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Five-year Stability Studies by GC and GC-MS of D-003 (a Mixture of C24:0 to C36:0 Fatty Acids) Alone and in 5 mg Film-coated Tablets

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SUMMARY. D-003, a product with cholesterol-lowering and antioxidant effects, is a mixture of very high molecular weight aliphatic acids ($C_{24:0}$ to $C_{36:0}$) purified from sugar cane *(Saccharum officinarum,* L.) wax, where octacosanoic acid is the major component. The stability of D-003 as such and in 5 mg film-coated tablets was determined following ICH guidelines. Validation studies of the GC method demonstrated good linearity (r > 0.9994, RSDs of the response factors and of the slopes < 5 and 2%, respectively), high accuracy (recoveries from 98.9 to 100.6%) and precision (RSD < 2% for repeatability and reproducibility). Specifications such as content, color, tablet weight, hardness, disintegration time, and microbiological content were assessed. Stress testing included acid hydrolysis, thermolysis, oxidation and photolysis. In addition, accelerated studies (12 months) under drastic conditions: 40 °C and 75% of relative humidity (RH) as well as long-term studies (60 months) at conditions of Climatic Zones IV (30 °C and 70% RH) and II (25 °C and 60% RH) were also conducted using three batches of AI and tablets. No significant changes or trends neither on the content nor on other specifications were found in any study. Overall, D-003 is very stable as such and in 5 mg film-coated tablets, with a shelf life of 5 years in climatic conditions of Zones IV and II.

RESUMEN."Estudios de Estabilidad por CG y CG-EM durante Cinco Años de D-003 (una Mezcla de Ácidos Grasos $C_{24:0}$ to $C_{36:0}$) Solo y en Forma de Tabletas Revestidas de 5 mg". El D-003 es nuevo producto con efectos reductores del colesterol y antioxidantes. Se trata de una mezcla de ácidos grasos alifáticos de alto peso molecular ($C_{24:0}$ a $C_{36:0}$) purificados de la cera de caña de azúcar *(Saccharum officinarum,* L.), donde el ácido octacosanoico es el componente mayoritario. Se determinó la estabilidad del D-003 en estado natural y en forma de tabletas recubiertas de 5 mg, siguiendo las guías de la ICH. Los estudios de validación de los métodos por CG demostraron una buena linealidad (r > 0,9994; CVs de los factores de respuesta y de las pendientes < 5 y 2%, respectivamente), elevada exactitud (recobrados desde 98,9 a 100,6%) y precisión (CV < 2% para la repetibilidad y la reproducibilidad). Se analizaron las especificaciones siguientes: contenido de D-003, color, peso de la tableta, dureza, tiempo de desintegración y contenido microbiológico. Los ensayos de estrés incluyeron hidrólisis ácida, termólisis, oxidación y fotólisis. Además, se llevaron a cabo estudios acelerados (12 meses) bajo condiciones drásticas: 40 °C y 75% de humedad relativa (RH), así como estudios a largo plazo (60 meses) utilizando tres lotes de IA y tabletas en las condiciones de las Zonas Climáticas IV (30 °C y 70% HR) y II (25 °C y 60% HR). En ninguno de los estudios se encontraron cambios significativos o tendencias, ni en el contenido de D-003 ni en las otras especificaciones. En general, el D-003 IA es muy estable así como las tabletas recubiertas con dosis de 5 mg, con un período de vencimiento de al menos 5 años en las condiciones climáticas de las Zonas II y IV.

INTRODUCTION

The purpose of stability studies is to provide the evidences on how the quality of a drug substance or a drug product varies with time under the influence of different environmental factors [1–3]. Thus, the final aim of these tests is to determine the period during which the drug meets the approved specifications when stored under defined conditions, being temperature, relative humidity, and light the most relevant. Fatty acids containing up to 24 carbon atoms, unsaturated or polyunsaturated have been linked to oustanding biological effects [4–5]. Previous to D-003, however, no drug containing primary fatty

KEY WORDS: D-003; GC; Octacosanoic acid, Stability studies; Tablets; Very long chain fatty acids.
PALABRAS CLAVE: Ácido octacosanoico, Ácidos grasos de elevado peso molecular, CG, D-003, Estudios de estabilidad, Tabletas.

