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Improvement of Solubility and Stability of Valsartan by Hydroxypropyl-β-Cyclodextrin

BRUNELLA CAPPELLO*, CLELIA DI MAIO, MARIA IERVOLINO and AGNESE MIRO
Dipartimento di Chimica Farmaceutica e Tossicologica Università degli Studi di Napoli Federico II, Via D. Montesano 49, 80131, Napoli, Italy

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Abstract

Aim of the present work was to investigate the effect of hydroxypropyl-β-cyclodextrin (HP-β-CD) on the solubility, dissolution rate and stability of Valsartan (VAL), a drug used orally for the treatment of hypertension. Phase solubility studies demonstrated the ability of the HP-β-CD to complex VAL and to increase drug solubility. The dissolved amount of VAL increased linearly with the addition of HP-β-CD according to an A_L type plot. The apparent stability constant of the complex, calculated supposing a 1:1 stoichiometry, was 296 ± 7 M^{-1}. VAL/HP-β-CD interactions were also studied by ^{13}C-NMR spectroscopy. Equimolar VAL/HP-β-CD solid systems were prepared by physical-mixing and freeze-drying, and their properties in the solid state studied by DSC and FT-IR analysis. The results provided clear indications of the formation of a new solid phase corresponding to the inclusion complex in the freeze-dried sample. The dissolution profiles of the drug from each solid system were affected by its physico-chemical properties, the freeze-dried being the most rapidly dissolving form. The thermal stability of the complex was studied, also determining the number and identity of the decomposition products of the drug. The stability studies revealed that the VAL/HP-β-CD complex significantly decreases the rate of VAL degradation. These results suggest that CD technology would be a very useful method to overcome the solubility and the stability problems of VAL.

Introduction

Valsartan, (S)-N-valeryl-N-{[2′-(1H-tertrazol-5-yl)biphenyl-4-yl]-metyl}-valine, (VAL) is a potent and specific competitive antagonist of the Angiotensisn II AT_1-receptor [1, 2].

The drug, used orally for the treatment of hypertension, exhibits a low bioavailability (AUC 23%), probably related to its poor water solubility [3]. According to the Biopharmaceutical Classification Scheme, VAL can be considered a class II compound, i.e. a water-insoluble, lipophilic and highly permeable compound. Therefore, it is possible to improve the VAL bioavailability by increasing its apparent solubility in water.

A useful approach seems to be the co-formulation of the drug with cyclodextrins (CDs). These cyclic oligosaccharides have the unique property to form inclusion complexes with a variety of organic/inorganic guest molecules of suitable size and polarity. Complexation by CDs affects many physico-chemical properties of the guest. Specifically, CDs are employed in pharmaceutical field for the improvement of the solubility in water, stability and bioavailability of hydrophobic drugs [4, 5]. Several CD derivatives with specific desirable properties have been developed. These modified CDs, in addition to native ones, can be chosen according to properties to be used as suitable host molecules [4].

In more general attempt to optimize the pharmaceutical properties and the pharmacokinetics of VAL formulations, this work was aimed to investigate the effectiveness of CD containing systems for improving the solubility and the dissolution rate as well as the stability of VAL.

Hydroxypropyl-β-cyclodextrin (HP-β-CD) was selected for its higher solubility than the parent compound. The interaction between VAL and HP-β-CD was studied in solution by the phase solubility method and ^{13}C-NMR spectroscopy. VAL/HP-β-CD solid systems in equimolar ratio were prepared by physical-mixing and freeze-drying and characterized by thermal analysis (DSC) and Fourier Transform Infrared analysis (FT-IR). The influence of the physico-chemical properties of the solid systems on dissolution rate have been highlighted and discussed.

* Author for Correspondence. E-mail: cappello@unina.it

The thermal stability of VAL alone and of VAL/HP-β-CD solid system (freeze-dried) was investigated and the number and identity of the decomposition products of the drug was determined.

Experimental

Materials

Valsartan (VAL) was kindly supplied by Novartis (East Hanover, NJ); Hydroxypropyl-β-cyclodextrin DS 0.61 (HP-β-CD) was purchased from Roquette (Lestrem, France). These chemicals were used as received without further purification.

All other chemicals and solvents were of analytical reagent grade purity. Double distilled water was used throughout the study.

