Article

Prednisone raw material characterization and formulation development

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Solid dosage forms for oral use, particularly tablets, are the most highly used dosage forms in therapy because they are easily administered, have high productivity and relatively low cost and provide a more stable drug to form a semi-solid net. Numerous parameters influence the quality of the final dosage form. In this study, the dissolution profile of 20-mg prednisone tablets bioequivalent to the reference product and three test formulations were evaluated using stability testing. During the study, prednisone tablets and the active pharmaceutical ingredient (API) prednisone from two different manufacturers were characterized with respect to their physical and physicochemical properties. The results showed that the dissolution profiles of the test batches and the reference product did not retain pharmaceutical equivalence throughout all the stability study. Notably, both samples of API prednisone were of the same crystal form, and any phase transition that occurred during the study could not be attributed to dissolution variation during stability.

Keywords: Prednisone/tablets/dissolution. Solubility. Formulation. Stability.

INTRODUCTION

Prednisone is a glucocorticoid that is extensively used in clinical practice (Romanholi, Salgado, 2007). Its therapeutic use is recommended for a variety of acute and chronic diseases, such as arthritis, hepatitis, allergic diseases, asthma, leprosy, and numerous other autoimmune and inflammatory diseases (Kaiser, Kley, 2002).

In Brazil, there are several industries commercialising prednisone in the form of oral immediate release tablets. Currently, Meticorten® is the reference product for these formulations at concentrations of 5, 20 and 50 mg (Anvisa, 2013).

Prednisone has the molecular formula $C_{21}H_{25}O_5$ and a molar mass of 358.43 g/mol (Farmacopeia Brasileira, 2010). It is an odourless white or almost white powder that melts at 233 °C with decomposition and may exhibit polymorphism. At room temperature, this drug is poorly soluble in methanol, ethanol, chloroform and dioxane, with low solubility in water (Farmacopeia Brasileira, 2010). Prednisone is a neutral molecule, and its pK_a values are not reported in the literature. Its solubility in water is 0.133 mg/mL at 25 °C, and three values for the partition coefficient (Log P) are reported in the literature: 1.46, 1.47 and 1.6 (Vogt *et al*., 2007; Passion, Gouveia, Morais, 2012).

Commercially active pharmaceutical ingredient (API) prednisone is an anhydrous, monohydrate or prednisone acetate salt. However, only the anhydrous and monohydrate forms are referenced in the Brazilian, European and United States Pharmacopoeia (European Pharmacopoeia, 2010; Farmacopeia Brasileira, 2010; USP, 2012).

According to parameters adopted by the International Pharmaceutical Federation (FIP), prednisone is classified as a class I drug of the Biopharmaceutical Classification System (BCS) and is highly soluble and permeable (Storpirtis *et al*., 2009). However, Vogt *et al*. (2007) argued in a review study that in the absence of conclusive data on permeability, absorption and solubility under the conditions recommended by the BCS, this drug does not have a classification defined in relation to the BCS.

Full characterization of the drug is an essential step for a formulation development study given that variations

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in characteristics, such as crystal structure, particle size, and fluidity, can significantly impact on bioavailability parameters, processability and stability (Fung, Ng, 2003; Iacocca, Burcham, Hilden, 2010).

Another issue that is rarely evaluated is related to the monitoring of the dissolution properties of formulations during stability. Although there is a regulatory prerogative requiring the completion of the dissolution test for the stability study, there is no requirement for an analysis of the dissolution profile. Few authors have demonstrated the manner in which it makes a difference (Ochi *et al*., 2016; Payghan *et al*., 2012; Vidal *et al*., 2010).

The objective of the present study was to characterize the active pharmaceutical ingredient (API) prednisone and the formulation of a solid dosage form (tablet) for registration as a generic medicine and to evaluate the dissolution stability of this formulation.

MATERIAL AND METHODS

Material

The reference drug product was Meticorten® tablets 20 mg (from Schering-Plough laboratory batch R023) produced in September 2010 and valid for two years. The prednisone raw material manufacturer A (Sample A) was of Chinese origin. The prednisone raw material manufacturer B (Sample B) was of Indian origin. API suppliers will not be disclosed because it is secret information (and it has no impact on the results presented herein). The excipients were corn starch (Quimica BPAR), croscarmellose sodium (Blanver), magnesium stearate (Magnesia), sodium starch glycolate (Amishi Drugs and Chemicals Ltd), lactose monohydrate (DFE Pharma), sodium lauryl sulfate (Stephan Philippines Inc.), mannitol (Shandong Tianli Pharmaceutical Co.), polyvinylpyrrolidone K30 (Nanhang Industrial Co.), and magnesium silicate (Talmag Pharma-S).

METHODS

Raw material characterization

Differential scanning calorimetry

Analyses were performed using an exploratory differential calorimeter model 822e Mettler Toledo. Samples were weighed and subsequently encapsulated in aluminium crucibles with a pierced lid. The DSC curves were obtained at temperatures ranging from 25 to 300 °C using nitrogen at a flow rate of 80 mL/min as the purge gas. Measurements were performed at the heating rates of 2.5, 5 and 10 °C/min.

Thermogravimetric analysis (TGA)

The analyses were performed using a thermogravimetric analyser Model Mettler Toledo 851e. Samples of approximately 3.5 mg were weighed and encapsulated in open aluminium pans. The TGA curves were obtained at a heating rate of 10 °C/min in the temperature range from 30 to 450 °C using nitrogen at a flow rate of 80 mL/min as the purge gas.

Powder X-ray diffraction

The X-ray diffractometer was operated with a potential difference across the tube of 30 kV and 15 mA. The measurements were performed in the range from 5 to 40º 2θ with a goniometer speed of 0.05 °/sec. The radiation used was CuKα (λ = 1.5418 Å). The experimental diffraction patterns were compared with a simulated diffraction pattern obtained from the Mercury software (Macrae *et al*., 2008), which was prepared using the deposited structure at the Cambridge Crystallographic Data Structure Database (CSD) (Allen, 2002).

Fourier transform infrared spectroscopy

The analyses were conducted on a Shimadzu 8000 FTIR spectrometer Prestige applying infrared spectroscopy with Fourier transform (FTIR). Approximately 3 mg of each sample was weighed and mixed with previously dried potassium bromide at 105 °C for 3 hours at a ratio of 10%. Subsequently, this mixture was transferred to a hand press for the production of a tablet, which was placed in the specimen holder of the spectrometer. The same procedure was performed for the chemical reference substances (SQR) of the USP and Brazilian Pharmacopoeia. The spectra were recorded at 4000-400 cm⁻¹.

