BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902023e23359

Article

Stability of sulfadiazine sugar-free oral suspensions for the treatment of congenital toxoplasmosis

Micheline Silva Dias[1], Amanda Maccangnan Zamberlan[2], Rebeca Lino Lourenço[2], Emanuele Saul Saraiva[2], Julya Sarmento Neis[1], Luana Mota Ferreira[1], Andréa Inês Horn Adams[1,2*]

[1]Pharmaceutical Sciences Graduate Program, Federal University of Santa Maria, Santa Maria, RS, Brazil, [2]Industrial Pharmacy Department, Federal University of Santa Maria, Santa Maria, RS, Brazil

This study aimed to develop and evaluate the stability of sulfadiazine sugar-free extemporaneous oral suspensions, focusing on treating congenital toxoplasmosis. The excipients were carefully chosen to obtain safe products for the pediatric population. Sulfadiazine suspensions (100 mg/mL) were prepared from the raw material or tablets, stored in amber glass bottles at 5±3ºC, and evaluated at 0, 14, and 30 days. An ultra-performance liquid chromatographic method was developed and validated to assay the drug. The particle size ranged from 29.3 to 50.6 µm, with some variation over the study; pH values around 7.0 and non-Newtonian behavior were observed without modification in the period. Formulations showed a fast dissolution rate (>80% in 15 minutes) without variation over the study. The drug assay was about 100% of the label claimed throughout the study, demonstrating the chemical stability and the preparations' dose homogeneity. The microbiological investigation indicated that both preparations met the requirements for the microbial count and absence of pathogens. In conclusion, the developed formulations can be used for 30 days when stored under refrigeration. The oral suspensions produced are easy to prepare and contain safe components, providing an alternative for congenital toxoplasmosis treatment in children.

Keywords: Pediatric formulation. Sulfadiazine. Suspension. Congenital toxoplasmosis. Extemporaneous suspensions

INTRODUCTION

Toxoplasmosis is a parasitic infection caused by the *Toxoplasma gondii* protozoan, which affects more than 40 million humans worldwide. Human infection occurs mainly through ingesting food or water contaminated (Khan, Khan, 2018; Kota, Shabbir, 2022). A worrisome infection is the congenital toxoplasmosis arising from vertical transmission during pregnancy from infected mothers to fetuses (Strang *et al.*, 2020). Impairments in the nervous system and eye disease development are the main risks of congenital toxoplasmosis in the fetus. Vertical transmission is important in the morbidity and mortality of fetuses, neonates, and children (Kota, Shabbir, 2022).

South America presents the highest burden of congenital toxoplasmosis and, even more worrying, the most pathogenic genotypes. A recent systematic review and meta-analysis of congenital toxoplasmosis in Brazil indicated 469 children with congenital toxoplasmosis in the period of January 2007 to December 2018; however, the data about the most significant worldwide toxoplasmosis outbreak (Santa Maria, Brazil) were omitted because any scientific paper on the follow-up of children was found (Strang *et al.*, 2020).

Congenital toxoplasmosis treatment for infants combines sulfadiazine (SDZ) and pyrimethamine with folinic acid supplementation (Strang *et al*., 2020). The therapeutic regimen can also comprise pyrimethamine, sulfadoxine, and folinic acid or pyrimethamine,

***Correspondence:** Andréa Inês Horn Adams. Departamento de Farmácia Industrial. Centro de Ciências da Saúde. Universidade Federal de Santa Maria. Av. Roraima, 1000, prédio 26, sala 1106. Santa Maria, Rio Grande do Sul, Brasil. CEP 97105-900. Phone: +55 55 3220 8661. E-mail: andrea.ih.adams@gmail.com. ORCID: http://orcid.org/0000-0002-8413-3710

azithromycin, and corticosteroids as an option for ocular toxoplasmosis management (Khan, Khan, 2018). To reduce disease severity, the treatment of congenitally infected children can begin in utero and continue through the first year of life (Strang *et al*., 2020). However, all these drugs are commercially available only as tablets, which limits their use in children under six years of age due to difficulties in swallowing and the requirements for dose adjustment based on the body surface (Belayneh, Tessema, 2021; Khan *et al*., 2022).

The scarcity of specific products for pediatric patients and the difficulties associated with the solid dosage forms administration contribute to the off-label use of drugs (Reis *et al*., 2021). Thereby, a non-labeled drug is defined as the use of medication at a different dose, indication, frequency regimen, administration route, or the use by an unapproved age group, requiring dose adjustments and extemporaneous formulation based on the available drug products (Allen *et al*., 2018; Reis *et al*., 2021; Silva *et al*., 2020).

In this context, extemporaneous preparations are one of the main strategies used to administer oral drugs in children and neonates. The manufacture of these formulations is based on crushing tablets or opening capsules for solubilizing or suspending the content in water, food, or other beverages (Belayneh, Tessema, 2021; Silva *et al*., 2020; Storpirtis *et al*., 2008). Notably, the preparation of an ideal pediatric formulation attempts to dose accuracy, suitable pharmaceutical form regarding age, and excipient compatibility, including their concentration (Belayneh, Tadese, Molla, 2020; Lam *et al*., 2014; Nakama *et al*., 2019; Silva *et al*., 2020).

The primary concerns about preparing extemporaneous formulations for pediatric use include formulation errors, microbial contamination, component miscalculations, and patient acceptance (Nakama *et al*., 2019; Silva *et al*., 2020). Hence, it is imperative to evaluate the physical, chemical, and microbiological features of the formulations, considering the active substance, excipients, production process, and type of package used for storing the final product (Attebäck, Hedin, Mattson, 2022; Belayneh, Tessema, 2021; Haywood, Glass, 2013).

In such context, the literature reports studies that evaluated the stability of SDZ in suspension prepared by crushing tablets or drug powder and dispersed in water for injection (Pathmanathan *et al*., 2004), commercial vehicle (Ferreira *et al*., 2016) and simple syrup and sorbitol (Costa *et al*., 2020). However, these formulations present some components that can limit their use by some pediatric patients. The use of syrup or sorbitol as a vehicle should be avoided in children, especially when the treatment is long-lasting, because a large amount of sucrose can alter the buccal pH, increase the dissolution of tooth enamel, and cause caries onset (Belayneh, Tadese, Molla, 2020; Niazi, 2009; Nakama *et al*., 2019). Sorbitol is indicated for patients with diabetes and should be avoided in pediatrics because it can cause diarrhea, impairing the absorption of the pharmaceutically active ingredient (Belayneh, Tadese, Molla, 2020; Nakama *et al*., 2019). Regarding the use of commercial vehicles, such as OraPlus® (Ferreira *et al.,* 2016), even though they are frequently used to suspend drugs (Silva *et al*., 2020), they present several excipients in their composition, increasing the formulation cost, the exposure to undesirable excipients and are not always available.

Therefore, considering that **I)** congenital toxoplasmosis is a significant cause of morbidity and mortality in infants; **II)** sulfadiazine composes the therapeutic regimen of congenital toxoplasmosis in neonates and children; **III)** the drug is only commercially available in tablet form, **IV)** and the formulations reported in the literature have limited stability or use inappropriate excipients for the age group, the purpose of this study was to develop a simple and sugar-free SDZ liquid formulation prepared from crushed commercial tablets or the active pharmaceutical ingredient (API) suitable for pediatric use. As a stabilizing agent, xanthan gum, a natural product recognized for its biocompatibility, non-toxic properties, and rheology control agent for aqueous systems (Nayak, Hasnain, Aminabhavi, 2021), was used. The formulation's stability was evaluated by chemical, physical, and microbiological characteristics.