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acids from 24 to 36 carbon atoms had been reported.

D-003 is a mixture of very high molecular weight aliphatic acids ($C_{24:0}$ to $C_{36:0}$) purified from sugar cane *(Saccharum officinarum,* L.) wax [6], in which each acid is within defined limits, octacosanoic acid being its main component (25.0-50.0%). Other acids present in the mixture are tetracosanoic (≤ 1.0%), pentacosanoic (0.3-1.5%), hexacosanoic (0.3-4.0%), heptacosanoic (0.3-4.0%), nonacosanoic (1.0-3.0%), triacontanoic (15.0-25.0%), hentriacontanoic (0.8-2.0%), dotriacontanoic (6.0-15.0%), tritriacontanoic (0.5-3.0%), tetratriacontanoic (5.0-15.0%), pentatriacontanoic (0.3-1.5%), and hexatriacontanoic (2.0-9.0%). D-003 has shown cholesterol lowering, antiplatelet and antioxidant effects in experimental and clinical studies [7-14]. Preclinical toxicological studies of D-003 have shown no drug-related toxicity, even orally administered at 1 g/kg for > 6 months [15–20].

In a previous paper, the authors carried out a drug-excipient compatibility study demonstrating that there was no chemical or physical interaction between D-003 and the analyzed excipients. They also proved by thermogravimetric analysis the high thermal stability of D-003, which melts without decomposition and is stable at temperatures as high as 220 °C [21]. However, those only are predictive results and are not recognized by the regulatory authorities, which in turn, mandate long term stability studies to establish the real stability and the expiration time of drug substance and drug products. Taking into account these facts, the aim of this study was to determine the stability of both the D-003 alone and its pharmaceutical form (5 mg film-coated tablets) through stressing test, accelerated and long term studies.

MATERIALS AND METHODS

Instruments

Gas Chromatographic (GC), as well as the GC/Mass Spectrometric (GC-MS) analyses were performed as previously described [22]. Briefly, a GC 14B gas chromatograph (Shimadzu, Japan) equipped with a flame-ionization detector and a BPX-5 wide bore column (25 m x 0.53 mm i.d, 1.0 µm film thickness) was used. Samples (1 µL) were injected at a column temperature of 220 °C by the "solvent flush" technique; then, the temperature was raised at 5 °C/min to 340 °C, with a final time of 10 min. The injector and detector were set at 320 and 340 °C respectively. Carrier gas flow (H_2) was 11.0 mL/min.

GC-MS analyses were carried out by using a gas chromatograph GC 8000, equipped with a mass selective detector MD800 (Fisons Instruments, England). An SPB-5 fused silica capillary column (30 m x 0.25 mm i.d., 0.32 mm film thickness) (Supelco, PA, USA) was employed. GC conditions: Injector port temperature, 320 °C; oven temperature gradient, from 100 to 200 °C at 40 °C/min, then increased by 10 °C/min from 200 to 320 °C and subsequently kept at 320 °C for 30 min. Injector parameters: split-splitless mode, septum purge flow-rate 5 mL/min, and a split-vent flow-rate at 45 mL/min, closed for 60 s. Portions of 1 mL were injected. Ion source and interface temperature were 250 °C and 280 °C, respectively. The ionization of samples was performed in electron impact mode (EI, 70eV). The mass spectrum was continuously acquired from 40 to 600 m/z with a scan speed of 1 s/decade in full scan mode. The carrier gas (helium) flow was 1 mL/min.