Intrinsic dissolution

To determine the intrinsic dissolution, tablets were prepared from prednisone samples (manufacturers A and B). The samples were subjected to different levels of compressive forces. Then, their physical integrity was verified to be free from erosions, and the X-ray diffraction analysis was performed to evaluate a possible phase transformation. The pressure selected for the experiment was 1200 psi because the inserts exhibited no erosion and the drug showed no phase transition under this condition. Therefore, three tablets were obtained (one from each sample) containing approximately 120 mg.

Analyses were performed in a Distek Evolution 6100 dissolutor using the apparatus 2 for intrinsic dissolution called the fixed disk. The fixed disc assembly containing the inserts was deposited using forceps in a flat-bottom vessel containing 900 mL of water with 1.2%

sodium lauryl sulfate (w/v). During the experiment, 10-mL aliquots were collected without medium replacement at 5, 10, 15, 20, 30, 45 and 60 minutes. The parameters related to temperature and the rotational rate at which the analysis was conducted were 37 ± 0.5 °C and 150 rpm, respectively. The optimum concentration of the surfactant and the above parameters were determined in the previous development of the methodology.

The analyses were performed in triplicate, and the amount of active drug released was obtained by reading the absorbance in a UV spectrophotometer at a wavelength of 242 nm. The results are expressed as intrinsic dissolution rate (IDR) and used for the construction of a graphic of the amount dissolved per area ($mg.cm^{-2}$) versus time (minutes).

Powder dissolution

To determine powder dissolution behaviour by dispersion, 20 mg of each sample (A and B) was added to the dissolution vessel containing 900 mL of purified water. The analysis was performed in triplicate with the same parameters used for the dissolution test for prednisone tablets described in the Brazilian Pharmacopoeia (Farmacopeia Brasileira, 2010) (temperature of 37 ± 0.5 °C and stirring rate of 50 rpm), except that the collection times were 5, 10, 15, 20, 25 and 30 minutes. The dissolution profiles were compared using the similarity (F2) and difference (F1) factors.

Scanning electron microscopy

Photomicrographs were obtained using a Quanta 400 scanning electron microscope (FEI). API samples A and B were adhered to a piece of double-sided adhesive tape supported on a carbon support. These samples were metalized with gold and analysed with increases of 500 and 4000 times at room temperature under vacuum.

Solubility

To determine the solubility of the samples (A and B), an excess of powder was added to the dissolution vessels containing 500 mL of the following three different media: hydrochloric acid buffer pH 1.2, phosphate buffer pH 6.8 and distilled water pH 5.5. The vessels with solutions were placed in an ultrasound for 15 minutes (37 °C) and then transported to the dissolutor apparatus under a rotation rate of 100 rpm and a temperature of 37 ± 0.5 °C for 24 hours. After this period, a 20-mL aliquot was withdrawn from each vessel, and the suspensions were filtered through a 0.45-µm membrane, diluted and then analysed by UV-Vis spectrophotometry at a wavelength of 242 nm (Okumu, Dimaso, Löbenberg, 2009; Wei, Löbernberg, 2006). Each experiment was performed in triplicate, and the pH of each sample was monitored during the experiment.

Evaluation of the API according to pharmacopoeic criteria

The assay for prednisone samples A and B was performed using ultraviolet absorption spectrophotometry according to the method (A) described in the Brazilian Pharmacopoeia 5th edition (Farmacopeia Brasileira, 2010).

Formulation

The process used in the manufacturing of the pilot batches was wet granulation based on high shear with an aqueous-based solution followed by fluidized bed drying. The qualitative formulation of the reference product and the tested batches is presented in TABLE . Three batches were produced: P0020020910, MEST01 and MEST02. All of the batches followed the same manufacturing process. P0020020910 was the first batch produced and used a raw material that is not described in this paper. This batch was used in the bioequivalence study. Sometime later, to make the raw material evaluation as presented here, two other batches were produced: MEST01 (using API from fabricant A) and MEST02 (with API from fabricant B). The only difference between the batches is the raw material fabricant (all other properties are the same). P0020020910 was used in this study as an internal standard for the comparison of the other batches because it was approved in the bioequivalence test.

Tablet characterization

Tablet evaluation according to pharmacopoeic tests

Average weight tests, hardness, friability, disintegration time, assay and humidity followed the recommendations in the Brazilian Pharmacopoeia 5th edition (Farmacopeia Brasileira, 2010).

Dissolution profile

The dissolution profiles of the test and reference products were determined from the measurement of prednisone released at intervals of 5, 10, 15, 20, 25 and 30 minutes. The apparatus 2 (paddle) was used with a stirring rate of 50 rpm. The dissolution medium used was 900 mL of distilled water at 37 °C. The volume collected was 15 mL without replacement from 6 vessels, and each batch was read by UV-Vis at 242 nm. An SR8 PLUS Hanson Research dissolutor was used. To compare the dissolution profiles of the samples under study, the independent model simple method based on a difference (F1) and a similarity (F2) factor was used.

TABLE I - Excipients present in the reference medicine and pilot batches

Excipients	R023	Test batches
Starch (corn)	X	X
Croscarmellose sodium	X	
Magnesium stearate	X	X
Sodium starch glycolate		X
Lactose monohydrate	X	
Sodium lauryl sulfate		X
Mannitol		X
Povidone (PVP)	X	X
Magnesium silicate		X

All batches were packaged in PVC blisters, as informed by the reference product manufacturer.

Stability study

Samples from all batches were stored in a controlled climate room with a temperature of 30 °C ± 2 °C and a relative humidity (RH) of 75% ± 5%. Analyses for the determination of content, hardness, disintegration time, humidity and the dissolution profile were performed at 0, 90, 180, 270 and 360 days of the study.

RESULTS AND DISCUSSION

Prednisone API characterization

Thermogravimetric analysis (TGA)

From the TG curves (Figure 1) obtained for samples A and B, at zero and 360 days, both samples were thermally stable up to approximately 261 °C. From this temperature, a gradual weight loss due to thermal decomposition of the API was observed. This result excludes the possibility of said samples when treating the monohydrate form or solvate with chloroform, as reported in the literature, because the samples studied exhibited no mass loss until the beginning of their thermal decomposition (Kuhnert-Bramdstätter, Gasser, 1971; Mesley, Johnson, 1965).

