Research article

Development and Validation of a Dissolution Test for 6 mg Deflazacort Tablets

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Abstract

The aim of this study was to develop and validate a dissolution test for the quality control of deflazacort (DEF) tablets, a drug mainly used for the prevention of organ transplantation rejection, labeled as containing 6 mg of active pharmaceutical ingredient (API), using an RP-HPLC method. After testing sink conditions and stability at 37°C, DEF was found to be unstable, and decomposed as a function of the dissolution media. However, in water the decomposition was not significant enough to interfere with the determination of dissolution for DEF. The best conditions to carry out the dissolution test were paddle at 50 rpm, with 500 mL of deaerated water at 37°C. Under these conditions, the *in vitro* release profiles of 6 mg DEF tablets produced by two different laboratories, representing all approved products in the national market, shown to be dissimilar. A correlation between disintegration and *in vitro* dissolution was found for the two investigated formulations. The developed dissolution test was adequate for its purpose and can be applied for the quality control of 6 mg DEF tablets.

Keywords

Quality control • *In vitro* release • RP-HPLC

Introduction

Drug absorption from a dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution and/or solubilization of the drug

under physiologic conditions, and the permeability across the gastrointestinal tract [1]. Based on these considerations, the *in vitro* dissolution test for immediate release solid oral dosage forms has gained significance as being a very important tool in order to characterize drug product performance, to assess batch-to-batch quality of drug products, as well as to guide the development of new drug formulations [1].

Deflazacort (11β,16β)-21-(acetyloxy)-11-hydroxy-2'-methyl-5'H-pregna-1,4-dieno[17,16-d]oxazole-3,20-dione, DEF) is a methyloxazoline derivative of prednisolone, that is used in rheumatoid arthritis, nephritic syndrome, organ transplantation rejection and juvenile chronic arthritis, among other diseases [2, 3]. It is a poorly water-soluble compound [4] with an oral bioavailability of about 70 %, which exhibits low mineralocorticoid activity [5] and was promoted as a relatively bone-sparing glucocorticoid when compared with other glucocorticoids [2, 6, 7]. Although DEF was patented in 1966 [8] and is commercialized as tablets containing 6-mg or 30-mg per dose unit, it is still not coded [9] in the European Pharmacopoeia, the United States Pharmacopoeia (USP) or the Argentinian Pharmacopoeia (FNA). Consequently, as far as we know, there is no monograph of this active pharmaceutical ingredient (API) and no official method for the assessment of the dissolution characteristics of DEF tablets.

A review of the literature found a reversed phase-liquid chromatography (RP-HPLC) method reported for the determination of DEF in raw materials, drug products, biological fluids and *in vitro* drug dissolution studies [10]. After optimization and validation, the method was applied for the determination of both the API content as the dissolution rate of DEF from tablets containing 30 mg of this API, using hydrochloric acid (HCL) 0.1 M as the dissolution medium. However, only three tablets were tested, with selection of conditions and validation assays for the dissolution methodology not being reported. In 2006, a novel application of Near Infrared Spectroscopy (NIRS) for the determination of dissolution profiles of DEF in intact tablets was reported [11, 12], and the results of the method were compared with the ones obtained by applying a dissolution method that used 900 mL of purified water containing 0.034 M NaCl and 0.087 M HCl at pH 1.1, and UV spectrophotometry as the quantification method. In addition, Cardoso et al. [13] have recently reported a dissolution test for tablets and capsules containing 30 mg of DEF, which uses 900 mL of 0.1M HCL and UV spectrophotometry as detection method.

A common feature of all these methods is the evaluation of the dissolution characteristics of DEF tablets using an acidic medium of pH 1.0–1.1. This is surprising since DEF is an ester prodrug that can be hydrolyzed in acidic media, as it occurs with aspirin whose hydrolysis to salicylic acid took place before the end of the dissolution test [14].

This present study describes the development and validation of a simple dissolution test for 6-mg DEF tablets, which was optimized on the basis of solubility and stability considerations. But, the most important objective was to compare the technical quality and dissolution characteristics of 6-mg DEF tablets commercialized in our country, since in Argentine renal and corneal transplanted patients use 6-mg DEF tablets daily for avoiding organ rejection, and differences in formulation could influence the release characteristics of the dosage forms questioning the interchangeability of the products.

