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Characterization of two new potential impurities of Valsartan obtained under photodegradation stress condition

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ABSTRACT

A photostability study of Valsartan (VAL) is reported. Exposure of the drug to UV–vis radiation ($\lambda > 320$ nm) yielded two previously unknown compounds, which were detected by HPLC. Preparative amounts of the new potential degradation products (DP-1 and DP-2) were obtained by submitting VAL bulk drug to extensive photodegradation. The impurities were isolated by preparative normal phase column chromatography. Analytical information from the infrared, nuclear magnetic resonance and mass spectral data of the degradation products revealed their structures as *N*-[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-*N*-isobutylpentanamide (DP-1) and *N*-(diazirino[1,3-*f*]phenanthridin-4-ylmethyl)-*N*-isobutylpentanamide (DP-2). DP-1 arose from decarboxylation of VAL, while DP-2 results from further loss of nitrogen from the tetrazole motif of DP-1, with concomitant cyclization to yield a tetracyclic diazacyclopropene derivative.

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1. Introduction

Valsartan (VAL) is (*S*)-2-(*N*-((2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl)pentanamido)-3-methylbutanoic acid (Fig. 1). The drug is a potent, orally active and highly selective antagonist of the angiotensin II AT1-receptor, which is widely employed for treatment of all grades of hypertension [1]. VAL has recently acquired official status in the most relevant compendia, including European [2] and United States [3] Pharmacopoeias; there, the valsartan monograph describes two liquid chromatography determinations for limiting a total of three related compounds.

In view of its wide use, the determination of process and degradation impurities in VAL bulk drug [4–9] and drug products [10–12] has received much attention. Recently, the group of Krishnaiah reported a UPLC based stability-indicating method for the drug, which included the simultaneous detection and quantification of seven impurities [13].

The International Conference on Harmonization (ICH) guidelines [14,15] require stress tests to be carried out in order to elucidate the inherent stability characteristics of the active pharmaceutical ingredients. These tests help identifying potential degradation products and understanding plausible degradation

pathways, being also helpful for validating the stability-indicating power of the analytical procedures [16]. The understanding of the potential degradation pathways of the drug substances, resulting from these studies, allows optimization of storage conditions, which ultimately may lead to lower impurity levels. Photostability testing is an integral part of stress testing, which aims to provide evidence on how the quality of a drug changes with time under the influence of the light. This is relevant, as photodecomposition may result in loss of potency and the formed degradation products may cause adverse effects [17].

The photostability of VAL has been studied by various authors with different results. Some researchers concluded that the drug is photostable [18,19], even after irradiating at 254 nm [12], while others have noticed that VAL is photolabile, without elucidating the structure of the degradation products [20–22]. On the other hand, the group of Singh [23] has recently reported that in photo-neutral conditions VAL undergoes free-radical *N*-dealkylation, and also a radical-mediated cyclization in photo-acidic conditions.

During the HPLC analysis of different batches of VAL, two hitherto unreported impurities (DP-1 and DP-2, Fig. 1) were detected at levels of about 0.2–1% (Fig. 2, run B). The degradation products were formed in solutions of VAL (1.0 mg ml⁻¹ in water: *tert*-butanol 2:1, v/v) exposed to UV–vis radiation from a metal halide lamp at $\lambda > 320$ nm under the conditions suggested by the ICH photostability guideline [14]. Hence, this study was undertaken towards the preparation and isolation of the impurities to facilitate their characterization by infrared, NMR and mass spectroscopic techniques.

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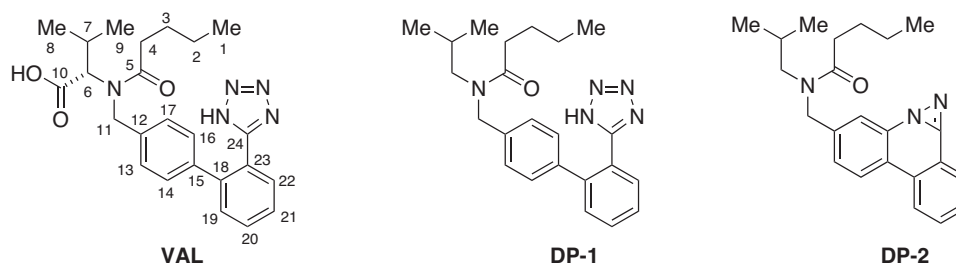


Fig. 1. Chemical structures of Valsartan (VAL) and its photodegradation products DP-1 and DP-2.

