

Characterization of Fosinopril Natrium-hydroxypropyl- β -cyclodextrin Inclusion Complex

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Fosinopril is the most hydrophobic substance among the angiotensin-converting enzyme (ACE) inhibitors, exhibiting a poor bioavailability following oral administration. Cyclodextrin complexation was performed in order to augment the bioavailability of the substance. The binary system ACE inhibitor – hydroxypropyl- β -cyclodextrin was prepared using the kneading method, in 1:1 molar ratio. The identity of the obtained compound was confirmed by thin-layer chromatography, thermal analysis methods and X-ray powder diffraction.

Keywords: fosinopril, hydroxypropyl- β -cyclodextrin, thermal analysis, thin-layer chromatography, X-ray powder diffraction.

Fosinopril, (2*S*,4*S*)-4-cyclohexyl-1-(2-{[2-methyl-1-(propanoyloxy)propoxy]}(4-phenylbutyl)phosphoryl)acetylpyrrolidine-2-carboxylic acid, (fig. 1), a phosphinic acid derivative, is an ACE inhibitor widely used in the treatment of essential hypertension, congestive heart failure, diabetic nephropathy and post myocardial infarction [1, 2]. It is a prodrug, thus hydrolyses to the active diacid product, namely fosinoprilat [1]. Fosinopril is characterized by a very high lipophilicity (logP = 4.75) and poor bioavailability following oral administration (36%) [3].

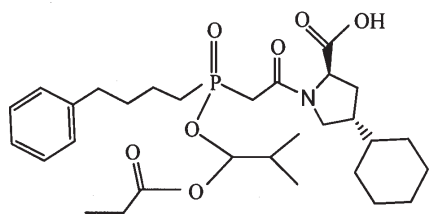


Fig. 1. Fosinopril: chemical structure

Cyclodextrins are cyclic oligosaccharides consisting of glucopyranose units linked by α -(1,4) bonds having a hydrophilic external surface and an inner hydrophobic cavity [4-6]. Due to their particular geometry, the cyclodextrins have the capacity of forming inclusion complexes (IC) by encapsulating into their cavity, entirely or partially, a wide variety of molecules, with drug substances among them. As a result of the cyclodextrins encapsulation, some physico-chemical and bio-pharmaceutical properties of the included compounds can favorably be modified, by enhancing solubility, stability and bioavailability as well as mitigating volatility, unpleasant taste and smell [4-7].

Inclusion complexes of various pharmaceutical substances within natural and semisynthetic cyclodextrins

were already analyzed: imidazole derivatives [7-11], sulphonamidic diuretics [12-16], pentacyclic triterpenes [17], repaglinide [18], ascorbic acid [19], orotic acid [20], pyridoxine [21]. Regarding the ACE inhibitors, the inclusion complexes of such compounds with β -cyclodextrin are also presented [22-26].

The aim of this work is to investigate the molecular encapsulation of the fosinopril sodium in the hydroxypropyl- β -cyclodextrin cavity by using thin-layer chromatography (TLC), thermal analysis and X-ray diffraction (XRD).

Experimental part

Hydroxypropyl- β -cyclodextrin (HPBCD) was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). Fosinopril sodium (FOS) was obtained from Terapia-Ranbaxy (Cluj-Napoca, Romania). The substances were used as received. The used solvents are in compliance with the analytical grade requested by the Romanian Pharmacopoeia 10th ed [26] and by European Pharmacopoeia 5th ed [27]

Preparation of the inclusion complex

The binary system fosinopril sodium – hydroxypropyl- β -cyclodextrin was prepared using the kneading method (kneaded products, KP). To this end, the amounts of guest substance and cyclodextrin were weighed according to molar ratio 1:1. The resulted mixture was pulverized in a mortar and then kneaded with a quantity of ethanol-water (50:50, w/w) equal to the sum of the amounts of the two substances, until the bulk of solvents have been evaporated. After drying at room temperature, the product was also dried in oven at 105°C. Afterwards, it was pulverized and sieved (100 μ m).

Thin-layer chromatography

The chromatographic investigations were performed at room temperature (22 \pm 2°C), by employing ascendant

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Table I
SAMPLES APPLIED ON THE CHROMATOGRAPHIC PLATE

Spot 1	Spot 2	Spot 3	Spot 4
2 mg/ml fosinopril sodium in ethanol	5 mg/ml Hydroxypropyl- β -cyclodextrin in distilled water	Fosinopril sodium and hydroxypropyl- β -cyclodextrin solution forming an "in situ" mixture	2 mg/ml FOS in binary system fosinopril sodium - hydroxypropyl- β -cyclodextrin in distilled water

TLC, using Kieselgel 60 chromatographic plates (Art 5748 DC-Plasstikfolien Kieselgel 60, Merck, Darmstadt, Germany), 20 X 20 cm, having a width of 0.25 mm. The plates were spotted with 5 μ L of the samples to be examined, at a distance of 2 cm between them according to table I. The composition of the used mobile phase was *i*-Propanol:Acetone 80:20 (v/v) whereas the hR_f value of fosinopril sodium was 61. The migration distance was 10 cm from the start line. The chromatographic plates were air dried after both development and revelation stages. Detection was performed by exposing the plates to iodine vapours.

