Host - guest System of Zofenopril and Randomly Methylated β - cyclodextrin

Preparation, characterization and solubility

LUCRETIA UDRESCU[1,2*], ADRIANA FULIAS[1], IONUT LEDETI[1], GABRIELA VLASE[3], PAUL BARVINSCHI[4], LUDOVIC KURUNCZI[1,2], LAURA SBARCEA[1]

[1] "Victor Babeş" University of Medicine and Pharmacy, Faculty of Pharmacy, 2 Eftimie Murgu Sq., 300041 Timişoara, Romania

[2] Institute of Chemistry Timisoara of the Romanian Academy, 24 Mihai Viteazul, 300223 Timisoara, Romania

[3] West University of Timisoara, Research Center for Thermal Analysis in Environmental Problems, 16 Pestalozzi, 300115 Timisoara, Romania

[4] West University of Timi'oara, Faculty of Physics, 4 Vasile Pârvan, 300223 Timisoara, Romania

Zofenopril calcium (ZOF) is one of the newest angiotensin converting enzyme (ACE) inhibitor, highly lipophilic and with low water solubility. This research investigates the interaction between ZOF and randomly methylated β-cyclodextrin (RAMEB), in order to prove the formation of an inclusion complex that has an enhanced water solubility profile for ZOF. This paper reports, for the first time, the physicochemical characterization and the solubility profile of an inclusion complex between ZOF and RAMEB, which is prepared using the kneading method. Different spectroscopic techniques, namely powder X-ray diffraction, ATR FT-IR spectroscopy, were applied in order to prove the formation of the ZOF/RAMEB inclusion complex, both in water and in solid state. The complex formation is further backed by thermal analysis (TGA/DTG/HF). The obtained results confirm that the physicochemical properties of the ZOF/RAMEB binary system, prepared using the kneading method, are different in comparison both with the parent substances and the corresponding physical mixture, thus suggesting that an inclusion complex was formed. After the formation of the inclusion complex with RAMEB, the solubility test has indicated that the water solubility of ZOF was increased by 4 times.

Keywords: zofeopril calcium, ATR-FTIR, PXRD, thermal analysis, solubility

Zofenopril calcium (ZOF), chemically named *calcium (2S,4S)-1-[(2S)-3-benzoylsulfanyl-2-methylpropanoyl]-4-phenylsulfanylpyrrolidine-2-carboxylate*, is a sulfhydryl-containing ACE inhibitor, as presented in figure 1a. Based on its selective cardiac ACE inhibition, ZOF is an effective antihypertensive agent and a therapeutic option in congestive heart failure and in acute myocardial infarction [1-3]. ZOF is an ester prodrug of zofenoprilat (fig. 1b), which is the bioactive acid form containing a sulfhydryl group [4]. The presence of sulphur in the ZOF molecule is responsible for its remarkable antioxidant and cardio protective properties [5, 6]. ZOF exhibits high lipophilicity (log P = 3.5) [1], while its major drawback is the low water solubility (0.3 mg/ml reported by [1]), since this is the actual utilized form in the oral formulations. Moreover, zofenopril calcium exhibits the tendency of forming numerous polymorph forms [7], thus resulting in decreasing its water solubility.

The cyclodextrins are a class of cyclic oligosaccharides consisting of six, seven or eight α-D-glucopyranose units, named α-, β-, and γ-cyclodextrin, respectively. Their particular hydrophobic cavity presents biomedical and pharmaceutical interests, because they are able to form inclusion complexes with guest molecules (*i.e.* drugs or their lipophilic moieties) with an improved solubility, stability or other physicochemical properties [8, 9]. The attained amorphous solid state following the complex formation using cyclodextrins [8, 10] has a potential benefit for the oral formulations containing ZOF, due to the increased water solubility.

Up to date, the studies on ZOF [11] and ZOF inclusion complexes with native β-cyclodextrin [12] are scarce; moreover, there is no scientific report on ZOF inclusion complex with RAMEB. In this paper, the formation of the inclusion complex between ZOF and RAMEB is investigated, as a consequence of ZOF molecularly encapsulation within hydrophobic cyclodextrin cavity.

