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Comparative physical–chemical characterization of encapsulated lipid-based isotretinoin products assessed by particle size distribution and thermal behavior analyses

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ABSTRACT

Isotretinoin is the drug of choice for the management of severe recalcitrant nodular acne. Nevertheless, some of its physical–chemical properties are still poorly known. Hence, the aim of our study consisted to comparatively evaluate the particle size distribution (PSD) and characterize the thermal behavior of the three encapsulated isotretinoin products in oil suspension (one reference and two generics) commercialized in Brazil. Here, we show that the PSD, estimated by laser diffraction and by polarized light microscopy, differed between the generics and the reference product. However, the thermal behavior of the three products, determined by thermogravimetry (TGA), differential thermal (DTA) analyses and differential scanning calorimetry (DSC), displayed no significant changes and were more thermostable than the isotretinoin standard used as internal control.

Thus, our study suggests that PSD analyses in isotretinoin lipid-based formulations should be routinely performed in order to improve their quality and bioavailability.

1. Introduction

While isotretinoin was first developed to be used as a chemotherapy medication for the treatment of some cancers because of its ability to kill rapidly dividing cells, it is also considered as one of the most effective drug approved in 1982 to treat, by oral route, severe recalcitrant nodulocystic acne, in spite of its potentially severe side effects, particularly its teragenocity [1–7]. Several generic formulations for oral use have recently been introduced, in addition to the brand formulations Roaccutane® and Accutane™ (Roche). This development, considering the high risk of teratogenicity associated with oral isotretinoin use, has led the European Commission and the European Medicines Agency (EMEA) to release a directive towards the harmonization of the Summary of Product Characteristics (SPC). Isotretinoin (13-*cis*-retinoic acid), a vitamin A derivate, is a very lipophilic molecule, almost insoluble in water, only partially soluble in oil and is known to be labile to heat, light and air [8,9]. Thus, a simple dry powder formulation would not have a sufficient shelf-life and the development of new formulations needs to overcome those physical–chemical barriers. Interestingly, an accelerated photostability study, revealed that co-inclusion of isotretinoin in urea improved solubility, stability (i.e., delay of the photo-degradation) and reduced handling problems associated with the drug [10]. The emergence of novel excipients (i.e., semi-solid dosage form such as soft-gelatin capsule) with acceptable regulatory and safety profiles coupled with advances in formulation technologies have also greatly improved the potential for successful formulations (i.e., lipid-based formulations such as oil suspensions or emulsions) that enhance the drug stability and bioavailability [11–14]. Nevertheless, the bioavailability can not only be improved by the lipophilic microenvironment provided by an oil suspension (i.e., lipid-based excipients) but also

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can be further enhanced by decreasing the particle size of the active ingredient, and generally depends on the composition as well as on the dissolution rate of the capsule shell [15]. Those properties are known to affect the pharmaceutical processing, the therapeutic efficacy, toxicity, stability and bioavailability [16]. Thereby, in addition to the amount determination of the active drug the patient is exposed to, ones of the most important and variable therapeutic determinants between generic and original products [17] are the analyses (particle size distribution, thermal analysis) of the solid-state of drug candidates. In such context, particle size distribution and thermal behavior should be required by the Pharmacopeia to all the pharmaceutical industries in order to ensure reliable development of pharmaceutical formulations, especially for suspensions of lipophilic molecules.

