

Dissolution Stability of Cephalexin Extemporaneous Suspensions During an Accelerated Stability Study

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ABSTRACT

The aim of the present study was to evaluate the influence of accelerated aging conditions on drug content and in vitro dissolution stability of three cephalexin (CE) extemporaneous suspensions obtained from pharmacies and a local state laboratory. The specific gravity, pH, and visual appearance of CE oral extemporaneous suspension were also studied. Dissolution profiles were performed according to FDA recommendations, since there is no official dissolution method codified. Analyses were performed throughout the administration period of the reconstituted suspension (7 days at room temperature storage or 14 days under refrigeration), at time zero, and during twelve months of storage of the powders under ICH accelerated aging conditions. Dissolution Efficiency (DE) values and assay results were analyzed via analysis of variance (ANOVA) to evaluate the effect of aging conditions on the stability of each formulation. From a clinical point of view, the storage conditions applied did not affect dissolution or chemical stability of CE oral extemporaneous suspensions.

INTRODUCTION

Cephalexin (CE) oral suspensions are useful and effective pharmaceutical formulations for patients hypersensitive to penicillin and for patients who have difficulty swallowing solid dosage forms. Cephalosporins are active pharmaceutical ingredients used worldwide in oral formulations because of their proven track record of broad-spectrum antibacterial activity and excellent safety over more than thirty years of clinical use (1, 2). CE is a first-generation cephalosporin antibiotic used to treat infections of the urinary tract, respiratory tract, skin, and soft tissue (3). CE is almost completely absorbed from the gastrointestinal tract and produces a peak plasma concentration one hour after a 500-mg oral dose. Absorption may be delayed if taken with food. CE is widely distributed in the body, and its half-life is about one hour. It is not metabolized, and about 80% or more of a dose is excreted unchanged in the urine (4).

Physical stability of extemporaneous suspensions is a parameter not often considered but of crucial evaluation due to the consequences it can have on drug bioavailability. Stability studies carried out on extemporaneous products only monitor changes in chemical stability, visual appearance, pH, caking, and redispersability, with no focus on in vitro dissolution stability (5–7).

Suspensions can be considered analogous to the disintegrated forms of tablets and capsules. If in vitro dissolution evaluation is a priority for the latter, it is logical to extend the requirement to suspensions as a limiting step to absorption and bioavailability (6–8). However, fewer than 10% of codified oral suspensions have a dissolution test described (9, 10), and to the best of our knowledge,

among the few studies carried out on suspension dissolution, almost none of them consider changes in dissolution during the administration period of the reconstituted form (11–18). If a product is stored under the conditions specified on the label, it is expected to maintain its initial dissolution behavior throughout its life, which is known as Dissolution Stability (19). This concept is a critical biopharmaceutical parameter because of the potential impact it may have on the bioavailability of the product. Therefore, it is essential that the dissolution characteristics of a pharmaceutical formulation, whether tablet, capsule, or even suspension (5), remain unchanged throughout its shelf life.

In a previous work (20), the appropriate general conditions for performing dissolution profiles of extemporaneous suspensions were established. Our research attempted to evaluate if ICH accelerated storage conditions (21) also significantly affect the dissolution stability of another cephalosporin antibiotic, such as CE. The aim of the present work was to evaluate and compare the influence of these aging conditions on the drug content and in vitro dissolution stability of three different CE suspensions in the Argentinean market during the administration period and during twelve months of powder storage.

MATERIALS AND METHODS

Reagents and Samples

Analytical grade potassium dihydrogen phosphate and HPLC grade methanol, acetonitrile, and water (J. T. Baker, USA) were used for chromatographic determinations. Distilled water was used as dissolution medium. CE monohydrate reference standard was purchased from INAME (ANMAT, Argentina). Two CE extemporaneous oral suspensions, manufactured by different pharmaceutical compa-

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nies, were purchased from pharmacies in Argentina. A third formulation was kindly provided by a public production laboratory. They all contained 250 mg CE per 5 mL.

At each sampling time, extemporaneous CE suspensions were reconstituted with a given volume of tap water according to instructions on the label and mixed by a standardized procedure. All tests were performed within product expiration dates, which were similar for all formulations.

