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Investigation of the Chemical Stability of Reconstituted Cefadroxil Oral Suspension Using Two Type of Water at Different Storage Conditions in Aden, Yemen

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ABSTRACT

Cefadroxil for pediatric use comes in the form of an oral powder, which has to be reconstituted before administration. Concerns have been raised about home storage conditions and the appropriateness of the temperature of the water used to reconstitute oral powders such as Cefadroxil into suspensions, within the recommended shelf life during the period of use, in-use stability refers to products in multi-dose containers that are at risk of losing their contents because of repetitive opening and closure. To investigate the effect of temperature on Cefadroxil oral suspension (250 mg/5ml) reconstituted in water at different temperature storage conditions. The Method was performed by using (HPLC): JASCO, model LC-Net II/ADC, C18 (250 mm × 4.6 mm i.d.) Column MS-II, with an isocratic mobile phase of Acetonitrile and Phosphate buffer pH 5.0 (4:96) with run time 15 minutes, the determinations were performed at a flow rate of 2.0 ml/min, and UV detector set at 230 nm. this study was carried out by selection of two brands of Cefadroxil dry suspension) imported and local brands), exposed to different conditions (refrigerator conditions (2-8°c) and ambient temperature (at room temperature 25 – 29°c) after reconstitution by two waters (boiled water and distilled water) then the assay test was performed using HPLC for fourteen days 0 day, 3rd, 7th, 10th and 14th day. HPLC results showed a decrease in Cefadroxil concentration in samples prepared by heated water as compared to those prepared by cooled water at 25°C. The statistical analysis result of the one way ANOVA test revealed that there was a significant difference ($P<0.05$) in the drug content of the Cefadroxil brands evaluated in the study, because Cefadroxil from the 0 day was higher than the limit in pharmacopeia (90-120%). There was a significant difference ($P<0.05$) in the drug content of the Cefadroxil stored under various in-home storage conditions refrigerator 2 – 8°c and room temperature 25 – 29°c evaluated in the study. Reconstituted oral Cefadroxil suspension is stable at temperatures between 2 and 29°C for a period of 3 days; reconstituted with distilled water also showed stability until the 7th day. This is seen in the concentrations of samples A and B. As a result of statistical comparison, the concentrations of Cefadroxil in a reconstituted oral suspension which were kept under different storage conditions were found to be statistically different at 0 to 14 days. It was concluded that after the preparation of the Cefadroxil reconstituted oral suspension, the storage conditions at different temperatures did cause degradation of the active compound.

Keywords: Cefadroxil, antibiotic, oral dry suspension, stability, HPLC.

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Introduction

Cefadroxil, which is one individual from cephalosporins, it arranged as dry powder for reconstitution by water, then it should be kept in unambiguous capacity conditions to keep up with adequacy what's more, soundness, likewise it ought to be utilized in somewhere around fourteen days to keep away from corruption of the dynamic fixing then, at that point, decrease in action to get the ideal advantage from the medication and keep away from harmfulness. Nonetheless, numerous patients don't have any significant bearing this data for various reasons, for example, there is no cooler and unpredictable power supply that might bring about different levels of debasement of the item [1].

Cefadroxil is among the most widely used antibiotics in the world, unfortunately, this drug is non-biodegradable and has been detected in discharges from wastewater treatment plants due to its low removal efficiency (25.8 %) [2]. Cefadroxil is a semi-engineered anti-infection having a place to the class of original cephalosporins. Its instrument of activity is because of hindrance of the amalgamation of the cell mass of primarily gram-positive microorganisms being broadly utilized for the treatment of contaminations like pharyngitis, tonsillitis, gonorrhea, skin, delicate tissue, ear and urinary lot [3]. Synthetically, it is assigned as 5-thia-1-azabicyclo [4.2.0] oct-2 enecarboxylic corrosive,7 - [[amino[4-hydroxyphenyl) acetyl] amino]-3-methyl-8-oxo-, monohydrate, [6R[6-alpha, 7-beta(R*)]]) (Figure 1), [4]. Various strategies are detailed for the assurance of cefadroxil including HPLC and spectrophotometry) [5].

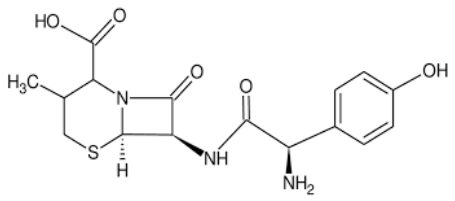

Figure 1: Chemical Structure of Cefadroxil

Cefadroxil follows up on microorganisms by restricting to explicit penicillin-restricting proteins (PBPs) present inside the bacterial cell wall, it causes hindrance of bacterial cell wall combination. Cell lysis happens via autolytic proteins of bacterial cell wall, for example, autolysins; it is viewed as that cefadroxil slows down an autolysin inhibitor. Cefadroxil monohydrate is an original cephalosporin anti-microbial showed for the treatment of urinary plot diseases, upper respiratory parcel contaminations, and skin and delicate tissue diseases in patients. The atomic recipe is $C_{16}H_{17}N_3O_5S.H_2O$ and sub-atomic weight is 381.40 g/mol. It is dissolvable in methanol. It is true medication in US Pharmacopeia 2020. The writing survey uncovers that not very many have been created for the assessment of cefadroxil alone and in mix with different medications [6].