The particle size distribution (PSD) is known to influence the bulk physical properties and determines the ability of powders to flow, mix, granulate and dissolve. It is also often a requirement in pharmaceutical manufacturing processes that particle size measurements are performed on raw materials [18]. Moreover, the particle sizing has a direct bearing on absorption of the drug from the gastrointestinal tract to the blood circulation. Indeed, it has been shown that the smaller particles are completely and more rapidly absorbed than larger particles which are released over a longer time period, resulting in lower plasma concentrations [19,20]. Thus, a reduced gastrointestinal absorption process can profoundly compromise the efficacy of drugs and, more importantly, the patient safety due to adverse effects generated by subsequent increased dose of the medication to obtain an expected result [21]. The analysis of the PSD would ensure the quality of the final dosage forms and should be considered as a pre-requisite, even before choosing the right drug delivery systems [22]. The emergence of a range of novel particle engineering technologies and the availability of new sophisticated characterization methods allow one to consider the "design by first intent" of particles with tailored physicochemical character and functionality [20]. The analysis of the PSD by polarized light microscopy is among the essential techniques commonly used in the pharmaceutical research and development field because it is considered as a suitable method for observing, measuring and determining the shape of individual particles [22]. Nevertheless, it is admitted that polarized light microscopy is not very reliable for sizing and should always be used in combination with other techniques such as laser diffraction, a preferred standard technique to complete microscopic observations. Indeed, laser diffraction has many features including its short analytical time, robustness, high precision and reproducibility, wide measurement range and flexibility of operation using liquid, spray or dry dispersion attachments [22].

Besides the PSD analysis, thermal analytical methods have been shown to be effective and suitable tools to study or control stability, bioavailability and drug–excipient interactions of raw materials and pharmaceutical products [23]. The thermal analysis refers to a group of relatively well-established techniques in which a physical property of substance and/or its reaction products is measured as a function of a controlled temperature program [23]. The thermal analytical methods mostly used are the thermogravimetry (TG), the differential thermogravimetry (DTG) and the differential scanning calorimetry (DSC), which evaluates the physical properties of drugs, including melting and vaporization temperatures with corresponding enthalpies, glass transitions, vapor pressures, yielding results rapidly and efficiently [24].

The aim of the present study was to newly characterize the physicochemical properties of three isotretinoin encapsulated products in oil suspension marketed in Brazil, through laser diffraction, polarized light microscopy to estimate their respective PSD and through TG/DTG and DSC analyses to determine their respective thermal behavior.

2. Materials and methods

2.1. Compounds

The isotretinoin standard, *aka* 13-*cis*-retinoic acid, (crystalline powder, 99.1% purity) was provided from the United States Pharmacopeia Reference Standard (USPRS) [25]. Three products named Product A (reference product), Product B (generic brand product) and Product C (generic brand product) were all soft-gel capsules containing 20 mg of isotretinoin suspension in oil suspension and commercialized by independent pharmaceutical companies. The nature of the soft-capsules for all products is the same. All precautions regarding storage and use of the isotretinoin were carefully followed according to the USP recommendations [25]. The reference product A in oil suspension (named as A, reference) is derived from the standard crystalline powder that is the sample without oil suspension (A, raw standard). The thermal analysis the raw powdered crystalline was compared to the reference one to enlighten the effect of the oil suspension in lipid formulation.

2.2. Particle size distribution (PSD)

The morphology of isotretinoin products was observed by polarized light microscopy (Carl Zeiss, Germany) at 20× magnification. The median particle size D [4.3] analysis in isotretinoin products was measured by laser diffraction, using a Mastersizer S long bed Ver. 2.19 with MS and a small volume sample dispersion unit QS (Malvern®, UK) capable of measuring samples in the range of 0.05–3500 μm. The content of one capsule for each product of isotretinoin was dispersed in acetone before the operation took place at 22 °C and at a focal length of 300 mm in order to achieve a particle concentration between 05% and 25%. For each assay, three series of ten separate determinations were made to guarantee the statistical representation of the analysis.

2.3. Thermogravimetry (TG) and differential thermal (DTG) analyses

All TG/DTG experiments were performed on a thermo-balance model TGA-50 (Shimadzu) using platinum crucibles in which approximately 04 mg of each individual sample were heated between 25 and 800 °C at the rate of 10 °C/min, under dynamic N_2 atmosphere at the flow rate of 50 mL/min. The decomposition was monitored as a function of temperature and weight loss. The TGA-50 equipment was calibrated with a standard reference of calcium oxalate.