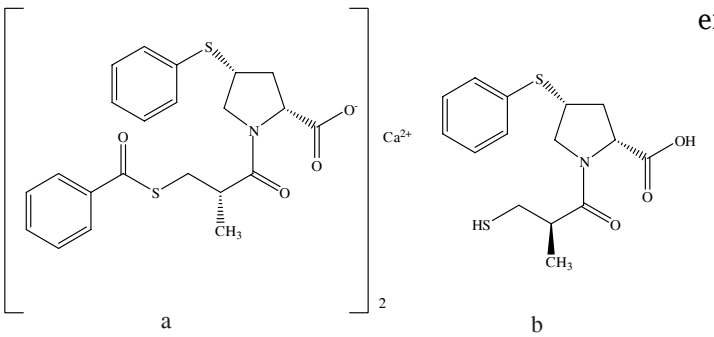

Fig. 1. The chemical structures of: (a) zofenopril calcium and (b) zofenoprilat

* email: udrescu.lucretia@umft.ro

Therefore, we applied spectroscopic techniques and TGA/DTG/HF thermal analysis as the most accurate and reliable methods in the study of the host-guest interaction.

Experimental part

Apparatus

The spectrophotometric measurements were performed by using a Spectronic Unicam-UV 300 UV-Vis double beam spectrophotometer, with 1 cm matched quartz cells.

X-ray diffraction studies of the pure substances (ZOF and RAMEB) and of their binary systems (the corresponding physical mixture and kneaded product) were performed using a Bruker D8 Advance powder X-ray diffractometer, in the range of 5-45° angular domain (2θ), with CuK radiation generated at 40 mA, 40 kV, and a Ni filter.

Thermal analysis was made by using a Perkin-Elmer Diamond simultaneous TGA/DTA instrument. The DTA curves (in μV) were changed with the heat flow (HF) curves (in mW) in order to determine the heat effects. The thermal behaviour of the substances was studied under an air atmosphere, at a flow rate of 100 mL/min and non-isothermal conditions, by increasing the ambient temperature up to 350 °C with a constant heating rate of 10 °C/min.

The FTIR spectra were yielded using a Bruker Vertex 70 spectrometer equipped with a platinum ATR unit, type Bruker Diamond A225/Q. Each spectrum represents 64 co-added scans, at a resolution of 2 cm^{-1}, in the 4000-400 cm^{-1} wavenumber range.

Materials and reagents

Zofenopril calcium was a gift sample from Berlin-Chemie Menarini (Berlin, Germany). Randomly methylated β-cyclodextrin (average formula weight 1303.4, DS~12) was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). The substances were used as received. All other chemicals and reagents were of analytical grade. All experiments were performed using distilled water.

Binary systems preparation

The accurate weight of RAMEB for a 1:1 molar ratio ZOF:RAMEB was triturated with an appropriate quantity of water, at the ambient temperature, for 10 min, up to homogenization. Then ZOF was slowly added to the paste. The dissolution of the drug was assisted by adding small quantities of water in the mixture. The paste was subject to kneading for 1 h. The paste that was obtained by performing this process was dried in the oven at 40°C, for 24 h. Then, the dried kneaded product, named ZOF/RAMEB KP, was pulverized and passed through a 75 μm size sieve.

The stoichiometric quantities of ZOF and RAMEB corresponding to a 1:1 molar ratio were gently mixed in a mortar, at the ambient temperature, for 10 min, in order to get a homogenous blend, thus obtaining the corresponding physical mixture at the same ratio as for the inclusion complex (ZOF/RAMEB PM). This binary system was used for comparison with ZOF/RAMEB KP.

Calibration curve of ZOF and the solubility profile of the kneaded product

A set of ZOF aqueous solutions was prepared, with concentrations ranging between 9 –72 μg/mL. The absorbance (*A*) values, recorded at 248 nm, in UV, at 25 °C, were represented as a function of ZOF concentration values (*C* in μg/mL), in order to achieve the calibration curve of ZOF.