Chemicals

All chemicals were of analytical reagent grade: hydrochloric acid (37%), methanol, chloroform, toluene, hydrochloric acid (0.1 mol/L), hydrogen peroxide (30%), and sodium hydroxide (0.1 mol/L); (Merck, Darmstadt, Germany). Internal standard (IS) solution: 1 mg/mL, 1-nonadecanoic acid ($C_{19:0}$, Sigma, USA) in chloroform. Stock solution in chloroform, was prepared with 1-tetracosanoic ($C_{24:0}$), 1-pentacosanoic ($C_{25:0}$), 1-hexacosanoic ($C_{26:0}$), 1-heptacosanoic ($C_{27:0}$), 1-octacosanoic ($C_{28:0}$), 1-nonacosanoic ($C_{29:0}$), 1-triacontanoic ($C_{30:0}$) and 1-hentriacontanoic ($C_{31:0}$) acids; all > 99% GC, (Sigma, St. Louis, MO, USA) to give final concentrations of 0.07, 0.05, 0.14, 0.12, 1.50, 0.08, 0.8, and 0.05 mg/mL, respectively. D-003 working standard solution in chloroform, at 1.22 mg/mL. The methylation solution (MSoln) was prepared with HCl-methanol 5 % (v/v). All these solutions were found to be stable for at least 1 month when stored at + 4 °C.

Experimental design of the stability studies

The stability studies have followed the regulation of the Cuban State Center for Drug Quality, based on the guidelines of the International Conference on Harmonization (ICH) [2].

Batches

Stability data was obtained from 3 pilot scale batches of AI [990701, 990702 and 990703 (86.7%, 87.8% and 86.5% of purity, respectively)] provided by CNIC (Havana City, Cuba) and 5

mg film-coated tablets (000501, 000502 and 000503) manufactured in Laboratorios MedSol (Havana City, Cuba), which fulfilled specifications for batch release. Batches were packaged in the same container closure system as proposed for storage and distribution. The sealant integrity of the thermo-sealed polyvinyl chloride (PVC)-aluminum blisters was assessed visually and using the vacuum test with methylene blue.

The proposed formulation of D-003 5 mg film-coated tablets, contained lactose, cornstarch, gelatin, microcrystalline cellulose, magnesium stearate, and sodium carboxymethylstarch as excipients. Tablets coating was performed with a mixture of cellulose acetophthalate, polyethylene glycol 20000, special talc for tablets, titanium dioxide, and blended indigotine plus quinoline yellow.

Specifications

The studies assessed how quality parameters could change from initial values and deviate from specified acceptance limits. Thus, relevant appearance, microbiological control, as well as physical and physicochemical specifications: color, weight, hardness, humidity and disintegration times were assessed according to methods of USP XXVI [23]. For reaching specifications, hardness should be > 3 kgf, and disintegration time should be < 30 min.

For determining the content of sugar cane wax fatty acids in the AI (D-003), 10 mg of test material were accurately weighed in a 1.8 mL vial and 1 ml of IS solution was added, then the solvent was evaporated to dryness at 80 °C under a gentle air flow. It was removed and cooled to room temperature. One ml of freshly made MSoln was added; afterwards, the vial was tightly capped and placed into a block heater at 80 °C for 90 min, with occasional shaking. After cooling, the vial was opened and the sample was evaporated to dryness. Then, 1 mL of toluene was added to the dry methyl ester mixture and the vial was once again tightly closed and heated at 80 °C for 3 min, this way the sample was ready to GC analysis (1 µL injection volume). Validation of this method is included in this paper.

The tablet's content was measured through a previously validated analytical method [22]. In brief, an amount of powdered tablets equivalent to 5 mg of D-003, 0.5 mL of the IS solution and 3 mL of chloroform were added to a 10 mL test tube with screw cap; and the tube was heated at 80 °C for 15 min with occasional shaking. The extract was hot filtered to another test tube, and 1.0 mL of the filtrate was transferred to a 1.8 mL crimp vial and was evaporated to dryness at 80 °C with the help of an air flow. Then, 0.5 mL of the MSoln was added and the vial was sealed and heated at 80 °C for 90 min. The content of the vial was evaporated to dryness at 80 °C with air flow, and 150 µL of toluene were added. The vial was sealed and heated at 80 °C for 3 min and 1 µL portions were examined by GC. The mass (mg) of each acid in the tablets was obtained by the internal standard method. The total mass of D-003 was determined by adding the masses of all the components. This value was corrected by taking into account sample mass and tablet average mass.

The identification and quantitation of individual acids were conducted through the internal standard method [24]. The analytical methods used were validated following the ICH guidelines [25], which included the peak purity confirmation by GC-MS.