MATERIAL AND METHODS

Chemicals and reagents

SDZ standard (≥ 99.0%, CAS 68-35-9) was purchased from Sigma-Aldrich (São Paulo, Brazil). Sulfazina® tablets 500 mg (lot 703725, Sobral Laboratory, Floriano, Brazil) were acquired locally. Sodium saccharin was obtained from Vetec (Rio de Janeiro, Brazil), and methylparaben was purchased from Delaware (Porto Alegre, Brazil). Xanthan gum was donated by CPKelco (Limeira, Brazil). The strawberry essence was acquired from the local trade. Propylene glycol was obtained from Dinâmica (São Paulo, Brazil). To prepare the formulations, distilled water was used. Ultrapure water was prepared by Simplicity Water Purification System (Millipore, Billerica, USA). Other reagents used in the study included: acetonitrile (Merck, Germany), acetic acid (Vetec Química Fina, Rio de Janeiro, Brazil), sodium hydroxide (Merck, Darmstadt, Germany), hydrogen peroxide, and hydrochloric acid (Alphatec, São José dos Pinhais, Brazil). All chemicals and solvents were used as received.

Preparation of SDZ extemporaneous oral suspensions

The SDZ suspensions were prepared from the API (suspension A) or by crushing the commercial tablets (suspension B) (Table I). The concentration of the xanthan gum was first studied (0.2, 0.3, or 0.4%) to prevent the sedimentation of the particles. Then, to determine the pH most favorable to the drug stability, a preliminary study was conducted using different buffers in the range of 4.0 to 8.0 (ANVISA, 2019a), stored under refrigeration (5°C ± 3°C) and analyzed over 14 days.

TABLE I – Optimized composition of SDZ 100 mg/mL oral suspensions, prepared from API (suspension A) or from crushed tablets (suspension B)

Components	Suspension A	Suspension B
SDZ	API, 10 g	SDZ crushed tablets (n=20)
Suspending agent	0.4 g	
Preservative solution	2.0 mL	
Sweetner	0.2 g	
Buffer solution	until pH 7.0	
Distilled water	enough to 100 mL	

The SDZ tablets or API, xanthan gum, and sodium saccharin were crushed to a fine powder using a mortar and pestle to prepare and optimize formulations. Then, the preservative solution (methylparaben prepared in propylene glycol at 100 mg/mL), the strawberry essence, and part of the vehicle were added and mixed until a smooth paste was formed. The pH of the formulation was adjusted to 7.0 using a buffer solution (phosphate buffer pH 8.5) (ANVISA, 2019a), the mixture was transferred to a graduated flask, and then the remaining distilled water was added until the final volume adjustment (100 mL). The preparations were homogenized, transferred to an amber glass bottle, and stored at 5°C ± 3°C. Both formulations were prepared at an SDZ concentration of 100 mg/mL (n=3/formulation).

Analytical procedure

Instrumental and chromatographic conditions

The analytical method was developed using an Ultra Performance Liquid Chromatography equipment (UPLC)

(Japan, Shimadzu) equipped with a binary gradient pump (LC-20A), a photodiode array detector (SPD-M20A), an auto-sampler (SIL-20AC), a column oven compartment (CTO-20AC) and a communication module with the computer (CBM-20A).

The separation was achieved at a C18-column (2.1x50 mm i.d., 2 µm, GIST-HP (G), Shimadzu, Japan), coupled to a C18-guard column (2.1x10 mm i.d., 2 µm, Shimadzu, Japan). Gradient elution was performed using mobile phase A (ultrapure water acidified with glacial acetic acid, pH 4.0) and mobile phase B (acetonitrile). The mobile phase ratio was adjusted as follows: 0 to 3 minutes, 85:15 (A:B); 3 to 5 minutes, 75:25 (A:B) and 5 to 8 minutes, 85:15 (A:B). The flow rate was 0.20 mL/minute. The sample injection volume was 1 µL, the column and the autosampler were kept at 25ºC, and the SDZ detection was at 267 nm.

Standard and sample preparation

The SDZ standard stock solution (1 mg/mL) was prepared in 0.025 M NaOH. The work concentration of 10 µg/mL was obtained by further diluting the standard stock solution with a diluent composed of a mixture of ultrapure water acidified with glacial acetic acid (pH 4.0) and acetonitrile (85:15, v/v).

The suspensions' relative density at 20ºC was first determined using a pycnometer (ANVISA, 2019a). Then, an equivalent amount of 10 mg of the suspension was weighed and diluted to 1 mg/mL with 0.025 M NaOH, followed by sonication (5 minutes), and further diluted to a final concentration of 10 µg/mL with the diluent.

UPLC method validation

The UPLC method was validated according to current guidelines (ANVISA, 2017; ICH, 2022). Formulation B was used, given the most complex matrix. Method selectivity was determined by the forced degradation study exposing the formulation to different stress conditions, and the SDZ peak purity was checked to determine the non-interference of the degradation products. In addition, a placebo containing all the excipients of the SDZ tablet (starch, talc, magnesium stearate, croscarmellose sodium, and microcrystalline cellulose) and the excipients used to prepare the suspension was also analyzed.

Linearity was assessed by analyzing three independent analytical curves (1, 2.5, 5, 10, 15, 20, and 25 µg/mL). The data were fitted and evaluated for regression and linearity deviation by the analysis of variance (ANOVA, α=0.05). Limits of detection (LOD) and quantification (LOQ) were estimated based on the standard deviation of the y-axis intercepts of regression analysis (σ) and mean slope (α). They were calculated from the following equations: LOD = 3.3 (σ/α) and LOQ = 10 (σ/α) (ICH, 2022).

Precision was assessed at the level of repeatability by analyzing six independent sample solutions at 10 µg/mL (analyst A, day 1), and intermediate precision by analyzing six other samples on a different day by a second analyst (analyst B, day 2). The SDZ content (%) in each sample solution was determined, and the relative standard deviation (RSD) was calculated; RSD values $\leq$ 2.0% were considered acceptable (Shabir, 2003).

Accuracy was determined by the recovery method, adding known amounts of standard solution to the sample solution and obtaining final concentrations of 8, 10, and 12 µg/mL (80, 100, and 120% of the working concentration, respectively). The difference between the results found in the non-spiked sample solution must correspond to the amount of standard added at each level. A 98 to 102% recovery interval was considered acceptable (Shabir, 2003).

Minor modifications in the optimized chromatographic conditions (pH value, flow rate of mobile phase, and oven temperature) were performed to study the effect of variation in the analytical factors. The SDZ concentration (%) and chromatographic parameters were evaluated.

Physical stability

The SDZ suspensions A and B were stored at 5°C ± 3°C for 30 days (n=3 for each preparation). At predetermined intervals (0, 14, and 30 days), aliquots were collected and analyzed regarding physical, chemical, and microbiological parameters.

The pH was determined using a calibrated potentiometer (Denver Instrument, Brazil) directly immersed in the formulations. The laser diffraction technique evaluated particle size by dispersing an amount of the formulations in distilled water until laser obscuration reached a range of 10-15% (Mastersizer 2000, Malvern Instruments Ltd., United Kingdom). The morphology of the SDZ particles suspended in the formulation was verified by analysis under an optical microscope (Olympus, Japan) with a digital camera (10x objective lens). A Brookfield viscometer (Brookfield, USA) was used for the rheological analysis with an S63 spindle. Approximately 34 mL of each formulation were added to the collection vessel and subjected to different rotation speeds at room temperature (25°C ± 1°C).