Thermal analysis

Fosinopril sodium, hydroxypropyl- β -cyclodextrin and their inclusion compound were analyzed by thermogravimetry (TG), derivative thermogravimetry (DTG) and differential thermal analysis (DTA) using a TGA/SDTA 851-LF 1100 METTLER apparatus. The thermal behaviour of the substances was studied under a nitrogen flow of 50 mL \cdot min⁻¹ in the temperature range of 25-250°C for the heating rate of 5°C \cdot min⁻¹. Samples with mass of about 35-50 mg were packed in Pt crucibles of 150 μ L.

X-ray powder diffraction

X-ray diffraction patterns were recorded at room temperature with a Bruker D8 Advance powder X-ray diffractometer in the $2\theta = 3-45^\circ$ angular domain using CuK α radiation (40 kV, 40 mA) and a Ni filter.

Results and discussions

In order to point out the inclusion complex character between fosinopril sodium and hydroxypropyl- β -cyclodextrin several methods were employed: thin-layer chromatography, thermal analysis and X-ray powder diffraction.

Thin-layer chromatography

The chromatogram of fosinopril sodium and its KP with hydroxypropyl- β -cyclodextrin is presented in figure 2. The chromatogram examination reveals the fact that in the presence of the used solvents system, the active substance migrates on the chromatographic plate, with a hR_f value of 61, whereas the cyclodextrin migrates at a very small distance, its hR_f value being significantly smaller than its fosinopril sodium counterpart; hydroxypropyl- β -cyclodextrin presents a long-oval shaped spot. The "in situ" mixture acts exactly the same as the two substances

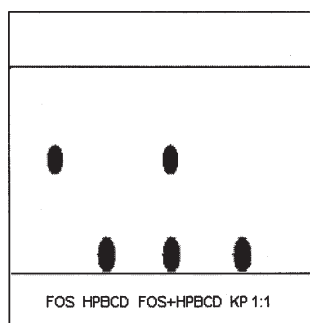


Fig. 2. TLC of fosinopril sodium and its KP with hydroxypropyl- β -cyclodextrin

individually, hence proving the existence of only one physical mixture and excluding the possibility of complex formation on the chromatographic plate. The binary compound fosinopril sodium - hydroxypropyl- β -cyclodextrin migrates similarly to the cyclodextrin, having the same chromatographic behaviour, which endorses the hypothesis of the existence of an inclusion complex between the two components.