2.4. Differential scanning calorimetry (DSC)

All DSC experiments were carried out on a DSC-50 cell (Shimadzu) using aluminum crucibles in which about 0.2 mg of each individual sample were heated between 25 and 800 °C at the rate of 10 °C/min, under dynamic N_2 atmosphere at the flow rate of 50 mL/min. The DSC-50 cell was calibrated with indium (mp 156.6 °C; $\Delta H_{fus} = 28.54\ J\ g^{-1}$), and zinc (mp 419.6 °C).

3. Results

3.1. Comparative particle size distribution and morphology of isotretinoin in soft-gel capsule products

The particle size distribution data for samples A, B and C (obtained from oil suspension) and characterized by laser diffraction are summarized in Table 1 and presented in Fig. 1.

In Table 1, the denomination D (v, 0.1) indicates that 10% of particles contained for the same quantity analyzed for the refer-

Table 1
Quantitative determination of particle size distributions (PSD) (μm) of isotretinoin products.

Sample	D (v, 0.1)	D (v, 0.5)	D (v, 0.9)	D [4.3]
Product A (reference)	2.94	10.04	22.74	11.66
Product B (generic)	8.26	25.57	64.93	33.37
Product C (generic)	7.82	29.27	55.30	30.80

D (v, 0.1) indicates that 10% of particles are of this size or smaller.
D (v, 0.5) indicates that 50% of particles are of this size or smaller.
D (v, 0.9) indicates that 90% of particles are of this size or smaller.
D [4.3] represents the average particle size.

ence product (A) and for both commercial generic products (B and C) corresponds to the evaluated particle size or smaller (Table 1). Similarly, D (v, 0.5) indicates that 50% of particles are of the particular size evaluated; D (v, 0.9) indicates that 90% of particles are of the specific size evaluated for all products size or smaller and D [4.3] represents the median particle size.

We can observe that for the reference product A, the median particle size D [4.3] is of 11.63 μm while for the generic products B and C, the median particle size is of 33.37 and 30.80 μm, respectively. The median particle size for both generic products (B and C) had a greater proportion (~three-fold) of larger particles than the reference product (A). It is worth noting that 90% of the particles content have a median size of 64.93 and 55.30 μm, respectively. In comparison, the particle size of the reference product (A) has a median particle size of 22.34 μm.

In Fig. 1, the size-distribution curves, obtained on a logarithmic scale, were quite similar between the two generic products (B and C) and indicated a broader PSD than the reference product (A). The shape of the PSD curves of all the analyzed products, was quite similar (S form) and all were quasi inter-parallel.

The three isotretinoin products (A, B and C) were further characterized by polarized light microscopy (Fig. 2) in order to observe eventual morphological changes in the particles. In accordance with the data obtained by laser diffraction, the size of the particles

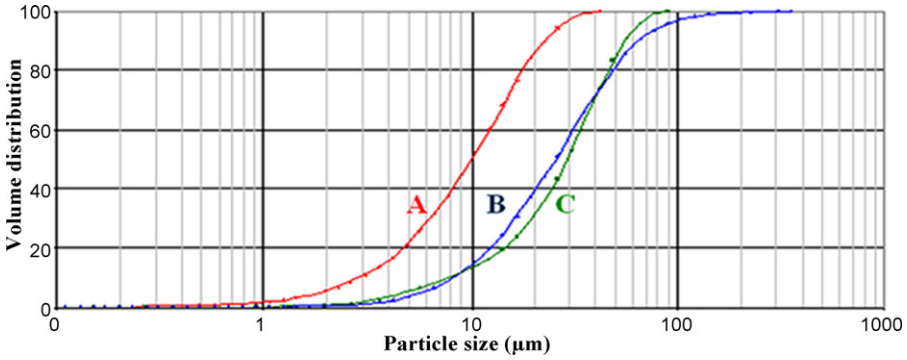

Fig. 1. Comparative particle size distribution of isotretinoin lipid-based products assessed by laser diffraction. (A: reference product, B: generic product and C: other generic product).