2. Experimental

2.1. Chemicals and materials

The experiments were performed with pharmaceutically certified Valsartan (Saporiti, Buenos Aires, Argentina) and analytical grade reagents (Merck & Co., Darmstadt, Germany). Kieselgel 60H (particle size 40–63 μm) was employed for column chromatography. Double distilled water and HPLC-grade solvents (J.T. Baker, Mexico City, Mexico) were employed for the chromatographic analyses and for sample preparation.

2.2. Instrumentation

The ^1H , ^{13}C (proton decoupled) and 2D NMR spectra (COSY, HSQC and HMBC) of the impurities were acquired on a Bruker Avance 300 (Bruker BioSpin GmbH, Karlsruhe, Germany) spectrometer (300 MHz for ^1H ; 75 MHz for ^{13}C) at 25 $^\circ\text{C}$ in CDCl_3 . Spectral data of VAL were obtained in $\text{DMSO}-d_6$. The chemical shift values are reported in the δ scale, in ppm relative to TMS internal standard (δ 0.00 ppm) for ^1H and relative to the solvent residual peak (δ_{CDCl_3} 77.0 ppm and $\delta_{\text{DMSO}-d_6}$ 39.5 ppm) for ^{13}C signals. Coupling constants (J) are expressed in Hertz. Signals are abbreviated as follows: s = singlet; d = doublet; t = triplet; m = multiplet; b = broad signal. HSQC spectra allowed to distinguish methyl and methine

carbons (positive peaks) from methylene carbons (negative peaks). The ^{15}N NMR data of DP-2 [gHMBC experiment (J_{HX} 8 Hz)] were obtained with a Bruker Avance II 400 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). Chemical shifts are expressed in ppm, downfield from nitromethane used as the internal standard.

The infrared spectra (FT-IR) of the impurities DP-1 and DP-2 were recorded in a Perkin Elmer Spectrum One spectrometer (Perkin Elmer, Inc., Shelton, CT), with the samples as dispersions in dry KBr. The high resolution mass spectra were obtained with a Bruker micrOTOF-Q II instrument (Bruker Daltonics, Billerica, MA). Detection of the ions was performed in electrospray ionization, in positive ion mode.

The HPLC separations were carried out with an in-house developed method, employing a Varian Prostar 210 liquid chromatograph (Varian, Inc., Walnut Creek, CA) equipped with two pumps, a Rheodyne injector fitted with a 20 μl loop, a 250 mm \times 4.6 mm cyano column (Luna, 5 μm particle size) thermostated at 30 $^\circ\text{C}$ in a column oven and a Varian Prostar 325 variable dual-wavelength UV–vis detector set at 226 nm. The mobile phase was a mixture of acetonitrile and potassium monobasic phosphate (pH 3.0; 0.02 M) (40:60, v/v), pumped at a flow rate of 1.0 ml min^{-1} . The chromatograms were recorded and analyzed employing Varian Star software v. 6.41. The peak purity studies were performed under the same conditions, with a HP 1100 liquid chromatograph (Agilent Technologies, Inc., Wilmington, DE) equipped with a photodiode array detector and controlled by the Chemstation software.

2.3. Photostability studies

2.3.1. Analytical scale studies

The studies were conducted at 35 $^\circ\text{C}$ in a 40 cm \times 30 cm \times 30 cm stability chamber fitted with four Philips G4T5 short-wavelength UV-lamps (4 W each) and with a 400 W metal halide lamp (Philips Lighting, Turnhout, Belgium), with a UV-A radiant output of 410 $\mu\text{W/cm}^2$ at 15 cm distance. The samples were solutions containing 1.0 mg ml^{-1} VAL in a mixture of water:tert-butanol (2:1,

