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Research Article

Development and Validation of a Discriminative Dissolution Test for Betamethasone Sodium Phosphate and Betamethasone Dipropionate Intramuscular Injectable Suspension

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Abstract. The intramuscular administration of the injectable suspension betamethasone sodium phosphate (BSP) and betamethasone dipropionate (BD) has immediate therapeutic activity due to solubilized BSP and prolonged activity resulting from the slow release of BD micro-crystals. The purpose of this study was to develop and validate a dissolution method for BD in intramuscular injectable suspensions with detection by high-performance liquid chromatography (HPLC) method. Five commercial products presented a distribution of particle sizes, ranging between 7.43 and 40.25 μm as measured by laser diffraction. It was also found that particle sizes differed between batches of the same product. The different products were tested using the paddle apparatus, with stirring speeds of 25 and 50 rpm in 300 mL of phosphate buffer; simulated body fluid, muscle fluid, and synovial fluid were used as biorelevant dissolution media at 37±0.5°C. It was verified that not only does average particle size affect the dissolution rate, but also the mode and the polydispersity index of the particles. Discriminatory power was obtained using the *in vitro* dissolution method with 0.1 M sodium phosphate buffer pH 7.4 containing 0.1% sodium lauryl sulfate and a stirring speed of 50 rpm. The HPLC-method is linear, precise, selective, and accurate for the quantification of BSP and BD in dissolution profile testing. This dissolution method can be utilized as a method to control the quality of these injectable suspensions.

KEY WORDS: dipropionate betamethasone; dissolution test; intramuscular injectable suspensions; simulated muscular fluid; sodium phosphate betamethasone.

INTRODUCTION

Betamethasone is a corticosteroid which mainly acts as a glucocorticoid. The injectable suspension is a relevant pharmaceutical form which contains the combination of esters of betamethasone, betamethasone sodium phosphate (BSP) and betamethasone dipropionate (BD), which have anti-inflammatory, anti-allergic, and anti-rheumatic effects. The immediate therapeutic activity in betamethasone is provided by the soluble ester BSP, which is rapidly absorbed (1,2). The microcrystals of BD form a drug depot that slowly releases betamethasone and is responsible for prolonging drug activity, thus controlling symptoms over a longer period of time (2). The t_{max} of the major metabolite of BSP was at 2.8±1.7 h and of BD was 15±9 h, after intramuscular injection (2). BD must first dissolve in the intercellular space fluid of muscle fibers before it can diffuse into the vascular space. The $t_{1/2}$ observed of the major metabolite for these drugs is 9.6±3.6 h and 80.8±22.7 h for BSP and BD, respectively (2).

BD is practically insoluble in water, becoming essentially a suspension of submicron particle size which is present in the injectable formulation and which ensures sustained release of the drug (3,4). More than 20 products are formulated using this drug association and are commercialized as injectable suspensions. The commercially available products contain formulations of different compositions; therefore, these formulations can present different physicochemical characteristics.

The *in vitro* dissolution studies are an indispensable tool during several stages of pharmaceutical formulation development, thus enabling evaluation of its stability and effectiveness (5–7). During pharmaceutical production and quality control, the results obtained by the dissolution test can be employed to verify variances that occur during manufacturing as well as ensure batch to batch reproducibility. In addition, this method enables comparison between batches obtained from different production sources (7–9).

Although the *in vitro* dissolution test has initially been developed for immediate release of solid oral dosages, its use was extended to formulations which had controlled and modified drug release profiles. Recently, the application of these tests was also extended to include a variety of dosage forms, such as patches, suspensions, and injectable microparticulate formulations (10–12). Suspensions are dispersed systems containing drugs with low solubility, thus the drug absorption is limited by its dissolution rate, thus providing different drug plasma profiles when different formulations are compared

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(12–14). The *in vitro* dissolution test for injectable suspensions systems is indispensable, since the effective absorption of the drug depends on the dissolution of particles at the absorption site (4,8,15,16).

Application of *in vitro* dissolution tests to evaluate the quality of suspensions is recommended in only nine monographs of the U.S. Pharmacopoeia for the oral suspensions of cefdinir, cefuroxime, phenytoin, ibuprofen, indomethacin, megestrol, meloxicam, mycophenolate, and nevirapine. However, dissolution testing for injectable suspensions cannot be found in any of the pharmacopoeia. Despite the lack of *in vitro* dissolution tests for injectable suspensions described in official compendia or in scientific articles, regulatory agencies are increasingly interested in establishing methods that evaluate dissolution of injectable drugs.

In this context, the purpose of this study was to develop and validate a dissolution method to evaluate the dissolution of BD in intramuscular injectable suspensions. It is important to accurately predict the performance of injectable suspensions by routine quality control analysis. Commercial suspensions with different physicochemical properties were analyzed in order to select the most discriminating conditions for the dissolution test.

MATERIALS AND METHODS

Materials

Commercial injectable suspensions that contain both BSP and BD were obtained from different manufacturers and purchased at the local market and are described as products A, B, D, and E. Different batches of the products B, D, and E were also purchased and identified by different letters. Among the suspensions studied was the reference product along with similar and generic brands. The raw materials, BSP and BD, product C (reference), and the placebo were kindly provided by Mantecorp Chemical and Pharmaceutical Industry (Rio de Janeiro, Brazil). The BSP (batch no. L0G223) and BD (batch no. L0G377) reference standards were purchased from U.S. Pharmacopeia.

The product contains 2 mg of BSP and 5 mg of BD in a 1-mL ampule, and the formulation excipients are: sodium phosphate dibasic, sodium phosphate monobasic, edetate disodium, benzalkonium chloride, PEG 300, PEG 400, water for injection (product A); disodium edetate, carboxymethylcellulose, polyethylene glycol 4000, monosodium phosphate, disodium phosphate, benzalkonium chloride, water for injection (product B); dibasic sodium phosphate, sodium chloride, disodium edetate, polysorbate 80, benzyl alcohol, methylparaben, propylparaben, carboxymethylcellulose sodium, polyethylene glycol, water for injection (product C); benzyl alcohol, edetate disodium, sodium chloride, macrogol 4000, polysorbate 80, sodium carboxymethyl cellulose, dibasic sodium phosphate, hydrochloric acid, sodium hydroxide, water for injection (product D); and sodium bisulfite, creatine, sodium citrate, methylparaben, sodium, povidone, water for injection (product E). The placebo was composed of the same constituents as the reference product.