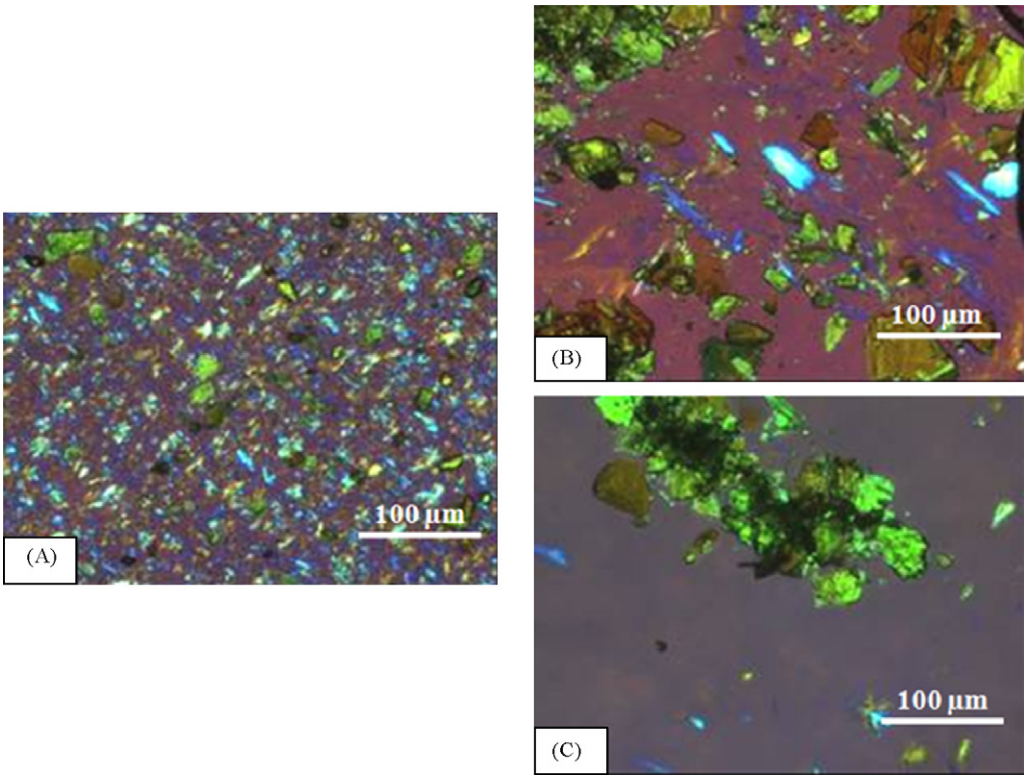

Fig. 2. Qualitative characterization of isotretinoin particles (lipid-based) assessed by polarized light microscopic images. Magnification 20×. Bar denotes 100 μm. (A) Reference product, (B) generic, and (C) generic.