Results and discussion

I. Solubility determination and dissolution test optimization

Drug solubility and stability are important properties to be considered when selecting a dissolution medium [15]. In addition, it is generally considered advisable to use dissolution media with sink conditions, i.e. when the amount of substance dissolved in the dissolution medium at the end of the dissolution test did not exceed 30 % of the saturation concentration) [16]. In order to test such conditions a preliminary study on DEF stability in Milli-Q® water, simulated gastric fluid without pepsin (SGF) and simulated intestinal fluid without pancreatin (SIF) [17], was carried out using TLC (silica gel, dichloromethane-ethyl acetate 9:1). No DEF degradation products were detected in water during 6 h of testing. After that, a main degradation product, attributable to 21-desacetyldeflazacort, was noted by TLC analyses of the supernatant, indicating that DEF was partially degraded. On the other hand, In SGF and SIF, DEF degradation products were detected after 20 min, indicating a poor stability of this API in both media. The low solubility in SIF was notable, with no DEF being detected in the supernatant even after 24 h. Thus, these two media appear not suitable for a simple dissolution method, and In the case of studying the dissolution rate of DEF in HCL 0.087 M or 0.1 M [10, 11, 13], the decomposition of DEF should not to be ignored. Interestingly, McCormick et al. [14] developing a dissolution method for tablets containing aspirin and warfarin found that aspirin was unstable in phosphate buffer (the optimum medium for warfarin), and in order to quantitate the amount of aspirin that was originally present in tablets, the amount of salicylic acid formed by hydrolisis was converted to its aspirin equivalent and added to the amount of aspirin remaining in the sample to obtain the total amount of aspirin. A similar procedure should be followed for DEF in aqueous HCL; however, neither the decomposition pathways of DEF in aqueous media nor its degradation products are known at present. This will constitute our next task.

Hence, taking into account that from a routine quality control point of view the dissolution method should be as simple as possible, water appear to be an appropriate dissolution medium. Thus, the aqueous solubility of DEF at 37°C was determined by using a validated HPLC method (as it will be discussed in the following section) and the concentration free of degradation products was taken as a measure of its saturation concentration [18-20]. The value at 6 h was found to be 108 µg/mL. Moreover, DEF continued to dissolve even after 16 h and at this time, its solubility was slightly higher. However, the experiment was then stopped since the presence of degradation products prevented the solubility equilibrium being reached [18]. Hence, although water is not an ideal medium [21], it was chosen as the dissolution medium on the basis of no degradation products were detected at least at 6 h, which was sufficient time for dissolution profiles determination.

Considering the dose unit (6 mg) and the total volume of the dissolution apparatus (900 mL), DEF complete dissolution in water would correspond to ~ 6.2% (6.67µg/mL) of the DEF solubility. So, even with water at 500 mL, sink conditions prevailed. Thus, in order to improve sensitivity, a volume of 500 mL was chosen. The dissolution rate of DEF from tablets of product A (taken as the reference product) was assessed at 50 and 75 rpm, the recommended speeds for apparatus 2 (Table 1). At 50 rpm, product A exhibited a very rapid dissolution, i.e. it released >85% of DEF in 10 min. Under these conditions, "conning" was not observed and the pH of the medium remained the same during the analysis. At 75 rpm, product A improved its dissolution, as expected; however, the dissolution resulted too

rapid, releasing 99.6% of DEF at 7.5 min. The statistical *t*-Student test at 0.05 confidence level (paired media) was applied to compare the cumulative percentage of DEF released (%R), using 50 and 75 rpm. The *P*-value presented was slightly greater than the significance level (0.05), indicating that there was not statistically significant difference between the %R at both stirring speeds. But, considering that a very rapid dissolution can mask differences among individual tablets and that at 50 rpm "conning" was not observed, a speed of 50 rpm was chosen.

Tab. 1. Effect of the paddle rotation speed on the cumulative percentage of DEF released (% R) from tablets of product A in 500 mL of deaerated water at 37° C.

Time, min	% R (± SD[a])		*t* test	*P*
	50 rpm	**75 rpm**		
0.0	0.0	0.0		
5.0	51.6 (3.5)	78 (13)		
7.5	74.7 (9.9)	100 (15)		
10.0	86.1 (3.1)	100.0 (6.1)	−2.534	0.052
15.0	96.4 (7.2)	100.1 (3.0)		
20.0	99.7 (9.9)	101.3 (1.6)		
30.0	100.2 (5.2)	101.6 (2.4)		

[a] standard deviation.