Analyses Schedule

The analyses were performed during the administration period of the reconstituted formulation at time of reconstitution (0), after 7 days of storage at room temperature (7RT), and after 14 days of storage under refrigeration (14R); throughout the powder storage period at time zero and after 3, 6, 9, and 12 months (0M, 3M, 6M, 9M, 12M) under ICH accelerated aging conditions for Argentinean climatic zone, 40 °C/75% RH (21). These conditions were obtained using a stability chamber (SCT Pharma, model ICH 830 L, Argentina).

Visual Inspection

During the stability study, all formulations were visually inspected for signs of caking, color and odor changes, and redispersion problems.

pH

The pH of each reconstituted suspension was measured with an Altronix TPX-I pH meter at every stage of stability evaluation (10).

Assay

During the administration period throughout the storage of the powders, the effect of aging conditions on the chemical stability of CE was examined using HPLC (10). Reversed-phase HPLC was performed on a system consisting of a quaternary gradient pump (Spectra System P4000), a vacuum membrane degasser (Spectra System SCM1000), a Rheodyne injector (model 9125) with a 20- μ L loop, a UV-vis detector (Spectra System UV2000) set at 254 nm, and a chromatography workstation (ChromQuest).

The mobile phase consisted of a mixture of methanol, acetonitrile, a solution of potassium dihydrogen phosphate at 13.6 g/L, and water (2:5:10:83; v/v/v/v). Fresh mobile phase was prepared daily, filtered through a 47-mm nylon membrane (0.45- μ m pore size, μ clar, Argentina), and vacuum degassed before use. Separation was performed at room temperature on a Hypersil C18 reversed-phase column (Waters), 10- μ m particle size, and 250 \times 4.6 mm i.d. The column was equilibrated for at least 45 min with mobile phase flowing through the chromatographic system before starting the assay. All analyses were performed under isocratic conditions at a flow rate of 1.5 mL/min.

Standard solutions were prepared on a weight basis using HPLC grade water as diluent, sonicated for 10 min

at room temperature, and suitably diluted. An appropriate volume was filtered through a 25-mm nylon membrane disposable filter (0.45- μ m pore size, μ clar, Argentina). They were injected in triplicate (RSD < 2.0%), and the results averaged. Sample solutions were also prepared on a weight basis using 5 mL of suspension from each bottle after mixing with a standardized manual procedure, and then treated as the standard solutions. Samples mimicked the usual product dose. In both cases, the theoretical concentration of CE injected was approximately 20 μ g/mL, and all solutions were used on the day prepared.

The assay results were compared using ANOVA to evaluate the effect of aging conditions on the chemical stability of CE in oral suspensions.

Dissolution Stability Study

Dissolution studies were performed according to FDA recommendations (22) because there is no codified method for dissolution of CE extemporaneous suspension (9, 10).

A suspension sample equivalent to a typical dose (5 mL) was taken on a weight basis using a suitable syringe-cannula system and quantitatively transferred to the dissolution vessel midway between the surface of the dissolution medium and the top of the rotating blade. Before sampling, the reconstituted suspension was mixed by manual agitation (20 upendings in 90 sec). To calculate the exact weight of suspension added to the vessel, syringe and cannula were weighed at three stages: empty, filled with the suspension, and after the sample was expelled into the dissolution vessel. The specific gravity of each formulation was determined to express the percentage of drug dissolved in the sampled volume (250 mg/5 mL).

Dissolution testing was carried out on a suitably calibrated USP Apparatus 2 (Erweka DT60) at 25 \pm 1 rpm, under sink conditions, in 900 mL of deaerated distilled water at 37 \pm 0.5 °C. Samples (10 mL) were withdrawn at 2, 5, 10, 20, and 30 min, with replacement of the same volume of fresh medium after each withdrawal, and filtered through a 25-mm nylon membrane disposable filter (0.45- μ m pore size, μ clar, Argentina). Samples were suitably diluted with HPLC grade water and analyzed using assay methodology. The concentration of each sample was calculated from a CE standard calibration curve. Results were averaged, and cumulative drug release percentages were calculated for dissolution profile estimation.