The current report involves 4 stability studies of D-003 AI and 5 mg film-coated tablets, including stress testing, accelerated study, and two long-term stability studies conducted in the climatic condition of both type IV and type II climatic zones.

Stress testing

Stress testing is aimed at knowing the stability of the drug substance under extreme conditions, which can help to identify degradation products, degradation pathways and the intrinsic stability of the drug. For assessing these tests, D-003 AI (batch 990702) and a fine powder of crushed tablets (batch 000503), were placed in neutral glass ampoules, and submitted to the following treatments:

Acid hydrolysis. Samples were suspended in 0.1 mol/L hydrochloric acid, approximately 1 g in 10 mL. The ampoule was flushed with nitrogen, sealed, and placed for 1 day in a stout container, inside an oven, at 108 ± 2 °C. Thereafter, the content was neutralized, filtered and washed several times with water, and then dried.

Oxidative degradation. Samples were suspended in 30% hydrogen peroxide, approximately 1 g in 10 mL. The ampoule was flushed with nitrogen, sealed, and placed in a stout container in the dark, at 25 ± 2 °C for 7 days. Thereafter, the content was neutralized, filtered and washed several times with water, and then dried.

Photolytic degradation. The ampoule, containing tablets, was flushed with nitrogen, sealed, and placed in an UV light cabinet (254 nm) at 25 ± 2 °C for 7 days.

Thermal degradation. The ampoule, contain-

ing tablets, was flushed with nitrogen, sealed, and placed for 3 months in a stout container, inside an oven at 55 ± 2 °C.

In all cases, samples were analyzed by GC. All assays were performed in triplicate. The samples were also submitted to GC-MS analysis in order to verify the occurrence of degradation products.

Accelerated studies

Accelerated studies are those conducted in a way that they mimic the effects of short-term storage outside the label storage conditions and their impact on product quality, situations that can occur during shipping. These studies are normally conducted at 40 ± 2 °C and 75 ± 5% RH for 12 months, recommended testing frequency being every 3 months. Thus, a random sample of 100 g of each AI batch and 2880 tablets from batches 000501, 000502 and 000503 were taken and stored at such conditions. Then, 10 g from each AI batch and 3 blisters (60 tablets) from different packages were taken at the initial time (t_O), as well as at 1, 3, 6, 9 and 12 months. In each sampling 3 blisters from different packages were taken.

For AI, 100 g from each batch were placed into PVC envelopes and sealed. Afterwards, they were placed into plastic tanks and hermetically closed. Those tanks were put into an incubator.

Long-term stability studies

Two long-term studies were performed for both AI and 5 mg film-coated tablets: at the climatic conditions of zone IV, including Cuba, and at climatic conditions of Zone II.

A random sample (500 g of each batch of AI) and 2 880 packaged tablets from each batch were taken and stored at 30 ± 2 °C and RH 70 ± 5 %, conditions simulating climatic zone IV (Study # 3), as well as at 25 ± 2 °C and RH 60 ± 5%, conditions simulating climatic zone II (Study # 4). A Web Winder incubator (Web, Germany) provided these last conditions, and temperature and humidity were checked twice a day, at 10:00 am and 3:00 pm, throughout the entire study. Samples were taken at starting (t_O) as well as after 3, 6, 9, 12, 24, 36 48 and 60 months. At each sampling time, 10 g of each AI batch and 15 blisters (300 tablets) were taken from different unopened containers to make the composites used for the analyses.

Data analyses and evaluation

Results were statistically analyzed with the t-test for dependent samples (matched paired), for comparing the data obtained at each time versus initial time (t_O) [2]. An a = 0.05 was *"a priori"* assumed for statistical significance. A Bonferroni adjustment for multiple comparisons in a single experiment was applied. In our case, an adjustable α = 0.01 and α = 0.0056 for accelerated and long-term stability studies, respectively were used. The quantitative results were normalized (percent of label claim) and regression analysis between D-003 content (%) and time was carried out for both AI and tablets. A shelf life was estimated by determining the earliest time at which the lower one-sided 95% confidence limit for the mean intersects the proposed acceptance criterion (a 5% change from starting values and/or failure to meet any quality specification of specifications). The statistical analyses were conducted with 6.0 Statistics program, from Statistics for Windows.