Solubility studies

Phase solubility studies were performed in unbuffered water, according to the Higuchi and Connors method [6]. An excess amount of VAL (12 mg) was added to 5 ml of water or HP-β-CD aqueous solutions (from 1×10^{-3} to 5.5×10^{-2} M) in screw-capped glass vials; these were mechanically shaken (SS40-D Grant shaking bath) at 25 °C until equilibrium was achieved (4 days). Aliquots were withdrawn, filtered (filter HA-0.45 μm, Millipore) and spectrophotometrically analysed for VAL content (Shimadzu UV-1204 spectrophotometer) at 247 nm. The presence of HP-β-CD did not interfere with the spectrophotometric assay of the drug. Each experiment was performed in triplicate; the coefficient of variation (CV) associated with each measurement was never greater than 3%.

^{13}C-NMR studies

^{13}C-NMR spectra were recorded on a Bruker AMX-500 spectrometer. Solutions containing 2×10^{-2} M of VAL or equivalent amount of VAL/HP-β-CD (1:1 mol/mol) were prepared in D_2O-DMSO-d_6 solution (1:1 v/v). Spinning tubes of 4 mm i.d. containing 0.5 ml of solution were employed. Tetramethylsilane was used as external reference and no correction was made for the susceptibility of the capillary. Chemical shifts were calibrated with an accuracy of 0.01 ppm.

Preparation of solid binary systems

Solid systems drug/CD were prepared by physical-mixing and freeze-drying as these methods are known to yield products with distinctive properties in the solid state [9].

VAL and HP-β-CD were sieved (IG3/WET/MS, Giuliani, Torino, Italy) and the corresponding 75–150 μm granulometric fractions collected. The VAL/HP-β-CD solid systems were prepared in 1:1 (mol/mol) stoichiometric ratio.

For the preparation of the physical mixture (PM), VAL and HP-β-CD were blended in a mortar until an homogeneous mixture was obtained. For the preparation of the freeze-dried product (FD), 1 g of PM was dissolved in 600 ml of water containing 0.06 g l^{-1} ammonium hydroxide to ensure drug solubilization. After 24 h of agitation at room temperature, the solution was frozen at −70 °C and freeze-dried in a Modulyo Edwards apparatus, obtaining the FD product. No residual ammonia (Nessler's test) was detected in FD product.

Differential scanning calorimetry

DSC measurements were carried out using a Mettler DSC 30 apparatus equipped with a TC II probe. Samples were weighed (2 mg) (Mettler M3 microbilance) in Al pans pierced with a perforated lid, and scanned at 10 °C min^{-1} in the 25–200 °C temperature range. Dry nitrogen was used as purge gas.

Infrared spectroscopy

FT-IR spectra (KBr disk) were obtained on a Bruker IFS-48 apparatus applying Fourier transformation of eight scans.

Dissolution studies

The dissolution studies of VAL and VAL/HP-β-CD solid systems were performed in unbuffered water (pH $\approx$ 6) at 37 ± 0.5 °C according to the dispersed amount method. In this procedure, 15 mg of drug or equivalent VAL/HP-β-CD blends were added to 50 ml of water (non-sink conditions), in a 100 ml beaker. A glass three-blade propeller was centrally immersed in the beaker and rotated at 100 rpm. Suitable aliquots were removed at different time intervals, filtered and spectrophotometrically analysed for VAL content, as for solubility studies. A correction was calculated for the sampling. Each test was performed in triplicate (CV < 3%).

Dissolution was characterized through the percent of the drug dissolved after 2 min (calculated with respect to the highest solubilized drug amount) and the relative dissolution rates of the drug-CD systems, calculated as the ratio of the amount of drug dissolved at 2 min to that obtained with the pure drug.

Stability studies

Thermal stability of VAL and its solid system with HP-β-CD (freeze-dried sample) was studied at 80 °C. The vials containing 1 mg of VAL or equivalent amount of VAL/HP-β-CD blend were placed in heated chamber (Biraghi, Naples, Italy) and stored up to 48 weeks. At certain intervals the samples were picked up, solubilized in the mobile phase and analysed for remaining VAL content by HPLC method as described below. The tests were performed in triplicate (CV < 3%).