The effect of the dissolved gases in the medium on DEF dissolution was investigated at 50 rpm. As shown in Figure 1, non-deareation of the medium caused an important decrease in the amount of DEF dissolved from tablets of product A, and an increase in the variability of the results. Therefore, a deareated medium is necessary for evaluating the dissolution behavior of DEF.
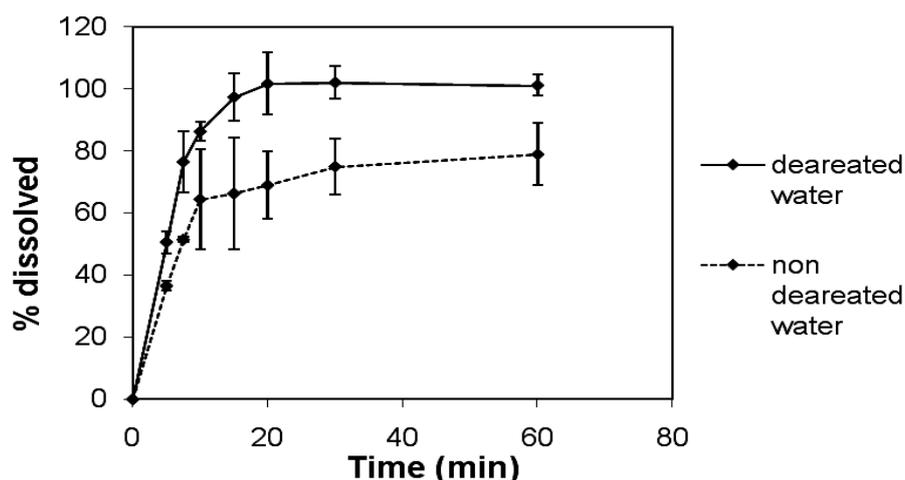
Fig. 1. Comparison of the DEF tablet´s dissolution profiles (Product A) using deareated and non-deareated water (500 mL). Bars indicate the standard deviation.

In consequence, 500 mL of deareated water appears an adequate medium for a routine quality control method, i.e. the decomposition of DEF is not significant enough to interfere with the dissolution determination, the pH of the medium remained unchanged after the end of the test, and also sink conditions prevailed.

II. Dissolution test and HPLC validation

Taking into account the instability of DEF, the analytical method was optimized in order to separate DEF from its degradation products. After several trials, it was noted that internal standards with retention times lower than that of DEF interfered with its degradation products, while, standards with higher retention times increased excessively the run times. Thus, the external standard method and a mixture of methanol and water (80:20 v/v) were chosen for testing. In order to verify that the equipment was adequate for the analysis to be performed, system suitability tests were carried out. They were defined based on the results obtained in replicate injections of a standard solution of DEF (20.0 µg/mL). The obtained R.S.D. value for the peak area of the analyte was 0.26%, indicating an acceptable system precision. The capacity factor ($k´$) was >2 (2.47), the number of theoretical plates (N) was >2000 (4200) and the tailing factor, measured at 5% of the peak height, was <2.00 (1.46). The results of system suitability testing, in comparison with specifications [17, 22], indicated that the proposed chromatographic conditions achieved reasonable retention and symmetric peak shapes for DEF. In fact, DEF degradation peaks were observed after acid, alkaline, light and thermal treatments, and resolved from the DEF one (Supporting information). Table 2 shows the percentages of DEF recovered after such treatments. In addition, no interference from excipients was observed in the tablet dosage forms (Supporting information). The dissolution test specificity also confirmed these results, as no interference from the excipients was found, i.e. the statistical t-test at the 0.05 significance level indicated no significant difference (P = 0.49) between the %R from tablets and the content of a 12 µg/mL DEF standard solution.

Tab. 2.   Degradation of DEF in selected conditions.

Condition	Time, h	Recovery, %	RRT[a] of Degradation products
DEF standard solution + HCl 0.1 M (RT[b])	0.5	97.7	3.45; 5.40
DEF standard solution + HCl 0.1 M (RT[b])	5	83.2	3.45; 5.40
DEF standard solution + NaOH 0.1M (RT)	5	85.8	3.52; 5.30
DEF standard solution, dark (RT)	5	100.0	None detected
DEF standard solution, daylight (RT)	5	98.5	3.95; 5.38
DEF standard solution, dark (4°C)	48	95.9	3.52; 5.30
DEF standard solution, 80 °C	5	76.8	2.60; 4.68; 5.43

[a] RRT: relative retention time. [b] RT: room temperature.

To assess the linearity, three standard curves for DEF were constructed, plotting micrograms injected versus peak areas on the 1–20 µg/mL concentration range. As the nominal concentration of DEF in 500 mL should be 12 µg/mL, the above range brackets this level. The regression equation was: $y = (2678 ± 21)10^3 . x + (6.6 ± 4.5)10^3$, where x is µg injected and y is peak area. The relative standard deviation (RSD of the slope was 0.78%, the coefficient of determination (R^2) was 0.9991 and the y-intercept was not

significantly different from zero at the 95% confidence level. The data was confirmed by means of analysis of variance (ANOVA), which demonstrated a significant linear regression and non-significant linearity deviation ($P < 0.05$).

