

THIN-LAYER CHROMATOGRAPHY ANALYSIS FOR CYCLODEXTRINS INCLUSION COMPLEXES OF FOSINOPRIL AND ZOFENOPRIL

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Abstract

The aim of this paper is to emphasize the inclusion complex character between fosinopril, zofenopril and natural and semisynthetic cyclodextrins (β -cyclodextrin, hydroxypropyl- β -cyclodextrin and randomly methylated- β -cyclodextrin), using thin-layer chromatography.

Fosinopril and zofenopril are the most hydrophobic substances among the angiotensin-converting enzyme inhibitors; the cyclodextrins are used to improve the physico-chemical properties of pharmaceutical substances in order to enhance some biopharmaceutical properties of the drugs.

Binary systems angiotensin-converting enzyme inhibitor – cyclodextrin were prepared using the kneading method, in 1:1 molar ratio. Thin-layer chromatography reveals that the inclusion complexes show lower R_F values compared to those of the angiotensin-converting enzyme inhibitors, as an index of molecular interaction between the components, due to the formation of inclusion complexes.

Rezumat

Obiectivul lucrării este evidențierea prin cromatografie pe strat subțire a complexului de incluziune dintre fosinopril, zofenopril și ciclodextrinele naturale și de semisinteză (β -ciclodextrină, hidroxipropil- β -ciclodextrină și β -ciclodextrină metilată aleator).

Fosinoprilul și zofenoprilul sunt cei mai hidrofobi reprezentanți ai clasei inhibitorilor enzimei de conversie a angiotensinei; ciclodextrinele sunt utilizate pentru îmbunătățirea unor proprietăți fizico-chimice ale substanțelor medicamentoase, în scopul optimizării unor proprietăți biofarmaceutice.

Sistemele binare inhibitor al enzimei de conversie - ciclodextrină au fost preparate utilizând metoda malaxării, în raport molar de 1:1. Analiza prin cromatografie pe strat subțire relevă faptul că produșii de complexare prezintă valori R_F mai mici decât cele

ale substanțelor active individuale, punând astfel în evidență existența unei interacțiuni la nivel molecular între componente, datorită formării complexilor de incluziune.

Keywords: fosinopril, zofenopril, cyclodextrins, thin-layer chromatography.

Introduction

The angiotensin-converting enzyme (ACE) inhibitors act on the rennin-angiotensin-aldosterone system, which is known as a key element in the adjustment of the blood pressure and hydro-electrolyte homeostasy. Today they are widely used in the treatment of essential hypertension, congestive heart failure, diabetic nephropathy and after myocardial infarction [6].

Fosinopril, (2S,4S)-4-cyclohexyl-1-(2-{{[2-methyl-1-(propanoyloxy) propoxy](4-phenylbutyl)phosphoryl}acetyl}pyrrolidine-2-carboxylic acid, is the only representative of the class of phosphorus-containing inhibitors. It has a very high lipophilicity, low water solubility and poor bioavailability following oral administration [2, 8].

Zofenopril, (2S,4S) - 1 - [(2S) - 3 - (benzoylsulphanyl - 2 - methyl propanoyl]-4-phenylsulphanylpyrrolidine-2-carboxylic acid, is characterized by the presence of sulphur in its molecule, which is responsible for the antioxidant properties of this compound. It presents selectivity for ACE within the cardiac tissue. Zofenopril has a high lipophilicity and its water solubility is 0.3 mg/mL [3, 9].

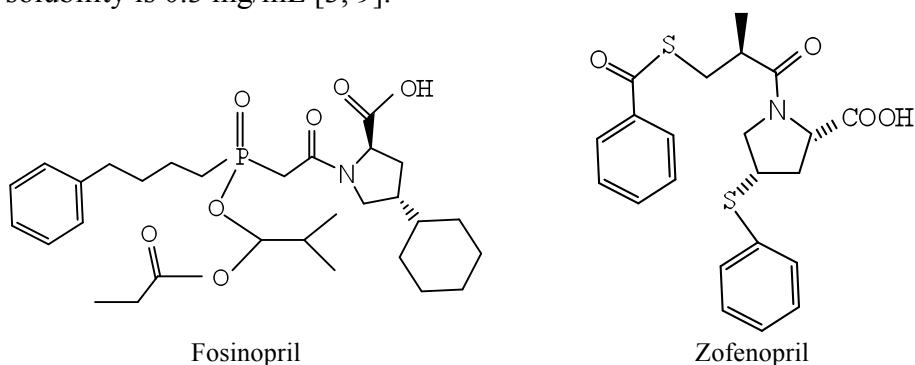


Figure 1
Chemical structure of fosinopril and zofenopril

Cyclodextrins (CD) are cyclic oligosaccharides consisting of glucopyranose units linked by α -(1,4) bonds obtained by the enzymatic modification of starch. They have the capacity of forming inclusion complexes (IC) by inclusion in their cavity of several pharmaceutical substances. As a result of encapsulation in cyclodextrins, some physico-

chemical and biopharmaceutical properties of the included compounds can be favorably modified: enhancing of solubility, stability, reduction of volatility, unpleasant taste and smell and improved bioavailability [4, 5].

