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Physico-Mechanical Characterization and Interactions with Excipients of D-003, a Mixture of Fatty Acids Isolated from Sugar Cane Wax

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SUMMARY. D-003 is a new product consisting of a mixture of fatty acids of very high molecular weight (from C24:0 to C36:0) in a reproducible proportion each, with cholesterol-lowering, and antiplatelet effects. As part of the formulation studies of D-003 a physico-mechanical characterization and its interaction with tablet excipients were carried out. The present study used differential scanning calorimetry (DSC) and thermogravimetry (TG) in order to evaluate the characteristic transitions and thermal stability of D-003 in presence of several excipients. Other properties, such as the angle of contact, solubility, wettability, particle size distribution, flow properties, tapped, bulk and at high pressure densities, as well as volume reduction under compression were measured. These studies, to our knowledge, have not been previously reported to these fatty acids. DSC studies demonstrated that there was no chemical or physical interaction between D-003 and the investigated excipients. Also, thermogravimetric analysis proved the high thermal stability of D-003, which melts without decomposition and is stable at temperatures as high as 220 °C. Industrial batches of D-003 showed adequate purity and uniform particle size distribution, good flow properties, compressibility and compactability, which support a suitable powder for manufacturing film-coated tablets as finished forms. Nevertheless, the very low solubility of D-003 in most solvents, especially in water, is the most critical aspect for developing the finished form, being relevant to decide the coating formulation.

RESUMEN. "Caracterización Físico-Mecánica e Interacciones con Excipientes del D-003, una Mezcla de Ácidos Grasos aislados de la Cera de la Caña de Azúcar". El D-003 es un nuevo producto que consiste en una mezcla de ácidos grasos de elevado peso molecular (desde C24:0 hasta C36:0), donde cada uno de ellos está en una proporción reproducible. Este producto presenta efectos antiplaquetarios y como reductor del colesterol. Como parte de los estudios de formulación del D-003 fueron llevadas a cabo la caracterización físico-mecánica y su interacción con excipientes para tabletas. El presente estudio utilizó la calorimetría diferencial de barrido (CDB) y la termogravimetría (TG) con el objetivo de evaluar la estabilidad térmica y las transiciones características del D-003 en presencia de diversos excipientes. Fueron medidas otras propiedades, tales como el ángulo de contacto, solubilidad, humectabilidad, distribución del tamaño de partículas, propiedades de fluido, densidades por asentamiento, por vertido y de alta presión, así como la reducción de volumen bajo compresión. Estos estudios, según nuestro conocimiento, no han sido previamente reportados para estos ácidos. Los estudios por CDB demostraron que no hubo interacción química o física entre el D-003 y los excipientes investigados. También, los análisis termogravimétricos probaron la elevada estabilidad térmica del D-003, el cual es estable y funde sin descomposición hasta temperaturas tan elevadas como 220 °C. Los lotes industriales estudiados mostraron adecuada pureza y distribución uniforme del tamaño de partícula, buenas propiedades de fluido, compresibilidad y cohesión, lo cual sustenta que sea un polvo apropiado para la elaboración de tabletas revestidas como forma terminada. Sin embargo, la muy baja solubilidad del D-003 en la mayoría de los disolventes, especialmente en agua, es el aspecto más crítico para el desarrollo la forma terminada, lo cual es importante para decidir la formulación del revestimiento.

INTRODUCTION

D-003 is an active ingredient purified from sugar cane (*Saccharum officinarum,* L.) wax with cholesterol-lowering, antiplatelet and antioxidant effects [1-11] that consists in a mixture of very long-chain fatty acids wherein octacosanoic acid is the main component (25-50%), but also contains tetracosanoic (≤ 1.0%), pentacosanoic

KEY WORDS: D-003, Differential scanning calorimetry, Drug-excipients interaction, Fatty acids, Sugar cane wax, Thermogravimetry.
PALABRAS CLAVE: Ácidos grasos, Calorimetría diferencial de barrido, Cera de caña de azúcar, D-003, Interacción droga-excipientes, Termogravimetría.

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(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%). The composition of this mixture is highly reproducible form batch to batch. Considering the good results of preclinical pharmacology [1-5] and that toxicology studies have not shown evidences of D-003-related toxicity [12-15].

To develop the pharmaceutical formulation to be used in clinical trials, several properties of the active ingredient were studied. The thermal characterisation is required to know the thermal transitions of D-003 and excipients to predict some pharmaceutical incompatibility. Therefore, the thermal behaviour of the active ingredient by differential scanning calorimetry (DSC) was investigated [16]. This is a method that offers advantages over traditional compatibility screenings, since no long-term storage of the mixture is required prior to evaluation, [16-21] and changes in the thermogram of a mixture, such as unexpected shifts, depressions, new or missing peaks are considered as relevant for the analyses.