Reagents were obtained from different local distributors. High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Tedia (Rio de Janeiro, Brazil); hyaluronic acid from Galena (São Paulo, Brazil); phosphoric acid, sodium bicarbonate, calcium chloride, magnesium chloride, potassium chloride, monobasic potassium phosphate, dibasic sodium phosphate, sodium hydroxide, imidazole, sodium lauryl sulfate, sodium sulfate, polysorbate 20, and polysorbate 80 from Vetec (Rio de Janeiro, Brazil); and sodium chloride, dibasic potassium phosphate, and monobasic sodium phosphate from Merck (Rio de Janeiro, Brazil).

For the filtration procedures 10-μm polyethylene filters from Hanson Research (São Paulo, Brazil) were used; 0.45 and 0.1 μm polyvinylidene fluoride filters were obtained from Millex Millipore® (São Paulo, Brazil). Distilled deionized water was obtained using the Milli-Q water purification system, Millipore (Bedford, Massachusetts, USA).

Particle Size Analysis

Analysis of BD particle size distribution in the injectable suspension was conducted by laser diffraction using the Malvern Mastersizer 2000 analyzer with the Hydro MS module (Worcestershire, UK). Distilled water was used as the dispersing media at a stirring speed of 1,500 rpm.

BD Solubility Study in the Dissolution Media

BD sink conditions were determined for the different dissolution media as presented in Table I. The solubility was determined using sodium lauryl sulfate (SLS), polysorbate 80 (P80), and polysorbate 20 (P20) at concentrations between 0.05 and 1.5% (*w/v*) and without the addition of surfactants during these measurements. The pH of the dissolution media was adjusted using 3 M sodium hydroxide or phosphoric acid solution (1:1), as presented in Table I.

The solubility studies were performed in a beaker containing 10 mL of the test media by adding approximately 150 mg of BD to ensure an excess of drug in the system. The solutions were stirred constantly using a magnetic stirrer at a speed of 500 rpm for a 24-h period. Thereafter, the solutions were centrifuged for 15 min in an Eppendorf 5430 R centrifuge (Hamburg, Germany), at rotation speeds of 5,000 rpm. The supernatant was filtered through a 0.45 μm membrane, and the filtrate was assayed by HPLC, according to the methods developed for quantifying raw materials described by U.S. Pharmacopoeia (17). The test was performed in duplicate for each dissolution media tested.

In Vitro Dissolution Study

BD is practically insoluble in water (2), and its solubility in different aqueous solutions was used as a criterion for selecting the media utilized in the dissolution tests. The dissolution media used were: sodium phosphate buffer (SPB) pH 7.4 containing 0.05, 0.1, 0.5% SLS (*w/v*) and 0.5, 1% P80 (w/v); SBF pH 7.4 containing 0.1% SLS (*w/v*) and 0.5% P80 (*w/v*); SMF pH 7.4 containing 0.1% SLS (*w/v*) and 0.5% P80 (*w/v*); and SSF pH 7.4 containing 0.1% SLS (*w/v*) and 0.5% P80 (*w/v*).

The BD dissolution profiles were studied in injectable suspensions, and the measurements were obtained using the Dissolutor Hanson Research Model SR6 (Chatsworth, USA),

Table I. Dissolution Media Composition

Dissolution media		Composition	Reference
SPB	Sodium phosphate buffer	50 mM NaH_2PO_4, 39.1 mM NaOH, H_2O q.s., pH7.4±0.05	(34)
SBF	Simulated body fluid	136.8 mM NaCl, 4.2 mM $NaHCO_3$, 3 mM KCl, 1 mM $K_2HPO_4.3H_2O$, 1.5 mM $MgCl_2.6H2O$, 2.5 mM $CaCl_2.2H_2O$, 0.5 mM Na_2SO_4, H_2O q.s., pH7.4±0.05	(25)
SMF	Simulated muscular fluid	180 mM NaCl, 10 mM imidazole, H_2O q.s., pH7.0±0.05	(27,28)
SSF	Simulated synovial fluid	136 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, 7.5 mM $(C_{14}H_{20}NNaO_{11})_n$), H_2O q.s., pH7.4±0.05	(25,35)

The dissolution media were tested without the addition of surfactants and with sodium lauryl sulfate (SLS), polysorbate 80 (P80) and polysorbate 20 (P20) at concentrations ranging from 0.05 to 1.5% (w/v)

with the paddle apparatus described in *Method 2* of U.S. Pharmacopoeia. The agitation speeds used were 25 and 50 rpm (±4%), which are the recommended conditions for dissolution methods applied in suspensions (9,16,17). The volume of dissolution media was 300 mL, maintained at 37±0.5°C. Sampling was performed manually at the following time points 5, 10, 15, 30, 45, 60, 90, and 120 min.

After homogenization, the suspension was removed from the ampule using a syringe and needle and transferred to the vessel, thus allowing the dissolution test to be started immediately. Two milliliters of samples was collected without subsequent replacement of the media. The sample was filtered directly into a vial for analytical quantification by HPLC as previously described. The calculation of the cumulative amount of dissolved drug was performed based on the mass of the drug added to the vessels previously quantified in the batch.

Evaluation of Dissolution Profiles

The dissolution profiles of the products were evaluated by statistical analysis of the difference factor (f_1), the similarity factor (f_2), and one-way analysis of variance (ANOVA) using the Tukey's multiple-comparison method. The statistical analyses were performed with GraphPad Prism Software Inc (La Jolla, California, USA).