Cephalosporins antibacterial gathering acts by restraint of bacterial cell wall blend and are regularly more impervious to deactivation by β-lactamases.The viability of cephalosporins, in normal with other b-lactam compounds, is firmly connected with the time over which the dynamic drug focus surpasses the bacterial least inhibitory fixation (t > MIC). Cefadroxil, is an original semisynthetic cephalosporin expected for oral organization. In vitro, it shows action against most types of *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella sp*., and both penicillin-vulnerable and safe *Staphylococcus aureus*. Cefadroxil makes long-acting restorative difference, high dissolvability and moderately expansive range of hostile to bacterial movement [7].

Cefadroxil (CFDL) is an original cephalosporin semi-engineered β-lactam drug. The *in vitro* concentrated on demonstrated that its action was basically the same as cephalexin. It is utilized for a few diseases set off by miniature living beings including contaminations of focal sensory system,

genitourinary, gynecology, upper respiratory lot, urinary, post-careful and skin. It is much of the time endorsed by specialists for the bacterial contaminations and promptly accessible in market in various business trademarks in the types of cases, tablets, and powder for suspension. It is quickly assimilated into the body liquids after its oral application (around in something like three hours). It is seen that 10 to 20% of CFDL regulatory measurements can be amassed by the body liquids while rest of its portion discharged through pee. The quality predominance of CFDL for the deals and organization is profoundly impressive in light of its creation which might extraordinarily affect its quality. The got information has an extraordinary significance for the synthesis of medication material and the improvement of insightful strategies .Several logical techniques like elite execution fluid chromatography (HPLC), fluid chromatography mass spectrometry (LC-MS), and electro chromatography, fine zone electrophoresis with chemiluminescence, spectrophotometry and electroanalytical procedures [8].

Cephalexin, a first-generation cephalosporin, is the first-line oral treatment for kids with outer muscle contaminations because of methicillin-defenseless *Staphylococ-cus aureus* (MSSA). Cefadroxil, a comparable first-generation cephalosporin, is an appealing option in contrast to cephalexin given its more extended half-life [9]. In Yemen because of the epileptic power circumstance, elevated degree of neediness and lack of education, coordinating patients on legitimate capacity of reconstituted anti-microbials at home for ideal advantage represents a significant test for drug specialists. The results of conveying less than ideal dosages of the anti-toxin or poisonous bye results of corruption of the medication because of unfortunate stockpiling are quite self-evident.

This, not entirely settled to find out the effect(s) of in-home capacity conditions on the soundness of Cefadroxil with the end goal of suggesting elective capacity conditions and proper drug specialists' guidelines to the patient when sufficient refrigeration is unattainable, Subsequently, the point of this review raised from the conceivable impact of temperature of water utilized for reconstitution or the stockpiling states of this medication during the suggested timeframe of realistic usability after reconstitution, 14 days, The tried boundaries were substance boundaries. Actual strategies were utilized to survey any substance changes which thusly could prompt changes in Cefadroxil suspension ways of behaving. After reconstitution under different in-home capacity conditions for 14 days, by determination of two brands of Cefadroxil dry suspension imported and neighborhood brands, presented to various circumstances fridge conditions 2-8°c and encompassing temperature at room temperature 25 - 29°c after reconstitution by two sort of water bubbled water and refined water then the measure test was performed involving HPLC for fourteen days at multi day, third, seventh, tenth and fourteenth day. HPLC strategy is utilized subjectively to assess Cefadroxil dynamic constituents in contrast with a reference standard and quantitatively to quantify any adjustment of Cefadroxil content. The current study aimed to investigate the effect of temperature on reconstituted Cefadroxil oral suspension (250 mg/5ml) at different storage conditions, and analyze the samples by HPLC technique.

Methods

Materials

The pure analytical standard of Cefadroxil (purity 99.8%, CAS Number: 16001971) was obtained from Sigma-Aldrich, Germany. A local brand of Cefadroxil monohydrate (A 250 mg/5 ml), and an imported brand of Cefadroxil monohydrate (B 250 mg/5ml), was purchased. It is available as a dry powder containing 250 mg/5 ml Cefadroxil for reconstitution in water for oral use and within the stated expiry dates at the time of this study. All reagents such as dipotassium hydrogen phosphate anhydrous (K_2HPO_4), orthophosphoric acid (H_3PO_4) and

potassium hydroxide and (KOH) were of analytical grade (Sigma-Aldrich, Germany) while Acetonitrile and methanol was of HPLC grade (Sigma-Aldrich, Germany). Ultra-pure water was obtained from Milli-Q (Millipore) water purification system.

Instruments

High-performance liquid chromatography (HPLC) system: JASCO, model LC-Net II/ADC. UV/VIS detector, model U.V-2070 plus. Quaternary gradient pump model P4-2089 plus. Colum oven model Co 2065 plus. ChromNav chromatography data software provided with the system. Ultrasonic bath (RoHS, model JP-031, China), electronic balance (RADWAG, model AS 220.B, Poland), pH meter (Adwa, model AD8000, Romania), vacuum pump (Jenway, model 8515, Engeland), and digital hygro-thermometer (Nimomed, SH-101, China. Accuracy: ±1C, ±5% RH).