The water solubility of ZOF within its inclusion complex was determined as follows: an excess amount of kneaded product was placed in 2 mL of distilled water, for obtaining a saturated solution. The mixture was shaken for 24 h, at 25°C, and then filtered on 0.45 μm cellulose acetate filter. The clear supernatant was properly diluted and its absorbance was measured at 248 nm in UV, at 25 °C. The residue dosing was performed by means of the calibration ZOF curve.

Results and discussions

PXRD analysis

PXRD technique allows the observation of the crystalline substance (i.e. ZOF) sharp peaks attenuation due to the inclusion interaction with the amorphous cyclodextrin, RAMEB. The PXRD patterns of the native ZOF and RAMEB and of their binary systems (the physical mixture and the kneaded product) are presented in figure 2.

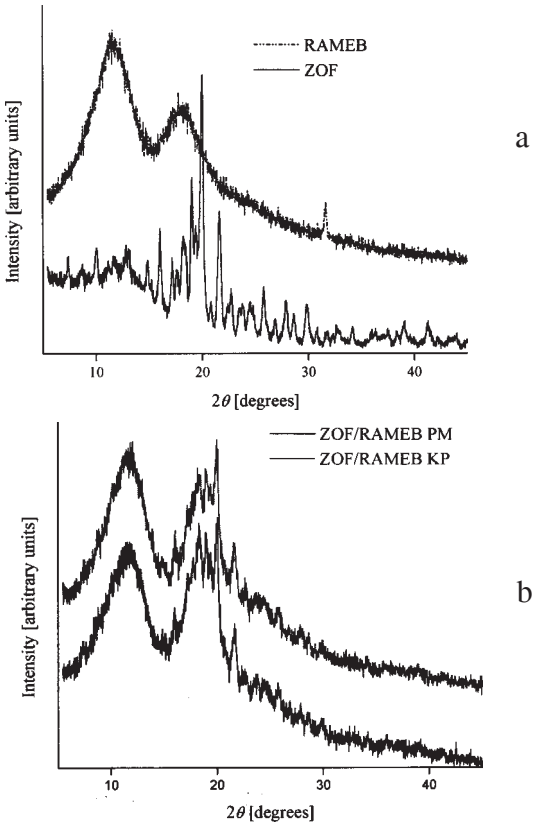

Fig. 2. PXRD patterns of the native substances (a), and their corresponding binary systems (b)

The PXRD spectrum of ZOF, presented in figure 2a, emphasizes its highly crystalline nature by characteristic diffraction peaks at 9.72, 13.74, 14.48, 16.00, 17.70, 18.42, 18.52, 19.00, 19.98, 20.56, 22.34, 24.60 2θ degrees [13]. The PXRD pattern of RAMEB, as shown in figure 2a, has two broad peaks and many diffused peaks with low intensities, which reveal its amorphous state. In figure 2b is presented the PXRD spectrum of ZOF/RAMEB PM (fig. 2b), which is almost an overlapping of the individual diffraction spectra. By contrast, the ZOF/RAMEB KP diffraction spectrum (fig. 2b) analysis reveals the diminished intensities of the ZOF characteristic sharp peaks (*i.e.* 16.00, 17.70, 19.00, 19.98, 21.93 2θ). This result emphasizes that the ZOF crystallinity is drastically reduced, due to the modifications in ZOF and RAMEB environment following the inclusion complex formation process [14, 15].