Also, other relevant characteristics for the technology and the formulation, like the angle of contact, solubility, wettability, particle size distribution, flow properties, density, and volume reduction under compression were studied [17]. These studies, to our knowledge, have not been previously reported to these kind of very long chain fatty acids. As part of the formulation studies of D-003, a mixture of fatty acids isolated from sugar cane wax, a physico-mechanical characterization and its interaction with tablet excipients were carried out.

MATERIALS AND METHODS

Batches of D-003 (# 990701, 990702, 990703 and 990704) were supplied by National Center for Scientific Research (Havana City, Cuba). Chloroform, methylene chloride, acetone, hexane and methanol (Merck, Darmstadt, Germany) were of analytical purity. All the excipients were of pharmaceutical quality: lactose, corn starch, sodium starch glycolate, polyvinyl pyrrolidone (PVP), sodium croscarmellose (acdisol), microcrystalline cellulose (microcell 101), gelatine, talc special for tablets, magnesium stearate, titanium dioxide, polyethylene glycol 20 000, and cellulose acetophthalate.

Thermal analysis

The DSC studies were carried out using a Mettler TA 3000 coupled with a thermal cell DSC-20 (Greifensee, Switzerland). The samples of D-003 (5-7 mg) were placed in an aluminium crucible (40 µL) and scanned at 10 °C/min and 30 mL/min of nitrogen flux, from 30 to 350 °C. Calorimeter was calibrated with an indium standard. To study pharmaceutical interactions, mixtures of D-003 with the selected excipients were prepared, using mass/mass ratios of 3:1, 1:1, and 1:3 that were obtained by mixing, for 10 min, 60 mg of D-003 with 20, 60 and 180 mg of excipients. Then, the mixtures were crushed and sieved at 300 µm. Samples were heated at 85 °C for 1 h, dried in a vacuum oven for 5 h, and cooled to 25 °C previous to the analysis.

Particle size analysis

Ethanolic suspensions were prepared at 1.0% of D-003 by weight. Sieves of 0.125, 0.045, 0.032, 0.020 mm and a fast filter paper were used, ordered by descending pore diameter sieve; and a collector was placed under the filter paper. The quantity of D-003 retained in each sieve and filter was calculated by difference between final and initial weights. The results were expressed in percentage of the total weight (n = 3).

Density at high pressure

D-003 (450 mg) was punched using a flat punch of 13 mm in an hydraulic press (SPECA, Model 15,011), at 130 kN for 30 s. Compacts were weighed in an analytical balance (with 0.01 mg of sensitivity), and their heights were also measured. Density (g/cm^3) was calculated as the ratio of mass to volume of the compacts (n = 3).

Flow properties

The flow properties of D-003 were determined by the Carr`s Index and the Hausner`s Index (n = 3) [18].

Bulk and Tapped density

Fifty g of D-003, weighed accurately up to 0.01 mg; were added to a glass cylinder of 250 cm^3 and the volume measured to determine the bulk density. Then, the cylinder was dropped 50 times from a 5 cm height over a soft surface and the tapped density was calculated. The final results (g/cm^3) were obtained considering the mean of three replicate analyses.

Compressibility and compactability

D-003 was mixed for 5 min with 0.5% magnesium stearate, and different tablets were produced using a 13 mm flat punch press (SPECAC Model 15.011) and compressing for 2 s with the following compression forces: 6.5, 13.0, 19.5, 26.0, 32.5 and 39.0 kN. The strength and volume of the compacts were determined (n = 3).

Contact angle

D-003 (500 mg) was compressed in 13 mm diameter flat punch press (SPECAC Model 15.011). The contact angles between solid and liquid were measured with a wettability tester, in which small drops of water, ethanol, chloroform, methylene chloride and acetone were taken with a microsyringe and placed on the compact surface (n = 5).

Solubility in organic solvents and water

D-003 (100 mg) was accurately weighed and transferred to a 1000 mL Erlenmeyer flask. The solvent (chloroform, methylene chloride, acetone, hexane, methanol and water) was added drop wise to dissolution, 1000 mL being the top quantity of solvent (n = 3). The solubility (mL/g) in each solvent was reported according to the descriptive terms of USP 26 [22].

RESULTS AND DISCUSSION

Table 1 summarizes the melting ranges of four batches of D-003, active ingredient. As can be seen, they ranged between 84 and 86 °C. Fig. 1 shows a characteristic DSC thermogram of D-003 (batch 9907002), where the expected endothermic melting transition near 86 °C can be observed.