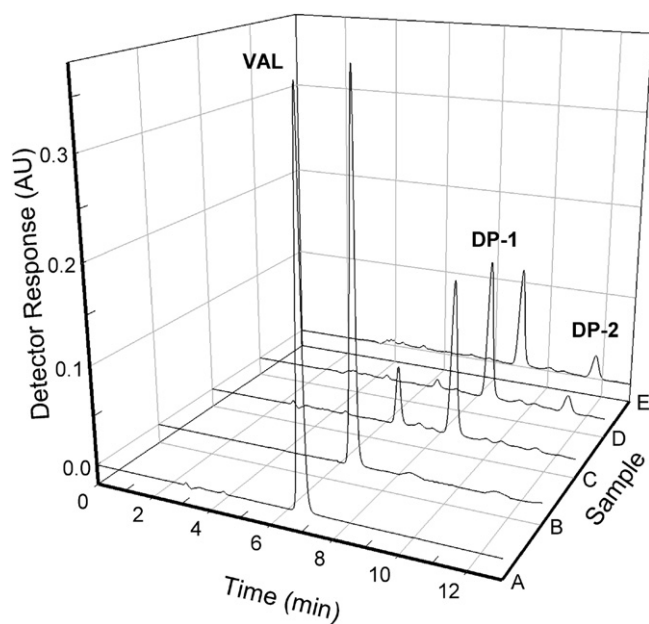


Fig. 2. Chromatograms of VAL and its photodegradation products. (A) Control sample (not exposed). (B) Sample of VAL (1.0 mg ml^{-1}) exposed to UV–vis radiation ($\lambda > 320$ nm) after the ICH guideline conditions. (C–E) Formation of the degradation products by exposure of a solution of VAL to the metal halide lamp, at different irradiation times (1.5, 3.0 and 4.5 h, respectively).

Table 1
Infrared and mass spectral data of DP-1 and DP-2.

Impurity	FT-IR (cm^{-1})	Mass spectra, m/z (%)
DP-1	3680–3230, 2957, 2871, 1736, 1642, 1533, 1469, 1263, 1100, 952, 820 and 770	414.2269 ($[\text{M}+\text{Na}]^+$, 100), 392.2445 ($[\text{M}+\text{H}]^+$, 54), 386.2191 ($[\text{M}-\text{N}_2+\text{Na}]^+$, 21, loss of N_2), 364.2361 ($[\text{M}-\text{N}_2+\text{H}]^+$, 3, loss of N_2), 357.2043 ($[\text{M}-\text{Me}_2\text{CH}=\text{CH}_2+\text{Na}]^+$, 5, loss of isobutylene) and 235.0971 ($[\text{M}-\text{C}_9\text{H}_{18}\text{NO}]^+$, 4, loss of the secondary amide moiety)
DP-2	2959, 2871, 1730, 1605, 1466, 1388, 1228, 1103, 1025, 996, 820 and 760	384.2040 ($[\text{M}+\text{Na}]^+$, 69), 362.2215 ($[\text{M}+\text{H}]^+$, 100), 278.1654 ($[\text{M}-\text{C}_5\text{H}_7\text{O}]^+$, 17, loss of the valeryl side chain), 205.0759 ($[\text{M}-\text{C}_9\text{H}_{18}\text{NO}]^+$, 61, loss of the secondary amide moiety)

Table 2
NMR spectral data of VAL and its photodegradation products DP-1 and DP-2^a.