RESULTS AND DISCUSSION

The validated method for D-003 AI showed good linearity between the calculated and the added masses [y = (0.865±0.02)x –(0.098±0.15)], r = 0.9996. Relative standard deviation of the response factors (RSDf) and of the slope (RSDb) were 2.15% and 0.88%, respectively; and absence of bias was found in the whole studied concentration range, from 30 to 150 % of the nominal mass (five points and n =3). The mean recoveries from spiked samples (Table 1) were all between 98.9 and 100.62% with good precision (RSD = 0.5–1.2%). Recoveries obtained and 100% value of theoretic recovery were not sig-

Amount added (mg)	Amount found (mg)			Mean recovery ± t x SD/$n^{1/2}$ (%)	RSD (%)	t_{exp}
	1	2	3			
1.120	1.111	1.122	1.117	99.70 ± 1.22	0.49	1.060
2.243	2.257	2.225	2.250	100.04 ± 1.85	0.75	0.092
3.363	3.375	3.358	3.326	99.70 ± 1.84	0.74	0.702
Total				99.82 ± 0.68	0.61	0.885

Table 1. Result of accuracy study of the analytical method for AI (n = 3).

Acid	Laboratory 1		Laboratory 2		Global	
	Mean (%) ± t x SD	RSD (%)	Mean (%) ± t x SD	RSD (%)	Mean (%) ± t x SD	RSD (%)
$C_{24:0}$	1.40 ± 0.04	1.37	1.36 ± 0.03	1.17	1.39 ± 0.04	1.99
$C_{25:0}$	1.04 ± 0.05	2.28	1.00 ± 0.03	1.46	1.03 ± 0.03	2.52
$C_{26:0}$	2.86 ± 0.04	0.63	2.79 ± 0.06	1.01	2.82 ± 0.06	1.52
$C_{27:0}$	2.53 ± 0.08	1.65	2.56 ± 0.08	1.54	2.54 ± 0.06	1.67
$C_{28:0}$	32.31± 0.65	0.99	32.11 ± 0.62	0.95	32.22 ± 0.42	0.98
$C_{29:0}$	1.72 ± 0.05	1.45	1.66 ± 0.05	1.56	1.68 ± 0.03	2.12
$C_{30:0}$	18.01 ± 0.38	1.03	17.98 ± 0.36	0.99	18.01 ± 0.25	0.98
$C_{31:0}$	1.13 ± 0.03	1.50	1.14 ± 0.07	2.97	1.13 ± 0.02	2.38
$C_{32:0}$	9.28 ± 0.24	1.29	9.13 ± 0.18	1.00	9.20 ± 0.16	1.41
$C_{33:0}$	1.40 ± 0.11	3.94	1.34 ± 0.08	2.86	1.38 ± 0.07	4.02
$C_{34:0}$	10.78 ± 0.29	1.32	10.84 ± 0.26	1.19	10.80 ± 0.19	1.25
$C_{35:0}$	0.58 ± 0.04	3.82	0.59 ± 0.12	9.62	0.58 ± 0.03	7.17
$C_{36:0}$	3.71 ± 0.14	1.92	3.85 ± 0.13	1.64	3.78 ± 0.10	2.57
Total	86.74 ± 1.78	1.00	86.35 ± 1.63	1.10	86.55 ± 1.11	1.06

Table 2. Results of precision study in two laboratories for AI (n = 8).

nificantly different, either for each concentration or for the total average recovery, according to the experimental t values, which were lower than tabulated t for p = 0.05 (4.303 and 2.306, respectively); ensuring the method's accuracy. Good precision (RSD = 0.92% for repeatability and RSD = 1.06% for reproducibility between two laboratories) were also obtained (Table 2) [25,26]. According to these results, it can be ensure that the validated procedure is appropriate for the quality control and stability studies of this AI.