The assessment of the method precision (Table 3) indicated that it was acceptable, i.e. the RSD values were all below 1%, with the intermediate precision also being found to be adequate (RSD values <1%). On the other hand, in the accuracy tests, the method was found to be accurate based on its intended use. As indicated in Table 4, the mean recoveries for both products ranged from 99.65% to 100.52% with RSD values < 1%.

Tab. 3. Precision of the assay method: repeatability and intermediate precision.

	Repeatability			Intermediate precision	
Sample Nº	**Product A[a] DEF (RSD), %**	**Product B[b] DEF (RSD), %**	**Day Nº**	**Product A[a] DEF (RSD), %**	**Product B[b] DEF (RSD), %**
1	98.60 (0.12)	102.20 (0.07)	1	98.60 (0.12)	102.20 (0.07)
2	98.54 (0.19)	101.52 (0.36)	2	99.33 (0.03)	103.67 (0.18)
3	98.69 (0.09)	101.85 (0.26)	3	98.29 (0.12)	104.83 (0.08)
4	99.29 (0.08)	103.70 (0.18)	4	99.15 (0.50)	103.50 (0.11)
5	98.86 (0.14)	101.69 (0.17)	5	98.86 (0.02)	104.50 (0.40)
6	98.75 (0.04)	103.53 (0.28)	–	–	–
Mean (RSD), %	98.79 (0.27)	102.42 (0.94)	Mean (RSD), %	98.85 (0.42)	103.74 (0.99)

[a] Batch 1A0814. [b] Batch A303.

**Tab. 4.** Recovery analysis for DEF tablets, using the HPLC method.

Product	*Concentration level, % w/w*		
	80	**100**	**120**
A	99.94	100.58	100.21
	99.80	100.48	100.10
	99.50	100.50	100.31
Mean (RSD), %	99.75 (0.23)	100.52 (0.05)	100.16 (0.36)
B	100.34	99.47	100.40
	99.99	99.69	100.78
	99.99	99.80	101.15
Mean (RSD), %	100.11(0.20)	99.65 (0.17)	100.18 (0.54)

[a] Batch 1A0814. [b] Batch A303.

The accuracy of the dissolution test was also verified by recovery experiments (Table 5).

Percentage recoveries of 100 ± 5 % have been previously recommended for the accuracy test [15]. As mean recoveries ranged from 100.1% to 103.5%, this showed that the dissolution method was accurate.