Dissolution profiles were compared using statistical evaluation of dissolution efficiency (DE). DE was calculated as the area under the dissolution curve at 30 min (measured using the trapezoidal rule) and expressed as percentage of the area of the rectangle described by 100% dissolution in the same time (23). DE values were compared by ANOVA to evaluate the effect of aging conditions on dissolution stability within each formulation.

Table 1. Reconstituted Suspension HPLC Assay, pH, and Specific Gravity throughout the Accelerated Stability Study

Storage ^a	Time ^b	Sample B			Sample C		
		% assay ^c ± SD	pH	Specific Gravity	% assay ^c ± SD	pH	Specific Gravity
0M	ORT	109.1 ± 2.4	6.3	1.103	105.5 ± 0.3	5.8	1.214
	7RT	96.5 ± 2.4	5.9	1.049	99.5 ± 1.5*	5.5	1.246
	OR	100.0 ± 0.7	6.3	1.076	104.0 ± 0.2	5.8	1.210
	14R	93.7 ± 2.7	6.1	1.028	97.2 ± 0.4*	5.6	1.211
3M	ORT	114.5 ± 3.8	6.3	1.085	97.0 ± 0.4	5.7	1.119
	7RT	104.6 ± 3.9	5.9	1.094	100.5 ± 0.6*	5.4	1.198
	OR	110.3 ± 0.1	6.2	1.087	106.3 ± 1.4	5.7	1.227
	14R	104.1 ± 4.8	6.1	1.084	105.5 ± 1.0	5.6	1.234
6M	ORT	113.9 ± 0.6	5.2	1.109	107.6 ± 1.0	5.3	1.238
	7RT	106.2 ± 1.5*	5.8	1.090	110.0 ± 3.0	5.5	1.223
	OR	115.7 ± 5.6	5.6	1.107	110.9 ± 0.6	5.3	1.255
	14R	108.3 ± 1.4	6.0	1.087	110.1 ± 0.2	5.4	1.233
9M	ORT	110.3 ± 4.7	6.3	1.068	110.0 ± 1.5	5.5	1.223
	7RT	101.9 ± 0.4	6.1	1.086	100.3 ± 0.8*	5.3	1.220
	OR	110.0 ± 5.6	6.1	1.080	106.2 ± 2.2	5.4	1.211
	14R	102.1 ± 0.9	6.1	1.060	106.7 ± 1.2	5.5	1.229
12M	ORT	100.1 ± 1.9	6.2	1.084	96.8 ± 2.2	5.3	1.232
	7RT	100.3 ± 0.9	6.1	1.078	91.9 ± 1.1	5.3	1.209
	OR	97.1 ± 0.5	6.1	1.080	106.0 ± 3.3	5.4	1.237
	14R	112.7 ± 0.8*	5.9	1.079	108.5 ± 3.1	5.3	1.223

^a Months of storage of powder for oral suspension under accelerated aging conditions

0M: Beginning of storage time

3M: After three months of storage

6M: After six months of storage

9M: After nine months of storage

12M: After twelve months of storage

^b Stages of analysis of reconstituted forms

ORT: Immediately after reconstitution (formulation to be stored at room temperature)

OR: Immediately after reconstitution (formulation to be stored under refrigeration)

7RT: Expiration date at room temperature storage

14R: Expiration date under refrigeration

^c Percent of label claim

* Significant differences (*p*-values between 0.01 and 0.05)

RESULTS AND DISCUSSION

The recorded assay results for formulation A at time zero were within the acceptance range, and a rapid dissolution with high values of *DE* was seen. The specific gravity values were between 1.137 and 1.159, and the lowest pH values were recorded for this formulation (range 4.34–4.50). After three months of storage under accelerated aging conditions, the study of this formulation was discontinued because of major changes in appearance (Figure 1). Microbiological tests were performed for this pharmaceutical form, without positive results. Chemical interaction tests are now being carried out using thermal analysis.