In that sense, the GC validated method for determining D-003 in 5 mg film-coated tablets [22], was also linear [(y = (1.01±0.04)x –(0.04±0.18), r = 0.9994, RSD_f =1.50% and RSD_b = 1.71%)] and accurate (total average recovery = 99.60%) over the studied range, from 38 to 150% of the nominal concentration (seven points and n =3). Repetability and reproducibility for two laboratories at the nominal dose (100%) were < 1.5%, fulfilling the Horwitz's criteria [26]. In the ruggedness study the injection volume must be carefully controlled, because it affected the resolution between $C_{28:0}$-$C_{30:0}$, however, the quantitation results were not significantly affected. The method was suitable for quality control and stability studies of these tablets.

Stress stability studies

Results of the stress testing studies (Table 3) showed no appreciable changes of D-003 content with regards to the initial value, which suggest that no degradation occurred in the simulated stresses situations. No extra-peak was observed in the chromatograms of degraded tablets (Fig. 1), which were similar to chromatograms of AI and original tablets, not presented. It was demonstrated by GC-MS analysis, which only showed the appearance and the structure of the fatty acids that compose D-003 ($C_{24:0}$ to $C_{36:0}$). Molecular peaks at m/z 382, 396, 410, 424, 438, 452, 466, 480, 494, 508, 522, 536, and 550 for $C_{24:0}$, $C_{25:0}$, $C_{26:0}$, $C_{27:0}$, $C_{28:0}$, $C_{29:0}$, $C_{30:0}$, $C_{31:0}$, $C_{32:0}$, $C_{33:0}$, $C_{34:0}$, $C_{35:0}$, and $C_{36:0}$, respectively, as well as the other typical fragments of fatty acid methyl esters (mostly m/z, 43, 57, 74, 87, 143) were observed. These results preliminarily suggest the good stability of D-003 AI and tablets, and also prove the good specificity of the proposed analytical methods.

The obtained results are in good agreement with those obtained from other authors, who demonstrated the high stability to oxidation of saturated fatty acids with lower chain lengths than those from composing D-003. They found, through DSC studies, that stearic acid ($C_{18:0}$) oxidation begins above 180 °C [27].

Treatment	Without treatment	Acid hydrolysis	Oxidation	Photolysis	Thermolysis
AI	100	99.06	99.25	99.31	99.64
Tablets	100	99.8	99.4	99.4	99.8

Table 3. Average normalized content (%) of D-003 in stress testing of AI (batch 990701) and 5 mg film-coated tablets.

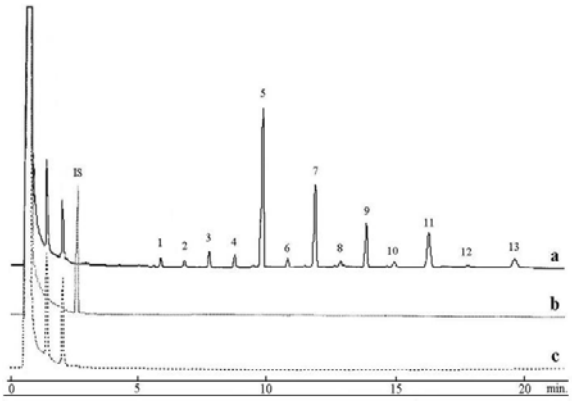

Figure 1. GC profiles of (**a**) D-003 tablets after exposure to oxidation conditions: suspended in 30% H_2O_2, at 25 °C for 7 days; (**b**) internal standard (IS); and (**c**) placebo tablets, all of them analyzed as methylester derivatives. Peaks correspond to derivatives of I.S ($C_{19:0}$), (1) $C_{24:0}$, (2) $C_{25:0}$, (3) $C_{26:0}$, (4) $C_{27:0}$, (5) $C_{28:0}$, (6) $C_{29:0}$, (7) $C_{30:0}$, (8) $C_{31:0}$, (9) $C_{32:0}$, (10) $C_{33:0}$, (11) $C_{34:0}$, (12) $C_{35:0}$, and (13) $C_{36:0}$.