This paper presents the capacity of fosinopril sodium and zofenopril calcium to form inclusion complexes with cyclodextrins and emphasizes the character of inclusion complex of the binary systems obtained using thin-layer chromatography.

Materials and methods

Materials

- Fosinopril sodium salt (FOS) (Terapia-Ranbaxy, Cluj-Napoca, Romania)
- Zofenopril calcium salt (ZOF) (Berlin-Chemie Menarini, Berlin, Germany)
- β -cyclodextrin (β -CD) (Fluka Chemie GmbH, Germany)
- hydroxypropyl- β -cyclodextrin (HPBCD) (Cyclolab R&D, Budapest, Hungary)
- randomly methylated- β -cyclodextrin (RAMEB) (Cyclolab R&D, Budapest, Hungary)
- the substances and the solvents used are of analytical grade requested by the Romanian Pharmacopoeia 10th ed [12] and by the European Pharmacopoeia 5th ed [13]
- chromatographic plates Kieselgel 60 (Art 5748 DC-Plastikfolien Kieselgel 60, Merck, Darmstadt, Germany), 20 X 20 cm, with a width of 0.25 mm

Preparation of inclusion complexes

Binary systems FOS - cyclodextrins (β -CD, HPBCD, RAMEB) and ZOF - β -CD were prepared using the kneading method (kneaded products, KP). For this purpose, the amounts corresponding to the molar ratio 1:1 guest substance : cyclodextrin were weighed. The obtained mixture was pulverized in a mortar and then kneaded with a quantity of ethanol-water (50:50, w/w) in the case of fosinopril, and methanol-water (50:50, w/w) in the case of zofenopril, equal to the sum of the amounts of ACE inhibitor - cyclodextrin, until the bulk of solvents evaporated. After drying at room temperature, the products were dried in the oven at 105°C. Afterwards, they were pulverized and sieved (100 μ m) [4, 5, 11].

Thin-layer chromatography (TLC)

Planar chromatography, a rapid and inexpensive method is mentioned in the literature for investigating the chromatographic behavior

of ACE inhibitors [1, 7] as well as the inclusion complex character between some pharmacons with very low hydrosolubility like imidazole derivatives, sulphonamidic diuretics and cyclodextrins [10, 11].

Chromatographic investigations were performed at room temperature ($22 \pm 2^\circ\text{C}$), by the method of ascendant TLC. The plates were spotted with 5 μL of the samples to be examined, at a distance of 2 cm between them according to table I. The composition of the mobile phases used and the R_f values of the examined substances are shown in table II. The migration distance was 10 cm from the start line. The chromatographic plate was dried in air after both development and revelation. Detection was performed by exposing the plates to iodine vapour. TLC analysis conditions are characteristics of the guest substance [1, 7].

Table I
Samples applied on the chromatographic plate

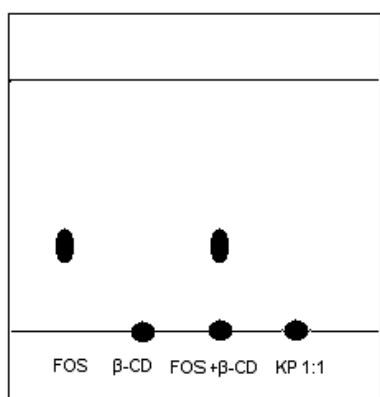
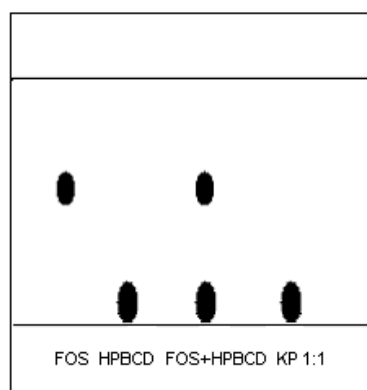
Substances	Spot 1	Spot 2	Spot 3	Spot 4
Fosinopril sodium	2 mg/mL FOS in ethanol	5 mg/mL β -CD in distilled water	FOS and β -CD solution forming an " <i>in situ</i> " mixture	2 mg/mL binary system FOS- β -CD in distilled water
Fosinopril sodium	2 mg/mL FOS in ethanol	5 mg/mL HPBCD in distilled water	FOS and HPBCD solution forming an " <i>in situ</i> " mixture	2 mg/mL binary system FOS-HPBCD in distilled water
Fosinopril sodium	2 mg/mL FOS in ethanol	5 mg/mL RAMEB in distilled water	FOS and RAMEB solution forming an " <i>in situ</i> " mixture	2 mg/mL binary system FOS-RAMEB in distilled water
Zofenopril calcium	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 " <i>in situ</i> " mixture	2 mg/mL binary system ZOF- β -CD in distilled water