Chemical stability

SDZ quantitation was performed following the aforementioned analytical method. According to the compendial monographs (ANVISA, 2019b; USP 39, 2016a), SDZ tablets should contain not less than 95% and not more than 105% of the labeled amount. For this reason, the criterion used for the evaluation was the SDZ assay, wherein values of drug content between these values were considered acceptable. The samples were prepared as previously described.

Dissolution test

The dissolution test was performed in manual dissolution equipment (Nova Ética, Brazil), using 900 mL of 0.1 M HCl, kept at 37°C ± 0.5°C and paddle apparatus at 50 rpm. The dissolution medium was the same recommended for SDZ tablets (ANVISA, 2019b; USP 39, 2016a). The chromatographic analysis was performed by the UPLC method, and the dissolution test was revalidated according to USP guidelines (USP 39, 2016b).

Five milliliters of the placebo suspension were transferred to vessels (n=3) containing 900 mL of dissolution medium at 37ºC ± 0.5ºC and stirred for 60 minutes at 150 rpm to evaluate the specificity. After this period, the aliquots (10 mL) were removed, filtered, successively diluted with 0.025 M NaOH and diluent solution, and analyzed. Furthermore, the stability of SDZ in the dissolution medium was also evaluated by analyzing a collected sample that was kept for 48 h at room temperature.

Linearity was evaluated by the analysis of three SDZ calibration curves with five concentration levels ranging from 3 to 15 µg/mL, which correspond to ± 20% below or above the lowest and the highest expected concentration, respectively (values obtained from the preceding analysis of the dissolution profile, *data not shown*). For this, a standard stock solution of 1 mg/mL SDZ was prepared in 0.025M NaOH, with subsequent dilution in the dissolution medium and the diluent solution. Linearity was estimated utilizing analysis for regression and linearity deviation using ANOVA.

The accuracy and precision were assessed by recovering known amounts of SDZ standard solution added to the placebo at three levels. Aliquots equivalent to 180, 500, and 585 mg were added to each vessel containing 5 mL of placebo suspension and dissolution medium. The system was preheated at 37ºC ± 0.5ºC and rotated at 50 rpm, as recommended by USP guidelines (USP 39, 2016a), for 120 min. In the end, 10 mL were withdrawn, and successive dilutions were made in 0.025 M NaOH and diluent solution, respectively, obtaining final concentrations of 4.0, 11.1, and 13.0 µg/mL (levels I, II, and III, respectively).

An aliquot of 5 mL of each suspension was placed in each vessel (n=6) and stirred for 15 minutes to perform the dissolution test. After that, 10 mL were sampled, filtered and 5 mL of the filtrate was diluted with 0.025 M NaOH in a 25 mL volumetric flask; then, one milliliter was transferred to a 10 mL volumetric flask, and the volume was made up with a diluent solution.

Microbiological stability

Before the microbiological evaluation, a validation step was performed to ensure that the antimicrobial action of SDZ and methylparaben would not interfere in the analysis. For this, the formulations were diluted in phosphate buffer pH 7.2 containing polysorbate 80 (3% v/v) (ANVISA, 2019a) and PABA (1:1 ratio of SDZ:PABA) (Costa *et al.,* 2020), both well-known inactivating agents

of the preservative and SDZ, respectively. The neutralizing action and absence of toxicity of both inactivating agents for microorganisms were evaluated by measuring their recovery using suspensions of test strains containing around 100 CFU of *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739) and *Candida albicans* (ATCC 10231). Soybean-Casein Digest Agar (TSA) was used for bacteria count (incubation at 30°C–35°C, 4 days), while Sabouraud dextrose agar medium was employed only for *Candida albicans* (incubation at 20°C–25°C, 5 days).

The recovery of microbial growth was investigated in three different test groups: inoculum, named CG (control group); neutralized sample with inoculum, named NSG (neutralized sample group) and neutralizing agents with inoculum, named NG (neutralizing group). The comparison between the microorganism growing in groups CG and NSG aimed to show the neutralization effectiveness, while the comparison between groups CG and NG should demonstrate the absence of toxicity of the neutralizing agents. A minimum microbial recovery of 50% was considered satisfactory (ANVISA, 2019a).

The microbiological evaluation of formulations was carried out by diluting the suspensions in phosphate buffer pH 7.2 containing polysorbate 80 at 3% (v/v) and PABA (ratio 1:1 of SDZ:PABA), aiming at obtaining dilutions at 10^{-1}, 10^{-2} and 10^{-3}. One milliliter of each dilution was transferred to sterile Petri dishes (n=2/dilution); then, 20 mL of sterile culture medium at 45°C were added, and after solidification, the plates were inverted and incubated. TSA was used for total aerobic bacteria count, and Sabouraud Dextrose agar for total molds and yeasts count (ANVISA, 2019a). Soybean-Casein Digest broth (I), MacConkey broth (II), and MacConkey agar were used for pathogen detection. The formulations and neutralizers were added to 100 mL of sterile broth I, which was incubated at 32.5ºC ± 2.5ºC for 24 h. Then, five milliliters were transferred from broth I to 45 mL of sterile broth II and allowed to incubate at 43ºC ± 1ºC for 48 h. Lastly, an aliquot of broth II was transferred to a Petri dish containing around 20 mL of solidified sterile MacConkey agar and incubated at 32.5ºC ± 2.5ºC for 72h.

Negative and environmental controls were simultaneously carried out to verify the experimental conditions. After incubation, the number of colony-forming units (CFU) was recorded. The requirements were total aerobic microbial count ≤ 10^2 CFU/mL, total combined yeasts/molds ≤ 10^1 CFU/mL, and Escherichia coli's absence (ANVISA, 2019a).

Statistical analysis

Formulations and analytical samples were prepared and analyzed in triplicate batches. The GraphPad Prism® version 8 software was used to perform the statistical tests. The results are expressed as mean ± standard deviation (SD). The results were analyzed using one-way or two-way ANOVA followed by Tukey's *post hoc* test. Values of p<0.05 were considered statistically significant.

RESULTS

Development of formulations

After preparation, both formulations showed a milky white appearance, with no visible precipitates or sedimentation and a characteristic odor of strawberry essence. Initially, three different concentrations of suspending agent (0.2, 0.3, and 0.4%) were tested. After visual analysis (*data not shown*), the highest concentration of xanthan gum was chosen, which is in accordance with other studies that also used xanthan gum as a stabilizing agent (Musko, Sznitowska, 2014; Provenza *et al.*, 2014).

Next, the preliminary stability study demonstrated that formulations prepared at pH 4.0 and 5.0 presented initial content below 95% (Figure 1), which can be related to the lower SDZ solubility in this range, reducing the dose homogeneity. Formulations with pH values in the range of 6.0 to 8.0 showed SDZ levels close to 100% during the study (p>0.05). The pH was adjusted to 7.0 due to the higher water solubility of the drug in this range and to avoid the methylparaben instability that could occur at pH 8.0 (Niazi, 2009).

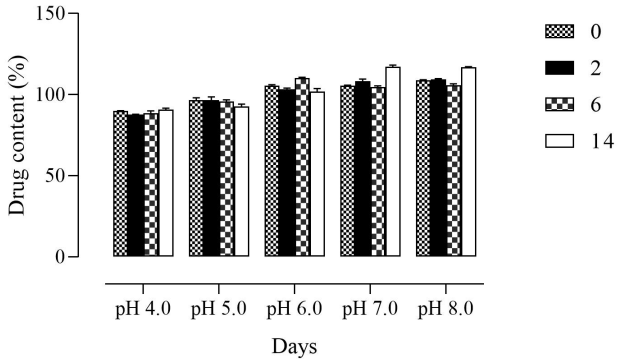

FIGURE 1 - Preliminary tests of SDZ stability in formulations prepared at different pH values. The results are expressed by mean with SD of triplicate. Data were analyzed by two-way ANOVA.