The ruggedness of the dissolution test was evaluated, and the results are shown in Figure 2. The effect of rotation speed was noticeable only at 5 min, where the disintegration and dissolution were favored by the increased stirring speed. Above 5 min, the variation in the %R was less than ± 5% [23] of that the optimal experimental conditions. The variation in the %R with the temperature change was also less than ± 5 % at all time points, confirming the ruggedness of the test.
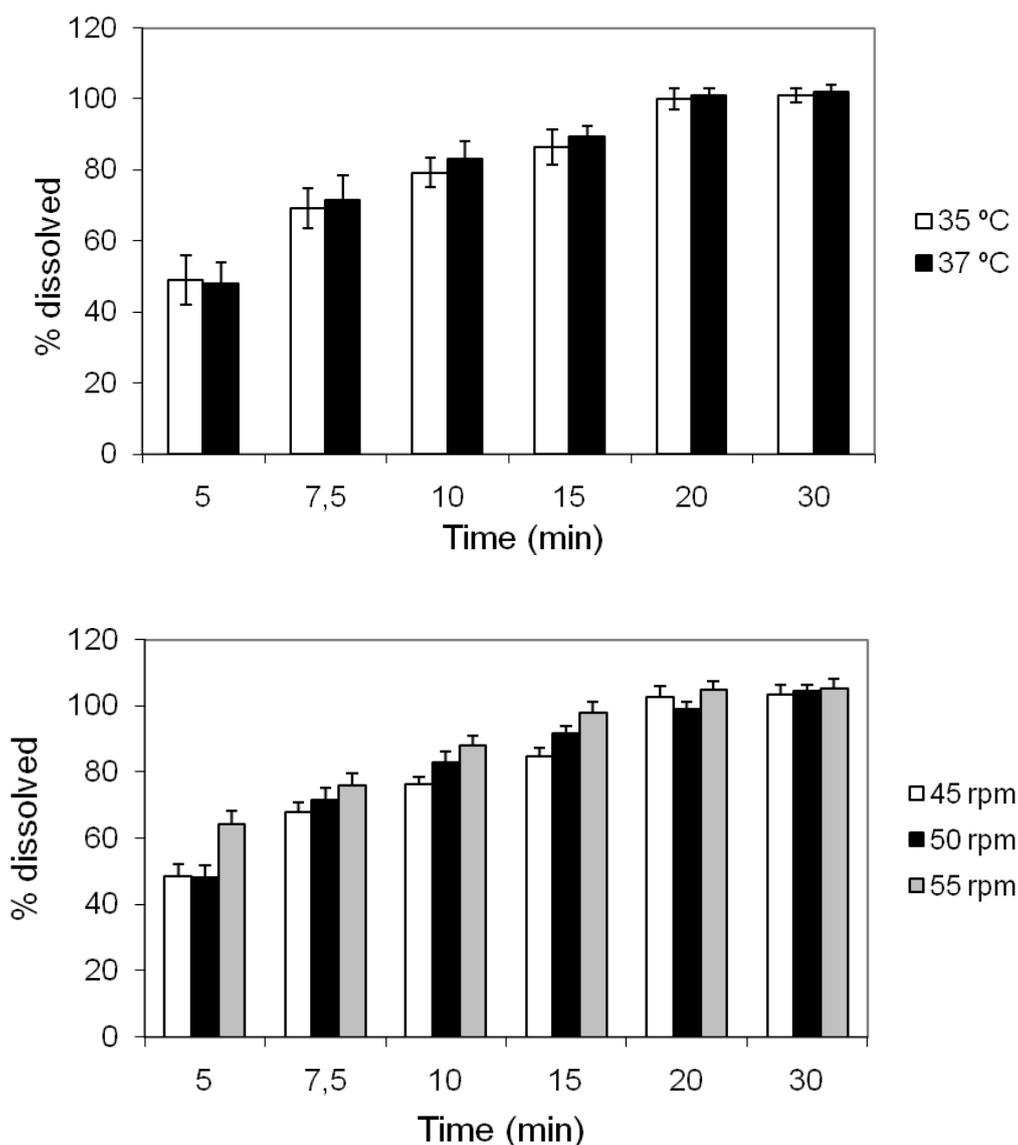

Fig. 2.   Ruggedness of the dissolution test against paddle rotation speed and temperature variation (Product A).

Tab. 5.   Accuracy data for the dissolution method.

Concentration level, % w/w	Recovery (%)[a]	Average recovered (RSD), %
80	103.50	
80	103.19	103.5 (0.24)
80	103.67	
100	99.37	
100	99.72	100.1 (1.0)
100	101.29	
120	104.96	
120	98.72	101.4 (3.2)
120	100.49	
Mean (RSD), %	101.6 (2.2)	

[a] Product A (Batch1A0814).

The dissolution profiles of products A and B in the optimized conditions are shown in Figure 3. A visual inspection of the resulting profiles clearly indicates that both products are inequivalent in their dissolution behavior. In accordance, One-way ANOVA [24, 25] indicated that the % R for products A and B were statistically different at each time point (P = 0.00). These results were confirmed by the Welch and Brown-Forsythe tests, which also gave P values = 0.00 at each time point, indicating that product A was significantly different from product B in its dissolution behaviour.
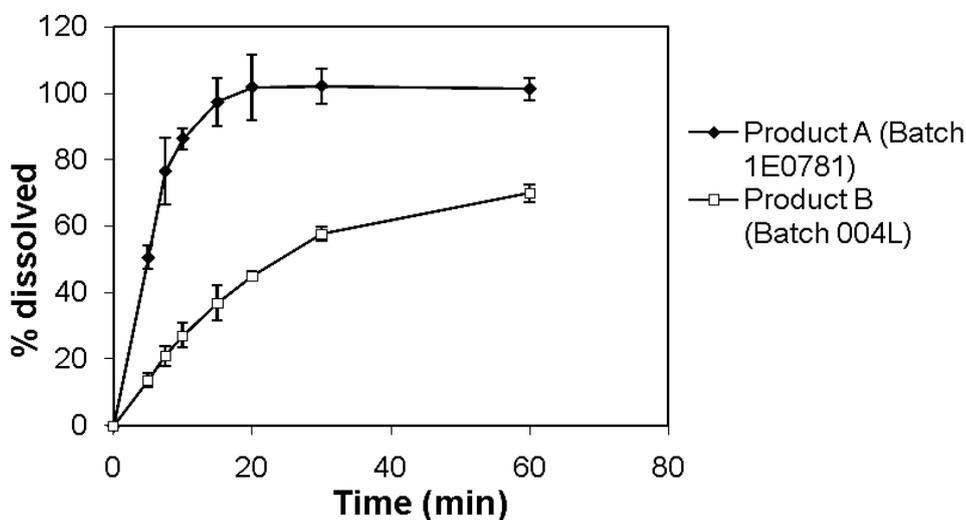
Fig. 3.   Mean dissolution profiles (n=6) of DEF from products A and B in 500 mL of deaerated water at 37 ºC and 50 rpm (Bars indicate the standard deviation).