	Valsartan			DP-1			DP-2		
	¹ H	¹³ C	COSY	¹ H	¹³ C	COSY	¹ H	¹³ C	COSY
1	0.74, t J=7.2	14.1 (CH ₃)	1.14	0.82, d J=6.5	13.8 (CH ₃)	1.25	0.97, t J=7.1	13.9 (CH ₃)	1.43
2	1.10–1.18, m	22.1 (CH ₂)	0.741.52	1.25, dq J=6.5, 7.7	22.5 (CH ₂)	0.82, 1.48	1.43, sextet J=7.7	22.6 (CH ₂)	0.971.73
3	1.52, sextet J=6.9	28.0 (CH ₂)	1.142.46	1.48, ddd J=7.1, 7.7, 8.3	27.6 (CH ₂)	1.25, 2.31	1.73, quintet J=7.6	27.6 (CH ₂)	1.432.48
4	2.41–2.51, m	32.9 (CH ₂)	1.52	2.31, bdd J=7.1, 8.3	32.9 (CH ₂)	1.48	2.48, bdd J=7.6, 7.6	33.0 (CH ₂)	1.73
5		172.4(C=O)			174.6(C=O)			174.1(C=O)	
6	4.03, d J=6.5	63.4 (CH)	2.17	3.04, dd J=7.1, 7.1	54.8 (CH ₂)	1.96	3.20, d J=7.5	55.3 (CH ₂)	2.02
7	2.17, septet J=6.5	27.2 (CH)	4.03	1.96, ddd J=7.1, 7.1, 7.3	26.9 (CH)	0.82,0.90,3.04	1.96–2.07, m	27.9 (CH)	0.973.20
8	0.73, d J=6.5	19.2 (CH ₃)	2.17	0.82, d J=7.3	20.1 (CH ₃)	1.96	0.97, d J=6.7	20.1 (CH ₃)	2.02
9	0.91, d J=6.5	20.5 (CH ₃)	2.17	0.90, d J=7.3	20.1 (CH ₃)	1.96	0.97, d J=6.7	20.1 (CH ₃)	2.02
10		174.2(C=O)							
11	4.41, d, J=13.6 4.49, d, J=13.6	49.1 (CH ₂)	4.494.41	4.49, s	48.6 (CH ₂)		4.86, s	48.8 (CH ₂)	
12		138.1 (C)			137.2 (C)			141.3 (C)	
13	7.05, bd J=8.0	126.7 (CH)	7.18	7.04, bd J=7.9	126.1*(CH)	7.15	7.77, d J=7.8	129.2 (CH)	8.75
14	7.18, d J=8.0	128.2*(CH)	7.05	7.06, bs	127.9*(CH)	7.15	8.75, dd J=1.5, 7.8	126.1 (CH)	7.77
15		138.2 (C)			138.4 (C)			118.5 (C)	
16	6.95, d J=8.0	128.7*(CH)	7.05	7.06, bs	128.0*(CH)	7.15		140.3 (C)	
17	7.08, dd J=8.0	126.7 (CH)	7.18	7.15, bd J=7.9	129.6*(CH)	7.06	8.45, d J=1.5	123.0 (CH)	
18		141.6 (C)			141.2 (C)			125.8 (C)	
19	7.48–7.59, m	129.2 (CH)	7.65	7.43, d J=6.8	130.7 (CH)	7.59	7.68, d J=7.8	127.8 (CH)	8.45
20	7.59–7.71, m	131.0 (CH)	7.54	7.59, dd J=6.8, 6.8	131.0 (CH)	7.43, 7.85	8.40–8.54, m	125.5 (CH)	7.687.88
21	7.48–7.59, m	127.4 (CH)	7.65	7.48, dd J=6.8, 7.4	129.3 (CH)	7.59, 7.85	8.40–8.54, m	124.3 (CH)	7.687.88
22	7.59–7.71, m	131.6 (CH)	7.54	7.85, d J=7.4	130.9 (CH)	7.48	7.88, d J=7.2	131.9 (CH)	8.45
23		123.8 (C)			123.2 (C)			129.8 (C)	
24		155.5 (C)			155.1 (C)			147.3 (C)	
NH	1.97, s			2.80, s					

^a Structures are numbered as in Ref. [26]. Each compound was observed as a mixture of rotamers; the signals of the most abundant rotamer are informed. The correlations shown in the ¹H and ¹³C NMR columns are in agreement with HSQC and HMBC spectra. In the COSY correlations, the centres of the multiplets are informed. The symbols “#” and “*” indicate pairs of assignments which may be interchanged.

v/v), placed in Pyrex containers at 15 cm from the source. For chromatographic analyses, 1.0 ml aliquots were periodically transferred to 10 ml flasks and diluted to the mark with mobile phase.

2.3.2. Isolation of DP-1 and DP-2

A solution of VAL (1.45 g) in a water:tert-butanol mixture (2:1, v/v, 315 ml) was placed in an Ace immersion photoreactor (Ace Glass, Inc., Vinland, NJ), fitted with a 100 W mercury lamp. The solution was purged with nitrogen and irradiated at 25 °C until approximately two thirds of the starting material was consumed, as judged by TLC [developed with 60:40 and 80:20 (v/v) CHCl₃:MeOH mixtures; the spots were detected by examination of the plates under UV-light (254 nm) or by exposure to vapors of iodine].