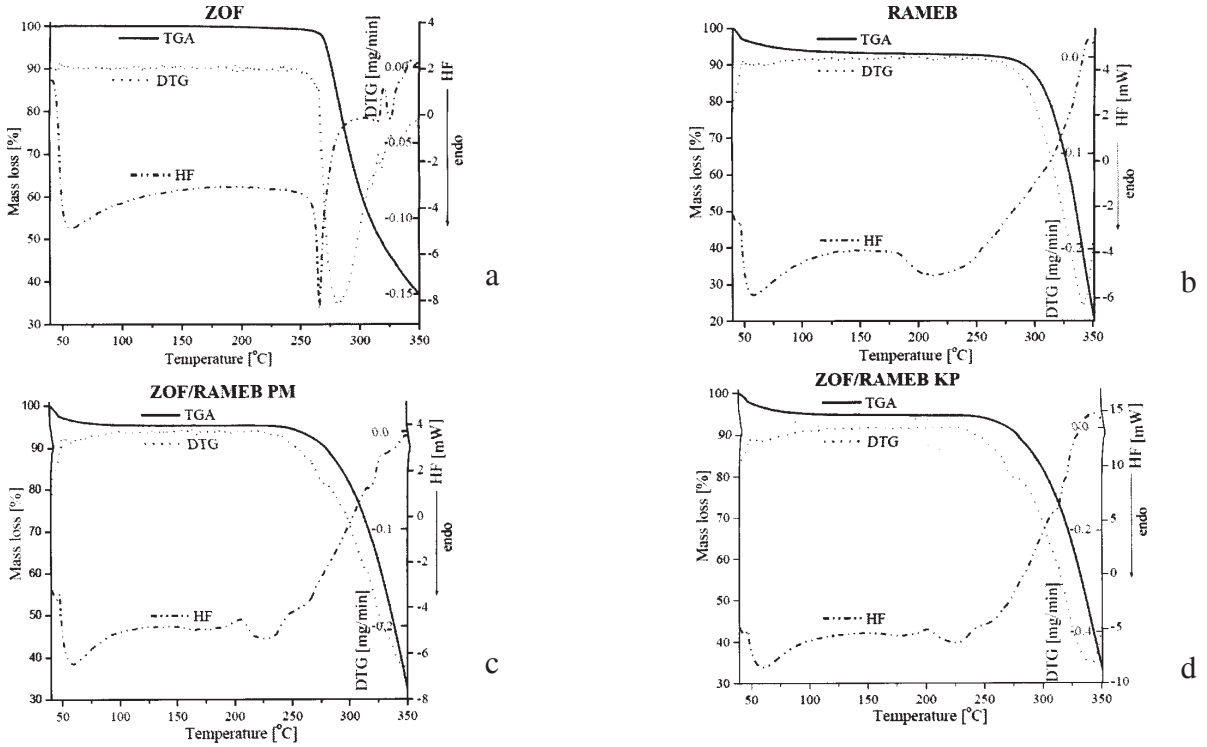

Fig. 3. The thermal profiles of ZOF (a), RAMEB (b), ZOF/RAMEB PM (c), and ZOF/RAMEB KP (d)