BSP and BD Quantification in Dissolution Tests

BSP and BD quantification in the dissolution testing was performed using the LaChrom Elite chromatograph system from Merck-Hitachi (Darmstadt, Germany) coupled to a diode array detector (DAD L-2130), quaternary pump (L-2455), column oven (L-2350), autosampler (L-2200), and EzeChrom software. The method used was based on the chromatographic conditions developed by our research group for the simultaneous quantification of BD and BSP in injectable suspensions (18). The stationary phase was the column C18 Kromasil 100–5 (4.6×150 mm, 5 mm), and the mobile phase was a ternary gradient, containing phosphate buffer (pH 4.5; 0.07 M), acetonitrile, and methanol, at a flow rate of 1.6 mL/min with a running time of 17 min (18). The injection volume was 99 μL; the diluent was the dissolution media, phosphate buffer (0.1 M) containing 0.1% SLS, and detection was performed at a wavelength of 254 nm.

Validation of the Quantification Method

The BSP and BD quantification method utilized in the dissolution test was validated according to current guidelines, according to the parameters established for quantification method of drugs in performance tests. Specificity, linearity, precision, quantification limit, detection limit, and accuracy were evaluated (19–22). The amount of drug that adsorbed to the filters was also verified (23). The dissolution media used in the dissolution test was 0.1 M SPB with 0.1% SLS.

Specificity was determined by the software EZChrom Elite through injection of BSP (6.6 μg/ mL) and BD (16.6 μg/mL) standard solutions, the product C (reference product), and placebo diluted in the dissolution media in the HPLC system. The placebo was composed of all constituents of the reference product without drugs. Overlaps of spectral scans were performed at the beginning, middle, and end of the chromatographic signal obtained from the drugs. The spectra similarity index was determined comparing the standards and spectra from product C. The ratiogram was determined by peak purity analysis. Representative chromatograms were used to demonstrate the specificity of the method (20).

The linearity was tested using the BSP and BD stock solution prepared by dilution in the dissolution media. The linearity of the method was evaluated through dilution of the stock solution into dissolution media at six different concentrations levels, equivalent to 10%, 25%, 50%, 75%, 100%, and 120% of the drug's working concentration (BSP 6.66 μg/mL and BD 16.66 μg/mL). The working concentration is equivalent to an ampule solubilized in 300 mL of dissolution media present in the vessels. The solutions were injected in triplicate over three consecutive days. The mean peak area *versus* concentration data was statistically analyzed with the least-squares linear regression and ANOVA using GraphPad Prism software, with a significance level of $\alpha<0.05$.

The determination of accuracy was accomplished by adding known amounts of the BSF and BD reference substances to the placebo solution of the reference product. Aliquots of this solution were added to vessels containing 300 mL of dissolution media at 37°C to obtain the following concentrations of: 0.6, 3.3, 6.6, and 8 μg/mL of BSP and 1.6, 8, 16, and 20 μg/mL of BD. After stirring for 90 min at 50 rpm, aliquots were collected, filtered, and analyzed by HPLC. Each concentration was prepared in triplicate, and the percentage of recovery was calculated by the ratio between the experimentally

determined concentration and the theoretical concentration. The repeatability (intraday) and the intermediate precision (interday) on consecutive days was established based on the relative standard deviation (RSD) of the obtained results.

The quantification limits (QL) and detection limits (DL) were estimated based on the standard deviation of the intercept and the slope of the calibration curve of the drugs using the equations QL=SD×10/*S* and DL=SD×3/*S*, where SD is the standard deviation of the intercept with the *y*-axis of three calibration curves and *S* is the average of the slopes of the respective curves (19,20).

To evaluate the possibility of drug absorption in the filters used in the dissolution test, two standard solutions, BD (1.6 and 16.6 mg/mL) and BSP (0.6 and 6.6 mg/mL), were prepared by dilution in the dissolution media. The filtration procedure was exactly the same as that employed for the dissolution test: The samples were passed through a polyethylene cylindrical filter connected to each cannula with a porosity of 10 μm (Hanson Research), followed by a second filtration step using a membrane filter with 0.1 μm pore size. The solutions prepared without the filtration procedure and after filtration were analyzed by the HPLC system to evaluate the possibility of waste due to drug adsorption to the filters. The percent recovery was calculated using the equation, *R*%= (drug content in the filtered solution/drug content in the unfiltered solution)×100 (22).

RESULTS AND DISCUSSION

Particle Size Measurements in Commercial Suspensions

The size of the particles in suspensions influences the physical properties of the formulation and has important implications for drug dissolution (12,14,23), thus it becomes important to control the size distribution of suspended particles in pharmaceutical products (15). The results of particle size distribution analysis of the products tested are shown in Fig. 1 and Table II, which present average diameter values for the respective populations (10%, 50%, and 90% of particles), the average diameter (AD), mode, and extension of the size distribution particles (Span). Among the products analyzed and the different batches of the same product, average particle sizes were observed ranging from 7.43 to 40.25 μm, with varying the Span values. In suspensions, polydispersity of particle size is a very important characteristic that indicates its quality. A high Span value associated with a high D90 indicates the presence of a wide distribution range of particle sizes. Also, the presence of large particles promotes slow

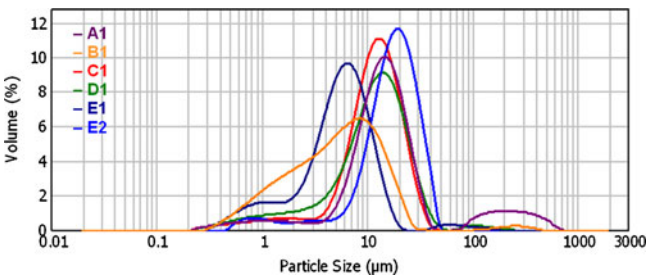

Fig. 1. Betamethasone dipropionate particle size distribution for the injectable suspensions studied in the dissolution test: products *A1, B1, C1, D1, E1*, and *E2*

dissolution of the drug (24). Given the influence of drug particle size in the dissolution process, it is of great importance to control the batch-to-batch variability of raw materials that are used in the production of a suspension. To avoid the lack of homogeneity between batches, which can impact on bioavailability, the drug granulometry must be monitored in routine quality control analyses.