Once the reaction was terminated, the solvent was removed under reduced pressure and the residue was chromatographed with mixtures of CHCl₃:EtOH, giving DP-1 (96 mg, 8%). Increasing solvent polarity furnished DP-2 (565 mg, 40%). The fractions containing pure isolated materials were pooled, concentrated under reduced pressure and stored at 4 °C.

3. Results and discussion

3.1. Generation, detection and isolation of impurities DP-1 and DP-2

VAL was stressed by exposure to radiation from a metal halide lamp, a light source that has been widely used in drug photostability research [24]. Its output spectrum is continuous from 315 to 2000 nm, being uniform in the 350–650 nm region and closely resembles that of sunlight [17,25]. Exposure to radiation of $\lambda > 320$ nm was ensured by confining the samples in Pyrex vessels.

Irradiation of diluted samples of VAL (1.0 mg ml⁻¹) resulted in the formation of two photodegradation products (DP-1 and DP-2). These could not be observed by HPLC in 20 min isocratic runs employing methods based on the use of C-18 columns, but were detected when in-house devised conditions were applied (Fig. 2). The same degradants were also found upon exposure of a solution of the drug (in a quartz container) to short wavelength UV radiation. In addition, traces of DP-1 and DP-2 were detected when the solid was exposed to the halide lamp during 24 h. The preparative pho-

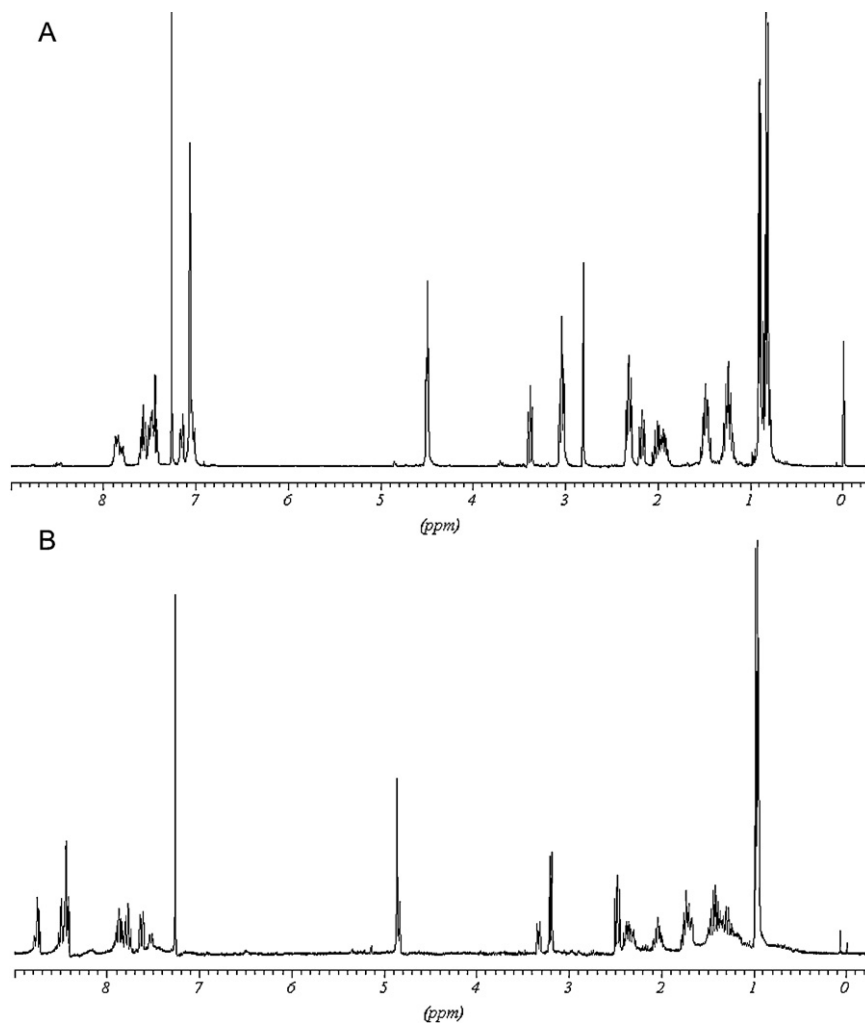


Fig. 3. ^1H NMR spectra of DP-1 (A) and DP-2 (B).

todegradation of VAL cleanly afforded DP-1 (8%) and DP-2 (40%) as relevant degradants. The impurities, which were isolated by normal phase preparative column chromatography, exhibited peak purities exceeding 0.995 by HPLC. The isolated products were used to

generate the spectral data employed for structural elucidation purposes. Their identity with the degradation products observed in the diluted analytical samples was confirmed by co-injection in the HPLC.