UPLC method validation

To optimize the previously developed chromatographic technique (Costa *et al.*, 2020), a UPLC method was validated using a gradient elution to provide separation of SDZ and methylparaben. With the optimization, the SDZ peak was detected at 2.6 minutes, and methylparaben was detectable at 7.3 minutes only when concentrated solutions were analyzed (*data not shown*), which is due to the difference in concentration between SDZ and methylparaben (Supplementary information; Figure S1).

SDZ concentration was not altered by basic, acidic, and thermal conditions (Supplementary information, Table SI). Despite the SDZ degradation by oxidation and UV-C radiation (8.5% and 2.6%, respectively), no extra peaks were detected in the chromatograms, and the SDZ peak purity index was >0.9999 in all conditions confirmed that no coelution occurred. Additionally, there was no excipient interference in the SDZ quantification as observed in the placebo suspension chromatogram (Supplementary information; Figure S1A). Thus, the method was considered specific for determining SDZ in the developed formulations during the stability study.

Method linearity was confirmed in the range of 10 to 250% of the usual concentration by the calibration curves, with a linear equation of y = 22482.7x - 2885.6, a correlation coefficient r=0.9996, linear regression ($F_{calculated}$=35116.57 > $F_{critical}$=4.6), without deviation of linearity ($F_{calculated}$=1.63 < $F_{critical}$=2.96). The LOD and LOQ were 0.31 µg/mL and 0.93 µg/mL respectively.

Regarding precision and accuracy evaluation, RSD values <2% were obtained in intra and inter-day analyses, suggesting the method's precision; recovery values between 98.0 and 102.0% were observed, confirming the method's accuracy (Supplementary information, Table SII). Changes in the mobile phase and column oven temperature promoted no relevant changes in the chromatographic parameters (Supplementary information, Table SIII). Although some method alterations (pH 4.5/26ºC) caused a decrease in the SDZ content, all values remained within the established limits of 95.0 – 105.0%, indicating the method's robustness.

Physical stability

The suspensions' odor and visual aspect remained unchanged, without any visible instability phenomena. Suspensions presented pH values around 7.0 during all storage periods (Table II) without significant statistical variation (p>0.05). Particle size ranged from 35.2 ± 5.26 µm at the initial time to 29.3 ± 0.61 µm after 30 days of storage for suspension A and 50.63 ± 2.65 to 38.4 ± 0.80 µm for suspension B. The particle size of formulation B was significantly larger than formulation A ($p<0.05$).

TABLE II - Results of pH, particle size, dissolution and SDZ content and of suspensions A and B over 30 days of study

Time (days)	pH	Particle size (µm)	Dissolution (%)	Drug content (%)
		Formulation A		
0	7.0 ± 0.02	35.20 ± 5.26#	91.73 ± 0.04	100.00 ± 1.20
14	7.1 ± 0.02	31.73 ± 3.96#	-	100.68 ± 4.74

TABLE II - Results of pH, particle size, dissolution and SDZ content and of suspensions A and B over 30 days of study

Time (days)	pH	Particle size (µm)	Dissolution (%)	Drug content (%)
30	7.1 ± 0.10	29.30 ± 0.61*#	92.00 ± 0.95	102.32 ± 3.21
Formulation B				
0	7.0 ± 0.02	50.63 ± 2.65#	85.01 ± 4.56	100.00 ± 0.71
14	6.9 ± 0.01	42.10 ± 1.65*#	-	99.64 ± 3.00
30	6.8 ± 0.02	38.40 ± 0.80*#	101.77 ± 7.57	101.86 ± 1.99

Notes: * denotes significant difference (p<0.05) in comparison to the day 0, for each formulation; # denotes significant difference (p<0.05) between formulation A and B, in the same day of analysis, using two-way ANOVA, followed by Tukey's *post-hoc*.

The microscopic analysis evidenced the presence of crystals in suspension A (Figures 2A and B), which could be attributed to the SDZ API (Figure 2E); however, the crystals did not hinder the physicochemical stability of formulation A.

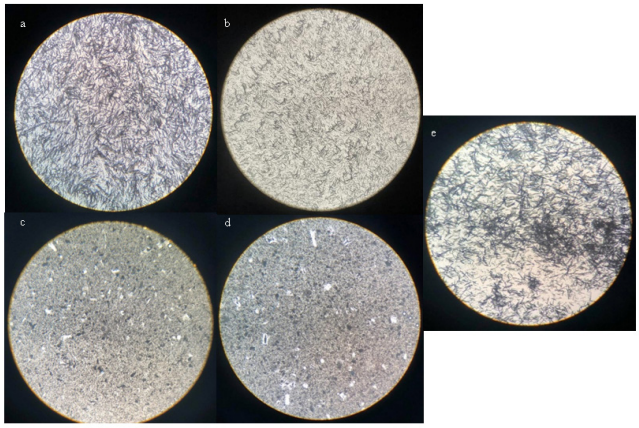

FIGURE 2 - Microscopic image in the 10x objective lens of suspension A and B. (a): Suspension A at the initial analysis time (day 0); (b): Suspension A at the final time (day 30) of analysis; (c): Suspension B at the initial time of analysis (day 0); (d): Suspension B at the final time (day 30) of analysis; (e): Powder of SDZ API.

Both formulations showed a reduction in viscosity as the shear rate was increased, indicating a non-Newtonian fluid behavior. The viscosity of the formulations did not change over the storage (Figure 3, p>0.05 - two-way ANOVA).

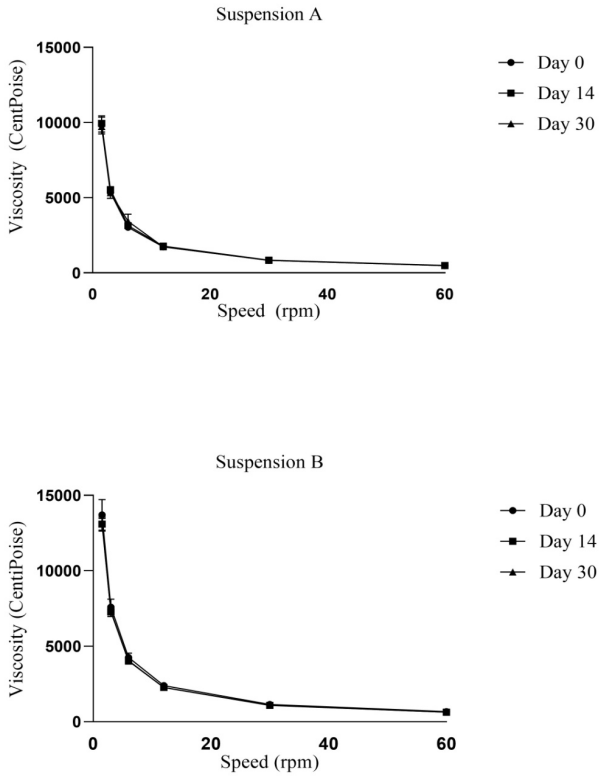

FIGURE 3 - Viscosity evaluations of SDZ suspensions A and B by Brookfield viscometer during the 30 days of stability study.