Sample collection

Cefadroxil monohydrate (250 mg/5ml) samples, of the same batch number, were collected from different pharmacies in the same city (Aden City). Two different brands were used in this study; one local brand (Yemen) and one imported brand (Egypt). Two Cefadroxil brands were included in this study with the same batch number and the same manufacturing date and expiry date from each brand (A and B).

Table 1: Cefadroxil monohydrate dry suspension brands (Strength of Samples 250 mg/5ml)

Code of Sample	Batch N.O	Manuf. Date	Exp. Date	Date of Open.
A	22B004	03/2022	03/2025	16-May-2023
B	01314920	08/2022	08/2025	16-May-2023

Reconstitution of samples

Eight (8) samples; consisting of four (4) samples of a local brand and 4 samples of B imported brands oral suspensions were freshly reconstituted with two types of water boiling water and distilled water. Powder was loosen from the bottom by the tapping against a hard surface. The specified amount of type of water was added, sometimes in two or more portions with shaking until all the dry powder was suspended, these suspensions were stored for 14 days at different storage conditions such as refrigerator condition 2-8°C , room temperature 25 - 29°C, and the amount of the suspension equivalent to 450 µg/ml of Cefadroxil was examined (for 8 samples) at zero time then stored in different condition for assay test at 3rd , 7th , 10th and 14th day [1, 19].

Preparation of analytical standard solutions

A stock standard solution of 1000 µg/mL was prepared by dissolving 100mg Cefadroxil monohydrate in 100.0 mL of mobile phase was prepared from Buffer: acetonitrile in a ratio of 96:4 respectively, This solution contains the equivalent of 1000 µg /mL of cefadroxil and subsequently diluted with Buffer: acetonitrile (96:4) to get thirteen standard solutions (10, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 300 and 350 µg/ml). The pH of the solution was maintained at 5.0. The solutions was filtered through a 0.22 µm pore size filter (Millipore, Bedford, USA) prior to injection [10].

HPLC validation method

This process ensures the suitability of the analytical procedure used for specific test and its intended use. The method was validated as stated by the International Conference on Harmonization ICH guidelines Q2, R1 (ICH, 2019) with respect to characteristics like linearity, accuracy, precision and its applicability.

Linearity

The standard solutions 10 , 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 300 and 350 µg/ml of the working concentration standard were prepared and diluted to the final volume with mobile phase. Three replicate measurements of each solution by (HPLC). The peak area response obtained for each solution was plotted against its corresponding theoretical concentration and a linear regression analysis was performed on the thirteen coordinates, Linearity plot for Cefadroxil was presented in Figure 3.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy was tested by calculating the percent recovery of the mean concentration of Cefadroxil monohydrate at different concentration levels in triplicate, and the relative standard deviation (RSD) was determined [11, 18]. The mean concentration value obtained for each level was compared to the theoretical value, which was considered to be 100%.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability (expresses the precision under the same operating conditions over a short interval of time), intermediate precision (expresses the precision within-laboratories variations: different days, different analysts, different equipment, etc.) and reproducibility (expresses the precision between laboratories-collaborative studies, usually applied to standardization of methodology). Here the precision was assessed at two levels: repeatability or intra-day variability and intermediate precision or inter-day variability. The repeatability was assessed by injecting the standard solution of Cefadroxil monohydrate at three different concentrations (40, 50 and 80 µg/mL) three times in succession. Intraday variability was performed by the same analyst over one day and intermediate precision was carried out by another independent analyst over 3 days [12].

Chemical Stability Study (Assay)

Assay test was performed using HPLC method as follow.

a) Preparation of the buffer solution

The buffer was prepared by dissolving 6.86 g of potassium dihydrogen phosphate anhydrous (KH_2PO_4) in 1000 mL of distilled water and adjusted with (10 N) KOH or dilute orthophosphoric acid to a pH of 5.0 [10, 20].

b) Mobile phase

The mobile phase was prepared from phosphate buffer: acetonitrile in a ratio of 96:4 respectively were selected as mobile phase exhibiting a rapid separation with retention time 3.198 ± 0.098 min. Retention time was short because of aqueous solubility and larger portion of the polar component in Mobile phase. pH was maintained at 5.0. The solution mixture was degassed in an ultrasonic water bath for 10 minutes and filtered through 0.22 micron pore size membrane filter under vacuum condition [10].

c) HPLC condition

Cefadroxil-monohydrate was assayed using HPLC method [10]. Reverse-phase chromatography with isocratic elution at a flow rate of 2.0 mL/min was used to produce chromatographic separation. Chromatographic analysis was carried out on a 15 x 0.45 cm, 5µm particle size octadecyl-silica) C18) column. The mobile phase was made up of a (96:4) combination of Phosphate buffer and acetonitrile. A UV-Detector with a wavelength of 230 nm was used to monitor the eluent. The column was kept at room temperature, and the sample solution and mobile phase were injected using a manual injector with a 20 µL loop. Prior to use, the mobile phase was filtered with a millipore 0.22 µm membrane filter and degassed with an ultrasonic bath.

d) Preparation of sample solution

After shaking the reconstituted samples, 0.05ml of the suspension produced was transferred into a 12.5 mL volumetric flask, then dissolved and diluted with mobile phase, shaken and brought up to volume before sonication and filtration 0.22 µm membrane filter, obtaining a concentration of about 200µg/ml. The filtrate 1.5 mL was transferred into a 2 mL-HPLC vial and 20 µl was injected into the HPLC system. Each analysis was performed in three replicates.