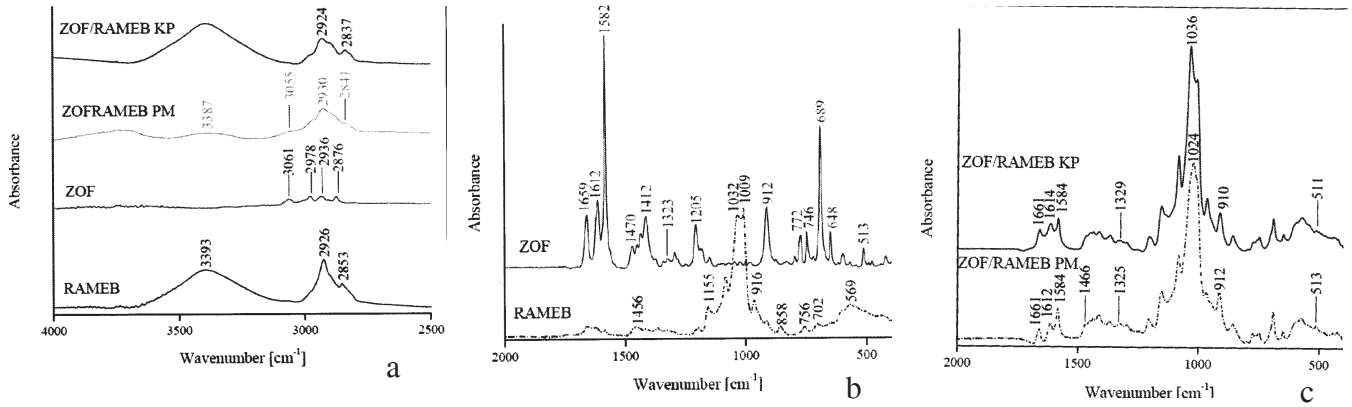

Fig. 4. ATR-FTIR spectra of: a) RAMEB, ZOF, ZOF/RAMEB physical mixture and ZOF/RAMEB kneaded product in 4000-2500 cm^{-1} spectral region; b) ZOF and RAMEB in 2000-500 cm^{-1} spectral region; c) ZOF/RAMEB PM and ZOF/RAMEB KP in 2000-500 cm^{-1} spectral region

Thermal analysis

Thermal analysis leads to valuable outcomes regarding cyclodextrin inclusion complexes formation. The thermal behaviour of the pure compounds and of their binary systems is depicted in figure 3.

According to the HF curve (fig. 3a), the ZOF melting point appears at 265 °C [13], this endothermic process being followed by decomposition, a process that is confirmed by the TGA curve. The RAMEB dehydration is revealed by the HF curve (fig. 3b), which presents a broad endothermic peak ranging between 40–140°C [16]. The water loss is also shown by the TGA curve, in the same interval of temperature. The melting point of RAMEB appears at a higher value than ZOF, as indicated by the DTG curve.

The thermal behaviour of ZOF/RAMEB PM (fig. 3c) reveals a diminished endothermic peak at 226°C, which corresponds to the ZOF melting process. The endothermic peak of ZOF melting process within ZOF/RAMEB KP is reduced in comparison with that of the corresponding physical mixture and it is shifted to a lower temperature, to 224°C (fig. 3d). Hence, a decrease in thermal stability of the ZOF/RAMEB kneaded product is observed, as a consequence of the amorphization of ZOF, as a guest compound, through the inclusion complex formation [17, 18].

ATR-FTIR analysis

The ATR-FTIR spectra of the pure ZOF and RAMEB, along with their corresponding physical mixture and kneaded product are presented in figure 4.

The ATR-FTIR spectrum of pure ZOF (fig. 4a, 4b), is characterized by the presence of peaks for the proline group (at 1470 cm^{-1}) [19], the anti-symmetric and symmetric stretching C=O vibrations at 1659 and 1612 cm^{-1}, respectively. The C_{ar}–H stretching vibration arises at 3061 cm^{-1}, the skeletal vibration of the aromatic ring appears at 1582 cm^{-1}, while the C_{ar}–H and C_{ar}–C_{ar} bending vibrations appear at 772 and 746 cm^{-1}, respectively [20]. The S-CH_2 stretching vibration is identified at 2876 cm^{-1} [19].

The FT-IR spectra of the inclusion complex prepared by kneading, as presented in figures 4a and 4c, reveal considerable differences in comparison with the corresponding physical mixture and the pure compounds. Almost all the characteristic peaks of ZOF are intact in the physical mixture spectrum, but they are shifted (or even absent) in the ZOF/RAMEB KP spectrum. By analyzing the spectral data, we found that the ZOF C_{ar}–H stretching