Chemical stability

Formulations A and B showed SDZ content close to 100% (Table II) over the study, meeting the established criterion of not less than 95% and not more than 105% of SDZ (ANVISA, 2019b; USP 39, 2016a). These data indicate that both formulations are chemically stable for 30 days of storage at refrigeration.

Dissolution test

The dissolution test was validated to evaluate the reliability of the method when applied to the analysis of the developed suspensions. Specificity was demonstrated by the placebo sample chromatogram which did not present any peak in the SDZ retention time. In addition, the SDZ content in the dissolution medium remained close to 100% after 48h, suggesting the drug stability in 0.1 M HCl.

Linearity was verified by ANOVA, with linear regression ($F_{calculated} = 8477.2186 > F_{critical} = 4.96$), absence of linearity deviation ($F_{calculated} = 0.2520 < F_{critical} = 3.71$), linear equation y = 22824x + 2214.2 and a correlation coefficient of r=0.999.

From the accuracy assay, recovery values of 99.23 ± 0.59%, 97.31 ± 1.48%, and 95.96 ± 0.15% for the three concentration levels were observed (Supplementary information; Table SIV); additionally, low RSD value (2.21%) was obtained in the intermediate precision analysis (Supplementary information; Table SV). These data met the USP requirements (recovery range of 95-105%) and indicated the accuracy and the precision of the method.

After analytical method validation, the dissolution test was performed for both formulations on day zero and 30 days of storage. High dissolution rates were observed in the dissolution profile study, even in the first minutes of the test (*data not shown*). Both formulations presented more than 80% of dissolution at 15 minutes, suggesting a faster dissolution than the official requirements for SDZ tablets. There was no difference in the SDZ dissolved amount at 30 days regarding the initial time, likewise between the formulations A and B (Table II, $p>0.05$).

Microbiological stability

The suitability of the microbiological method was proved through microbial recovery, which ranged from 97.50% to 161.22% for all the microorganisms tested in the NSG groups, suggesting the effectiveness of the neutralizing agents. Besides, the microbial recovery in the NG groups indicated no toxicity of neutralizing agents (Supplementary information, Table SVI). Regarding microbiological stability, the total aerobic microbial count and the total combined yeasts/molds count were <10 CFU/mL over 30 days; no contamination by *E. coli* was observed.

DISCUSSION

More than half of the newborns hospitalized in neonatal intensive care unit (ICU) received at least one prescription under different conditions from those authorized by regulatory agencies, reaching 100% when premature newborns and surgical patients are considered (Alonso *et al.*, 2019). In the pediatric ICU, 23.4% of the prescribed drugs include off-label use, and 12.6% are unlicensed drugs, highlighting the need to improve available information about the use of drugs in neonates and to monitor adverse effects (Ferreira *et al.*, 2012; Alonso *et al.*, 2019). Developing appropriate formulations for pediatric patients has been recognized worldwide as urgent, given that most drugs are available in solid dosage forms, leading to difficulty swallowing and dose adjustment (Reis *et al.*, 2021; Silva *et al.,* 2020). An alternative to circumvent such limitation is to prepare liquid extemporaneous formulations.

As mentioned, the SDZ formulations reported in the literature were prepared using some adjuvants that may be unsuitable for children. In this study, SDZ suspensions were prepared from crushed tablets or using the API as an alternative formulation devoid of other excipients. Unfortunately, just one brand of SDZ tablet was commercialized in the country, which impaired the comparison between manufacturers.

The suspensions are sugar-free and were formulated using xanthan gum, which is recognized because of its biocompatibility and non-toxic properties (Nayak, Hasnain, Aminabhavi, 2021). The usual concentration is 0.1 – 0.5%, providing non-Newtonian fluid with pseudoplastic behavior (Provenza *et al.*, 2014; Sivaneswari *et al.,* 2016). In studies that address the toxicity of excipients in children, xanthan gum is not cited among the most likely to cause damage and side effects in this population, so we believe that it is a safe substance for children in the dose used in our study (Rouaz *et al.*, 2021).

Despite being commonly used in pediatric formulations, propylene glycol toxicity is a primary concern, mainly when exposure to high doses, that affects the central nervous system. The recommended doses are neonates, 1 mg/kg; under 5 years, 50 mg/kg; and adults, 500 mg/kg. Each milliliter of our formulation provides 0.02 mL of propylene glycol or 20.8 mg/kg, an appropriate concentration for children, excluding neonates (Rouaz *et al.,* 2021; Belayneh, Tadese, Molla, 2020).

Saccharin is used as a sweetener in the formulation, which is necessary to improve the palatability of the formulation and increase treatment adherence. There are reports of adverse effects caused by saccharin in children; however, this sweetener is often preferred as it is 300–600 times stronger than sucrose, thus allowing lower doses. The recommended dose is 2.5-5 mg/kg/day, and our formulations contain 2 mg (Belayneh, Tadese, Molla, 2020), following the recommendation.

Parabens are the most used preservatives in pharmaceutical products, cosmetics, and foods. They prevent microbial growth and, consequently, drug degradation and the possible changes in the organoleptic characteristics of the formulation. The maximum recommended dose is 10 mg/kg/day (Rouaz *et al.,* 2021); our formulations provide 2 mg/kg/day; thus, they can be considered safe for children.

The preparations were produced at a concentration of 100 mg/mL (Costa *et al.*, 2020; Ferreira *et al.*, 2016) to achieve the therapeutic dosage with the smallest volume of the formulation, considering the dosage for the treatment of congenital toxoplasmosis is 100 mg/kg/day divided into two administrations. Additionally, formulations with smaller volumes are usually better tolerated by children (Khan *et al.*, 2022; Nakama *et al.,* 2019; Reis *et al.*, 2021).

Considering the SDZ pKa values, at the pH established for formulations (pH 7.0), the aromatic amine group will be predominantly non-ionized (pKa 1.8). In contrast, the sulfonamide group, whose pKa is 6.5, will be partially ionized, leading to higher water solubility. This pH value is also favorable to the preservative stability since parabens are subject to hydrolysis in aqueous solution at pH values higher than 8.0 (Niazi, 2009).

Although official monographs for SDZ tablets recommend the assay by HPLC technique, we developed and validated a UPLC method to assess the stability of the formulations due to some advantages of this technique, such as lower solvent consumption and waste generation in comparison to HPLC methodologies (Castro *et al.,* 2021), greater resolution and shorter analysis time (Costa *et al.,* 2020; Gumustas *et al.*, 2013). Conversely, the presence of the preservative in the formulations demanded some adjustments in the UPLC isocratic method previously reported (Costa *et al.*, 2020) because, under those conditions, the retention time of methylparaben was 14.00 minutes, which is probably due to its lipophilicity (log P = 1.96). The optimized conditions using gradient elution resulted in a shorter analysis (total run time of 8.00 minutes) without losing separation efficiency.

Collectively, the data obtained suggest a specific, linear, accurate, precise, and robust analytical method. Regarding the forced degradation, our results corroborate those Ferreira *et al.* (2016) found, with SDZ degradation rates of approximately 3% for all conditions evaluated (NaOH, HCl, UV, and heat). They are also similar to those obtained by Costa *et al.* (2020) for oxidative degradation (<10%).