Stability method

The dry powder (A and B 250mg/5ml), batch no. (22B004) and (01314920) respectively, ready for

reconstitution and the prepared suspension (50 and 60 mL) respectively, were individually subjected to a short-term stability study for 14 days according to ICH guidelines (ICH, 2019. Two reconstituted bottles of the brand from both brands (A and B 250mg/5ml) were used for the study. The specified amount of type of water (boiled water and distilled water) was added, sometimes in two or more portions with shaking until all the dry powder was suspended and the amount of the suspension equivalent to 200µg/ml) of Cefadroxil was examined (for 8 samples). The variable storage conditions commented on were; refrigerator condition (2 – 8°c), room temperature (25 – 29°c).

Analytical Procedure

Analytical standard curve of concentration vs. absorbance of the UV detector at 230 nm. The concentration of Cefadroxil (in µg/ml) in the reconstituted samples was determined using the regression equation **Y= 10164.60 * X + 1055.97** with an R^2 value of = 0.99979, where y is absorbance and x is the concentration.

Statistical Analysis

Data were analyzed using origin 8.5.1. Data was analyzed by SPSS software to determine the significance of differences related to storage conditions, the results of day 0 to day 14 were statistically compared by One-way analysis of variance (ANOVA). Differences were considered significant at P-values < 0.05.

Results

Simulated in-Home Study

The results from the in-home study show the respective peak area obtained under the two simulated storage conditions post-reconstitution. These peak area values were used to determine the concentrations of the two Cefadroxil remaining after 14 days as shown in Tables 5 and 6.

Method validation: Linearity

Peak area responses were plotted against the concentration of Cefadroxil. The plot revealed good linearity over a concentration range of 10 to 350 µg/mL with regression equation Y= 10164.60 * X + 1055.97 and a correlation coefficient of 0.99979 (Figure 2) with confidence interval at P = 0.05. Figure 2. The calibration curve for the analytic standard of Cefadroxil showed a good linear response between the peak area and the concentration as shown in Table 2.

Table 2: Standard calibration curve for Cefadroxil linear response over the concentration range of 10 – 350 µg/ml using peak area data at λ= 230 nm

Conc. (µg/ml)	Peak Area (µV.S)		t_R (min)	
	Mean	RSD%	Mean	RSD%
10	118665	0.126	3.225	0.000
25	252830	0.154	3.233	0.263
50	481276	0.555	3.197	0.661
75	734306	0.571	3.142	3.454
100	1011520	0.580	3.306	4.086
125	1270970	0.815	3.295	4.865
150	1529620	3.406	3.336	2.159
175	1746650	1.510	3.167	0.000
200	2008120	0.803	3.233	0.263
225	2282400	2.057	3.197	0.661
250	2525450	2.439	3.230	5.902
300	3098390	2.171	3.158	1.606
350	3556900	4.956	3.200	1.556
R^2	0.99979		3.225±0.058	

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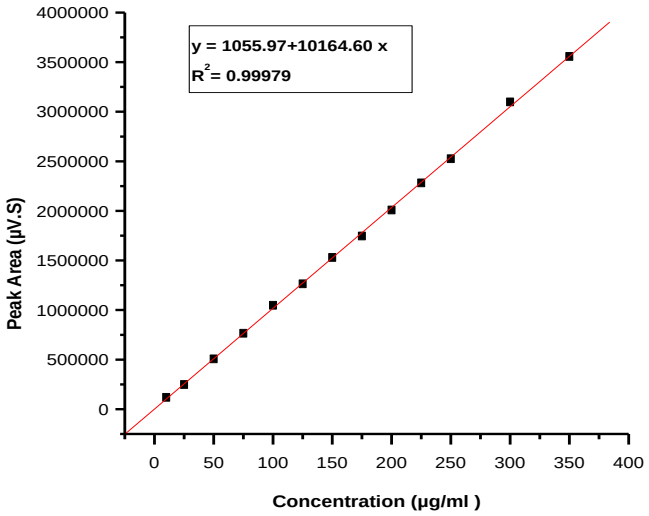

Figure 2: Standard calibration curve for Cefadroxil linear response over the concentration range of 10 – 350 µg/ml using peak area data at λ= 230 nm

Accuracy

The results for accuracy were expressed as percentage drug recovered and percentage RSD, after addition of 50 percent (15 µg), 100 percent (30 µg) and 150 percent (60 µg) of standard solution to sample solution (30 µg) (Table 3). Results depicted percent recoveries range from 96.43 to 100.80 percent and percent RSD range from 0.74 to 1.42 percent, which complies with the acceptance criteria proposed. Percent recovery should be in a range of 80 to 120 percent, according to that was stated in the International Conference on Harmonization ICH guidelines Q2, R1 [13].