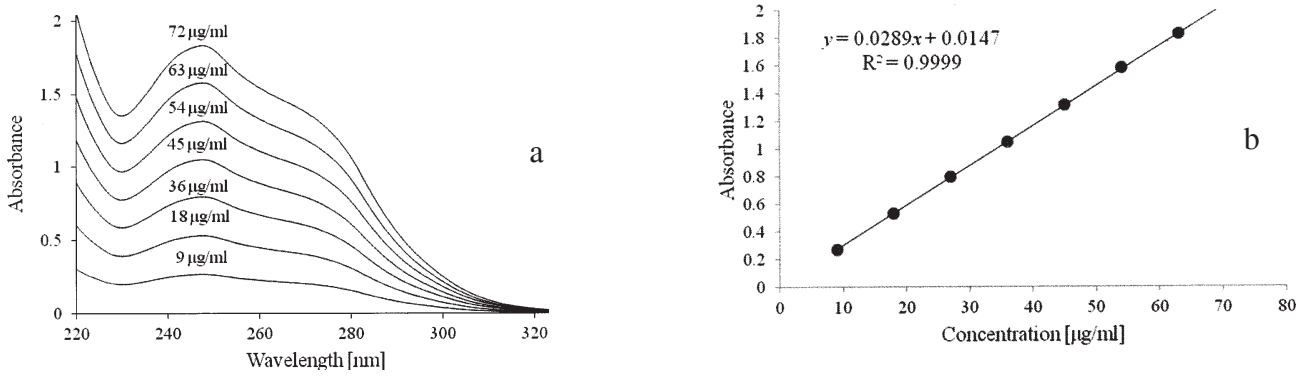

Fig. 5. a) UV absorption spectra of ZOF in distilled water, with concentrations ranging between 9 and 72 µg/mL; b) the calibration curve of ZOF in distilled water.

vibration is shifted to 3055 cm^{-1} in ZOF/RAMEB PM, and it is no more present in ZOF/RAMEB KP, thus indicating that one of the two ZOF aromatic rings is entrapped within the RAMEB cavity. ZOF's C=O stretching vibrations are still present, although shifted in both binary systems. The proline group vibration, which is identified at 1470 cm^{-1} in the ZOF spectrum, is shifted at 1466 cm^{-1} in ZOF/RAMEB PM, and absent in the ZOF/RAMEB KP. At the same time, the ZOF peak which corresponds to the S-CH_2 stretching vibration is absent from both binary systems, thus suggesting that the inclusion complex was formed and the 4-(phenylthio)pyrrolidyne is enclosed in the RAMEB cavity.

Solubility

The testing of the water solubility of our ZOF/RAMEB inclusion complex is a valuable purpose of our experimental approaches. Therefore, the saturation shake-flask method [21, 22] was applied in order to measure the ZOF water solubility, at 25 °C, as it is in ZOF/RAMEB KP, using the calibration curve of ZOF in water, at 25 °C (fig. 5).

The calibration curve of ZOF is characterized by equation $A = 0.0289 \cdot C + 0.0147$ ($R = 0.9999$), where A stands for absorbance, measured at 248 nm, and C is ZOF concentration in µg/mL.

The determination of the water solubility of the ZOF/RAMEB KP was achieved by preparing a concentrated solution, which was properly diluted [21, 22]. The UV spectrophotometric measurements have indicated that the water solubility of the included ZOF is 1.199±0.014 mg/mL, as an average value of five experimental determinations. This result indicates that the water solubility of ZOF is increased by four times in comparison with the free ZOF (0.3 mg/mL), due to the solubilising effect of RAMEB.

The next step was to perform of a standard control experiment. Therefore, a mass of 4.7 mg of ZOF/RAMEB KP, which is equivalent to 1.199 mg of ZOF, was dissolved in distilled water and a clear solution was obtained. This result indicates that the solubility of the ZOF/RAMEB inclusion complex is adequate for tablet dosage form [21, 22].

Conclusions

This paper reports for the first time the physico-chemical characterization of an inclusion complex between ZOF and RAMEB. It also stresses the solubilising role of RAMEB in improving the water solubility of the calcium salt of zofenopril. By applying the kneading method, an inclusion complex between ZOF and RAMEB was formed, as proven by ATR-FTIR spectroscopy, PXRD, and thermal analysis. The water solubility of ZOF/RAMEB KP was enhanced by four times in comparison with pure ZOF.