The suspensions presented a milky white color, a characteristic odor of the strawberry essence, and no cake formation. Furthermore, the pH of the formulations remained unchanged over the study, which was expected since the formulations were buffered. Parameters such as formulation texture in the oral cavity, macroscopic appearance, and rheological properties also represent essential factors in children's acceptance of the preparation (Khan *et al.*, 2022; Lopez *et al.*, 2018). In this context, particle size can influence the texture and palatability of formulations (Khan *et al.*, 2022). In our study, formulation B presented larger particles than formulation A, possibly due to the tablet excipients. Previous studies also reported a particle diameter of around 50 µm, when the extemporaneous oral suspension was developed from crushing tablets (Mendes *et al.*, 2013; Provenza *et al.,* 2014). When analyzing the particle morphology, the presence of crystals from the API was identified in suspension A; however, this characteristic was not changed over time.

Therefore, the formulations showed no change in viscosity after 30 days of storage and presented non-Newtonian fluid behavior, attributed to the presence of xanthan gum in the formulations, which usually offers plastic or pseudoplastic flow (Nayak, Hasnain, Aminabhavi, 2021; Provenza *et al.*, 2014). It is worth mentioning that xanthan gum is a polysaccharide widely applied in the food and pharmaceutical industries because it has excellent thickening characteristics, appropriate water solubility, and physicochemical stability in a wide pH range (3 – 12) and temperature (Kulkarni, Shaw, 2016; Nayak, Hasnain, Aminabhavi, 2021; Provenza *et al.*, 2014).

The SDZ content in the formulations was about 100% throughout the study, demonstrating the chemical stability and dose homogeneity after adequate agitation. The suspensions have greater chemical stability than formulations reported in the literature. Pathmanathan *et al.* (2004) obtained SDZ suspensions with only 3 days of chemical stability, while Costa et al. (2020) demonstrated chemical stability for SDZ suspension over 14 days.

Regarding dissolution test, the assessment of *in vitro* dissolution is important considering that it allows predicting the drug bioavailability (Brevedan, Varillas, Vidal, 2012). The official requirement for SDZ tablets is at least 70% of drug dissolution over 90 minutes (ANVISA, 2019b). More than 80% of SDZ dissolved in 15 minutes, indicating that the suspensions behave like a solution and should not have any bioavailability problems (FDA, 1997). Additionally, the absorption and therapeutic efficacy of a drug in a suspension compounded from crushed tablets are unlikely to differ from those of the original dosage form used in its compounding (Zaid *et al.,* 2017). The fast dissolution could be associated with the particle size reduction because of the crushed tablets. The particle size effect in the dissolution and absorption is more evident in the case of poorly water-soluble drugs (Bonamici, 2009), such as SDZ. Besides, the drug dissolution from a suspension is favored because the disintegration step is not required.

The preservative in aqueous formulations is required to warranty the microbiological stability. The most used preservatives are benzoic acid and parabens; however, they are not indicated for pediatric formulations due to allergic reactions, such as urticaria and anaphylaxis (Khan *et al.*, 2022; Rouaz *et al.*, 2021). Conversely, parabens have a broad spectrum of action at minimal concentrations and have been used in pediatric formulations (Niazi, 2009; Souza *et al.*, 2014). Therefore, the preservative used in our study was methylparaben, which can be applied individually or in combination with propylparaben or other parabens (Tonazio *et al.*, 2011).

Regarding the microbiological stability, the results indicated that the microbial limits were met over the stability study because less than 10 CFU/mL of aerobic microbial count and total combined yeasts/mold were observed beyond the absence of *E. coli* growth. These results demonstrated microbiological stability over 30 days. In sildenafil suspensions for adult and pediatric uses, a concentrated solution (4%) of parabens (methylparaben and propylparaben) was used as a preservative, ensuring formulations with microbiological stability of 90 days (Sae Yoon *et al.*, 2015). These data indicated that using only one paraben could be an alternative for pediatric suspensions, preserving infants from exposure to two preservative substances and avoiding microbial contamination of formulations for a reasonable time. As a limitation of our study, the absence of evaluation under different storage conditions, including temperature and types of packaging, which future studies could support.

CONCLUSIONS

Two new oral suspensions of SDZ for pediatric use have been developed from raw materials and tablets. From the results, it could be concluded that both formulations were chemically, physically, and microbiologically stable for 30 days and stored in a refrigerator. The formulations are alternatives to provide the required dosing flexibility to meet the specific demands of patients. In addition, the studied formulations present desirable characteristics for use in pediatrics, kept as simple as possible, using the lowest concentration and quantity of safe adjuvants, and are easy to prepare. In conclusion, these formulations can be a promising alternative for SDZ administration in treating infants and children affected by congenital toxoplasmosis.

ACKNOWLEDGEMENTS

This study was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brasil) – Finance code 001, and FIT (UFSM). We thank CAPES for their fellowship support to Micheline Silva Dias and Luana Mota Ferreira.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Micheline Silva Dias, Amanda Maccangnan Zamberlan, Rebeca Lino Lourenço, Emanuele Saul Saraiva, Julya Sarmento Neis, and Luana Mota Ferreira. The first draft of the manuscript was written by Micheline Silva Dias, Luana Mota Ferreira, and Andréa Inês Horn Adams, and all authors commented on previous versions. All authors read and approved the final manuscript.

REFERENCES

Agência Nacional de Vigilância Sanitária. Anvisa. Farmacopeia Brasileira. v. I, 6ª ed. Brasília: Anvisa; 2019a.

Agência Nacional de Vigilância Sanitária. Anvisa. Farmacopeia Brasileira. v. II, 6ª ed. Brasília: Anvisa; 2019b.

Agência Nacional de Vigilância Sanitária. Anvisa. Resolução de Diretoria Colegiada no. 166, de 24 de julho de 2017. Dispõe sobre a validação de métodos analíticos e dá outras providências. Diário Oficial da União 25 jul 2017.

Allen HC, Garbe MC, Lees J, Aziz N, Chaaban H, Miller JL, et al. Off-Label Medication use in Children, More Common than We Think: A Systematic Review of the Literature. J Okla State Med Assoc. 2018;111(8):776-83.

Alonso AS, Avila-Alvarez A, Eiriz MC, Roca CM, Gómez PY, López AC, et al. Use of off-label drugs in neonatal intensive care. An Pediatr. 2019;91(4):237-43. https://doi.org/10.1016/j.anpede.2018.12.005.

Attebäck M, Hedin B, Mattsson S. Formulation optimization of extemporaneous oral liquids containing naloxone and propranolol for pediatric use. Sci Pharm. 2022;90(1):1-18. https://doi.org/10.3390/SCIPHARM90010015.

Belayneh A, Tadese E, Molla F. Safety and biopharmaceutical challenges of excipients in off-label pediatric formulations. Int J Gen Med. 2020;2020(13):1051-66. https://doi.org/10.2147/IJGM.S280330.

Belayneh A, Tessema Z. A Systematic Review of the Stability of Extemporaneous Pediatric Oral Formulations. Sci World J. 2021;2021:8523091. https://doi.org/10.1155/2021/8523091.

Bonamici D. Sistema de classificação biofarmacêutica e bioisenções. [Dissertação] – São Paulo: Universidade de São Paulo, Faculdade de Ciências Farmacêuticas; 2009.

Brevedan MIV, Varillas MA, Vidal NLG. Dissolution stability of cephalexin extemporaneous suspensions during an accelerated stability study. Dissolution Technol. 2012;19(3):23-7. https://doi.org/10.14227/DT190312P23.

Castro AEA, Gusmão RS, Souza DAG, Sa HPA, Pereira RMS, Santos ML. Surgimento e Evolução do Cromatógrafo Líquido de Ultra Alta Eficiência (UPLC), sua Aplicabilidade, Vantagens e Desvantagens. Uniciências. 2021;25(2):86-92. https://doi.org/10.17921/1415-5141.2021v25n2p86-92.