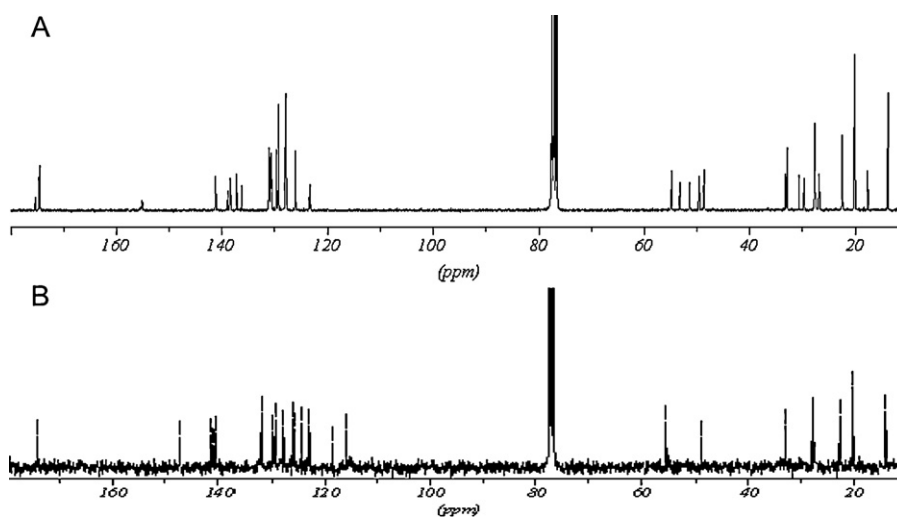


Fig. 4. Proton decoupled ^{13}C NMR spectra of DP-1 (A) and DP-2 (B).

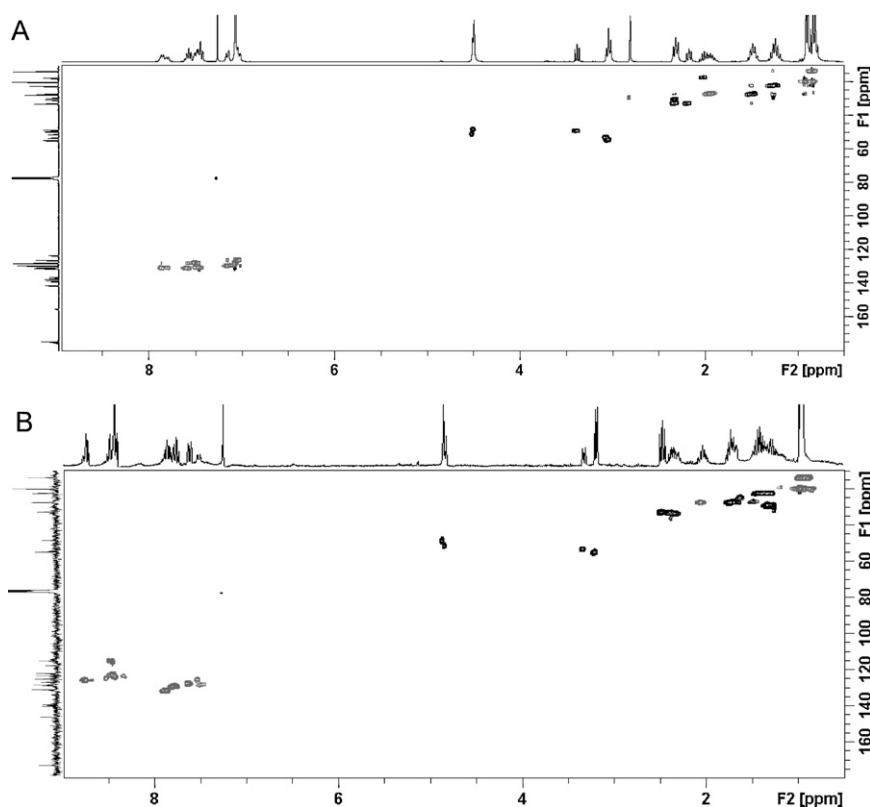


Fig. 5. HSQC spectra of DP-1 (A) and DP-2 (B).