VAL's degradation products were analysed by LC-MS/MS system. All the mass spectral analyses were performed on a LCQ ion trap mass spectrometer (ThermoFinningan, San Jose, CA) equipped with an electronspray ionization source in the negative ion mode. Full scan spectra were collected from m/z 500 to 1500 using a capillary temperature of 150 °C, a capillary voltage of −10 V, a tube lens offset of −60 V, a spray voltage of 4.5 kV, a sheath gas and an auxiliary gas flow of 80 and 20 (arbitrary units), respectively.

HPLC analysis

A high-performance liquid chromatograph Schimadzu LC-10 AD (Schimadzu Co., Kyoto, Japan) was used, equipped with a 7725 Rheodyne injection valve and a Schimadzu SPD 10A UV detector, set at a wavelength of 247 nm. The chromatograms were recorded by a Schimadzu C-R6A integrator. The mobile phase consisted of a 50:50 (v/v) mixture of aqueous phosphate buffer (0.01 M) at pH 2.8 and acetonitrile. The isocratic flow rate of the mobile phase was 1.1 ml min^{-1}. A Kromasil C18 column (250 × 4.6 mm) was used as stationary phase. For analysis, the solid samples containing VAL were solubilized in the mobile phase; 20 μl of the solutions were injected into the column. The retention time of VAL was approximately 4 min.

Results and discussion

Solution studies

The equilibrium phase solubility plot of VAL in aqueous HP-β-CD solutions at 25 °C is reported in Figure 1. In the absence of HP-β-CD the VAL solubility in water was 85 ± 2.1 μg ml^{-1} (2.0×10^{-4} M). In the presence of the carrier the drug solubility increased proportionally as the HP-β-CD molar concentration increased. At the highest HP-β-CD concentration tested (5.5×10^{-2} M), a 18-fold VAL solubility increase was obtained. According to the Higuchi and Connors classification [6], the diagram obtained was of A_L type, since it was characterized by a straight line pattern. This type of diagram indicates the formation of a soluble drug–CD complex, thereby increasing the total amount of drug in solution.

Assuming a 1:1 stoichiometry of the complex (this assumption is usually made for A_L type diagrams in the absence of additional information), the apparent stability constant of the complex ($K_{1:1}$) was calculated from the slope of the phase solubility diagram using the Higuchi and Connors Equation (1):

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the VAL water solubility. The calculated $K_{1:1}$ value was 296 ± 7 M^{-1}

^{13}C-NMR studies on VAL both alone and in the presence of HP-β-CD were performed since the NMR technique is known to yield useful information on the nature of the interactions between CDs and guest molecules [7]. The differences in chemical shift values between VAL in the free and complexed state are presented in Table 1. The negative sign of Δppm (i.e., the difference in VAL chemical shifts in the presence and absence of HP-β-CD) refers to an upfield shift, whereas the positive sign indicates a downfield shift. According to the Inoue model [7], carbon atoms deeply inserted in the CD cavity (from the secondary hydroxyl groups

Table 1. ^{13}C-NMR chemical shifts corresponding to VAL in the absence and presence of HP-β-CD

VAL carbon	VAL (δ_0)	VAL/HP-β-CD(δ)	$\Delta\delta$ ($\delta-\delta_0$)
C-14, 16	130.09	130.13	0.04
C-18	142.32	142.32	0.00
C-19	131.79	131.71	−0.08
C-20	132.86	132.83	−0.03
C-21	128.18	128.17	−0.01
C-22	129.25	129.25	0.00
C-23	123.52	123.71	0.19
C-24	156.39	156.59	0.20

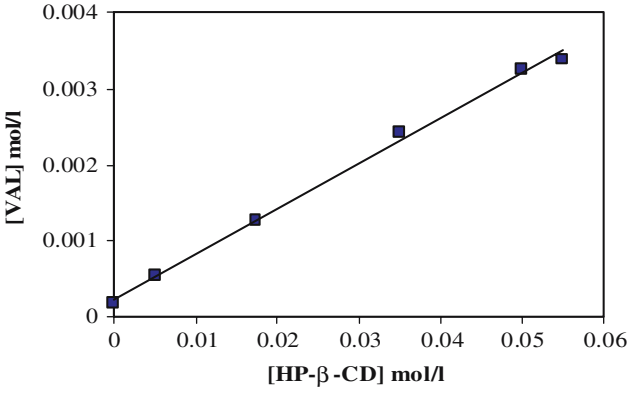
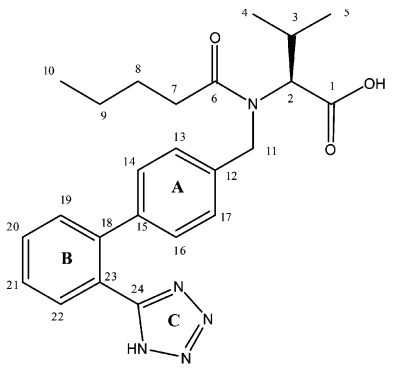