Costa BSR, Nascimento LP, Amorim MVP, Gomes APB, Veríssimo LA. Stability of extemporaneous sulfadiazine oral suspensions from commercially available tablets for treatment of congenital toxoplasmosis. Trop Med Int Heal. 2020;25(3):364-72. https://doi.org/10.1111/tmi.13354.

Ferreira AO, Polonini HC, Silva SL, Patrício FB, Brandão MAF, Raposo NRB. Feasibility of amlodipine besylate, chloroquine phosphate, dapsone, phenytoin, pyridoxine hydrochloride, sulfadiazine, sulfasalazine, tetracycline hydrochloride, trimethoprim and zonisamide in SyrSpend® SF PH4 oral suspensions. J Pharm Biomed Anal. 2016;118:105-12. https://doi.org/10.1016/j.jpba.2015.10.032.

Ferreira LA, Ibiapina CC, Machado MGP, Fagundes EDT. High prevalence of off-label and unlicensed drug prescribing in a Brazilian intensive care unit. Rev Assoc Med Bras. 2012;58(1):82-7. https://doi.org/10.1016/s2255-4823(12)70159-5.

Food and Drug Administration. FDA. Dissolution testing of immediate release solid oral dosage forms. 1997. Rockville, MD: FDA; 1997. [cited 2023 May 12]. Available from: https://www.fda.gov/media/70936/download.

Gumustas M, Kurbanoglu S, Uslu B, Ozkan SA. UPLC versus HPLC on drug analysis: Advantageous, applications and their validation parameters. Chromatographia. 2013;76:1365-1427. https://doi.org/10.1007/s10337-013-2477-8.

Haywood A, Glass BD. Liquid dosage forms extemporaneously prepared from commercially available

products - Considering new evidence on stability. J Pharm Pharm Sci. 2013;16(3):441-55. https://doi.org/10.18433/j38887.

International Conference of Harmonisation. ICH. Q2(R2), Validation of analytical procedures: text and methodology. Geneva: International Conference on Harmonization; 2022.

Khan D, Kirby D, Bryson S, Shah M, Rahman Mohammed A. Paediatric specific dosage forms: Patient and formulation considerations. Int J Pharm. 2022;616:121501. https://doi.org/10.1016/J.IJPHARM.2022.121501.

Khan K, Khan W. Congenital toxoplasmosis: An overview of the neurological and ocular manifestations. Parasitol Int. 2018;67(6):715–21. https://doi.org/10.1016/j.parint.2018.07.004.

Kota AS, Shabbir N. Congenital Toxoplasmosis. [cited 2023 May 12]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545228/.

Kulkarni VS, Shaw C. Use of Polymers and Thickeners in Semisolid and Liquid Formulations. Essent Chem Formul Semisolid Liq Dosages. Londres: Academic Press, 2016. 43-69 p. https://doi.org/10.1016/B978-0-12-801024-2.00005-4.

Lam JKW, Xu Y, Worsley A, Wong ICK. Oral transmucosal drug delivery for pediatric use. Adv Drug Deliv Rev. 2014;73:50-62. https://doi.org/10.1016/J.ADDR.2013.08.011.

Lopez FL, Ernest TB, Orlu M, Tuleu C. The effect of administration media on palatability and ease of swallowing of multiparticulate formulations. Int J Pharm. 2018;551(1-2):67-75. https://doi.org/10.1016/j.ijpharm.2018.08.021.

Mendes C, Costa AP, Oliveira PR, Tagliari MP, Silva MAS. Physicochemical and microbiological stability studies of extemporaneous antihypertensive pediatric suspensions for hospital use. Pharm Dev Technol. 2013;18(4):813-20. https://doi.org/10.3109/10837450.2012.693504.

Musko M, Sznitowska M. Use of compounded dispersing media for extemporaneous pediatric syrups with candesartan cilexetil and valsartan. Acta Pharm. 2014;64(4):463-74. https://doi.org/10.2478/acph-2014-0037.

Nakama KA, Santos RB, Serpa P, Maciel TR, Haas SE. Organoleptic excipients used in pediatric antibiotics. Arch Pediatr. 2019;26(7):431-6. https://doi.org/10.1016/j.arcped.2019.09.008.

Nayak AK, Hasnain MS, Aminabhavi TM. Drug delivery using interpenetrating polymeric networks of natural polymers: A recent update. J Drug Deliv Sci Technol. 2021;66:102915. https://doi.org/10.1016/j.jddst.2021.102915.

Niazi SK. Handbook of Pharmaceutical Manufacturing Formulations. Second Edition, New York: Informa Healthcare USA, Inc; 2009. https://doi.org/10.1201/b14437.

Pathmanathan U, Halgrain D, Chiadmi F, Schlatter J, Vermerie N. Stability of sulfadiazine oral liquids prepared from tablets and powder. J Pharm Pharm Sci. 2004;7(1):84-7.

Provenza N, Calpena AC, Mallandrich M, Halbaut L, Clares B. Design and physicochemical stability studies of paediatric oral formulations of sildenafil. Int J Pharm. 2014;460(1-2):234-9. https://doi.org/10.1016/j.ijpharm.2013.11.006.

Reis F, Pissarra R, Soares H, Soares P, Guimarães H. Off-label and unlicensed drug treatments in Neonatal Intensive Care Units: a systematic review. J Pediatr Neonatal Individ Med. 2021;10(2):e100213. https://doi.org/10.7363/100213.

Rouaz K, Chiclana-Rodríguez B, Nardi-Ricart A, Suñé-Pou M, Mercadé-Frutos D, Suñé-Negre JM, et al. Excipients in the paediatric population: A review. Pharmaceutics. 2021;13(3):1-44. https://doi.org/10.3390/pharmaceutics13030387.

Sae Yoon A, Sawatdee S, Woradechakul C, Sae Chee K, Atipairin A. Physicochemical and microbiological stability of the extemporaneous sildenafil citrate oral suspension. Sci Pharm. 2015;83(4):659-70. https://doi.org/10.3797/scipharm.1505-08.

Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J Chromatogr A. 2003;987(1-2):57-66. https://doi.org/10.1016/S0021-9673(02)01536-4.

Silva MRM, Dysars LP, Santos EP, Ricci Júnior E. Preparation of extemporaneous oral liquid in the hospital pharmacy. Braz J Pharm Sci. 2020;56:e18358. https://doi.org/10.1590/S2175-97902019000418358.

Sivaneswari S, Karthikeyan E, Veena D, Chandana PJ, Sumana PS, Ramya L, et al. Physiochemical characterization of taste masking levetiracetam ion exchange resinates in the solid state and formulation of stable liquid suspension for pediatric use. Beni-Suef Univ J Basic Appl Sci. 2016;5(2):126-33. https://doi.org/10.1016/j.bjbas.2016.04.004.

Souza A, Santos D, Fonseca S, Medeiros M, Batista L, Turner M, et al. Toxic excipients in medications for neonates in Brazil. Eur J Pediatr. 2014;173(7):935-45. https://doi.org/10.1007/s00431-014-2272-z.

Strang AGGF, Ferrari RG, Rosário DK, Nishi L, Evangelista FF, Santana PL, et al. The congenital toxoplasmosis burden in Brazil: Systematic review and meta-analysis. Acta Trop. 2020;211:105608. https://doi.org/10.1016/j.actatropica.2020.105608.

Storpirtis S, Mori ALPM, Yochiy A, Ribeiro E, Porta V. Farmácia clínica e atenção farmacêutica. Rio de Janeiro: Guanabara Koogan; 2008.