Differential scanning calorimetry (DSC)

The calorimetric profiles presented in Figure 2 indicate the possibility that two different crystalline forms exist because the melting peaks displayed different temperatures. The results of the tests at 2.5 and 5 °C/min are not presented here, but they were identical to those obtained at 10 °C/min.

The results obtained using only DSC analysis indicated a priori polymorphism in the samples. These data however contradict those obtained by X-ray diffraction.

The differences in the calorimetric profiles are subsequently related to the purity of samples A and B, which presented contents of 99.7% and 98.5%, respectively. The presence of small amounts of impurities in a substance may decrease the melting point and increase its melting range (Donnelly et al., 1990). In the literature, authors have also used DSC to determine the purity of a given substance (Ferguson et al., 2000; Schnitzler et al., 2001). However, this technique should not be used for this purpose when the analyte decomposes during its fusion (Dooren, Muller, 1984), such as for prednisone (Farmacopeia Brasileira, 2010). Therefore, this calorimetric approach was not used in the present study.

Powder X-ray diffraction

Despite the question raised by DSC regarding the presence of polymorphism in the samples, characterization by X-ray diffraction (Figure 3) confirmed the presence of the same crystalline phase in samples A and B. The peaks in the diffraction pattern are the same in both samples. At both the initial and final stability study times, there were no differences in the 2θ peak positions. The study also demonstrated that compared with an XRD pattern simulated from crystallographic data of an anhydrous crystalline form of prednisone deposited in the CSD (Allen, 2002), the samples had the same crystalline phase as that described here. Therefore, it is concluded that during the study period and at the temperature and relative humidity conditions used for storage, no crystalline phase transition was observed in the samples.

Infrared spectroscopy

The infrared absorption spectrum results demonstrated that at time zero, prednisone samples A and B alone exhibited absorption maxima at the same wavelength and the same relative intensities as those observed in the spectrum of samples of the USP and Brazilian Pharmacopoeia standards (Figure 4). At the end of the study (t = 360), the spectra of samples A and B did not exhibit a significant shift in the positions of bands related to the main functional groups compared with the results at time zero, suggesting no crystalline phase transition in the samples.

Intrinsic dissolution

The intrinsic dissolution rate (IDR) is an important tool to evaluate the differences between polymorphs and solvates, particularly when there is a small amount of sample available (Qiu, Chen, Zhang, 2009). This type of dissolution evaluates the API in an isolated form, i.e.,
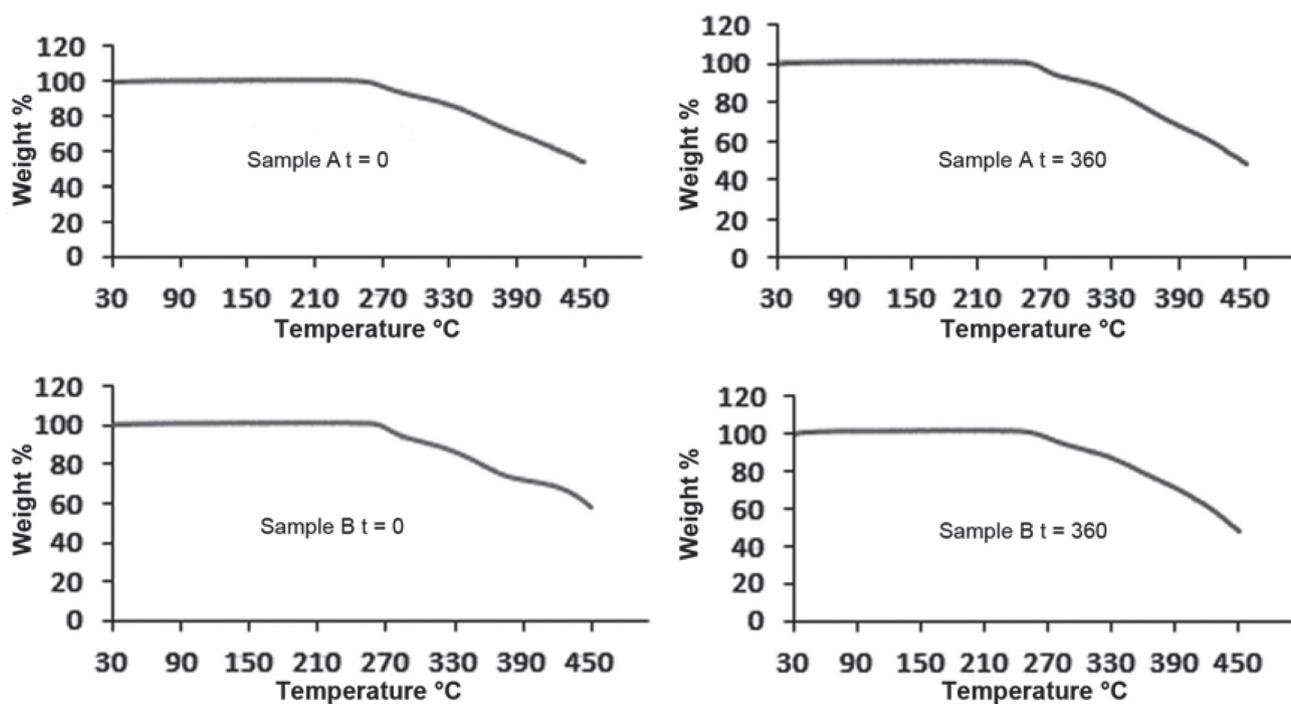
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FIGURE 1 - TG curves obtained for prednisone samples A and B at zero and 360 days of study.
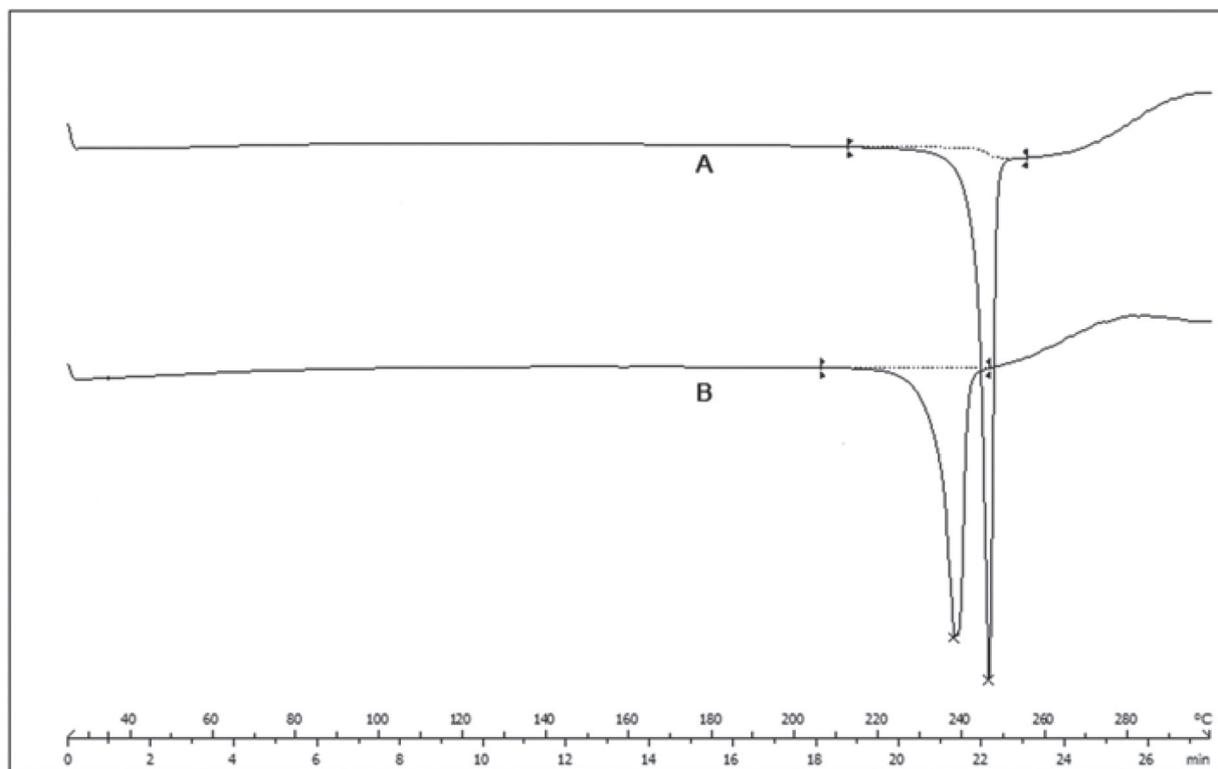