Accelerated stability studies

Results of the accelerated studies demonstrated that D-003 AI and tablets were also stable in such conditions after 12 months of exposure. No significant changes with regards to the initial value occurred in the studied batches, neither in D-003 content (p = 0.452715 for AI and p = 0.057191 for tablets) nor in other physical and physicochemical parameters (Tables 4 and 5). No appreciable changes between the results, before and after exposure, were detected for the rest of AI and tablet batches, clearly showing that any degradation occurred during experimental time were negligible. The absence of degradation could be explained due to the saturated long chain of these acids, which contribute to their chemical and physical inertness. It is in good agreement with other reports, where fatty acids decompose at temperatures over 180 °C [27], as well as with DSC studies of D-003, which also demonstrated no interaction with the employed excipients. Thermogravimetric analysis also proved the high thermal stability of D-003, which melts without decomposition and is stable at temperatures as high as 220 °C [21].

Since all values were quite stable, the usual kinetic study for predicting expiring date of the product was not performed, as recommended by ICH Q1AR guidelines [2]. Taking into account these results it may be estimated, even before starting log-term testing, that the shelf life of this product is at least 5 years [28].

Long-term stability studies of D-003 Active ingredient (Zones IV and II)

Results of the two long-term stability studies, conducted at the conditions of the Climatic Zones IV and II for 60 months showed no outstanding or significant variation in organoleptic characteristic and relative humidity (≤ 1.0%). Microbial limit test was also fulfilled for all samples at the several tested times.

The t-test applied to the results of the content from the active ingredient for the three studied batches in the two studied climatic zones, revealed no significant differences between the initial time and the other tested times. Figs. 2 and 3 show the relationship between the normalized content of D-003 and studied times for batch 990702 under zone IV and II conditions, respectively. It can be observed that the lower confidence limit does not intercept the

Time (months)	0	1	3	6	9	12
AI	100	99.7	98.8	98.7	99.4	99.7
Tablets	100	98.7	100.6	99.8	99.3	98.9

Table 4. Average normalized content (%) of D-003 in accelerated studies (T: 40 ± 2 °C, RH: 75 ± 5%) of AI (batch 990701) and tablets (batch 000502).

Study	Time (months)	Mass (mg)	Color	Hardness (kgf)	Disintegration (min.)*
A	0-12	125.2-126.3	Light green-very Light green	3.9-4.0	3-7
Z-IV	0-60	125.2-126.3	Light green	3.8-4.1	3-8
Z-II	0-60	125.2-126.3	Light green	3.8-4.0	3-8

Table 5. Ranges of the assessed specifications of D-003 5 mg film-coated tablets in the accelerated and long term stability studies * Time to total disintegration of the last tablet. **A**: Accelerated study (T: 40 ± 2 °C, RH: 75 ± 5%). **Z-IV** Long-term stability study under conditions of Climatic Zone IV (T: 30 ± 2 °C, RH: 70 ± 5 %). **Z-II** Long-term stability study under conditions of Climatic Zone II (T: 25 ± 2 °C, RH: 60 ± 5%).

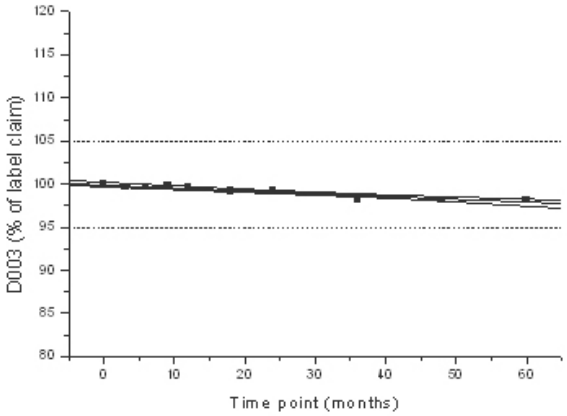

Figure 2. Long-term stability study under conditions of Climatic Zone IV. D-003 AI, batch 990702.