Tonazio L, Vilela MMP, Jesus RR, Pinto MAO, Amaral MPH. Reações Adversas dos Adjuvantes Farmacêuticos Presentes em Medicamentos para Uso Pediátrico. HU Rev. 2011;37(1):63-8.

UNITED STATES PHARMACOPEIA. USP. 39ed. Rockville: United States Pharmacopeial Convention, 2016a, p. 5941.

UNITED STATES PHARMACOPEIA. USP. 39ed. The Dissolution Procedure: Development and Validation. Rockville: United States Pharmacopeial Convention, 2016b, p. 1202-1222.

Zaid AN, Shtayah R, Qadumi A, Ghanem M, Qedan R, Daibes M, et al. Stability of extemporaneously prepared rosuvastatin oral suspension. Am J Heal Pharm. 2017;74(19):1579-83. https://doi.org/10.2146/ajhp160235.

Received for publication on 22nd May 2023
Accepted for publication on 20th July 2023

SUPPLEMENTARY INFORMATION

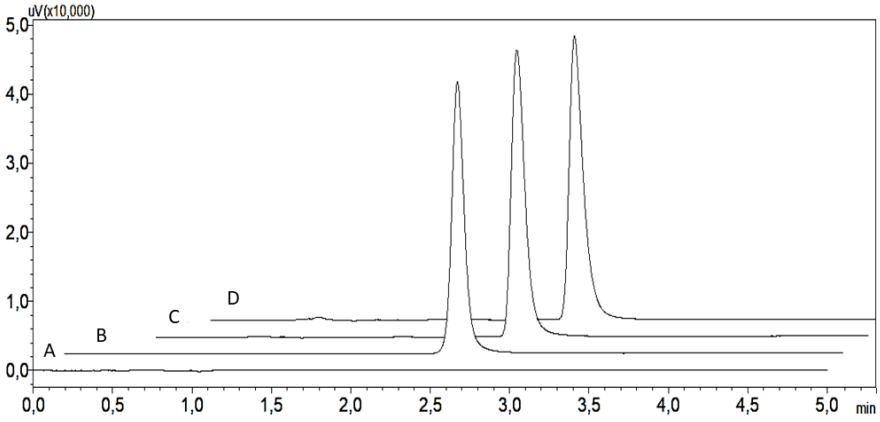

FIGURE S1- Typical chromatograms of the analytical solutions obtained using the optimized conditions: A) placebo formulation; B) SDZ standard stock solution; C) Suspension A; D) Suspension B.

TABLE SI - Residual SDZ content after exposure of suspension B to different stress conditions

Stress condition	Time of exposure (days)	Residual content (%) Mean ± SD
H_2O_2 35%	1	91.48 ± 1.25
UV-A	1	102.07 ± 0.05
UV-C	1	97.44 ± 0.99
HCl 1 M	5	100.42 ± 0.08
NaOH 1 M	5	99.64 ± 1.11
Heat 60°C	5	100.07 ± 0.04

Notes: SD – standard deviation

TABLE SII – Precision and accuracy data obtained in the validation of the UPLC method

Precision			
Drug assay (%) Day 1, analyst A	Mean ± SD[a] (RSD)	Drug assay (%) Day 2, analyst B	Mean ± SD[a] (RSD)
96.48		95.40	
95.59		95.54	
98.85	96.12 ± 1.55 (1.62)	95.44	95.20 ± 0.60 (0.63)
94.57		95.61	
94.81		94.01	
96.42		95.20	
Mean ± SD[b] (n=12)		95.66 ± 1.22	
RSD (n=12)		1.28	

Accuracy					
Level (%)	Added concentration (µg/mL)	Recovered concentration (µg/mL)	Recovery (%)	Mean ± SD[c]	RSD (%)
80	3	2.96	98.79	100.79 ± 1.82	1.88
	3	0.04	101.23		
	3	3.07	102.34		
100	5	5.13	102.63	101.86 ± 0.68	0.66
	5	5.07	101.36		
	5	5.08	101.58		
120	7	7.17	102.47	101.41 ± 1.46	1.44
	7	7.14	102.02		
	7	6.98	99.75		
Mean ± SD[d]				101.35 ± 1.30	1.28

Notes: SD – standard deviation; RSD – relative standard deviation; Mean ± SD[a]: n=6, repeatability; Mean ± SD[b]: n=12, intermediate precision; Mean ± SD[c]: n=3, accuracy in same level; Mean ± SD[d]: n=9, accuracy between levels.

TABLE SIII - Results of the robustness evaluation of the UPLC method

Variations	Retention time	Theoretical plates	Tailing factor	Capacity factor	Assay Mean ± SD
Optimized conditions[#]	2.56	5292	1.32	2.44	100.45 ± 1.32
Mobile phase flow					
0.19 mL/min	2.68	5097	1.37	2.77	98.85 ± 0.94
0.21 mL/min	2.46	4999	1.43	2.81	98.75 ± 0.04

TABLE SIII - Results of the robustness evaluation of the UPLC method

Variations	Retention time	Theoretical plates	Tailing factor	Capacity factor	Assay Mean ± SD
pH of aqueous phase					
3.5	2.64	4505	1.36	2.50	98.79 ± 1.14
4.5	2.67	4540	1.35	2.65	97.74 ± 0.42*
Oven temperature					
24 ºC	2.67	4264	1.67	2.80	99.02 ± 0.52
26 ºC	2.62	4732	1.46	1.68	97.98 ± 0.35*

Notes: SD – standard deviation; #Optimized conditions: mobile phase flow, 0.20 mL/min; aqueous phase, pH 4.0; oven temperature, 25 ºC; *Asterisks denotes the significant difference in comparison to optimized condition (p<0.05, one-way ANOVA, followed by Tukey's *post-hoc*).

TABLE SIV - Accuracy results of SDZ percentual recovery in the validation of the dissolution test

Levels	Day 1	Day 2	Mean ± RSD
I	99.65	98.81	99.23 ± 0.60
II	96.26	98.36	97.31 ± 1.48
III	96.06	95.85	95.96 ± 0.15

Notes: RSD – relative standard deviation

TABLE SV - Inter-day precision for the SDZ standard stock solutions in the validation of the dissolution test

	Concentration level		
	Level I	**Level II**	**Level III**
Day 1	101.59	98.36	97.37
Day 2	99.65	96.26	96.06
Day 3	98.81	94.17	95.85
Mean (%)	100.02	96.26	96.43
RSD (%)	1.43	2.18	0.85

Notes: RSD – relative standard deviation

TABLE SVI - Recovery of microorganisms obtained in the validation of the microbiological counting method

Microorganism (dilution)	CG (CFU/plate)	NG (CFU/plate)	NG recovery (%)	NSG (CFU/plate)	NSG recovery (%)
S. aureus (10^{-5})	40	39	97.50	44	110.00
P. aeruginosa (10^{-4})	112	116	103.57	129	115.18

TABLE SVI - Recovery of microorganisms obtained in the validation of the microbiological counting method

Microorganism (dilution)	CG (CFU/plate)	NG (CFU/plate)	NG recovery (%)	NSG (CFU/plate)	NSG recovery (%)
E. coli (10^{-5})	60	65	108.33	72	120.00
C. albicans (10^{-3})	49	79	161.22	61	124.49

Notes: CG: control group (inoculum); NG: neutralizing group (inoculum + neutralizing agents); NSG: neutralized sample group (inoculum + sample + neutralizing agents)