The distribution of particle size values in the injectable suspensions analyzed allowed identification of products with similar and different granulometric characteristics. The suspensions A1, C1, E1, and E2 were used as a tool for the development of a discriminating *in vitro* dissolution test. Thereafter, the products A to E (batch 1) were subjected to the dissolution test using the developed method.

Study of BD Solubility in Different Dissolution Media

The dissolution media must have composition and characteristics similar to the bodily fluid in which the drug dissolves *in vivo* (9,10,25). The choice of media tested was based upon the indication of intramuscular and intra-articular administration of the injectable suspensions (Table I) providing an increased biorelevance capacity, *i.e.,* prediction of the *in vivo* performance of a drug product (25). The pH of the SPB, SBF, and SSF media was adjusted to 7.4 in order to recapitulate the pH of arterial blood and the pH of the interstitial fluid, which is about 7.35 (26). The pH of the SMF media was 7.0, therefore similar to the intracellular pH of a muscle cell. The conditions of the proposed intramuscular media were based upon properties of the media which promote relaxation of muscle fibers (27,28).

Since BD is a weak acid with low water solubility, media were evaluated using surfactants in order to obtain the sink condition or the volume necessary to dissolve the drug without the need for large volumes of media (30). The administration routes indicated for this injectable suspension have reduced biological fluid circulation, and the sink condition has a different role than when formulations are absorbed in the gastrointestinal tract. In these cases, the injected particles form a depot exhibiting an extended dissolution process due to the low concentration gradient (non-sink condition). The volume of dissolution media used was 300 mL to recapitulate this condition. According to Food and Drug Administration recommendations, buffered solutions containing up to 5% (*w/v*) surfactant may be used as the dissolution media for *in vitro* dissolution studies (29). As seen in Table III, BD showed low solubility in media prepared without surfactant, and, as expected, the increase of the saturation concentration in the media was proportional to the concentration of surfactant in all media studied. Media that presented a saturation concentration for BD greater than 16.6 μg/mL were considered acceptable in this study, since 5 mg of BD contained in an ampule can be dissolved in 300 mL of media. The use of P20 did not significantly increase the solubility of BD, thus the SLS and P80 surfactants were selected for developing the dissolution method. To avoid compromising the discriminatory power of the test, concentrations exceeding 1% surfactant were not used.

Development of the Dissolution Test

In the pharmaceutical industry, drug dissolution testing is routinely used to provide critical *in vitro* drug release

Table II. Particle Size Distribution of the Injectable Suspensions

Product	Batch no.	Diameter (μm)			AD (μm)	Mode (μm)	RSD (%)	Span
		D10	D50	D90				
A	1	5.46	14.54	93.88	40.25	14.86	15.81	6.04
B	1	1.12	5.19	16.07	10.93	7.80	8.61	2.87
	2	1.04	4.70	26.25	14.91	6.57	8.13	5.46
	3	1.14	5.74	13.74	7.48	7.62	0.41	2.19
C	1	4.37	11.88	22.51	12.92	12.94	5.20	1.53
	2	5.10	12.17	22.21	13.04	12.94	2.99	1.40
	3	6.51	16.71	32.84	23.76	17.46	16.53	1.59
	4	4.79	16.12	35.70	26.18	18.66	17.58	1.90
D	1	2.35	11.64	25.01	13.82	14.25	12.87	1.95
	2	2.16	12.66	27.92	14.51	16.25	12.18	2.04
	3	1.80	10.30	23.08	13.50	12.45	16.62	2.06
E	1	1.25	5.20	11.55	7.43	5.91	8.75	1.98
	2	6.27	17.05	31.45	18.06	18.78	11.16	1.48

AD average diameter; *RSD* relative standard deviation, $n=3$

information for forms of solid oral dosages. In recent years, the dissolution test has been extended as a quality control method to assess oral suspensions and injectable dispersed systems (12,17). Commercial products denoted as A1, C1, E1, and E2 were selected for development of a dissolution test for BD injectable suspensions. The E1 and E2 products were chosen, due to their different particle size characteristics, for developing a method capable of discriminating products with significant differences in their physicochemical properties. The reference product (C1) was compared with a product with a similar mode value (A1) but with different average size particles in order to evaluate the influence of each of these on physicochemical parameters of the dissolution profile.

The influence of the surfactant and its concentration in the BD dissolution profile were initially verified in the SPB media (without addition of surfactants) and with the addition of SLS and P80. The choice of surfactants and concentrations were chosen based on the results of the solubility test.

As shown in Fig. 2, products C1 and E2 were tested using SPB with SLS concentrations of 0.05%, 0.1%, and 0.5% (*w/v*) and P80 at concentrations of 0.5% and 1% (*w/v*). The dissolution media 0.5% SPB with SLS and 1% P80 provided the sink condition according to the solubility of 145 and 47 μg/mL (Table III), respectively. However, the dissolution profiles of C1 (Fig. 2a) and E2 products (Fig. 2b) show that the high surfactant concentration promotes rapid dissolution of BD, thus compromising the discriminative power of the method. In Fig. 2a, it can be observed that product C1 in 0.05% SPB SLS media rapidly achieved its saturation concentration, causing paralysis of the drug dissolution process. According to data listed in Table III, the sink condition was not achieved using SPB media with 0.1% SLS and 0.5% P80; however, the complete dissolution of BD was obtained for product C1 (Fig. 2a), as well as for E2, where approximately 80% of the dose was released within 30 min. Both conditions, either 0.1% SLS or 0.5% P80 were found to be suitable during dissolution testing. Selection of these media that did not achieve the sink condition can be justified if demonstrated to be more selective (26). However, the surfactant SLS at a concentration of 0.1% was selected, since this condition presented satisfactory discriminative power and is widely used as a surfactant in dissolution media. The dissolution profile shown in Fig. 2c indicates that BSP is solubilized in the formulations tested.