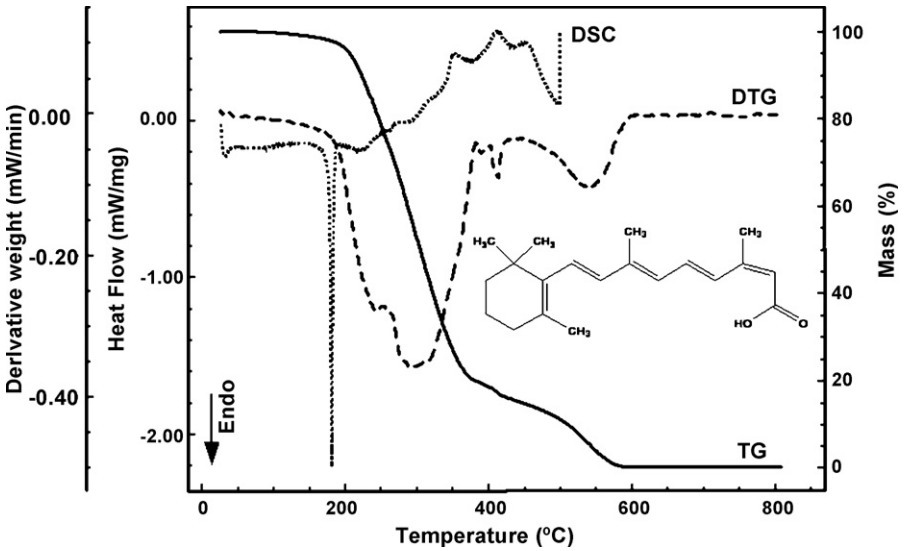

Fig. 3. TG/DTG and DSC curves of isotretinoin raw powdered crystalline standard product. The molecular structure of isotretinoin is shown in the figure.

observed in the generics (B and C) is larger and more heterogeneous than in the reference product (A). Comparatively to the reference product (A), the roughness appeared also more pronounced in the generics, especially in the generic product B, for which we could visualize clear irregularities.

3.2. Thermal behavior of raw (which design the molecule without oil suspension) pure isotretinoin versus lipid-based isotretinoin formulations

In order to enlighten the effect of oil suspension and consequently lipid-based isotretinoin formulations on the thermal properties, the thermal analysis of the raw powdered crystalline standard sample A was compared to the lipid-based isotretinoin formulations for the same sample A (under oil suspension), and the two others generic commercial products (B and C).

3.2.1. Thermal analysis of the raw isotretinoin standard compound without lipid formulation

The TG/DTG and DSC curves obtained with the isotretinoin (raw A) are presented in Fig. 3.

The TG/DTG curves show two major consecutive weight loss steps between 110 and 800 °C relative to the thermal decomposition of the organic groups of the molecule (see Inset, Fig. 3). The thermostability of the biomolecule is then up to 110 °C. The first weight loss starts at 110 °C and ends at 380 °C through a fast process and with a major mass loss (Δm = 80%). Subsequently, the second one begins with a slow process followed by a fast process, with a minor mass loss (Δm = 19.6%) between 380 and 600 °C. Both thermal decomposition steps are characteristic of the pure isotretinoin, can be noticed between 110 and 600 °C.

The DSC curve displays a single sharp endothermic peak as well as exothermic peaks. The endothermic peak at 181 °C, is typical of the pure crystalline isotretinoin substance and melting point in accordance to that reported in the literature [26]. Extra endothermic peaks between 360 and 550 °C are attributed to the thermal decompositions steps of the organic groups of the molecules that can be attributed to the condensation of the carboxylic group to form anhydride and the decomposition of the remaining organic part

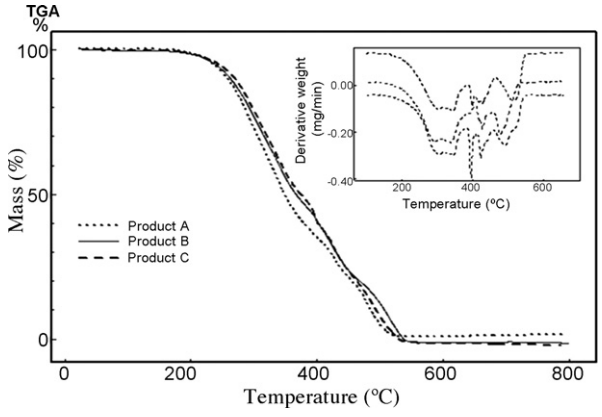

Fig. 4. TG/DTG curves of isotretinoin lipid-based products: A (reference), B (generic) and C (generic).

3.2.2. Thermal analysis of lipid-based isotretinoin formulations

The TG/DTG and DSC curves of the three lipid-based isotretinoin products (reference A, generics B and C) are respectively visualized in Figs. 4 and 5.