Figure 1. Phase solubility diagram of VAL at increasing amounts of HP-β-CD (mean of three experiments, CV < 3%, error bars omitted for the sake of clarity).



Structure of Valsartan

(Arbitrary numbers were assigned to the atoms in the structure for ease in presentation of data)

side) experience a negative shift that progressively decreases moving towards the secondary hydroxyl rim, passes through zero and then becomes positive when a carbon is found externally close to this rim. As a consequence, the upfield shifts of the C19, C20 and C21 carbon atoms of VAL (Table 1) suggest that the phenyl group, indicated as B-ring, is included within the HP-β-CD cavity. Since chemical shift changes for C18 and C22 are equal to zero, these atoms are just on the secondary hydroxyl rim and this confirms the inclusion of the B-ring. In addition, the downfield shifts of the C14–16, C23 and C24 carbon atoms indicate that these atoms are externally close to the rim.

Solid state studies

The DSC curves for VAL alone and its binary mixtures with the carrier are shown in Figure 2.

The thermal curve of VAL showed a melting endothermic peak at 116 °C. In the thermal curve of the PM the melting peak of VAL had a reduced intensity and was shifted to a lower temperature as a consequence of interaction between the drug and HP-β-CD [8]. However, this interaction could be induced by the thermal energy supplied to the sample in the DSC scan [9].

The complete disappearance of the drug endothermic effect was instead observed for the system obtained by freeze-drying. This phenomenon is indicative of a stronger interaction between VAL and the HP-β-CD in FD with respect to PM sample and provides clear indications for the formation of a new solid phase corresponding to the inclusion complex [10].

Infrared spectrum of VAL as well as those of its binary systems with HP-β-CD are presented in Figure 3.

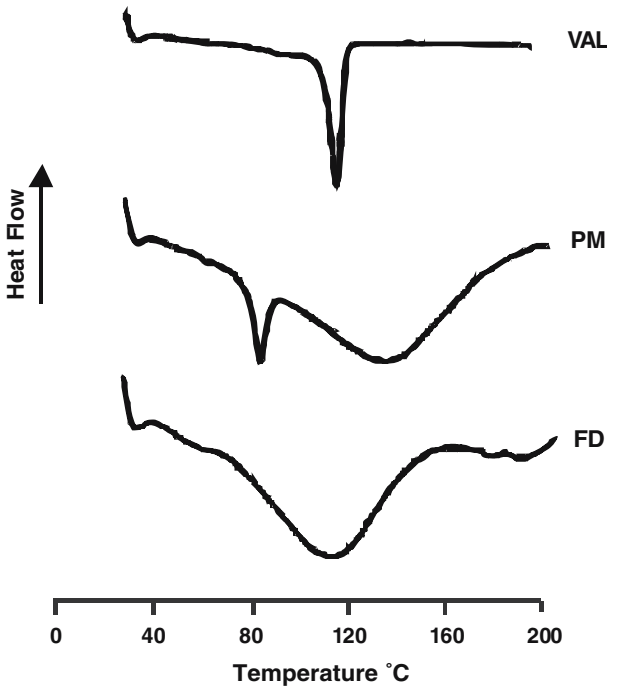


Figure 2. DSC curves of VAL and its equimolar PM and FD products with HP-β-CD.