In Fig. 3, the dissolution profiles obtained are presented using the following media, SBF, SMF, SSF, and SPB along with 0.1% SLS in the dissolution test of the C1 and E2 products, using a stirring speed of 50 rpm. The dissolution profiles of the C1 and E2 products that were

Table III. BD Solubility in Different Dissolution Media

Dissolution medium	Betamethasone dipropionate solubilized (μg/mL)								
	Surfactants % (p/v)								
	–	SLS 0.1%	SLS 0.5%	P80 0.5%	P80 1%	P80 1.5%	P20 0.5%	P20 1%	P20 1.5%
SPB	0.84	30.04	145.49	22.48	47.00	58.52	16.15	27.73	31.72
SBF	0.38	26.67	159.74	20.17	42.39	55.41	18.24	33.25	38.78
SMF	1.59	22.69	111.90	26.32	30.47	67.88	17.62	26.44	38.26
SSF	–	29.16	–	26.36	–	–	–	–	–

SLS sodium lauryl sulfate, *P80* polysorbate 80, *P20* polysorbate 20, *SPB* sodium phosphate buffer; *SBF* simulated body fluid, *SMF* simulated muscle fluid, *SSF* simulated synovial fluid

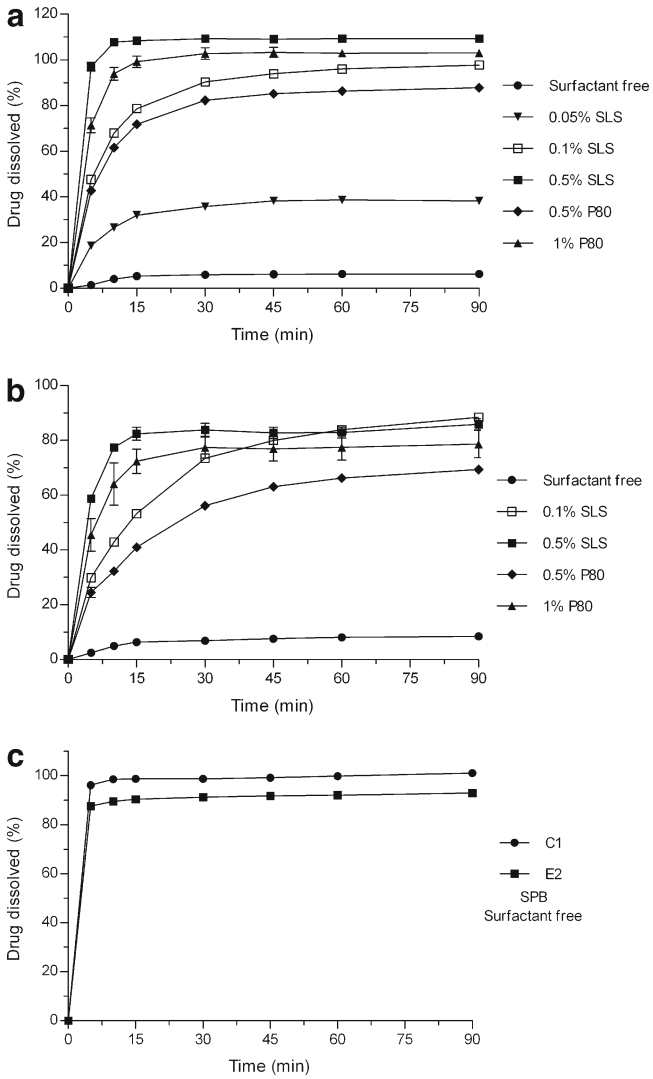

Fig. 2. Dissolution profiles of the C1 product (**a**), E2 product (**b**) in SPB dissolution media containing different concentrations of surfactant, SLS or P80, and BSP dissolved in surfactant free SPB medium (**c**), at a stirring speed of 50 rpm

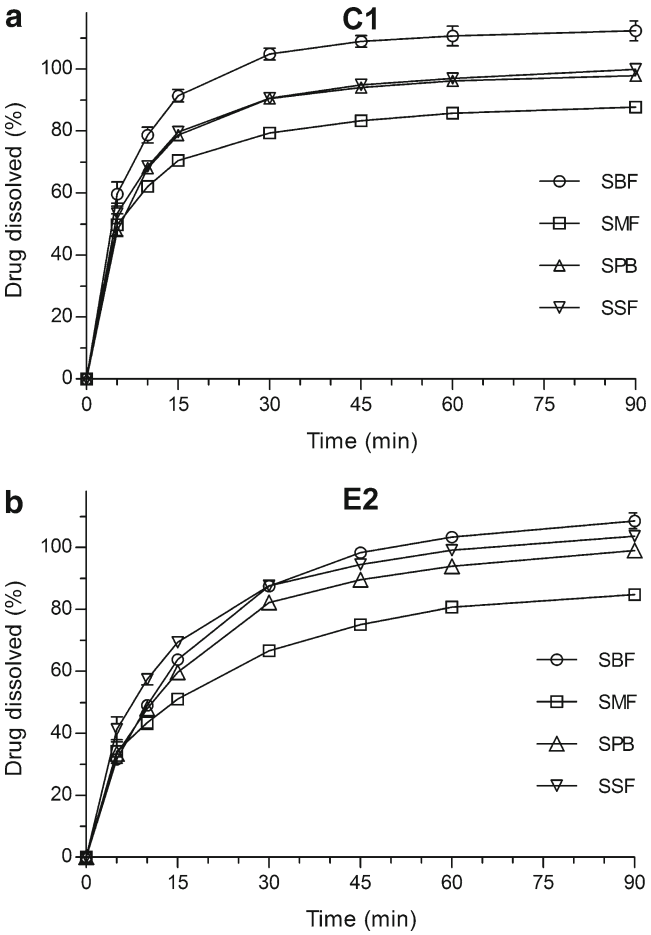

Fig. 3. Dissolution profile of the products C1 (**a**) and E2 (**b**) in different dissolution media containing 0.1%SLS, at a stirring speed of 50 rpm