In Fig. 4, the TG/DTG curves show that the lipid-based isotretinoin formulations are similarly all thermostable up to ~180 °C. Subsequently, a continuous mass loss, up to 550 °C, occurs. 550 °C represents the temperature for which a complete mass loss (Δm = 98.9%) of the products A, B or C is observed. The thermostability up to 180 °C suggests the important role on the thermal properties of lipid-based formulation compared to the raw product assessed (product A, see Section 3.2.1).

In Fig. 5, a similarity between the respective DSC curves of the three lipid-based products is concordantly noticed. For any of the analyzed products, the DSC curve do not reveal endothermic peaks but, interestingly, only a relatively sharp exothermic peak around 430 °C, which is attributed to the major thermal decomposition of the organic groups for the encapsulated isotretinoin compound. The absence of endothermic peak at 181 °C, as the one observed in the raw isotretinoin standard (A) can be explained by the oil suspension and the absence of the crystalline nature of the product.

4. Discussion

Isotretinoin is a very lipophilic molecule but, labile to heat, light and air imposing considerable limitations to develop therapeutic formulations. It has been shown that encapsulation technology

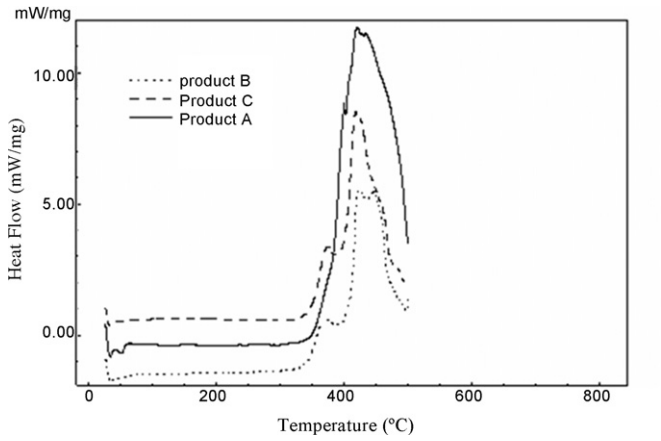

Fig. 5. . DSC curves of isotretinoin lipid-based products: A (reference), B (generic) and C (generic).

in oil suspension can enhance the drug bioavailability [10–14]. The thermal stability and the particle size distribution are two major physical–chemical criteria that influence the bioavailability [15,16,23]. Thus, the aim of our study was mainly to compare the particle size distribution (PSD) as well as the thermal behavior of three isotretinoin soft-gel capsule products containing the same dose (20 mg) of active ingredient in oil suspension. All those products have been provided from distinct laboratories in Brazil and were named, for confidentiality purpose, products A, B or C. Their characterizations have been performed, following the US Pharmacopeia recommendations regarding the precautions for use, through laser diffraction and polarized light microscopy to evaluate their PSD as well as through DSC and TG/DTG to determine their thermal behavior.

4.1. The particle size distribution of lipid-based formulations of isotretinoin

The drug bioavailability depends on many factors, among them, the PSD. Indeed, the drug bioavailability and efficacy can be enhanced by decreasing the particle size of the active ingredient, avoiding meantime to increase the dose of medication which may cause irreversible adverse effects [15,16]. Indeed, independently of the amount of the active drug used, the size of isotretinoin particles has also a direct bearing on absorption from the gastrointestinal tract [19–21]. The absorption of the active ingredient is a factor that can profoundly affect both the therapy efficacy and even may adverse the effects of the medication. Consequently, the determination of the appropriate particles size would increase the drug efficacy allowing the use of smaller dose of active drug in order to reduce potential adverse effects.