In Table 2 the wave numbers of the characteristic bands of VAL for the different samples analysed are listed. VAL alone shows two carbonyl absorption bands at 1732 and 1605 cm^{-1}, assigned to the carboxyl carbonyl and amide carbonyl stretching, respectively. These bands are of diagnostic value to elucidate drug–CD interactions. The two absorption bands of the pure drug appeared unchanged in the PM. On the contrary, in the FD product the characteristic carboxyl carbonyl stretching band was recorded to a lower wave number than the

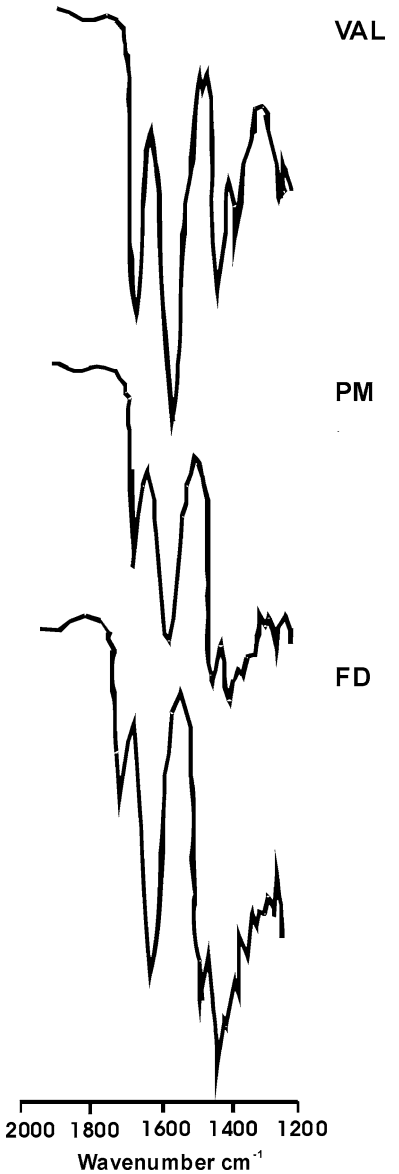


Figure 3. FT-IR spectra of VAL and its equimolar PM and FD products with HP-β-CD.

Table 2. Carbonyl stretching bands (cm^{-1}) of VAL and VAL/HP-β-CD equimolar systems: PM and FD

Sample	C=O acid	C=O amide
VAL	1732	1605
PM	1732	1606
FD	1723	1630

drug alone, whereas the amide carbonyl band appeared markedly shifted to higher wave number. This behaviour can be attributed to the interaction of the carrier with the drug molecule, involving hydrogen bonds [11].

Dissolution studies

The mean dissolution curves of VAL and its solid systems with HP-β-CD are reported in Figure 4. At each time point, the amount of VAL dissolved from the PM sample was higher with respect to VAL alone. The FD product provided a further increase of solubilized drug. Table 3 shows the relative parameters of the dissolution process, i.e. the percentage of VAL dissolved within 2 min (DP) in the absence or in the presence of HP-β-CD (PM and FD systems), and the relative dissolution rate (RDR) of the PM and FD samples calculated at $t = 2$ min.

The PM produced approximately a 2-fold increase of VAL dissolution rate (RDR 1.8). The highest amount of solubilized drug was obtained with the FD sample (DP 72.1 ± 1.7%), that exhibited a 5.5 RDR. The increase in VAL dissolved from the HP-β-CD containing systems can be ascribed to formation of a readily soluble complex in the dissolution medium [12] and to their different drug–CD interaction degree occurring at solid state.

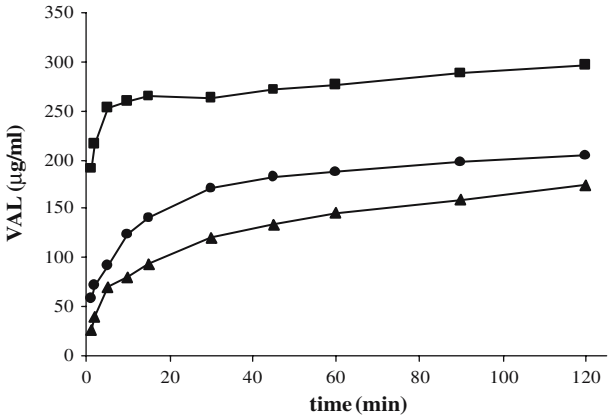


Figure 4. Dissolution curves of VAL (▲) and VAL/HP-β-CD equimolar systems: physical mixture (●) and freeze-dried product (■) (mean of three experiments, CV < 3%, error bars omitted for the sake of clarity).