carried out using SBF, SMF, or SPB media were not similar (Fig. 3) according to $f_1>15$ and $f_2<50$ (Table IV). It was not possible to discriminate between products that were tested using SSF media (Table IV). The products C1 and E2 presented different dissolution profiles in the 0.1% SPB SLS media, according to the f_1 and f_2 values. The 300 mL of 0.1% SPB SLS media was twice the saturation volume. Due to its discriminating power and ease of its preparation, SPB media at pH 7.4 containing 0.1% SLS was defined as the optimal dissolution media for the BD dissolution test.

In order to verify whether decreasing the rotation speed from 50 to 25 rpm, for the dissolution media SPB containing 0.1% SLS, could enhance the discriminatory power of the method, two batches of product E, which had different average particle sizes, were evaluated at the reduced rotation speed (Fig. 4a, Table II). According to the f_1 and f_2 results (Table IV), the method is discriminative for the two batches at the two rotational speeds tested. The product E1 showed an accelerated dissolution of BD compared with E2 at both rotational speeds. This result can be attributed to the smaller average particle size in product E1 (7.43 μm) compared with (6.18 μm) in product E2. The f_1 and f_2 values demonstrate that the same batch of product E, subjected to different rotational speeds yielded similar dissolution profiles. This indicates that both conditions are able to discriminate differences between batches of a product in regards to differing particle size distribution (Table IV).

The two rotational speeds were also employed while evaluating products A1 and C1 (Fig. 4b), with particle sizes of 14.9 and 12.9 μm, respectively. The f_1 and f_2 values showed no difference between the dissolution profiles of the products tested at the selected rotational speeds (Table IV). The P values calculated using ANOVA showed statistical differences between the dissolution profile of products A1 and C1 at a speed of 25 rpm. When the differences between the dissolution profiles of each product at the two rotational speeds tested were evaluated by f_1 and f_2, a difference was observed only for product A1 (Table IV); however, the P value shows a difference between both products. This difference could be explained by the higher average particle size and Span in product A1 (Table II). The dissolution profiles of product C1

Table IV. Statistical Analysis of the BD Dissolution Profiles of Injectable Suspensions

Products (stirring speed)	Dissolution profile analysis		
	f_1 (%)	f_2 (%)	Significance[a] P<0.05
Statistical analysis of the Fig. 3			
SBF	44.22	29.16	–
SMF	22.64	42.24	–
SPB	28.06	39.34	–
SSF	10.60	52.58	–
Statistical analysis of the Fig. 4			
E1 × E2 (25 rpm)	30.33	26.28	P<0.05*
E1 × E2 (50 rpm)	52.47	22.60	P<0.05**
E1 (25 × 50 rpm)	10.63	51.80	P>0.05
E2 (25 × 50 rpm)	9.31	59.24	P>0.05
A1 × C1 (25 rpm)	10.83	56.63	P<0.05*
A1 × C1 (50 rpm)	4.47	68.61	P>0.05
A1 (25 rpm) × A1 (50 rpm)	15.24	48.50	P<0.05**
C1 (25 rpm) × C1 (50 rpm)	10.05	55.90	P<0.05**
Statistical analysis of the Fig. 5			
A1 × B1	18.04	43.24	P>0.05
A1 × C1	4.47	68.61	P>0.05
A1 × D1	4.09	72.37	P>0.05
A1 × E1	33.56	29.18	P<0.05**
B1 × C1	11.71	51.36	P>0.05
B1 × D1	18.57	43.01	P<0.05*
B1 × E1	11.62	44.92	P>0.05
C1 × D1	7.04	61.78	P>0.05
C1 × E1	26.40	32.98	P<0.05*
D1 × E1	31.02	30.62	P<0.05**

f_1=difference factor (0–15%); f_2=similarity factor (50 – 100%)
[a] One-way ANOVA–Tukey's test (α=0.05)
*P<0.01; **P<0.001

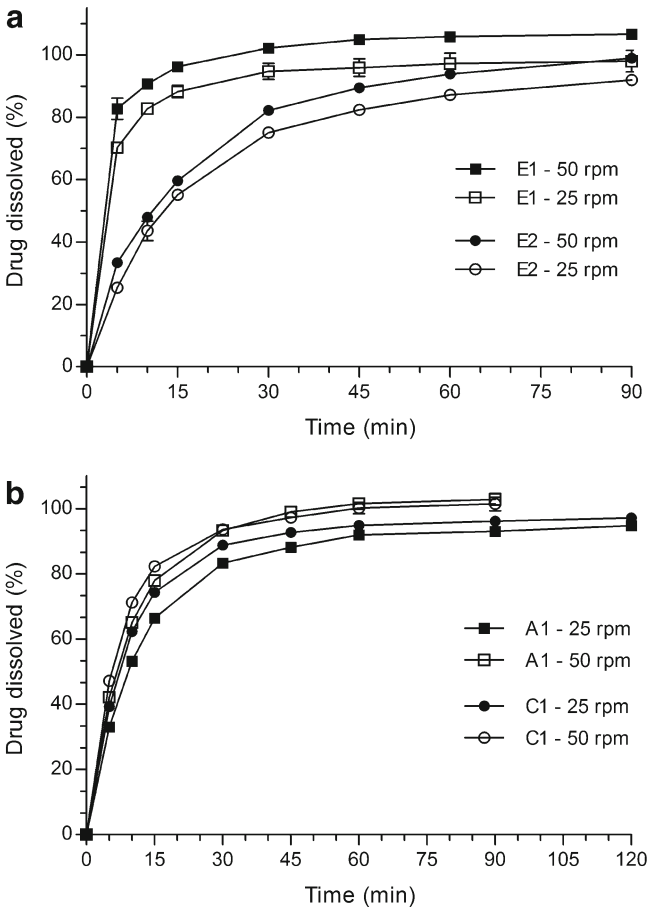

Fig. 4. BD dissolution profiles of product E1 *versus* E2 (**a**) and product A1 *versus* C1 (**b**) in SPB media containing 0.1% SLS, at stirring speeds of 25 and 50 rpm