FIGURE 2 - DSC curves of samples A and B at a heating rate of 10 °C/min.

without the presence of excipients (Gibson, 2009). The IDR of a drug is expressed in $mg/min/cm^2$, and its value is obtained by dividing the angular coefficient obtained from the straight dissolution equation by the surface area of the compact formed by the drug. The results obtained for samples A and B are presented in Figure 5.
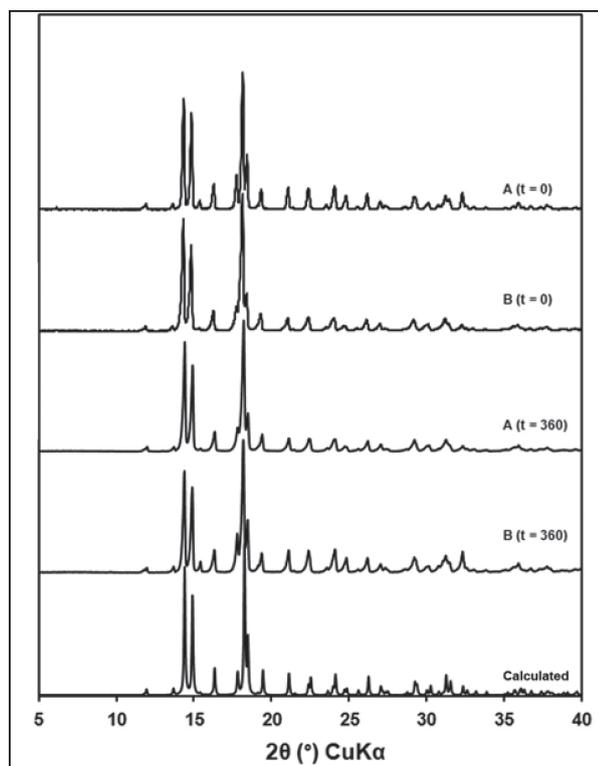
FIGURE 3 - Diffraction patterns of prednisone samples A and B at zero and 360 days of the stability study and the simulated pattern.

As shown in Figure 5, the intrinsic dissolution profiles revealed a pronounced linear behaviour because both samples had a coefficient of determination that was greater than 0.99. With this linearity, it was concluded that there was no phase transition during the analysis and that the sink condition was also maintained during all times considered in the analysis.

Samples A (6.71 mg/min/cm^2) and B (6.73 mg/min/cm^2) exhibited similar dissolution profiles and IDR. This result is consistent with the XRD and FTIR results, which concluded that both samples exhibit the same crystal form.