3.2. Structural elucidation of the impurities

Both photodegradation impurities were unequivocally characterized by analysis of their infrared, mass (Table 1), ^1H NMR and ^{13}C NMR (Table 2 and Figs. 3–5) spectral data, and by comparison with the spectra of the drug. Additionally, COSY and HMBC spectra of the degradation products further secured unequivocal assignment of the signals.

3.2.1. Structural elucidation of impurity DP-1

In its NMR spectra, DP-1 was observed as a mixture of rotamers. Integration of the signals of H-6 in its ^1H NMR spectrum indicated their proportion as 74:26. Reminiscing the behaviour of VAL [26], this suggested that the impurity had two conformations in solution, corresponding to the *trans* and *cis* isomers of the amide bond, exchanging slowly on the NMR time frame.

The key differences between these spectra and those of VAL (Table 2) evidenced the lack of the carboxylic acid moiety, which caused shielding of H-6 and C-6 (δ 3.04 and 54.8 ppm, respec-

tively), with respect to VAL (δ 4.03 and 63.4 ppm, respectively). This was confirmed by the FT-IR spectrum (Table 1), which lacked the characteristic carboxylic acid carbonyl (1740 cm^{-1}) and O–H stretching ($2300\text{--}3600\text{ cm}^{-1}$) bands, found in the spectrum of VAL. A broad band ($3230\text{--}3680\text{ cm}^{-1}$) with a maximum at 3444 cm^{-1} (N–H stretching), confirmed the integrity of the tetrazole moiety [27].

Therefore, the structure of DP-1 was proposed as *N*-[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-*N*-isobutylpentanamide. This was further confirmed by the HRMS of the impurity, which exhibited $[\text{M}+\text{H}]^+$ and $[\text{M}+\text{Na}]^+$ peaks at m/z 392.2445 and 414.2269, consistent with the expected molecular formulae $\text{C}_{23}\text{H}_{30}\text{N}_5\text{O}$ ($[\text{M}+\text{H}]^+$) and $\text{C}_{23}\text{H}_{29}\text{N}_5\text{NaO}$ ($[\text{M}+\text{Na}]^+$), respectively.

3.2.2. Structural elucidation of impurity DP-2

The NMR spectra of DP-2 also contained superimposed resonances, as a result of being a mixture (65:35) of rotamers in solution, as stemmed from the integration of the signals attributed to H-6 in its ^1H NMR spectrum. Comparative analyses of the ^1H and ^{13}C NMR

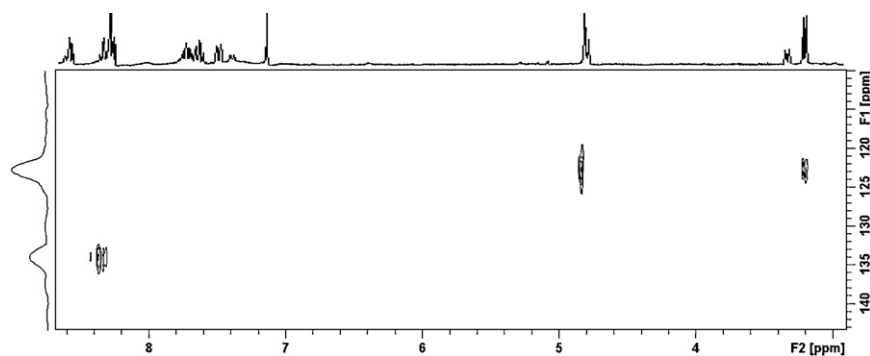


Fig. 6. ^{15}N gHMBC spectrum of DP-2.

spectra of VAL and DP-2 (Table 2) revealed the absence of the carboxylic acid group in the latter, which resulted in shielding of H-6 and C-6 (δ 3.20 ppm and δ 55.3 ppm, respectively) with regards to atoms in VAL, C-6 being observed as a methylene carbon in the HSQC experiment.

The FT-IR spectrum of the impurity, which displayed an absorption maximum at 1642 cm^{-1} (amide carbonyl), corresponding to the valeryl side chain but lacked absorption bands corresponding to the stretching of the O–H and C=O moieties, further confirmed the absence of a carboxylic acid moiety.