obtained at 25 and 50 rpm are significantly different (P< 0.001); however, according to the f_1 and f_2, no difference was found between the profiles. Products A1 and C1 have similar D10 value (5.46 and 4.37 μm, respectively), but differences were observed in D90, which represents the larger particles (93.88 and 22.51 μm, respectively). Although the mean diameters are quite different (40.25 and 12.92 μm, respectively), the mode values (14.86 and 12.94 μm, respectively) are more representative of the particle size distribution, since it is in accordance with the similarity found between the dissolution profiles of products A1 and C1.

The 50 rpm rotational speed provided higher hydrodynamic forces to the dissolution media compared with when tested at 25 rpm, with a decrease in the dust bowl at the bottom of the vessel and increased amount of drug dissolved. Thus, the use of the dissolution media SPB with 0.1% SLS at 50 rpm appeared to have suitable discriminatory power to evaluate suspensions with differing characteristics. This condition was used to evaluate five different commercial formulations (A, B, C, D, and E, Batch 1). The *in vitro* dissolution results obtained while testing the different products are shown in Fig. 5, and the statistical analysis is presented in Table IV.

As shown in Fig. 5, products A1, C1, and D1, despite their different mean diameters, showed similar dissolution profiles (f_2>>50; P>0.05). Product A1 has a bimodal distribution of particle sizes with an average size of 40.25 μm and a mode of 14.86 μm (Table II). In contrast, products C1 and D1 contained particles with a mean diameter similar to the mode (~13–14 μm). The similarity between product A1 and products C1 and D1 can be attributed to the mode value and the high polydispersity index of

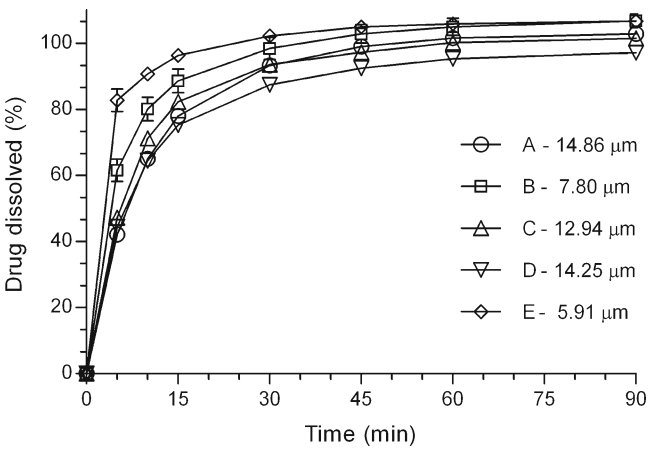

Fig. 5. Dissolution profiles obtained using the products *A, B, C, D*, and *E*, identified as batch no. 1, in SPB media containing SLS 0.1%, at 50 rpm stirring speed. The mode values are presented in the *inset*

product A1 (Span=6.04), which means that the D10 and D50 values are similar among the three products. Product E1 presented a BD dissolution rate significantly higher than the other products that were analyzed (f_2<< 50, P<0.05), which can be explained since they possess particles of the lowest average diameter whereas product B1 had intermediate particle size distribution values and a dissolution profile that is similar to C1, however statistically different from products A1, D1, and E1.

An increase in the superficial area of the particle in contact with the dissolution media initially promotes a rapid rate of BD dissolution from the suspension. This phenomenon was described by the Noyes and Whitney equation that gives a direct relationship between particle size and surface area and provides the rate of particle dissolution (30). Based on the dissolution profile of the reference product (C1) compared with products with similar (A1 and D1) and different (B1 and E1) size particle distributions, it can be suggested that the percentage of drug released (Q) between 60% and 80% in 15 min and Q>80% in 30 min as an acceptable criteria.

Accelerated *in vitro* release test methods with good discriminatory power are critical for quality control of extended-release products. In this case, the *in vitro* dissolution test for injectable suspensions happens at a faster rate than the release of *in vivo* drugs (31). Injection of dispersed systems generates a deposit at the application site, thus promoting the slow release of the drug into the bloodstream (31,32). Therefore, differences observed in the first 15 min of dissolution between the formulations may be greatly enhanced in the plasma profile (15,31). The medication reference materials describe that this association action occurs rapidly at the onset, whereby reaching its maximal effect at 1–2 h, with a duration of action lasting for 3.25 days. These data corroborate the t_{max} and $t_{1/2}$ values, 15±9 h and 80.8±22.7 h, respectively, measured with the major metabolites of BD after intramuscular injection (2). During the development and production of injectable suspensions, it is fundamental to maintain rigorous control over particle size, the degree of crystallinity, and the occurrence of drug polymorphism (15,33).

Validation of the Dissolution Method

The chromatographic method developed for the simultaneous measurement of BSP and BD in injectable suspension was validated for the quantification of these drugs in the dissolution tests (19).

No chromatographic peaks were observed in the dissolution media and placebo in the BSP (6.92 min) and the BD retention times (12.85 min), as shown in Fig. 6, which was confirmed by the three-dimensional chromatograms obtained by photodiode array detector analysis. UV spectra of the drugs chromatographic peaks obtained at three different times were overlaid, BSP (6.82, 6.92, 7.02 min) and BD (12.79, 12.85, 12.91 min), and in both cases, the only observed changes were intensity readings of 0.9999 to 1, a similarity index and peak purity of >0.999 for both drugs. The specificity of the method was demonstrated for testing of drugs in the presence of SPB with 0.1% SLS as the dissolution media as well as placebo.