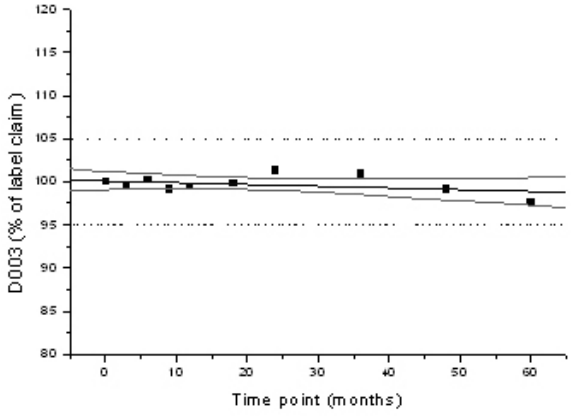

Figure 4. Long-term stability study under conditions of Climatic Zone IV. Tablets, batch 000503.

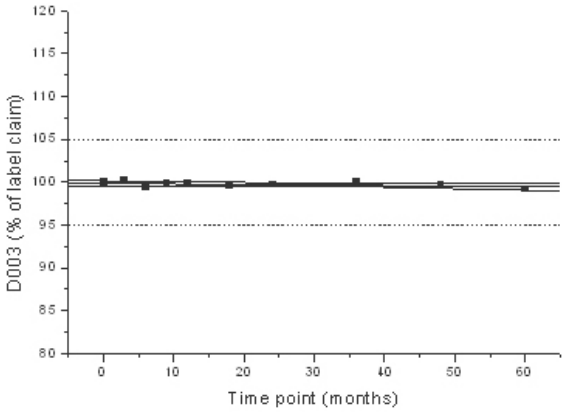

Figure 3. Long-term stability study under conditions of Climatic Zone II. D-003 AI, batch 990702.

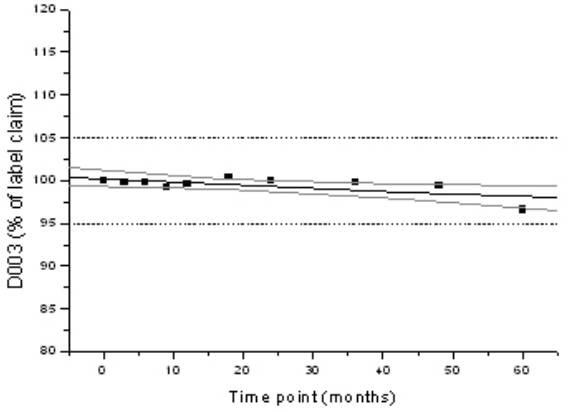

Figure 5. Long-term stability study under conditions of Climatic Zone II. Tablets, batch 000503.

lower acceptance criteria from 95 % of label claim, which means that no degradation occurred over the 60 months of study. A similar behavior was observed for the other studied batches. These findings were expected according to the previous obtained results in stress and accelerated studies, where no degradation was observed either. Such evidences point out the very high stability of D-003 AI, suggesting a 60 months period as the shelf life for this product.

Long-term stability studies of D-003 5 mg film coated tablets (Zones IV and II)

Results of the two long-term stability studies, conducted at the conditions of the Climatic Zones IV and II for 60 months, support the high stability of these tablets, since neither significant change nor out of limits specifications were observed (Figs. 4 and 5). Moreover, all batches maintained acceptable microbiological stability during the studied period. Thus, in the most aggressive of the researched conditions in long-term studies, those from the Climatic Zone IV, no outstanding or significant variation was observed either in the content of the active ingredient or in the other parameters.

Similar results were obtained at the conditions from the Climatic Zone II, which mean that the three studied batches showed the same behavior pattern. These behaviors can be appreciated in Figs. 3 and 5, where the lower confidence limit does not intercept the lower acceptance criteria from 95% of label claim. It suggests that no significant degradation occurred for the batch 000503 submitted to the conditions of both climatic zones. For such reasons, as occurred in accelerated studies, there were no applied kinetic studies for estimating the expiring date of the product. Therefore, these facts indicate the very high stability of this pharmaceutical form, and a shelf life period of 60 months can be proposed.

CONCLUSIONS

The GC validated method was found to be useful for the quality control and stability studies of D-003 active ingredient. The content, physicochemical and microbiological parameters of D-003 under the above storage condition changes within an acceptable range, showing no significant variation. D-003 as such and in tablet formulation is very stable according to the present results, which support a shelf-life of 5 y in climatic conditions of zones IV and II.

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