Table IIMobile phases used and hR_f values of the investigated substances

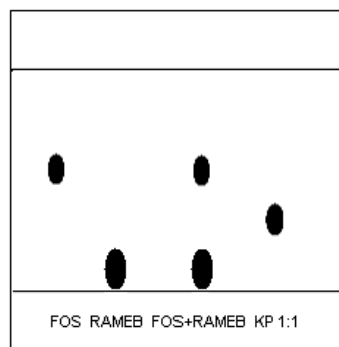
ACE inhibitors	Cyclodextrins	Mobile phase	hR_f values of ACE inhibitors
Fosinopril sodium	β -CD	<i>n</i> -Butanol	34
Fosinopril sodium	HPBCD	<i>n</i> -Butanol : Acetone 80:20 (v/v)	55
Fosinopril sodium	RAMEB	<i>n</i> -Butanol : Acetone 80:20 (v/v)	55
Zofenopril calcium	β -CD	Ethyl acetate : water 98:2 (v/v)	41

Results and discussion

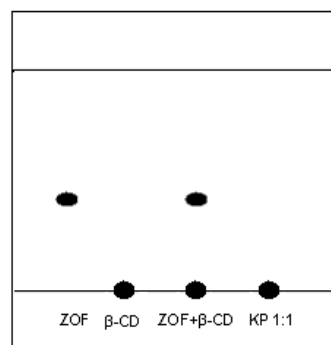
Figures 2-5 represent chromatograms of the investigated ACE inhibitors and their inclusion complexes with cyclodextrins.

**Figure 2**TLC of FOS, and its KP with β -CD**Figure 3**

TLC of FOS and its KP with HPBCD

**Figure 4**

TLC of FOS and its KP with RAMEB

**Figure 5**TLC of ZOF and its KP with β -CD

The examination of β -CD binary systems chromatograms discloses that FOS and ZOF, in the presence of solvents systems used, migrate on the chromatographic plate, while β -CD remains at the start line. For the “*in situ*” mixture, the guest substance migrates similarly to the ACE inhibitor alone and β -CD remains at the start line, which rules out the forming of an IC on the chromatographic plate. In the case of kneaded products, the ACE inhibitor is retained by β -CD at the start line, proving that an inclusion complex is formed between the ACE inhibitor and β -CD.

For the binary system FOS - HPBCD, one can notice that cyclodextrin migrates at a very small distance on the chromatographic plate, presenting a long-oval shaped spot. The “*in situ*” mixture acts exactly like the two substances, demonstrating the existence of only a physical mixture, excluding the possibility of complex formation on the chromatographic plate. The kneaded product FOS – HPBCD migrates as the cyclodextrin, having the same chromatographic behavior as cyclodextrin.

In the case of the binary system FOS – RAMEB, the cyclodextrin hR_f value is significantly smaller than the hR_f value of FOS; RAMEB presents a long-oval shaped spot. The “*in situ*” mixture behaves as the two substances separated, while the complexation product is characterized by a hR_f value between the ACE inhibitor and the cyclodextrin hR_f values, which sustains the hypothesis of the existence of an inclusion complex between the two components.

Conclusions

Thin-layer chromatography has proved to be useful and easily applied in emphasizing the inclusion complexes between fosinopril, zofenopril and cyclodextrins.

Binary compounds obtained through the kneading method have a chromatographic behavior which differs from that of the active substances, the hR_f value of complexes being smaller than that of the guest molecules.

This study also proves that β -cyclodextrin (β -CD), hydroxypropyl- β -cyclodextrin (HPBCD) and randomly methylated- β -cyclodextrin (RAMEB) may act as host molecules in order to form inclusion complexes with fosinopril and β -CD with zofenopril.

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