The validated dissolution method was also applied to the quality control of three batches of product A, as shown in Figure 4. According to ANOVA test, the profiles were not significantly different in terms of the % R at each time point (P >0.05), indicating good manufacturing reproducibility for product A.
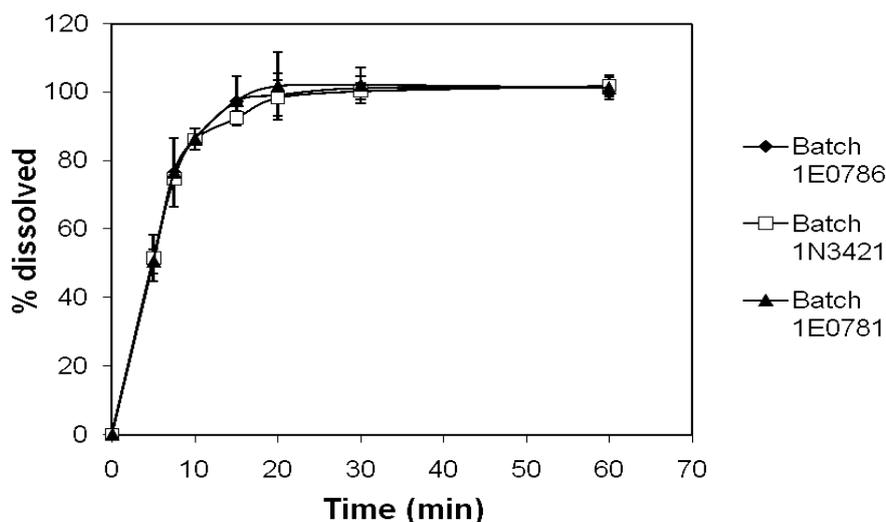
Fig. 4. Mean dissolution profiles (*n*=6) of three batches of product A in deaerated water at 37°C and 50 rpm (Bars indicate the standard deviation).

III. Pharmacotechnic tests

In order to evaluate the technical quality of DEF tablets, various physical tests were conducted and the results presented in Table 6. It was determined that both products fitted with the specified limitation for tablet weight variation (the mean weight ± 15%) and with the recommended hardness range (2-8 Kg). Moreover, the average tablet weight and hardness did not differ significantly between products (Table 6).

Tab. 6. Physical characteristics of the investigated formulations.

Characteristic	Product A[a]	Product B[a]
Tablet weight (mg)	186.2 (1.1)	193.0 (1.4)
Hardness (Kg)	5.58 (27)	5.02 (28)
Thickness (mm)	2.47 (0.89)	2.61 (2.0)
Diameter (mm)	12.74 (0.60)	13.23 (0.37)
Disintegration time[b]	2'50"	5'19"
Drug content (mg)	6.2 (1.2)	6.2 (2.0)
Batch	1E0781	004L

[a] Values expressed as mean (RSD%). [b] FNA criterion: the time corresponding to the last disintegrated tablet [26].

The average drug content of both products was identical, within the experimental error, and complied with the pharmacopoeial criteria for related corticoids (100 ± 10% of the labeled amount) [17]. In contrast, a marked difference was found in the tablet disintegration times, being evident a relation between disintegration time and *in vitro* dissolution. Product A showed the shortest disintegration time, which seemed to be reflected in its fast release *in vitro* (Figures 4 and 5). Meanwhile, product B exhibited a

lower dissolution, with its disintegration time being almost double. Thus, the dissolution differences were reflected in the disintegration behavior of the products.

In summary, a simple dissolution test for evaluating the *in vitro* biopharmaceutical quality of 6-mg DEF tablets was developed and validated. The method was found suitable to study the dissolution of this API, finding significantly different release profiles for the two products commercialized in our country, which indicates that the developed dissolution method has enough discriminatory power to reflect formulation differences. Taking into account that product A is a very rapidly dissolving product; it appears that there exist fewer ramifications for the DEF bioavailability in product A than in product B, which was found to be a very slowly dissolving product, so its interchangeability may be seriously considered. Nevertheless, *in vivo* bioavailability studies should be conducted in order to confirm any correlation with the *in vitro* performance of DEF products.