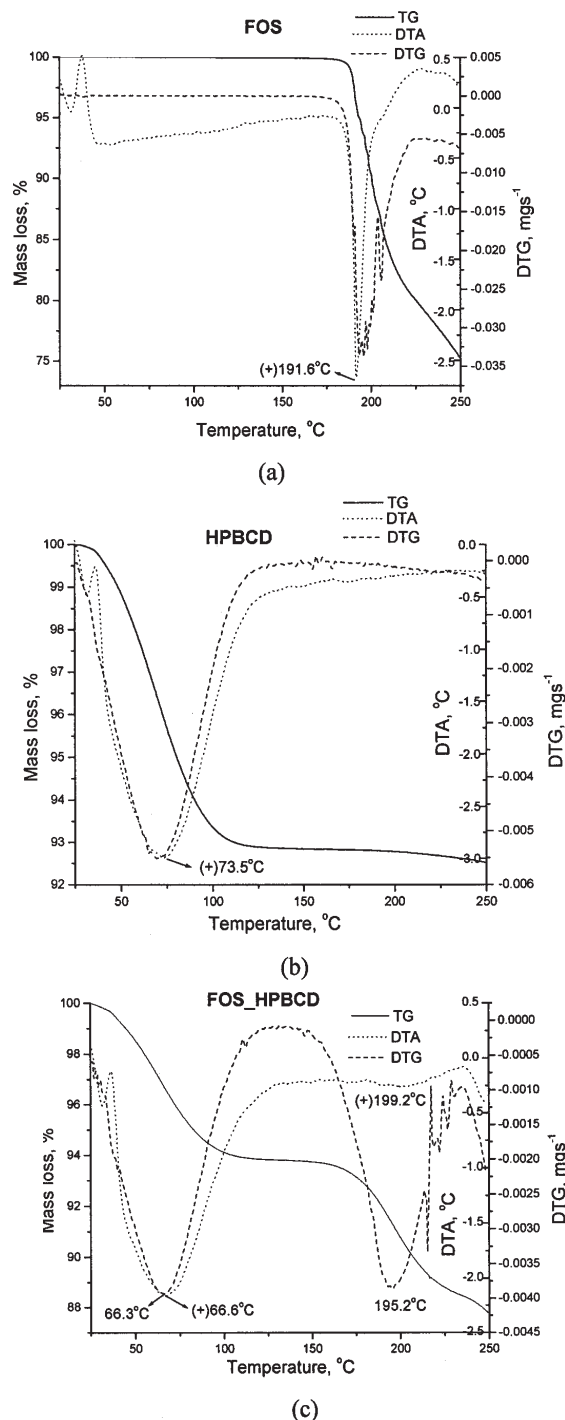


Fig. 3. TG, DTG and DTA curves corresponding to: fosinopril sodium (a), hydroxypropyl- β -cyclodextrin (b) and their KP (c)

Thermal analysis

The TG, DTG and DTA curves of fosinopril sodium, hydroxypropyl- β -cyclodextrin and their KP are presented in figure 3. The fosinopril sodium thermograms indicate a weight loss process between 180-240°C (TG) and an endothermic peak at 191.6°C (DTA) corresponding to the melting point of the drug followed by melt degradation (DTG). The thermograms of hydroxypropyl- β -cyclodextrin show a weight loss process between 40-180°C (TG) and an endothermic transition between 40-120°C with a maximum at 73.5°C (DTA) due to the loss of water molecules. By comparing the thermograms of the pure substances with those of the binary system, a marked reduction of area and broadening of the melting peak of fosinopril sodium in the inclusion compound has been noticed; the melting peak shifts towards a higher temperature in the KP. The area reduction and the shift towards a higher temperature of the melting peak of the fosinopril sodium in the KP are an indication of molecular interaction between the two substances.

X-ray powder diffraction

The X-ray diffraction analysis confirms the thermal analysis results. X-ray powder diffraction patterns of fosinopril sodium, hydroxypropyl- β -cyclodextrin and their KP were presented in figure 4. The fosinopril sodium has a crystalline structure and the hydroxypropyl- β -cyclodextrin has an amorphous structure. In Figure 4 it can be noticed that the KP and hydroxypropyl- β -cyclodextrin patterns are almost identical, which means that the fosinopril sodium has no longer the original crystalline structure, proving that an interaction between the two substances has occurred, due to the inclusion of fosinopril sodium into the cyclodextrin cavity.

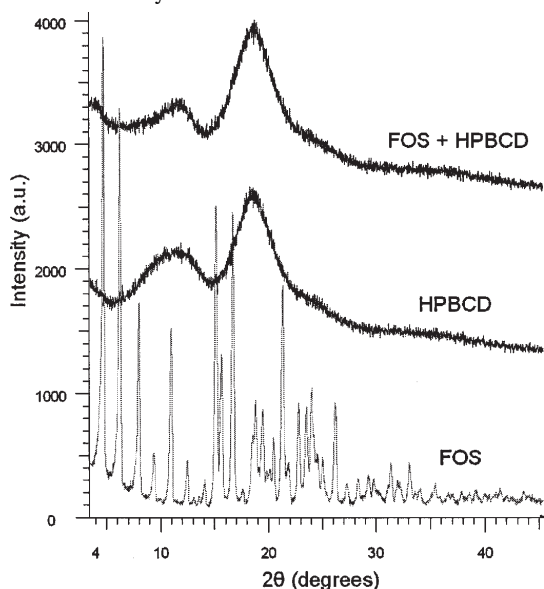


Fig. 4. X-ray patterns of fosinopril sodium (bottom), hydroxypropyl- β -cyclodextrin (middle) and their corresponding inclusion complex (top)

Conclusions

The TLC data underlines the existence of a molecular interaction between the ACE inhibitor and cyclodextrin. The identical chromatographic behaviour of the cyclodextrin and the binary product ACE inhibitor - hydroxypropyl- β -

cyclodextrin backs up the hypothesis of forming an inclusion complex of the two components.

The thermal analysis experiments confirm the inclusion compound formation because of the area reduction and the shift toward a higher temperature of the melting endothermic peak of the fosinopril sodium in the inclusion complex thermograms.

The X-ray powder diffraction certifies the inclusion complex fosinopril sodium - hydroxypropyl- β -cyclodextrin formation, through the loss of the crystalline structure of the fosinopril sodium in the KP and therefore confirms the effectiveness of the kneaded method in this case.

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