Similar to the results obtained in the solubility test to classify the samples as highly soluble according to the BCS, a study correlating the IDR of 15 drugs performed by Yu *et al*. (2004) considered that an IDR greater than 0.1 mg.min^{-1}.cm^{-2} indicates high solubility, and drugs with lower values exhibit low solubility. Thus, according to this study, both samples are classified as highly soluble. Zakeri-Milani *et al*. (2009) mentioned approximately 0.58, 0.63, and 3.45 mg/min/cm^2 for furosemide (solubility of 1.474 mg/mL), ketoprofen (2.12 mg/mL), and atenolol (16.87 mg/mL), respectively. Dezani *et al*. (2013) reported that the zidovudine (solubility of ~20 mg/mL) IDR is 0.75 mg/min/cm^2.
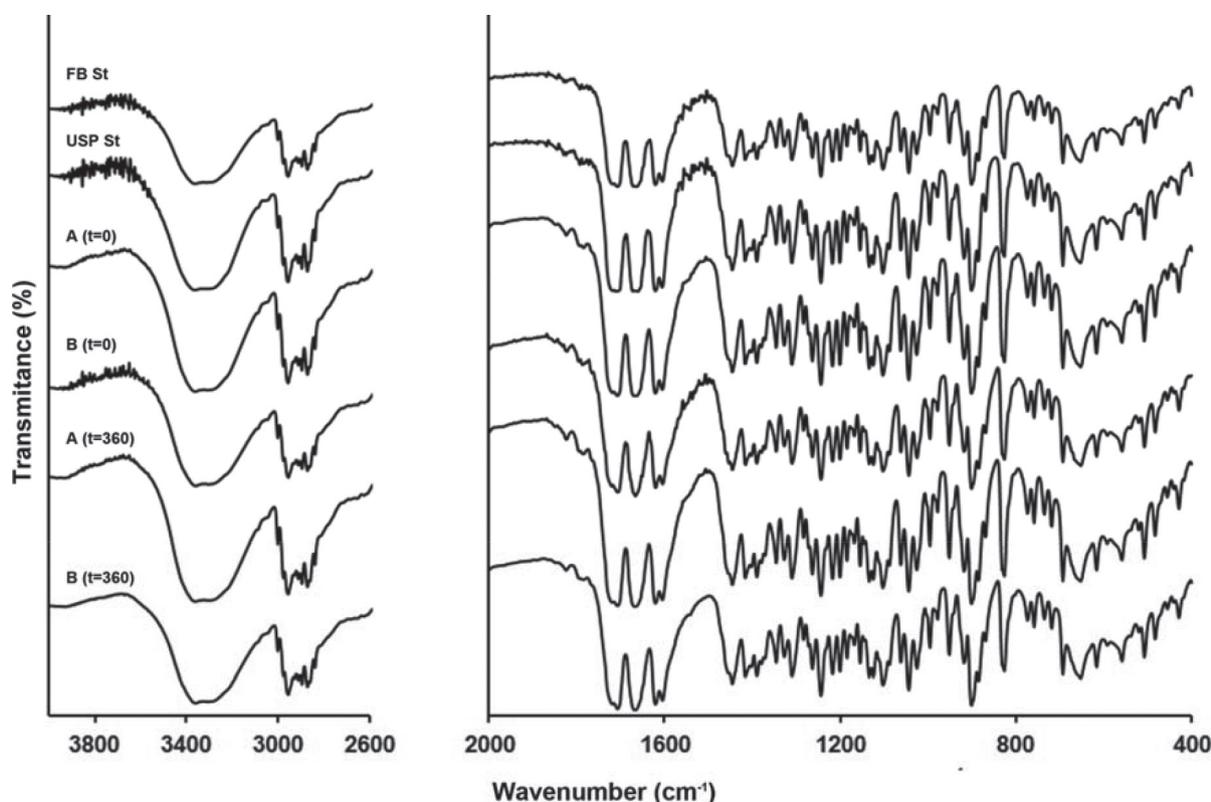

FIGURE 4 - Fourier transform infrared spectroscopy (FTIR) of prednisone samples A and B at zero and 360 days of the stability study and the USP (USP St) and Brazilian Pharmacopoeia (FB St) standards.
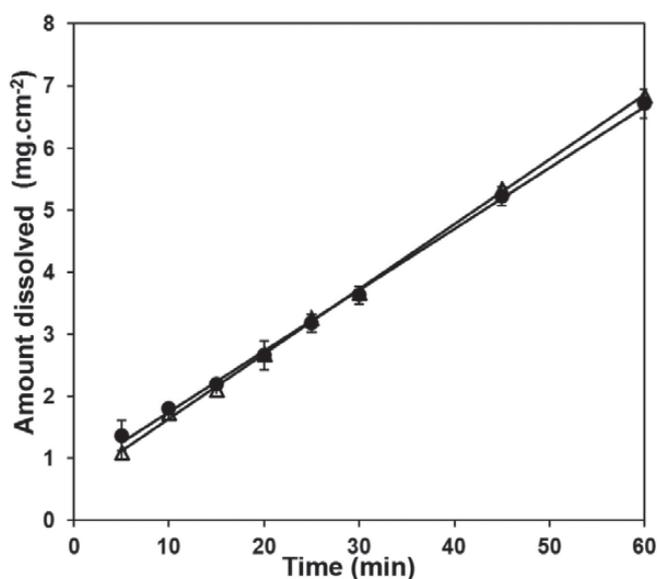
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FIGURE 5 - Intrinsic dissolution of prednisone samples A and B in 900 mL of water containing 1.2% sodium lauryl sulfate using apparatus 2 at 150 rpm.

Powder dissolution

The results of the powder dissolution for both samples are presented in Figure 6. Samples A and B had a mean dissolution of 87.7% and 85.1% at 30 minutes, respectively. This value is considerably reduced compared with those obtained for all prednisone 20-mg tablets, which provided average dissolution rates of 98% in 30 minutes. This discrepancy in the results may have occurred during the analysis because part of the active drug floated on the surface of the medium and/or attached to the apparatus (paddle), forming a cluster. Therefore, part of the active added mass did not come into contact with the environment and did not undergo the dissolution process. This observation is not so common in tablet dissolution because the excipients in this case can enhance powder wettability and lower static properties verified in the powder alone.

Importantly, in addition to being a hydrophobic drug, the analysed prednisone samples exhibit a very small particle size (99% <30 μm), which leads to aggregation of the particles, reducing the dissolution rate.
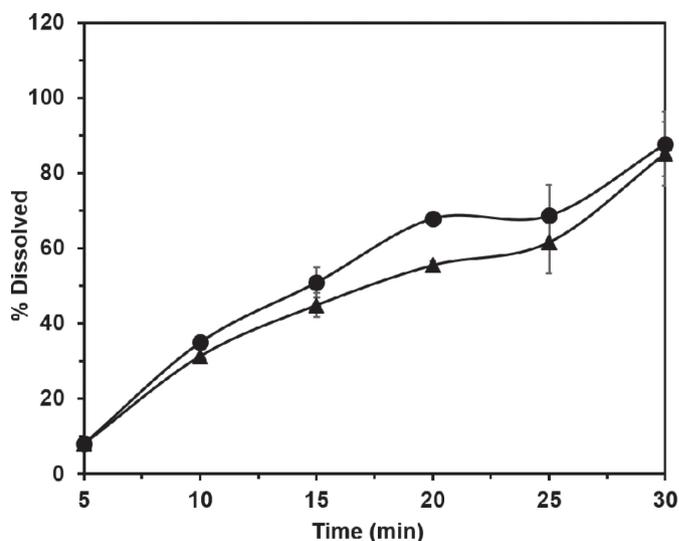

FIGURE 6 - Comparison between powder dissolution profiles of prednisone samples A (circle) and B (triangle).

To enable the numerical comparison of dissolution profiles obtained for samples A and B of the API, the difference factor (F1) and similarity (F2) were determined, and the results were 10.03 and 58.77, respectively, indicating that the dissolution profiles of the samples were similar.

Solubility (saturation)

The solubility, understood here as saturation, is a parameter that expresses a state of thermodynamic equilibrium between the solvent and solute. This equilibrium is dependent on a number of experimental conditions, such as temperature (Qiu, Chen, Zhang, 2009). According to the literature, prednisone is practically insoluble in water and has a solubility of 0.133 mg/mL at 25 °C (Vogt *et al*., 2007).