Experimental

I. Materials

DEF (99.2% purity) was further purified by recrystallization from ethyl acetate in order to obtain a working standard whose purity was then checked by several techniques, including DSC and TG (MDSC 2920 and TG 2950 analyzers, TA Instruments, New Castell, DE, USA), Hot stage microscopy (Leitz, Wetzlar, Germany) and Power X-ray diffractometry (D8 Advance, Bruker, Germany) studies. Milli-Q® water (Millipore, Bedford, USA) was used for both the dissolution media and the HPLC mobile phase. All other reagents and chemicals were of analytical or HPLC grade (Sintorgan S.A.). Nylon membranes (0.45-µm pore size, Pall Corporation) and Teflon filters (10-µm, Hanson Research, P/N 27-101-074) were commercially acquired. DEF tablets were obtained locally from pharmacies in Cordoba city. The investigated products were:

Product A: Azacortid® 6 (Aventis, Italy), labeled as containing 6 mg of DEF and the following excipients: lactose, microcrystalline cellulose, magnesium stearate and cornstarch.

Product B: Flamirex® 6 mg (Sanofi-Synthelabo, Argentine) labeled as containing 6 mg of DEF and the following excipients: corn starch, calcium phosphate dibasic, microcrystalline cellulose, polyvinyl pyrrolidone, crosscarmellose and magnesium stearate.

II. Methods

II.a Dissolution studies

Dissolution was conducted using a Hanson SR6 dissolution tester (Hanson Research, CA, USA) using USP Apparatus 2 at a temperature of 37.0 ± 0.5 ºC. For all experiments, filtered (Teflon filters fitted to the stainless steel cannulas of the dissolutor) aliquots (5 mL) were withdrawn with replacement at appropriate time intervals, using a 5mL syringe. All the sample solutions were protected from the light until being analyzed. Due to the limited number of samples, 3–6 tablets per formulation were tested in the development and validation stage, and six in the optimized conditions. The cumulative percentage of API released (%R) was determined by HPLC assay. Checks for adsorption to the filters were performed by preparing DEF solutions at the nominal concentration in dissolution media,

and then passing them through the filters. This procedure was repeated during 15 min. The results of these studies revealed no significant loss of API.

II.b Solubility experiments

An excess amount of DEF working standard (10 mg, mildly ground in an agate mortar) was introduced into screw-capped vials containing 7 mL of the appropriate solvent [Milli-Q® water, simulated gastric fluid without pepsin (SGF) and simulated intestinal fluid (SIF) without pancreatin (pH 6.8)] in a shaking water bath at 37°C. At suitable time intervals, samples were collected, filtered (0.45-µm), and analyzed by TLC (silica gel, dichloromethane-ethyl acetate 9:1, UV detection) to detect DEF degradation products. Residual solid materials were also analyzed post-assay using TLC. After that, the solubility of DEF was determined in water as described, but assessing the amount dissolved by HPLC. All tests were performed in duplicate.

II.c Chromatographic conditions

The HPLC system consisted of a Spectra System P2000 pump, a Rheodyne® 20 µL fixed loop injector (7125 model), a variable UV-Visible detector (Thermo Separation Products) and a Chromatopac integrator (Spectra System). Chromatography was performed on a Synergi-4µ-Fusion RP-80 C_{18} (Phenomenex, 250mm x 4.6mm, 4.5 µm) column. The mobile phase was a mixture of methanol and water (80:20, v/v), which was filtered (0.45-µm) and degassed using vacuum before use. All analyses were performed at room temperature (RT, 20–25°C) at a flow rate of 0.8 mL/min. Detection was made at 245 nm (0.01 a.u.f.s). Under these conditions, DEF retention time was roughly 6.5 min. Triplicate 20 µL injections were made for each analysis. Peak areas were integrated using the program Peak Simple®1.0 for Windows.

II.d Dissolution test and HPLC validation

The dissolution test and the HPLC method were validated through the determination of stability, specificity, linearity, accuracy/precision and ruggedness [15, 17, 27, 28].

Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. System suitability tests were carried out by making six replicate injections of a standard solution containing 20.0 µg/mL of DEF, and analyzing the chromatograms for DEF peak area, theoretical plates (N), capacity and tailing factors. Acceptable results for these parameters, calculated using reported equations [17, 22] were required before sample analysis.