Stability studies

VAL oral formulations available on the market have to be stored at controlled room temperature (USP 24) due to the limited drug thermal stability. Since CD complexation of drugs has been reported to increase both their chemical and physical stability [4, 13], we investigated VAL thermal stability in the absence and in the presence of HP-β-CD.

Table 3. Percent of active ingredient dissolved (DP) and relative dissolution rate (RDR) at $t = 2$ min of VAL and its PM and FD products with HP-β-CD (± indicates the SD of the respective values)

Sample	DP	RDR
VAL	13.1 ± 0.3	1
PM	23.9 ± 0.5	1.8
FD	72.1 ± 1.7	5.5

Figure 5 shows the thermal degradation, at 80 °C, of VAL both alone and in FD product with HP-β-CD as a function of time. From these data it was not possible to determine the reaction order for VAL degradation, as it often happens in studies on degradation kinetics in the solid state [14]. The diagrams show that thermal stability of VAL was much higher when it was associated with HP-β-CD. In fact, after 48 weeks the concentration of undegraded VAL was 73% for the drug alone, whereas it was 93% for the drug/HP-β-CD system. Therefore, enhancement of drug stability was approximately 4-fold in the presence of the carrier.

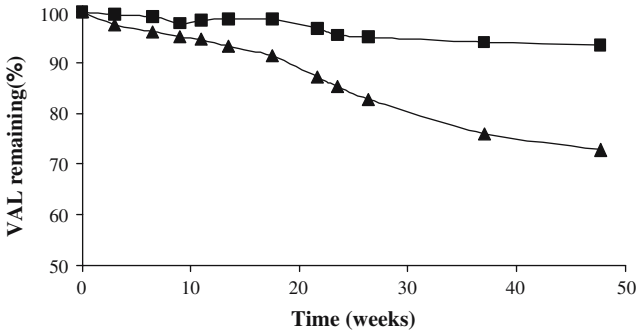


Figure 5. Thermal degradation of VAL as a function of time at 80 °C: VAL (▲), FD product (■) (mean of three experiments, CV < 3%, error bars omitted for the sake of clarity).

The HPLC analysis of the samples under investigation, stored at 80 °C, permitted us to determine the number of decomposition products.

The chromatograms of VAL alone revealed the presence of two decomposition products, which were identified by LC-MS/MS analysis, on the basis of the mass of the molecular ion and the fragment ions. The compounds are reported in the degradation scheme in Figure 6. From the results obtained it can be hypothesized that the degradation of VAL in the tested conditions follows different reaction mechanisms: (i) breakdown of the amide bond to give product I, that can (partially) undergo N-dealkylation and oxidation producing product II; (ii) direct N-dealkylation and oxidation of VAL to give product II. On the other hand, the HPLC analysis of the VAL/HP-β-CD sample (FD product) showed only one degradation product. This was identified as product II (Figure 6).

This means that HP-β-CD interferes with VAL decomposition, improving its thermal stability. The mechanism of the decreased VAL degradation as a consequence of HP-β-CD complexation is under investigation.