The thermogravimetric analysis of D-003 (Fig. 2) demonstrated that no mass loss occurred from 35 to 200 °C, which confirms that the transition near 86 °C previously detected was related to the melting process of the active ingredient, that occurs without decomposition. Nevertheless, mass loss of 88%, corresponding to a thermal decomposition process, was detected between 200 and 340 °C. The calculated energy of activation was high (295.35 kJ/mol), confirming the high thermal stability of D-003.

D-003 batch #	Melting temperature (°C)
9907001	84.2-85.6
9907002	84.6-86.0
9907003	84.4-86.0
9907004	84.0-85.8

Table 1. Melting temperature ranges of D-003.

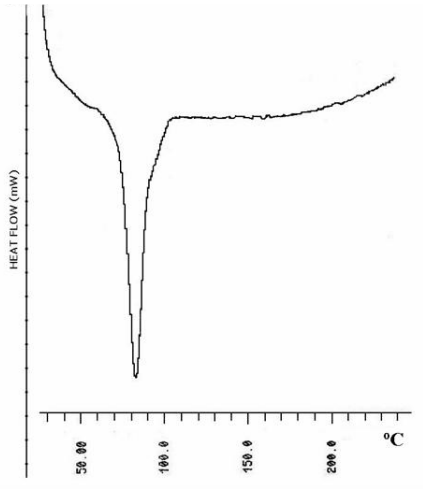

Figure 1. DSC thermogram of D-003.

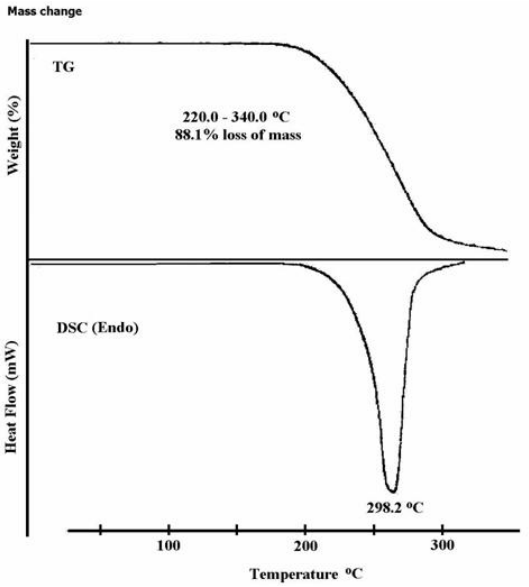

Figure 2. TG and DSC thermograms of D-003.

The DSC thermograms of the different mixtures of D-003 and the excipients demonstrated that there were no chemical or physical interactions. Fig. 3 shows, as example, the thermal curves obtained for the binary mixtures at 1:1 ratios, where the characteristic transitions of D-003 at 86 °C and those corresponding to each excipient were observed, no new transitions being detected. This result confirms the absence of chemical incompatibility between D-003 and the studied excipients.

Table 2 summarizes the results of the particle size distribution. The low coefficient of variation (CV) of each analysed fraction indicates that particle size distribution was homogeneous across the batches, a relevant aspect for the development of the formulation. Considering that the water solubility of D-003 is extremely poor,

Batch	9907001	9907002	9907003	9907004	Mean	SD	CV
Particle size (µm)		(%)			(%)		(%)
< 45	17.0	16.8	17.1	16.6	16.9	0.22	1.30
45-125	79.0	79.1	78.8	78.9	79.0	0.13	0.16
125-250	2.5	2.4	2.5	2.5	2.5	0.05	2.02
> 250	1.5	1.7	1.6	1.8	1.6	0.13	8.12
Mean diameter (µm)	80.7	81.0	81.2	82.0	81.22	0.56	0.68

Table 2. D-003: Particle size distribution.

Batch	9907001	9907002	9907003	9907004	Mean
Bulk density (g/cm³)	0.38	0.39	0.38	0.38	0.38
Tapped density (g/cm³)	0.50	0.50	0.49	0.47	0.49
High Pressure density (g/cm³)	0.96	0.96	0.95	0.96	0.96
Carr's Index (%)	24.0	22.0	22.4	18.9	21.8
Hausner's Index	1.32	1.28	1.29	1.23	1.28

Table 3. Relevant physico-chemical properties of D-003.

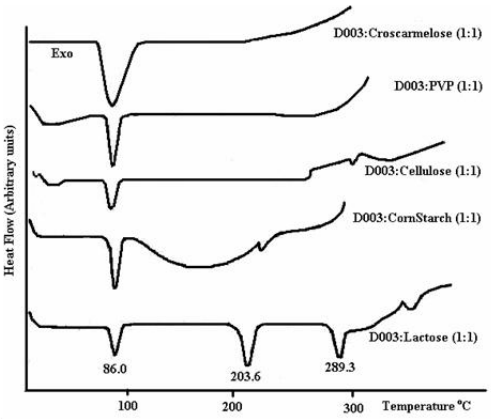

Figure 3. DSC thermograms of D-003 and excipients.

