

Substances	Spot 1	Spot 2	Spot 3	Spot 4
Lisinopril	2mg/ml LIS in water	5 mg/ml β-CD in distilled water	LIS and β-CD solution forming an "in situ" mixture	2 mg/ml binary system LIS-β-CD in distilled water
Fosinopril	2mg/ml FOS in ethanol	5 mg/ml β-CD in distilled water	FOS and β-CD solution forming an "in situ" mixture	2 mg/ml binary system FOS-β-CD in distilled water
Zofenopril	2 mg/ml ZOF in ethanol : chlorhidric acid 1N 1.98:0.0459(v/v)	5 mg/ml β-CD in distilled water	ZOF and β-CD solution forming an "in situ" mixture	2 mg/ml binary system ZOF-β-CD in distilled water

Table 3

The hRf values of the studied substances on silica gel

Substances	Mobile phases used[a]									
	1	2	3	4	5	6	7	8	9	10
Lisinopril	3	29	18	28	36	0	0	0	0	0
Fosinopril	84	90	91	92	88	16	71	58	68	55
Zofenopril	77	85	89	87	83	38	79	57	67	24

a The composition of the mobile phases is listed in Table 1

It is known that, the retention of the investigated substances, under the condition of planar chromatography on silica gel, is the result of the hydrogen bond formed with the silanol groups of the sorbent, dipole-dipole and other electrostatic interaction (Aleksic et al., 2001).

The bigger adsorption observed for LIS, compound with diacid function, is probably caused by its capacity of forming four hydrogen bonds with the silanol group of the sorbent. The FOS and ZOF compound, having 1-oxo-proline as common structural part, present in their molecule, beside the oxygen of the carboxyl group which can form hydrogen bonds, the phosphorus of the phophinic group and the sulphur of thiolic groups, which, due to their lower electronegativity, have a weaker interaction with the sorbent.

Figures 2-4 represent chromatograms of the investigated ACE inhibitors and their inclusion complexes with β-CD.

Examination of chromatograms shows that the active substances migrate over a different distance on the chromatographic plate, the hRf value being 61 for LIS, 86 in case of FOS and 92 for ZOF, while β-CD remains at the start line.

For the "in situ" mixture, both the guest substance and β-CD acts similarly individual compounds, which rules out the forming of an IC on the chromatographic plate. In the case of binary systems, ACE inhibitor is retained by β-CD at the start line, proving that the active substance is included in β-CD cavity, forming an inclusion complex.

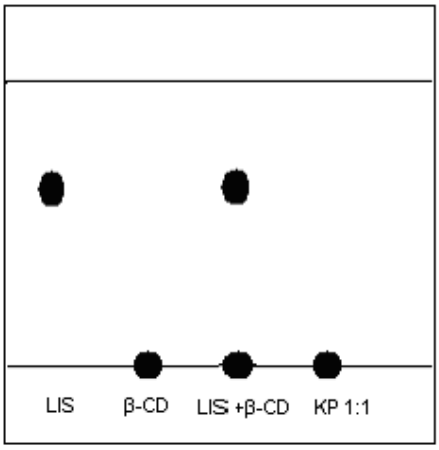

Fig. 2 TLC of LIS and its IC with β-CD

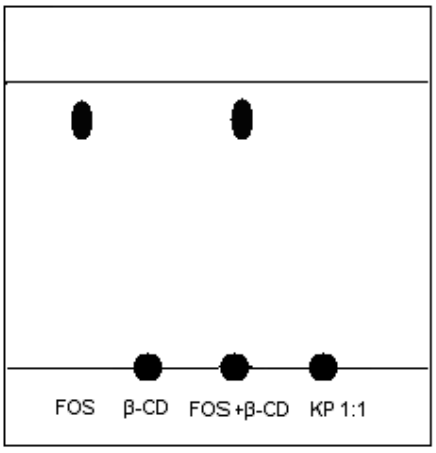

Fig. 3 TLC of FOS and its IC with β-CD

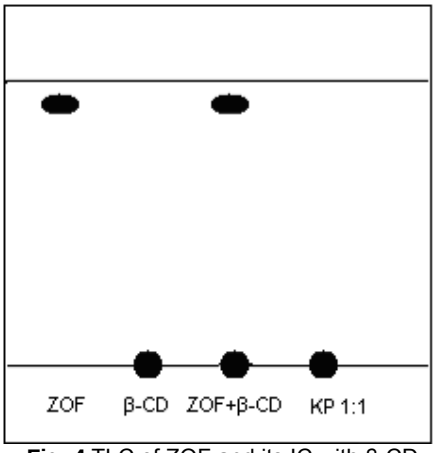

Fig. 4 TLC of ZOF and its IC with β-CD

CONCLUSIONS

Thin-layer chromatography has proved to be a simple, rapid and inexpensive method useful in analyses of ACE inhibitors and their inclusion complexes whit β-CD.

Binary compounds obtained by kneading method have a chromatographic behaviour which differs from that of the active substances, the hRf value of complexes being smaller than that of the guest molecules. Inclusion complex character of the complexation products has been also pointed out using this method.

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