Table 3: Accuracy – Recovery Studies

A%	Concentration B	Concentration C	Peak Area (µV.S)	Drug Recovered (µg/ml) ± SD	% drug Recov.	% RSD
50	30	45	461904 463301 454879	45.36± 0.44	100.80	0.97
100	30	60	593853 610318 597805	59.20± 0.84	98.67	1.42
150	30	90	887688 880798 874823	86.79± 0.64	96.43	0.74

*(N=3); **A:** % of Standard added to sample; **B:** Sample concentration (µg/ml); **C:** Total drug (Sample + Standard) (µg/ml).

Precision

Precision was assessed at two levels: repeatability or intra-day variability and intermediate precision or inter-day variability. Results for the interday and intraday precision study were expressed as %RSD (Table 4). Result revealed RSD below 1.51 percent for intra-day variability and for inter-day variability it was less than 1.49 percent which complies with the acceptance criteria proposed according to that was

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stated in the International Conference on Harmonization ICH guidelines Q2, R1 [13].

Table 4: Intra and inter-day variations of the HPLC method for determination of Cefadroxil.

Conc. (µg/ mL)	Repeatability precision (intraday)		Intermediate precision (interday)		
	Measured Conc. ± SD (µg/ ml) (N=3)	% RSD	Days	Measured Conc. ± SD (µg/ ml)	% RSD
40.0	40.53±0.61	1.51	1	41.56±0.34	0.82
			2	40.64±0.44	1.08
			3	39.82±0.60	1.31
50.0	50.34± 0.67	1.33	1	50.36±0.71	1.41
			2	50.74±0.32	0.63
			3	49.31±0.53	1.07
80.0	79.80± 0.94	1.18	1	79.48±0.89	1.12
			2	80.04±1.19	1.49
			3	79.94±1.12	1.40

Limit of detection and Limit of quantification

In the present study, limit of detection (LOD) and limit of quantification (LOQ) with acceptable precision and accuracy was calculated from standard deviation(SD) of the response Peak Area (µV.S) and the slope of linear regression (Y = 10067 * X - 1085 and R^2 = 0.99929) obtained from specific calibration curve of six different concentrations i.e. 10, 25, 50, 75, 100 and 125 µg/mL, (Table 5 and Figure 3) in the low end region of proposed range (ICH, 2019). LOD was found to be 5.489 µg/ml while LOQ was 16.634 µg/ml.

Table 5: Calibration curve of Cefadroxil for LOD and LOQ

Conc. (µg/ml)	Peak Area (µV.S)		tR (min)	
	Mean	RSD%	Mean	RSD%
10	118665	0.126	3.225	0.000
25	252830	0.154	3.233	0.263
50	481276	0.555	3.197	0.661
75	734306	0.571	3.142	3.454
100	1011520	0.580	3.306	4.086
125	1270970	0.815	3.295	4.865
R2	0.99943		3.233±0.061	

A Limit of detection LOD =3.3× (SD /Slope) and limit of quantization LOQ =10× (SD /Slope). SDYX=16922, Slope=10067

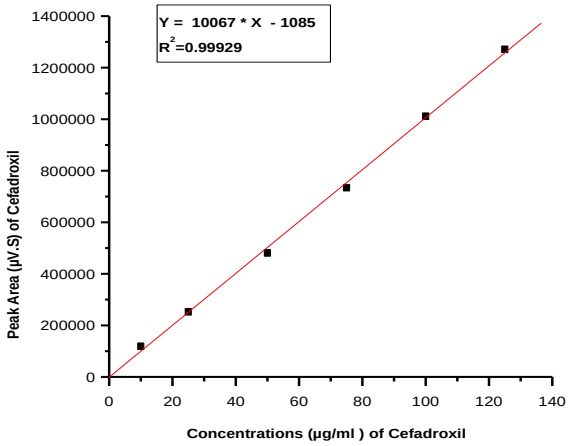

Figure 3: Calibration curve of Cefadroxil for LOD and LOQ

Chemical tests (Assay test) for Cefadroxil suspension

Table 6 shows the results of assay test for A (Cefadroxil 250 mg/5 ml) has the same batch no. (22B004) after reconstitution by two different types of water during 14 days at different conditions. Cefadroxil for oral suspension must contain not less

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than 90.0% and not more than 120% of labeled amount of Cefadroxil [10].

The peak area (µV.S), retention time (min), mean ±SD concentrations (µg/ml) and % Content of the oral suspensions reconstituted with distilled and boiled water from different in-home storage conditions (refrigerator condition (2 – 8°c) and at room temperature (25 – 29°c) for 14 days, are given in table 6, figures 4 and 5.

The results of assay test in table (6) show that all samples of A brand which were reconstituted with two water (D.W and B.W), during the test period, condition (refrigerated between 2-8°c) showed stability for all antibiotics for the first three days. The local antibiotic (Brand: A) reconstituted with distilled water also showed stability until the seventh day. The local antibiotic (Brand: A) reconstituted with distilled water also showed assay result was above the upper limit. The sample of this brand consider rejected at zero point because of the assay result was above the upper limit 127.18% at refrigerator. This is may be due to some issues in this brand itself may be during manufacturing or formulation or transportation and shipping of this brand. Since, the samples which was reconstituted with distilled water have better assay results than same samples reconstituted with boiled water. Almost this is because the high degradation in ambient temperature.