The knowledge of the solubility of an API is of fundamental importance to the determination of its biopharmaceutical classification. In accordance with the BCS, the solubility should be determined at 37 °C and in an aqueous medium with different pH values. Table II presents the solubility values experimentally obtained according to the BCS for prednisone samples A and B.

TABLE II - Solubility values experimentally obtained according to the BCS for prednisone samples A and B

Media	Solubility (mg/mL) at 37 °C		Volume (mL) necessary to solubilize 50 mg	
	Sample A	Sample B	Sample A	Sample B
HCl buffer, pH 1.2	4.48	4.51	11.03	11.08
Water, pH 5.5	4.53	4.54	11.04	11.01
Phosphate buffer, pH 6.8	4.55	4.57	10.98	10.94

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Samples A and B exhibited a solubility of approximately 4.5 mg/mL at 37 °C for all tested pH values. This result is consistent with the information obtained from the literature that ranks prednisone as a neutral molecule; therefore, changes in pH do not affect its solubility (Vogt *et al.*, 2007).

Based on these results, both samples can be classified as highly soluble, and the volume of medium required to dissolve 50 mg of prednisone (a larger dose available for domestic dosage) was approximately 11 mL at all pH values tested, which is significantly less than the 250 mL recommended by the BCS.

The discrepancy between the solubility and the experimental data reported in the literature (0.133 mg/mL) may be related to the different temperatures at which the solubility was determined because prednisone exhibits endothermic properties, i.e., its solubility increases with increasing temperature (Vogt *et al.*, 2007).

Scanning electron microscopy

The photomicrographs obtained for both samples A and B (Figure 7) reveal irregular and small particles that are mostly smaller than 30 micrometres. Note that
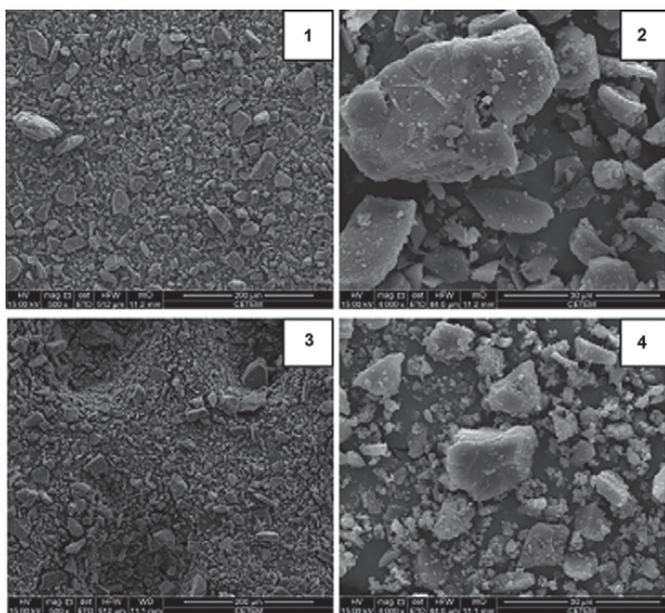

FIGURE 7 - SEM photomicrographs of sample A (1 and 2) and sample B (3 and 4).

these data are consistent with the results (99% ≤ 30 mM) obtained by laser diffraction provided on the certificate of analysis (CoA) of the samples supplied by the manufacturers of both samples.

Active pharmaceutical ingredient evaluation using pharmacopeic criteria

Samples A and B were approved in all pharmacopeial tests. The contents found for the raw materials A and B at different time intervals are presented in Table III.

The contents of both samples showed no significant variation over the entire course of the study.

Evaluation of prednisone tablets

It is important to evaluate some basic physico-chemical parameters of the tablets in addition to stability. During the development of drug products, quality control of the dosage forms must be evaluated from a variety of different aspects (Table IV).

According to the limits recommended by the Brazilian Pharmacopoeia 5th edition to assess weight change in uncoated tablets, all samples were within the established limit. Notably, in general, all lots had a small increase in average weight and values for the maximum and minimum weights observed. This finding may be related to the small increase in moisture that occurred in all lots during the stability study.

Regarding disintegration, lots P0020020910, MEST01 and MEST02, which were produced with the same formulation, presented maximum values of 3 minutes and 20 seconds.

Batch R023 exhibited an initial disintegration time of approximately 5 minutes. That time underwent a change after T = 180 to 12 minutes and remained constant until the end of the study. This increase may be related to the excipient povidone present in the formulation, as this polymer can form a film on particles of the granulate and consequently the induration of the tablets over time, thereby jeopardizing the process of disintegration and dissolution (Blok, 2007; Murthy, Guebre-Sellassie, 1993).

The Brazilian Pharmacopoeia 5th edition does not recommend tests for moisture in prednisone tablets, but

TABLE III - Assay results (%) of samples A and B at 0, 90, 180, 270 and 360 days of the stability study

Samples	Time zero	90 days	180 days	270 days	360 days
A	99.7	99.8	99.6	99.8	99.6
B	98.5	98.8	98.7	98.6	98.6

TABLE IV - Compilation of the results of pharmacopoeic parameters of the formulations evaluated during the stability study

Parameter	Result			
	R023	P0020020910	MEST01	MEST02
Average weight (mg)				
T = 0	303.4	142.1	143.2	140.7
90 days	303.7	142.3	143.4	140.9
180 days	303.6	143.1	143.6	141.2
270 days	304.1	143.0	143.7	141.3
360 days	304.3	143.4	144.0	141.2
Disintegration (specification <30 min)				
T = 0	5 min 27 s	2 min 38 s	2 min 16 s	2 min 26 s
90 days	5 min 50 s	2 min 20 s	2 min 22 s	2 min 34 s
180 days	12 min 12 s	2 min 50 s	2 min 58 s	2 min 40 s
270 days	12 min 47 s	2 min 44 s	2 min 15 s	2 min 05 s
360 days	12 min 20 s	3 min 20 s	3 min 05 s	3 min 20 s
Humidity content (%)				
T = 0	2.53	5.71	4.71	4.67
90 days	2.62	5.85	4.78	4.70
180 days	2.75	6.26	4.65	4.73
270 days	2.68	6.32	4.77	4.87
360 days	3.23	6.25	5.20	4.96
Hardness (N) (average ± RSD)				
T = 0	57.4 ± 8.7	53.1 ± 8.1	21.6 ± 6.1	33.0 ± 6.5
90 days	60.5 ± 5.1	55.6 ± 10.5	23.4 ± 10.7	33.6 ± 11.3
180 days	64.9 ± 9.5	56.8 ± 5.6	23.9 ± 5.9	35.5 ± 6.7
270 days	65.8 ± 5.6	56.2 ± 7.5	25.2 ± 7.4	38.5 ± 5.9
360 days	66.4 ± 6.8	55.8 ± 5.7	25.7 ± 5.6	38.7 ± 7.3
Friability (specification < 1%)				
T = 0	0.42	0.33	0.72	0.58
90 days	NR	0.36	0.67	0.43
180 days	NR	0.29	0.54	0.49
270 days	NR	0.25	0.45	0.33
360 days	NR	0.21	0.20	0.24
Assay (specification 90-110%) (average ± RSD)				
T = 0	106.7 ± 0.3	100.6 ± 0.4	100.4 ± 0.4	101.7 ± 0.2
90 days	106.0 ± 0.2	100.4 ± 0.2	100.0 ± 0.9	101.2 ± 0.3
180 days	105.9 ± 0.5	100.1 ± 0.4	100.7 ± 0.2	101.3 ± 0.3
270 days	105.7 ± 0.3	100.2 ± 0.7	100.3 ± 8.4	100.7 ± 0.5
360 days	105.8 ± 0.3	99.9 ± 0.2	100.1 ± 0.7	100.4 ± 0.2