Stability and specificity: To investigate the stability of DEF and the ability of the HPLC method to separate it from its degradation products, forced degradation studies were performed, using acid, base, heat (80 °C) and daylight. Degradation samples were obtained by transferring 1mL of a 180 µg/mL DEF stock standard solution (in mobile phase) into screw-capped vials, and adding 9 mL of HCL 0.1M, NaOH 0.1 M or mobile phase (heat and light effect). Also, the stability of the stock solution was evaluated for 48 h at 4 °C in the dark. After suitable time intervals, the solutions were neutralized (where corresponding), filtered and assayed, verifying the chromatograms obtained (peak areas and degradation products formation). The dissolution test specificity was assessed by determining the %DR from tablets (n=3) in 500 mL of water at 37.0 °C with a stirring speed

of 150 rpm. Sample aliquots were withdrawn, filtered, analyzed by HPLC and compared with a DEF standard solution of about 12 µg/mL

Linearity: Aliquots of a 200 µg/mL DEF stock solution were transferred to 10-mL volumetric flasks to obtain six standard solutions (1–20 µg/mL range). The solutions were prepared on different days (*n*=3) in order to test inter-day linearity and they were injected in triplicate every day. Linearity was evaluated by the least squares regression method and ANOVA.

Precision: For the method precision, the repeatability and intermediate precision were determined by assaying samples of product A and B at the same concentration, under the same experimental conditions, during the same day (preparing six sample solutions) and in five different days, respectively. Thus, ten tablets of each product were crushed to fine powder. An accurately weighed amount of powder equivalent to 8 mg of DEF was placed in a 50-mL volumetric flask and methanol (2 mL) was added to favor the solubilization of the drug. Then, 10 mL mobile phase were added, the mixture sonicated for 15 min, and the volume was completed with the same diluent. This mixture was then mixed and filtered. The first 10 mL were rejected, and 1 mL of the filtrate was transferred to a 10-mL volumetric flask and diluted with mobile phase. The solution was filtered before the HPLC analysis. Precision was evaluated based on the RSD of the results.

Accuracy: it was assessed at 80, 100 and 120% of the assay of DEF by recovery experiments, where the assay method was applied to portions of powdered tablets spiked with known amounts of DEF. The accuracy of the dissolution test was evaluated by recovery tests of known amounts of DEF (at 80, 100 and 120% of the label claim) added to tablets. Accurately weighted portions of DEF were dissolved in methanol (3 mL) and quantitatively transferred to vessels containing water (preheated at 37 °C) to a final volume of 500 mL. Then, tablets of product A were dropped and rotated for 15 min at 150 rpm. Aliquots were withdrawn, and suitably diluted with mobile phase before being assayed by HPLC.

Ruggedness: It was evaluated through small, deliberate variations of some critical parameters such as paddle rotation speed and temperature. The stirring speed was varied in the range of 45.0–55.0 rpm, and two temperatures were assayed (35.0 and 37.0 °C).

II.e Applied methods to compare dissolution profiles

Where appropriate, Student *t*-test or Univariate ANOVA was used to compare the data [24, 25]. In ANOVA, the percentages dissolved were tested separately at each time point to see if there were differences among the conditions or products. *Post hoc* procedures were also applied to determine when the differences arose. The data were considered to be significant when $P < 0.05$.

II.f Pharmaceutical quality control tests

Tablets of each formulation were subjected to the following tests: a) *Weight variation:* Each tablet (*n*=10) belonging to each formulation was weighted on an electronic balance (Mettler H-72). b) *Hardness:* This test was applied with a tablet hardness tester (AVIC DU-4 07MR98, Argentine). Six tablets of each product were individually put into the tester and the 'resistance to crushing' was measured. c) *Thickness:* the thickness of tablets (*n*=6) from each product was measured with the micrometer included on the tablet hardness

tester. d) *Assay:* it was performed following the procedure described under precision. e) *Disintegration:* Tablets (*n*=6) of each formulation were subjected to a disintegration test according to the FNA 7° Edition (37.0 ± 0.5 °C, water and basket rack assembly) [26] using a QC-21 Hanson disintegrator tester.

Acknowledgements

Supporting Information

4 typical chromatograms for (a) DEF standard, (b) Product A, (c) Product B and (d) DEF standard after acid degradation (aqueous HCL 0.1 M) are available in the online version (Format: PDF, Seize: < 0.1 MB): http://dx.doi.org/10.3797/scipharm.0904-05.

Authors' Statement

Competing interests

The authors declare no conflict of interests

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