HPLC assay results and values for pH and specific gravity throughout the accelerated aging study of formulations

B and C are recorded in Table 1. All pH values for formulation C remained in the range of 3.0–6.0, satisfying pharmacopeial specifications (10). However, pH values for formulation B were slightly higher than the upper specification limit in almost all cases. No important changes in pH and specific gravity values were recorded throughout the stability study. During aging of constituted suspensions, color changes and an unpleasant odor were observed, but there were no visual signs of caking, and all formulations were easily redispersed even after twelve months of storage of the powders.

CE chemical stability was evaluated by HPLC analysis. The decrease in the CE peak area and the appearance of new peaks were monitored in each run for all



Figure 1. Appearance of sample A after three months of storage under accelerated aging conditions.

tested tablets, but only the CE peak area was quantified. Chemical stability of CE in the stressed formulations was examined against a CE reference standard, which was run simultaneously each time samples were evaluated. The CE concentration measured in the assay of each formulation remained within 90% of the labeled amount throughout the stability study (Table 1). ANOVA was used to establish differences among the assay average results during the storage period. Statistically significant differences were only recorded in few cases (two for formulation B and four for formulation C). All of these results indicate that the analyzed powders, as well as the reconstituted forms, are chemically stable during the administration period at room temperature and under refrigeration, according to pharmacopoeial specifications (10).

Both formulations showed a high dissolution rate with large percentages of drug release at early time points of the dissolution profile. In all cases, more than 70% was dissolved in 2 min for both formulations, and dissolution levels greater than 100% were achieved in most cases. Dissolution profiles were compared in terms of *DE*. Almost all *DE* values were more than 100%, which indicates an excellent dissolution performance. Variability associated with *DE* in terms of standard deviation was less than 5% in almost all cases, with three values in the range 5–10% and only one result higher than 10% (OR, 12M, formulation B). ANOVA was used to compare *DE* values (dissolution stability). Statistical comparison of *DE* values showed differences during the administration period of the suspension stored under room temperature or refrigeration in only in a few cases (Figure 2).

CONCLUSIONS

CE concentrations in formulations B and C remained within 90% of labeled amount throughout the stability

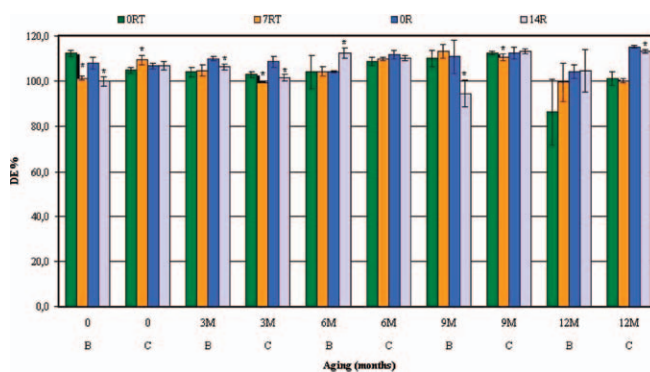


Figure 2. Dissolution efficiency (*DE*) evolution throughout the administration period of the reconstituted forms and powder aging (under accelerated aging conditions).

* Significant changes

ORT: Immediately after reconstitution (formulation designated for storage at room temperature)

OR: Immediately after reconstitution (formulation designated for storage under refrigeration)

7RT: Expiration date at room temperature storage

14R: Expiration date under refrigeration

0: Beginning of storage time

3M: After three months of storage

6M: After six months of storage

9M: After nine months of storage

12M: After twelve months of storage

study. No significant changes in pH, specific gravity, or redispersion of the formulations were recorded. However, important changes in odor and color were found. Both formulations showed a high dissolution rate with high dissolved percentages at early time points of the dissolution profile. Although some statistical differences were found among *DE* values throughout administration period, they do not have important clinical significance.

Throughout the product aging study, the reconstituted forms B and C were chemically stable and had acceptable dissolution stability during the administration period. Formulation A was not stable after three months of storage under accelerated aging conditions, and it was removed from the study. Possible interactions between drug and excipients are being analyzed.

Accelerated aging conditions do not affect the dissolution stability of all cephalosporin antibiotic suspensions in the same way. The instability shown for cefadroxil extemporaneous oral suspensions (20) was not seen in CE formulations, which were chemically and physically stable after a year of storage under these conditions.

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