References

1.SUBISSI, A., EVANGELISTA, S., GIACHETTI, A., Cardiovasc. Drug. Rev., **17**, 2, 1999, p. 115.
2.AMBROSIONI, E., Am. J. Cardiovasc. Drug., **7**, **1**, 2007, p. 17.
3.BORGHI, C., CICERO, A. F. G., AMBROSIONI, E., Vasc. Health Risk Manag. **4**, 3, 2008, p. 665
4.SINGHVI, S. M., FOLEY, J. E., WILLARD, D. A., MORRISON, R. A., J. Pharm. Sci. **79**, 11, 1990, p. 970
5.EVANGELISTA, S., MANZINI, S., J. Int. Med. Res. **33**, 1, 2005, p. 42
6.PASINI, A.F., GARBIN, U., NAVA, M. C., STRANIERI, C., PELLEGRINI, M., BOCCIOLETTI, V., LUCHETA, M. L., FABRIZZI, P., LO CASCIO, V., COMINACINI, L., Am. J. Hypertens. **20**, 4, 2007, p. 443
7.LOZANO, R., JOSEPH, J. M., KLINE, B. J., J. Pharm. Biomed. Anal. **12**, 2, 1994, p. 173
8.LOFTSSON, T., BREWSTER, M. E., MASSON, M., Am. J. Drug Deliv. **2**, 4, 2004, p. 261
9.STELLA, V.J., HE, Q., Toxicol. Pathol. **36**, 1, 2008, p. 30
10.SEMALTY, M., PANCHPURI, M., SINGH, D., SEMALTY, A., Curr. Drug. Discov. Technol. **11**, 2, 2014, p. 154
11.SBÂRCEA, L., UDRESCU, L., DRĂGAN, L., TRANDAFIRESCU, C., BOJIŢĂ, M., Rev. Chim. (Bucharest), **63**, no. 6, 2012, p. 562
12.SBÂRCEA, L., UDRESCU, L., DRĂGAN, L., TRANDAFIRESCU, C., SZABADAI, Z., BOJIŢĂ, M., Farmacia **58**, 4, 2010, p. 478
13.AHMED, K.M., SINGH, Y.R., RASIKLAL, T.N., MAHADEO, L.S., (Glenmark Generics Limited), 2010, Patent no WO 2010084515 A2
14.MISIUK, W., ZALEWSKA, M., Carbohydr. Polym. **77**, 3, 2009, p. 482
15.MARIAN, E., JURCA, T., VICAS, L., KACSO, I., MICLAUS, M., BRATU, I., Rev. Chim. (Bucharest), **62**, no. 11, 2011, p. 1065
16.CUNHA-FILHO, M.S.S., DACUNHA-MARINHO, B., TORRES-LABANDEIRA, J.J., MARTÍNEZ-PACHECO, R., LANDÍN, M., AAPS PharmSciTech, **8**, 3, 2007, E60
17.DOILE, M.M., FORTUNATO, K.A., SCHMUCKER, I.C., SCHUCKO, S.K., SILVA, M.A.S., RODRIGUES, P.O., AAPS PharmSciTech. 9, 1, 2008, p. 314
18.SBÂRCEA, L., UDRESCU, L., DRĂGAN, L., TRANDAFIRESCU, C., SASCA, V., BARVINSCHI, P., BOJITĂ, M., Rev. Chim. (Bucharest), **62**, no. 3, 2011, p. 349
19.BARTH, A., ZSCHERP, C., Q. Rev. Biophys. **35**, 4, 2002, p. 369
20.RUČMAN, R., ZUPET, P., (Silverstone Pharma), 2013, Patent no WO2013095307 A1
21.CHEN, W., YANG, L.J., MA, S.X., YANG, X.D., FAN, B.M., LIN, J., Carbohydr. Polym. **84**, 4, 2011, p. 1321
22.MA, S. X., CHEN, W., YANG, X. D., ZHANG, N., WANG, S. J., LIU, L., YANG, L. J., J. Pharm. Biomed. Anal. **67–68,** 2012, p. 193

Manuscript received: 15.10.2014