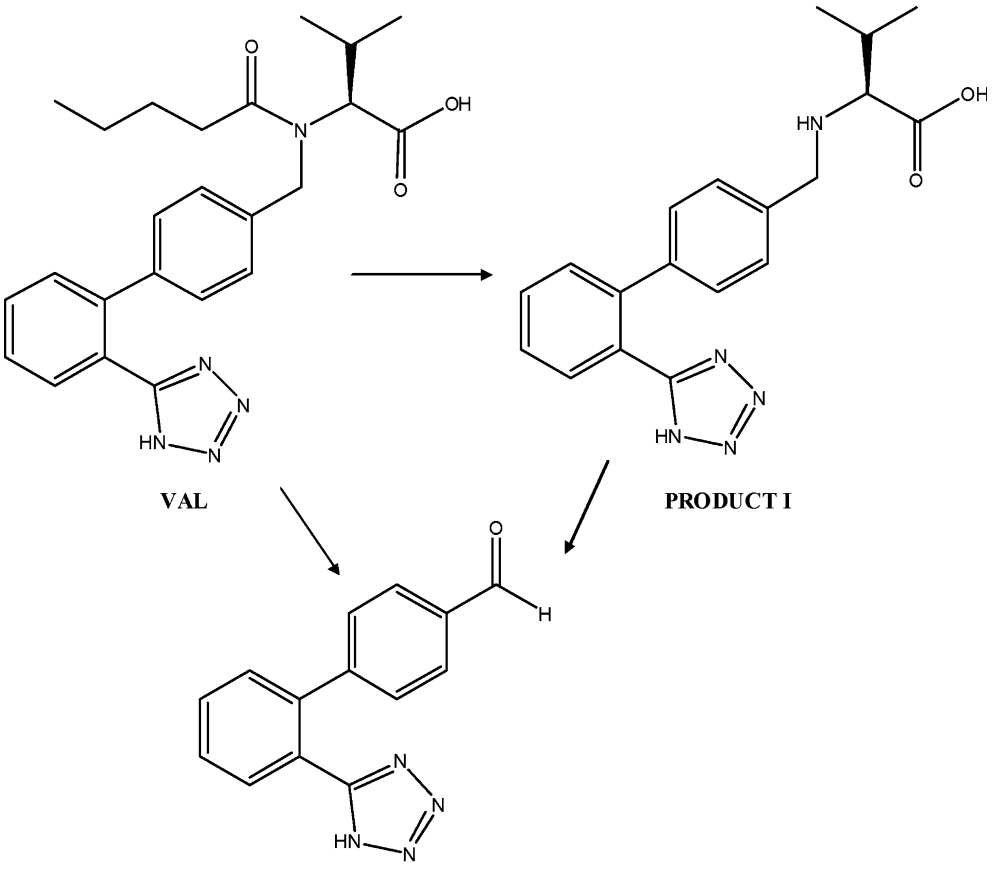


Figure 6. Scheme of degradation of VAL.

Conclusions

The HP-β-CD can be used successfully to improve the solubility in water and dissolution of VAL. Enhancement of the solubility of VAL was approximately 18-fold at 7.6% (5.5×10^{-2} M) HP-β-CD. In addition, a 5.5-fold increase of VAL dissolution rate was obtained with the VAL/HP-β-CD freeze-dried sample (1:1 molar ratio). These results were attributed to the formation of an inclusion complex as indicated by DSC, FT-IR and ^{13}C-NMR studies.

This work also reveals the potential of HP-β-CD from the stability point of view, since the inclusion complex VAL/HP-β-CD exhibits a greater thermal stability than the neat drug.

The improvement of VAL solubility, dissolution and stability implies the possibility to develop oral dosage forms of the drug with higher bioavailability and longer shelf-life.

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References

1. L. Criscione, M. De Gasparo, P. Buehlmayer, S. Whitebread, H.P. Ramjouè, and J.M. Wood: *Br. J. Pharmacol.* **110**, 761 (1993).
2. R. Dina and M. Jafari: *Am. J. Health-Syst. Pharm.* **57**, 1231 (2000).
3. G. Flesch, P. Müller, and P. Lloyd: *Eur. J. Clin. Pharmacol.* **52**, 115 (1997).
4. T. Loftsson and M.E. Brewster: *J. Pharm. Sci.* **85**, 1117 (1996).
5. K. Uekama, F. Hirayama, and T. Irie: *Chem. Rev.* **98**, 2045 (1998).
6. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instrum.* **4**, 117 (1965).
7. Y. Inoue: *Ann. Rep. NMR Spectros.* **27**, 59 (1993).
8. K.H. Kim, M.J. Frank, and N.L. Henderson: *J. Pharm. Sci.* **74**, 283 (1985).
9. P. Mura, G.P. Bettinetti, A. Manderioli, M.T. Faucci, G. Bramanti, and M. Sorrenti: *Int. J. Pharm.* **166**, 189 (1998).
10. P. Mura, M.T. Faucci, P.L. Parrini, S. Furlanetto, and S. Pinzauti: *Int. J. Pharm.* **179**, 117 (1999).
11. Y. Nakai, K. Yamamoto, K. Terada, and K. Akimoto: *Chem. Pharm. Bull.* **32**, 685 (1984).
12. O.I. Corrigan and T. Stanley: *J. Pharm. Pharmacol.* **34**, 621 (1982).
13. B. Cwiertnia, T. Hladon, and M. Stobiecki: *J. Pharm. Pharmacol.* **51**, 1213 (1999).
14. H.H. Tønnesen: *Int. J. Pharm.* **225**, 1 (2001).