In addition, the shielding of the ^{13}C signals of C-15 and C-17 with concomitant deshielding of those attributable to C-18 and C-23 were indicative of the functionalization of the aryl group supporting the benzylamido moiety at C-16, which in turn was observed as a quaternary carbon resonating at δ 140.3 ppm. Furthermore, shielding of C-24 (δ 147.3 ppm) was also detected, suggesting the transformation of the tetrazole moiety into a smaller nitrogen-bearing ring system.

A ^{15}N NMR gHMBC experiment (Fig. 6) was run in order to gain more structural information and assess the formation of a C-16–N bond. This exhibited a cross-peak between the resonance of the amidic nitrogen (δ_{N} 122.7 ppm) and its nearest protons H-6 and H-11, confirming the presence of the secondary amide motif [28]. For the sake of comparison, the chemical shift of the amidic nitrogen of VAL (in DMSO- d_6) was found at δ_{N} 121.1 ppm. A second cross-peak between H-17 and another nitrogen atom (δ_{N} 133.9 ppm), confirmed the attachment of this nitrogen atom to the neighbour C-16.

On the other hand, the HRMS of DP-2 exhibited molecular ion signals at m/z 384.2040 ($[\text{M}+\text{Na}]^+$) and m/z 362.2215 ($[\text{M}+\text{H}]^+$). The value of M^+ was consistent with $\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}$ as a molecular formula for the impurity, and unveiled that the degradation took place with loss of the elements of nitrogen. This observation finally helped to postulate the structure of DP-2 as *N*-(diazirino[1,3-*f*]phenanthridin-4-ylmethyl)-*N*-isobutylpentanamide. Analysis of the ionic fragments (Table 2) further confirmed that DP-2 contained valeryl (m/z 278.1654) and isobutyl side chains, and that they were bound to the same nitrogen atom (m/z 205.0759).

3.3. Pathways of formation of the impurities

According to the proposed structures, DP-1 arised from light-induced decarboxylation of VAL. Photolytic decarboxylation as a degradation pathway has numerous precedents among pharmaceutically relevant compounds [29] and many drugs have been shown to be naturally degraded by a sunlight-mediated decarboxylation process [30].

On the other hand, DP-2 might have resulted from further decomposition of the tetrazole moiety with loss of nitrogen and cyclization of the reactive intermediate species onto the neighbour aromatic ring. Alternatively, cyclization could have preceded the loss of nitrogen. Interestingly, the group of Singh [23] recently characterized a photodegradation product of VAL, resulting from cyclization of its tetrazole moiety onto the neighbour ring of the biaryl system. Upon submission to mass spectral analysis, the degradation product underwent sequential loss of nitrogen, yielding a diazirino[1,3-*f*]phenanthridine intermediate, which further decarboxylated to furnish a fragment ion (m/z 360.2062) congruent with the calculated $[\text{M}-\text{H}]^+$ for DP-2 (m/z 360.2076). However, despite succeeding with the isolation of the product resulting from decarboxylation of VAL (DP-1), in our experiments we were unable to detect such a degradation product.

Diazirino[1,3-*f*]phenanthridines resulting from cyclizations similar to that leading to DP-2 have been detected during the mass spectral studies of candesartan cilexetil and irbesartan [31,32], structurally related to VAL. In addition, an analogous ring-forming

outcome was observed during the photolysis of telmisartan, when its biphenyl 2-carboxylic acid moiety was photocyclized to a benzo[*c*]chromen-6-one derivative [33]. Furthermore, the related photochemical cyclizations of 8-phenyltetrazolo[1,5-*c*]pyrimidine and 2-azido biphenyls to 9*H*-carbazole derivatives have also been described [34].

4. Conclusions

For the better understanding of the intrinsic stability of an active pharmaceutical ingredient it is necessary to identify all the relevant impurities formed under different conditions. A photostability study of Valsartan was performed, yielding two previously unknown compounds as new potential degradation impurities of the drug.

The products were isolated by normal phase column chromatography and characterized by analysis of their infrared, NMR and mass spectral data. DP-1 was the product of decarboxylation of VAL, while DP-2 resulted from additional loss of nitrogen from the tetrazole moiety with concomitant cyclization onto the neighbour aromatic ring.

Acknowledgments

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