NR = not realised

this parameter was evaluated during stability studies to assess a possible correlation with the mechanical strength test and the dissolution profile of the tablets studied because these data directly affect these parameters (Aulton, 2005). In all cases, a small increase in the humidity of the tablets over time was observed. This pattern was also observed

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for the reference drug product, and a humidity variation slightly greater than the reference remains for the test drug. For a PVC, i.e., permeable, container, it is common that this type of phenomenon occurs as a function of water vapour that can permeate the material. Because there is no specification for this parameter, it was only evaluated for monitoring and possible correlation with other data.

According to the Brazilian Pharmacopoeia, the hardness test is informative but does have existing approval parameters (Farmacopeia Brasileira, 2010). Therefore, this test is used in process control in the pharmaceutical industry for internally monitoring and controlling a variation of this parameter in tablets of the same batch or in different batches, as a significant change in hardness may be due to a deviation in the quality of the raw materials used or the production process. However, the mechanical strength of the tablets can also be altered during storage. Such changes can be caused by numerous factors, including moisture absorption, changes in crystal structure and plastic deformation of particles (Aulton, 2005).

The results obtained during the stability study indicate a small increase in hardness for all batches. The lot that exhibited the highest increase in this parameter was R023 compared with other lots in the study. Similar to disintegration, the increase in hardness was potentially triggered by the association of factors, such as increased moisture absorption and the use of povidone in the tablets

(Blok, 2007; Murthy, Guebre-Sellassie, 1993).

The friability results reveal an increase in the mechanical resistance to attrition of the tablets during the stability study, which may be associated with the moisture absorbed by the tablets. This moisture would lead to the formation of solid bridges between particles, increasing their resistance (Aulton, 2005). Importantly, the friability for the R023 batch was not assessed at all study times due to the unavailability of that lot on the market, but it has no impact on the statements presented here.

All results of content and uniformity of content are approved. However, a certain strangeness was noted in the content values for the reference product. Despite falling in the range of approval, these values are well above average, tending towards the upper range limit. This type of result suggests that one cannot categorically state that there is an addition of excess active ingredient during manufacturing, a practice known as overage, which is not recommended by regulators. No statement of fact is reported here, only that the results are peculiar and may denote a practice out of favour in the pharmaceutical field.

Dissolution profiles of the tablets

The individual results of the dissolution profiles of lots R023, P0020020910, MEST01, and MEST02 at zero, 90, 180, 270 and 360 days of the stability study are presented in Figure 8.
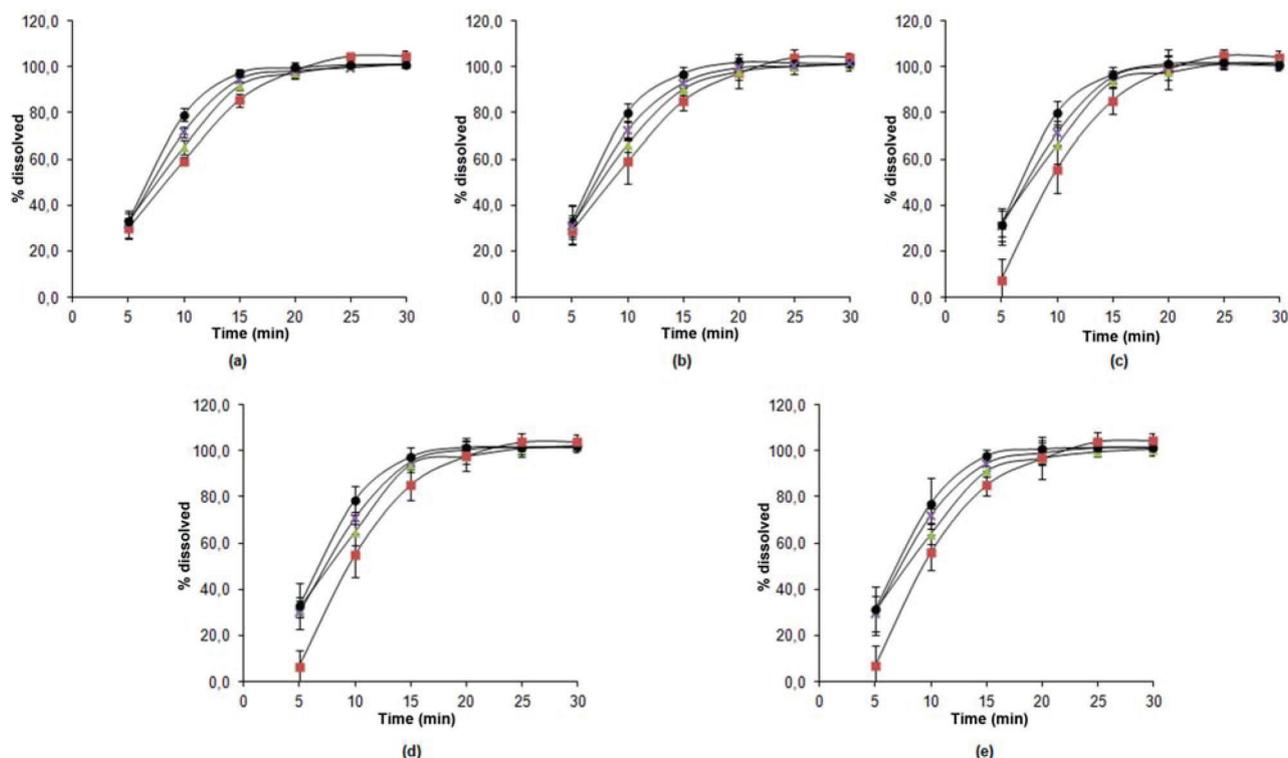

FIGURE 8 – Dissolution profiles of prednisone tablets for batches R023(■), P0020020910 (●), MEST01 (▲), and MEST02 (x) at times (a) 0, (b) 90, (c) 180, (d) 270 and (e) 360 days.