In this regard, our study shows that there was a considerable difference (~three-fold) of the PSD between the isotretinoin soft-gel lipid-based products (Table 1 and Fig. 1). Indeed, while the median particle size D [4.3] distribution was similar for the generic product B (33.37 μm) and C (30.80 μm), the reference (original) product A (11.66 μm) displayed a smaller median particle size. Furthermore, in accordance with the PSD data obtained by laser diffraction, significant morphological changes between the three soft-gel capsule products were observed by polarized light microscopy. The particle size of the generics B and C displayed a pronounced roughness and were visibly larger than the reference product A (Fig. 5). It worth noting that for a better drug adsorption, the smaller particle size such as that of reference A would be much more appreciated. Therefore this PSD study was a very important parameter to ensure the quality control of some commercial drugs.

4.2. The lipophilic microenvironment provided by encapsulated isotretinoin in oil suspension influenced the thermal stability of the active ingredient

The thermal analysis is another important parameter for the quality drug control. Lipid-based formulations of products A, B, and C showed the influence of the oil suspension and the important role on the thermostability compared to raw samples.

It is well admitted that the bioavailability of the isotretinoin can be enhanced, not only by decreasing the particle size, but also by conferring a lipophilic microenvironment to the active ingredient (i.e., oil suspension) [11–14]. Indeed, the excipient composition of the capsule shell can influence the bioavailability [15]. Thus, the encapsulated isotretinoin in a lipophilic microenvironment would minimize chemical degradation and potentially enhance the drug bioavailability. In such context, the lipid-based formulation is important and the associated physical–chemical characterization of the encapsulated biologically active biomolecule associated to lipid is therefore necessary.

In accordance with this concept, our data demonstrate that the thermal behavior analysis (which is an important parameter to check) for all the isotretinoin soft-gel capsules in oil suspension (A, B and C products) were all more thermostable than the powdered isotretinoin reference standard measured for (A). Indeed, the raw isotretinoin standard (A) was thermostable up to ~110 °C (Fig. 3) whereas all the isotretinoin soft-gel capsule products in oil suspension were thermostable up to ~180 °C (Fig. 4). In fact, in addition of the bioavailability enhancement, the lipid formulation acts as protective barrier to thermal degradation.

5. Conclusions

The physical–chemical properties of any drugs can affect the therapeutic efficacy, toxicity, bioavailability, pharmaceutical processing and stability. Quality control, characterization and comparative studies are important parameters that permit to ensure the final product quality or enhance a particular feature (i.e., stability, bioavailability). For the first time, using lipid-based isotretinoin formulations marketed in Brazil, we undertook a comparative physical–chemical characterization in terms of PSD and thermal behavior analysis.

Particle size analysis is one major parameter to ensure the quality control within production environments in order to keep a tight control on the specification of the drugs. Based on the mean size particle characterization, a factor that is known to affect absorption from the gastrointestinal tract, our study showed that there was a significant difference between the isotretinoin soft-gel generics (products B and C) and the reference original product (product A). Nevertheless, it is important to keep in mind that the similarity in PSD between the two generics (products A and B) does not necessarily mean that they are bioequivalent, unless *in vivo* bioequivalence assays are performed. Together, our results suggest that the control of the particle size distribution in general and in isotretinoin generics in particular, shall be routinely considered in order to ensure the final product quality and preserves the patient's safety.

Thermal analysis methods are applied in pharmaceutical industry for over 35 years with the goal to evaluate the thermostability of drugs. The TG/DTG as well as the DSC analyses allowed us to comparatively control the thermal behavior of marketed isotretinoin products. Thereby, we report that the thermal behavior of semi-solid lipid-based formulations of isotretinoin (i.e., soft-gel isotretinoin capsules in oil suspension) confers a higher thermostability than the isotretinoin powered compound (i.e., standard raw pure isotretinoin), reinforcing the role of the lipophilic microenvi-

ronment of the drug for its stability and certainly its bioavailability. To the best of our knowledge, it is the first time that such comparative thermal analyses are performed on semi-solid lipid-based formulations of isotretinoin marketed in Brazil.

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