Table 6: Results of Assay Test mean ±SD Concentrations (µg/ml) and % Content for A suspension at room temperature and refrigerator conditions after reconstitution by two types of water for 14 days.Brand: A – Yemen

Brand: A - Yemen					
Day	Assay Test	Refrigerator (2 – 8°C)		Room temperature (25 – 29°C)	
		D. water	B. water	D. water	B. water
0 day	Conc.	251.86±8.16	236.32±0.23	230.86±1.58	224.41±1.40
	% Content	127.18%±4.12	119.33%±0.11	116.57%±0.80	113.31%±0.71
3rd day	Conc.	219.66±0.06	200.88±0.92	219.14±0.34	199.59±0.57
	% Content	110.91%±0.03	101.43%±0.46	110.65%±0.17	100.77%±0.28
7th day	Conc.	210.27±0.18	162.99±0.64	156.97±3.91	152.97±0.93
	% Content	106.17%±0.10	82.29%±0.32	79.24%±1.98	77.22%±0.47
10th day	Conc.	168.36±1.41	130.15±0.00	130.74±0.25	134.33±0.10
	% Content	84.99%±0.71	65.69%±0.00	65.99%±0.13	67.81%±0.05
14th day	Conc.	136.81±0.46	90.80±0.88	67.91±0.39	68.45±0.49
	% Content	69.06%±0.23	45.82%±0.45	34.25%±0.20	34.53%±0.25

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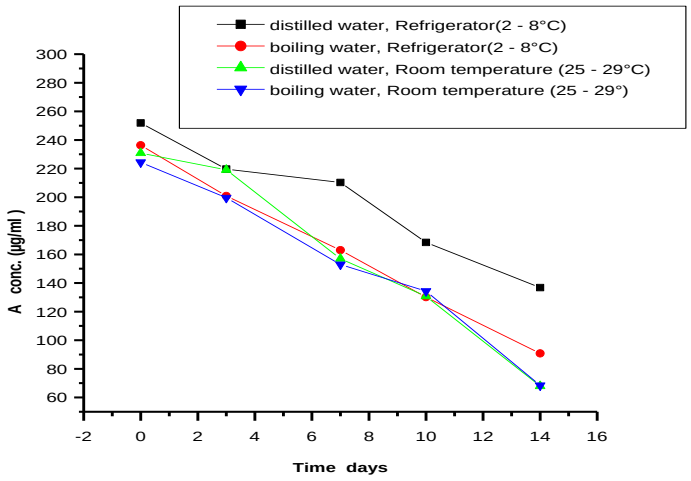

Figure 4: Degradation (concentrations (µg/ml) of the oral suspensions reconstituted with distilled and boiled water versus time period under different in –home storage conditions

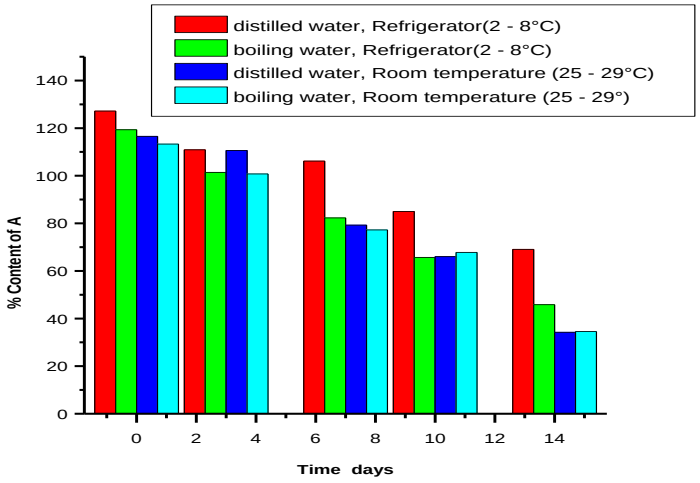

Figure 5: Degradation (% Content) of the oral suspensions reconstituted with distilled and boiled water versus time period under different in –home storage conditions

Storage under condition (kept in a cupboard at a temperature 25-29 °C) on the other hand, showed the least level of stability. Degradation was observed on the 7th day in all antibiotics. Analysis on the 10th day, and the 14th day showed extensive degradation. The results of assay test for B brand has the same batch no. (01314920) after reconstitution by two different types of water during 14 days stored at refrigerator (2-8°C) and at room temperature are illustrated in table (7).

The peak area (µV.S), retention time (min), mean ±SD concentrations (µg/ml) and % Content of B oral suspensions reconstituted with distilled and boiled water from different in-home storage

conditions (refrigerator condition (2 – 8°C) and at room temperature (25 – 29°C) for 14 days, are given in table 7, and figures 6 and 7.

The results of assay test in table (5) showed that all samples of B brand which were reconstituted with two type of waters (D.W and B.W), during the test period, condition (refrigerated between 2-8°C) showed stability for all antibiotics for the first three days. The local antibiotic (Brand: B) reconstituted with distilled water also showed stability until the 7th day. Hence, the samples which were reconstituted with distilled water, have better assay results than same samples reconstituted with boiled water. Almost this is because the high degradation at ambient temperature.