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The F1 and F2 values, comparative indices used for the evaluation of the similarity and difference between two dissolution profiles were also calculated to better verify the monitoring of lots for stability. The results are presented in Table V.

Batch P0020020910 exhibited an average release rate that was greater than 85% within 15 minutes for all time points in the stability profile, which was maintained during the entire study. Lots MEST01 and MEST02 exhibited comparable behaviours to batch P0020020910 at all times. Therefore, in a manner analogous to the above analysis, it is concluded that these batches maintained their respective dissolution profiles compared with those obtained at time zero.

Although batch R023 presented an average release rate exceeding 85% within 15 minutes and values less than 15 for F1 and greater than 50 for F2 at all times of the stability study, the results presented in Table 5 and Figure 24 indicate that this batch underwent a significant change in its dissolution profile. This change began at t = 180 and remained at t = 270 and t = 360, when an active release within five minutes of 7.5%, 6.6% and 6.7%, respectively, was observed. These values differ from the percentage release rates observed at time t = 0 and t = 90, which were 29.9% and 28.8%, respectively, within five minutes. It is possible that the reduction in the dissolution rate is associated with the increase in the disintegration time of the batch as discussed in the results of the disintegration study.

Comparison of dissolution profiles is a useful tool when the behaviour of two drugs must be known before conducting a study of relative bioavailability/ bioequivalence or pharmaceutical equivalence to claim exemption from such studies or for a possible post-registration change (Anvisa, 2011; 2010). However, when performed during the stability study, this comparison enables the evaluation of whether a formulation that has an initial dissolution profile equivalent to another formulation maintains that status throughout its shelf life.

Importantly, the rate at which a drug is released from its dosage form can be a limiting factor in the rate of absorption. Thus, a possible variation in this parameter during storage can cause a change in the beginning intensity or duration of a therapeutic response (Murthy, Guebre-Sellassie, 1993).

All batches of 20-mg prednisone tablets submitted to the study met the acceptance criteria established in the official monograph (Farmacopeia Brasileira, 2010), which must have a dissolution rate greater than 80% after 30 minutes of the test. According to the current Brazilian regulatory perspective, it is not necessary to calculate factor F1 or F2 for drugs of high solubility and rapid dissolution formulations. Therefore, batches P0020020910, MEST01 and MEST02 maintained their dissolution profiles equivalent to that of batch of R023 because these batches exhibited a dissolution that was greater than 85% for the first 15 minutes (Anvisa, 2010). However, during the study period, the F1 and F2 values calculated for lots P0020020910, MEST01 and MEST02 (Table V) showed a reduction of similarity and an increase in the difference between their dissolution profiles compared with batch R023. Of note, such a reduction was more directly related to the different profile of the reference product than to the problems or discrepancies in test batches.

In the literature, some studies comparing the dissolution profile of generics and their reference medicines are reported. Operto and collaborators (2008) compared 10 brands of paracetamol tablets produced in Argentina. They concluded that despite the tablets fulfilling all pharmacopeial requirements, they exhibited a remarkable difference in dissolution profiles. Kohler *et al*. (2009) evaluated the dissolution profiles of five generic 500 mg dipyrone tablets and concluded that none were equivalent to the reference drug product.

Similarly, Brum *et al*. (2012) analysed 8 brands of 750-mg paracetamol tablets available in Brazil and also found that one of the generic brands did not show a dissolution profile equivalent to that of the reference product. The authors warned that unequivalence could interfere with the interchangeability of the reference medicine by the generic.

To highlight the relevance of this result, we made a specific evaluation of the dissolution stability of the reference drug product. Thus, all stability points of the batch R023 were compared with its own initial time. This type of evaluation has been reported by Murthy and Guebre-Sellassie (1993) as an important tool to predict the performance of a tablet during stability and to demonstrate whether it retains the same quality during the shelf-life. Most regulatory recommendations (as in Brazil) exclusively claim the dissolution results for registration but not the dissolution profile.

As noted in Table V, based on F1 and F2 values, the reference medicine was stable during the first year of the stability study. However, F2 was reduced from 95.99 at 90 days to 50.79 in one year, which is at the borderline of the approval limit. In the next stability point, the drug would likely be disapproved. Given that the shelf-life declared in the package of the medicine is 2 years, it can be questioned whether a patient taking this product close to this time is actually consuming a medicine with assured quality and

TABLE V - F1 and F2 factors for all of the batches compared with R023 at t=0, t=90, t=180, t=270 and t=360 days of study

Days of study	Factor	Batches			
		R023*	P0020020910	MEST01	MEST02
Beginning (t=0)	F1	-	9.02	4.84	6.65
	F2	-	50.31	67.51	58.32
90	F1	0.97	9.87	4.52	7.49
	F2	95.99	48.98	68.20	56.15
180	F1	5.72	15.01	11.12	12.79
	F2	51.56	41.41	46.76	45.21
270	F1	6.09	15.58	10.57	12.60
	F2	50.59	40.64	46.88	45.33
360	F1	6.00	15.13	10.35	12.61
	F2	50.79	41.94	47.90	45.67

* This batch was evaluated by comparing its own results during stability to t=0.

clinical efficiency. Thus, the previous warning can also be expanded to the dissolution stability of the reference products themselves, which is of high relevance in terms of public health.

CONCLUSION

All techniques used in the characterization of prednisone samples A and B were helpful, providing information that indicated that both samples presented the same crystalline form and did not undergo transition over the stability study. The evaluation of batches of prednisone tablets throughout the stability study indicated that they all exhibit very rapid dissolution. Notably, when analysed individually, the dissolution profiles of all batches, except R023, exhibited minimal variation and remained similar during the entire study period. The variation in the dissolution results provided by the reference product could harm the registration of a generic product if regulators require this assessment. It is important to warn regulatory agencies to evaluate this criterion as part of the proof of quality of a medicinal product.

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