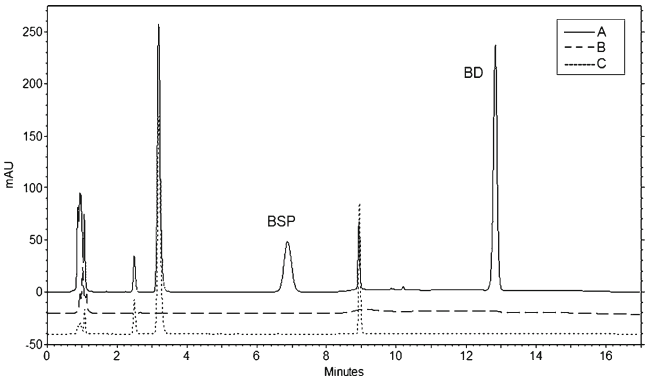

Fig. 6. Representative chromatograms of the reference drug (*C1*), Dipropan® (**a**), the dissolution media: SPB with 0.1% SLS (**b**); and the reference drug placebo (**c**). The samples were diluted with dissolution media. *BSP* betamethasone sodium phosphate; *BD* betamethasone dipropionate

The statistical analysis presented in Table V reveals a strongly linear correlation, with correlation coefficients above 0.999 for both drugs tested. The validity of the regression curve is represented by the F_{calc} value which was higher than the F_{tab} value which indicates that the slope of the curve is significantly different from zero; therefore, it is assumed that the slope is not zero and the linear curve fit is acceptable for both drugs since P< 0.0001. The detection and quantification limit values were calculated from the calibration curves to be 0.14 and 0.48 μg/mL for BSP and 0.29 and 0.98 μg/mL for BD, respectively. The method is sensitive and suitable for the detection and quantification of drugs from initial sampling points.

Accuracy, repeatability, and intermediate precision were determined at four different levels, and the results are presented in Table VI. The percentage of recovery and RSD

Table V. Statistical Analysis of the Linearity of the Quantification Method

				Statistic
Drug	Range	Parameters	Results	α<0.05
BSP	0.6 – 8 μg/mL	Slope	428367±7836	F calc=2.91 F tab=3.20
		Intercept	−11174±20564	F calc=1.71 F tab=3.19
		R^2	0.9997±1.80E-04	
		Regression	F calc=10199 F tab=4.49	P<0.0001
BD	1.6 - 20 μg/mL	Slope	375200±3736	F calc=2.60 F tab=3.20
		Intercept	52980±41179	F calc=0.39 F tab=3.19
		R^2	0.9994±3.51E-04	
		Regression	F calc=32343 F tab=4.49	P<0.0001

Table VI. Summary of Results of Repeatability, Intermediate Precision and Accuracy of the Quantification Method

Level (%)	Conc. (μg/mL)	Day	Mass (mg/Ampule)±SD	Intraday RSD (%)	Interday RSD (%)	Initial conc. (μg/mL)	Recovered conc. (μg/mL)	Recovery (%)±SD
Betamethasone sodium phosphate								
10	0.6	1	2.09±0.04	2.15	3.06	0.73	0.73	99.5±1.01
		2	2.01±0.05	2.39				
50	3.3	1	2.13±0.01	0.53	1.07	3.60	3.65	101.3±0.85
		2	2.09±0.02	0.74				
100	6.6	1	2.12±0.01	0.66	1.32	6.69	6.67	99.7±2.21
		2	2.17±0.02	0.73				
120	8	1	2.13±0.01	0.47	1.54	8.07	8.04	99.6±2.14
		2	2.17±0.04	1.76				
Betamethasone dipropionate								
10	1.6	1	6.45±0.14	2.30	3.34	1.68	1.69	100.3±1.36
		2	6.74±0.17	2.51				
50	8	1	6.49±0.03	0.46	0.86	9.12	9.19	100.8±0.66
		2	6.53±0.07	1.03				
100	16	1	6.46±0.03	0.50	1.44	16.94	17.01	100.5±1.35
		2	6.62±0.04	0.65				
120	20	1	6.47±0.02	0.35	1.37	20.84	20.67	99.2±1.54
		2	6.57±0.10	1.53				

Conc. concentration, *SD* standard deviation, *RSD* relative standard deviation

values are below the recommended limit, within the range of 100±5% (19,20), demonstrating the accuracy and precision of the method.

Adsorption of drugs to the membrane filter utilized for *in vitro* dissolution testing must be evaluated to ensure that no more than 5% of the drug adsorbed to the polymeric material of the filtering systems (22). The percentage of BSP lost by adsorption to the filter was 0.23± 0.09% when tested in a solution containing 8 μg/mL of BSD and 2.07±0.82% for a solution 0.6 μg/mL. The loss of BD was 0.41±0.18% for a solution at 16 μg/mL and 2.53±1.80% for a solution at 1.6 μg/mL. These results demonstrate that the filters used in the studies do not result in appreciable loss of drug during the dissolution tests *in vitro*.

CONCLUSIONS

The validation of the *in vitro* dissolution tests showed that this method is linear, precise, selective, and accurate for the quantification of samples containing low concentrations of BSP and BD collected during the *in vitro* dissolution testing.

The differences in particle size found between the commercial injectable suspensions were detectable by examining the obtained dissolution profiles. The *in vitro* dissolution studies indicated that formulations containing a higher percentage of smaller drug particles promote an increase in the dissolution rate. It was verified that not only does the average particle size affect the dissolution rate, but is also influenced by mode and the polydispersity index (Span) of the particles. The *in vitro* dissolution method using 0.1 M SPB pH 7.4 containing 0.1% SLS at a stirring speed of 50 rpm, presented discriminatory power capable of characterizing BD dissolution profiles from injectable suspensions. This dissolution condition can be used as a quality control method to evaluate these injectable suspensions.

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