Storage under condition (kept in a cupboard at a temperature of 25-29°C) on the other hand, showed the least level of stability. Degradation was observed on the 7th day in all samples. Analysis on the 10th and 14th day showed extensive degradation. Concentrations had fallen below 90% by the 10th day due to extensive degradation that occurred. Reconstituted oral suspensions should be used within 7 days if refrigerated or within 5 days if stored at room temperature in the absence of better alternatives. Generally, the above results assured the fact that the ideal condition for Cefadroxil storage is the standard storage condition of 2-8°C.

Table 7: Results of assay test (peak area (µV.S), Rt (min), mean ±SD concentrations (µg/ml) and % content for B samples at room temperature and refrigerator conditions after reconstitution by two types of water for 14 days

Brand: B - Egypt					
Day	Assay Test	Refrigerator (2 – 8°c)		Room temperature (25 – 29°c)	
		D. water	B. water	D. water	B. water
0 day	Conc.	216.92±0.69	222.08±1.78	201.71±3.32	196.28±0.08
	% Content	109.53%±0.35	112.14%±0.90	101.85%±1.68	99.10%±0.04
3rd day	Conc.	206.48±0.01	197.54±0.18	183.15±0.45	175.89±1.90
	% Content	104.26%±0.01	99.74%±0.09	92.47%±0.23	88.80%±0.96
7th day	Conc.	186.24±0.44	165.24±0.52	173.24±1.84	146.12±1.65
	% Content	94.03%±0.23	83.42%±0.26	87.47%±0.93	73.76%±0.83
10th day	Conc.	144.73±0.67	140.60±0.83	157.56±0.59	128.18±1.40
	% Content	73.06%±0.34	70.98%±0.42	79.54%±0.30	64.70%±0.71
14th day	Conc.	132.43±0.17	129.55±2.06	114.95±0.11	82.27±0.14
	% Content	66.85%±0.09	65.39%±1.03	58.02%±0.06	41.51%±0.07

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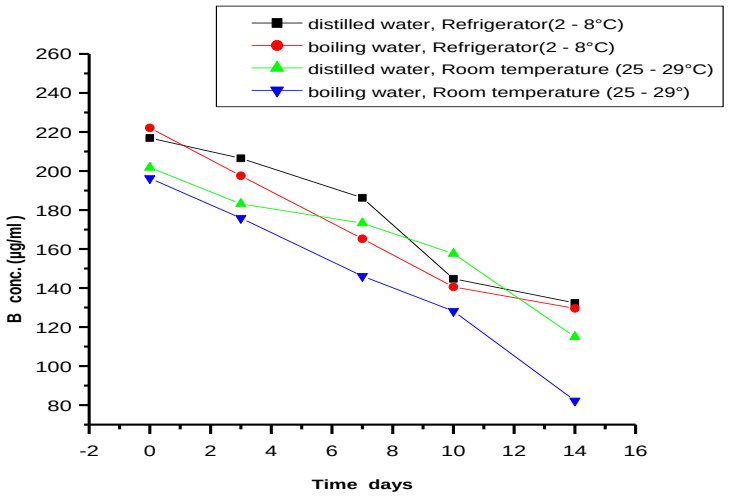

Figure 6: Degradation (concentrations (µg/ml) of B oral suspensions reconstituted with distilled and boiled water versus time period under different in –home storage conditions

The statistical analysis result revealed that there was a significant difference (P<0.05) in the drug content of the Cefadroxil brands evaluated in the study. The result showed that there was a significant difference (P<0.05) in the drug content of the Cefadroxil oral suspension which was reconstituted by boiled water and distilled water evaluated in the study. Furthermore, there was a significant difference (P<0.05) in the drug content of the Cefadroxil stored under various in-home storage conditions refrigerator 2 – 8°C compared to that kept at room temperature 25 – 29°C.

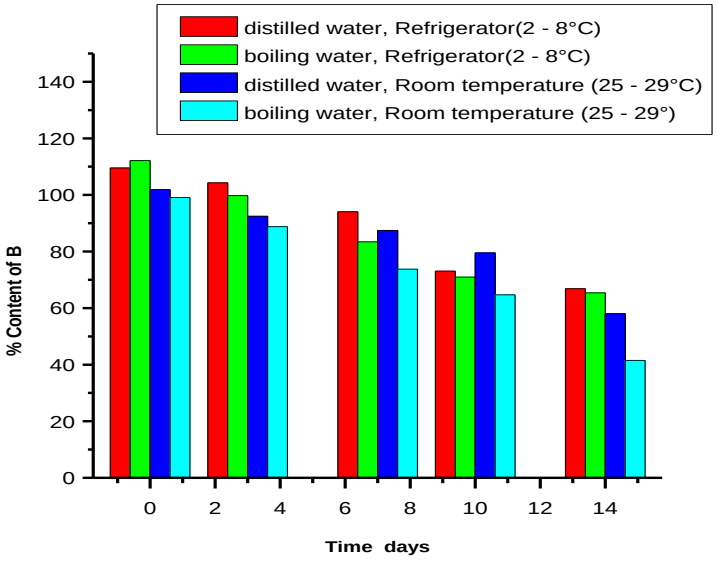

Figure 7: Degradation (% content) of B oral suspensions reconstituted with distilled and boiled water versus time period under different in –home storage conditions

Discussion

The objective of this study was to investigate the Chemical stability of Cefadroxil 250 mg/5 ml oral suspension reconstituted with distilled and boiled water versus time period under different in – home storage conditions over 14 days were investigated by the HPLC analysis method. As a result of the different storage conditions (refrigerator temperature and room temperature 25°- 29°C) applied, it was determined that there was significant difference between the chemical degradation rates in the samples and there was a change exceeding 50% in the samples according to the initial concentration.