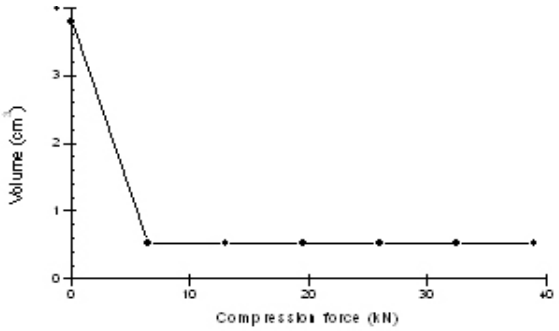

Figure 4. Compressibility of D-003.

a small particle size, like that found (81.2 µm) should improve the absorption of the substance after oral dosing.

Other relevant properties of D-003, like bulk density, tapped density, and density at high pressure were assayed (Table 3). D-003 has shown a low bulk density, being relevant for the formulation since the homogeneity of the

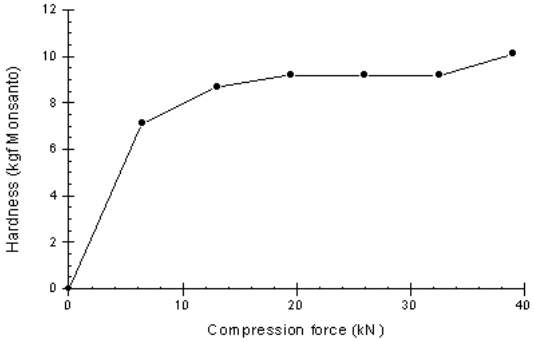

Figure 5. Compactability of D-003.

powder mixture is improved when active ingredient and excipeints have similar bulk density and particle size distribution. The determined values of D-003 powder were within the acceptance limits, according to Carr's and Hausner's indexes [18], to consider powder flow as good.

The volume decreased as the compression forces increased up to 6.5 kN, but higher forces did not cause further volume changes (Fig 4) being considered that D-003 is a powder compressible at low pressures. Also, the cohesion properties of D-003 were good (Fig. 5), since the obtained compacts had high hardness values that ranged from 7.14 kgf Monsanto (at 6.5 kN), to 10.2 kgf Monsanto (at 39 kN). There was no adherence of D-003 to the punches neither during the compressibility studies nor during the determination of the density at high pressure.

The solubility of D-003 in the solvents commonly used in the pharmaceutical industry is extremely low, in water being practically zero (Table 4). Therefore, according to these values

Solvent	Polarity	Classification*
Water	9.0	Insoluble
Ethanol	5.2	Insoluble
Acetone	5.4	Insoluble
Chloroform	4.3	Slightly soluble
Methylene chloride	4.2	Very slightly soluble
Hexane	0.0	Insoluble

* According to USP 26, at 25 °C

Table 4. Solubility of D-003 in organic solvents and water.

Solvent	Angle of contact
Water	59° ± 1.0°
Ethanol	17° ± 1.0°
Methylene chloride	7° ± 0.5°
Acetone	8° ± 0.5°
Chloroform	6° ± 0.5°

Table 5. Angle of Contact of D-003 in organic solvents and water.

and those of the contact angles (Table 5), D-003 has a very low wettability in water, a relevant characteristic for formulation studies that can affect the formation of homogeneous granulates, even in very low concentrations. The wettability of D-003 in different solvents followed the next order: chloroform ≈ methylene chloride > ethanol > acetone > water. These results should be also considered in the formulation of the coating suspension.

CONCLUSIONS

As per the results of the thermogravimetric analysis, D-003 has shown a high thermal stability, melting without decomposition and being stable at temperatures as high as 220 °C. Also, D-003 has not shown chemical or physical interactions with the investigated excipients. Industrial batches of D-003 have shown an uniform particle size distribution, good flow properties, compressibility and compactability, which support a suitable powder for manufacturing film-coated tablets as finished forms. The wettability of D-003 in the different solvents tested is: chloroform ≈ methylene chloride > ethanol > acetone >> water.

The results of physico-mechanical characterization and stability assays in the presence of excipients suggest that the D-003 could be an appropriate product for manufacturing film-coated tablets. Nevertheless, the very low solubility of D-003 in most solvents, especially in water, is the most critical aspect for developing the finished form, being relevant to decide the coating formulation.

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