The analysis indicated a marked decrease in the content of Cefadroxil in the samples which were prepared with hot water and compared to control (samples prepared with water at 25 °C). After 7 days of reconstitution, there was about 21% loss in the content of Cefadroxil as compared to 58% in 14th day. However, there was not any decomposition compound on HPLC chromatogram which might not be detected at the same used wavelength.

The result of this study is similar to the work done by [14], which investigated the effect of type of water for reconstitution and the temperature on stability of Cefadroxil dry oral suspension 250 mg/5 ml during the recommended in use shelf life. His study was carried out by selection of three brands of Cefadroxil dry suspension (imported and local brands), the results revealed that the distilled water was the best water used for Cefadroxil reconstitution. There is a significant effect of brand type used because brand A from the 0 day was higher than the limit in pharmacopeia (90-120%), however, B and C were within the limit at beginning then became lower than the limit after 2 weeks from reconstitution. Moreover, the storage conditions of Cefadroxil samples after reconstitution affected the Cefadroxil quality significantly; therefore, the samples were stored in refrigerator had less degradation rate than that were stored at room temperature. Cefadroxil showed good stability after reconstitution by the three types of water especially D.W. and it must be stored at refrigerator conditions to decrease the degradation of Cefadroxil during the recommended period [1].

These results are also in line with the work done by [15], carried out study on stability of powder for oral suspension containing 250mg Cefadroxil and 100mg Cefixime/5 ml. Cefadroxil in suspension is stable during 8 days in the refrigerator and 6 days at room temperature. Cefixime in suspension is stable during 8 days in refrigerator and 6 days at room temperature. The study shows that the suspension can be administered during 6 days if kept at 25°C, and 8 days if kept in refrigerator [15].

[14] carried out study on Cefuroxime axetil 125mg/5ml (A) oral suspension at room temperature (25°C) and stored in refrigerator (2-8°C).Reconstituted Cefuroxime axetil stored at refrigerator (2-8°C) is stable for 10 days over 90%, after that start to degradation. While concentration percentage of Cefuroxime axetil stored at room temperature were over 90% up to 5th day, degradation was extensive by 7th day with cefuroxime axetil concentration failing to 80% outside the acceptance limits. Cefuroxime axetil suspension after reconstitution was found stable for 10 days, if stored at refrigerated condition but 3 days only at room temperature [14].

A study was carried out by [16], carried out studies on augmentin by using HPLC to determine the chemical stability of amoxycillin and potassium clavulanate in 250/62 oral suspension. It was stored at room temperature (20°C) and 8°C over a period of 11 days, During the test period, the amoxycillin component was found to be more stable than the clavulanate. Amoxycillin was stable for 7 days at both temperatures. Potassium clavulanate maintained at least 90% of

its initial concentration for 7 days at 8°C but showed more than 40% degradation in the same time period at room temperature. For potassium clavulanate the shelf-life or time taken for the original concentration to drop to 90% of its value at room temperature was found to be 2 days [16]. A study conducted by [17] and carried out a research on stability of cefuroxime axetil oral suspension at different temperature storage conditions (stored at room 20°C and refrigerated 5°C conditions). Based on their findings, cefuroxime axetil oral suspension preserves its stability for 10 days after reconstitution under room and refrigerated conditions. It is obvious, according to the f2 value obtained on the 10th day, that there is a difference between the released ceforoxime axetil from oral suspension at room (87.68%) and refrigerated (92.35%) conditions. Concentration changes can be caused by the mechanisms associated with drug release and hydrolytical decomposition of the sample and higher temperatures during longer period of storage [17].

Conclusion

This study was performed on Cefadroxil oral dry powder for suspension. Cefadroxil is one of cephalosporin antibiotics first generation. Cefadroxil oral dry suspension needs reconstitution before use. The reconstitution process, storage conditions, type of water used in reconstitution are important factors for drug stability as much as the manufacturing process. This study included two brands of Cefadroxil, two types of water for reconstitution, and two different storage conditions then the assay test performed using HPLC system. Reconstituted oral Cefadroxil suspension is stable at temperatures between 2 and 29°C for a period of 3 days; reconstituted with distilled water also showed stability until the 7th day, this is seen in the concentrations of drug A and B results obtained. As a result of statistical comparison, the concentrations of Cefadroxil in a reconstituted oral suspension which were kept under different storage conditions were found to be statistically different at 0 to 14 days. It was concluded that after the preparation of the Cefadroxil reconstituted oral suspension, the storage conditions at different temperatures did cause degradation of the active compound.

The results for day one and 3rd day for all samples fall within the accepted range, and microbiological studies should be done on these samples to ascertain microbial stability. In addition, studies should be done to determine the concentration of drug, microbial, physical and toxicological stability of reconstituted Cefadroxil for a duration of 14 days of storage. Also, it is necessary to carry out further studies in which the reconstituted oral suspension will be evaluated in terms of aesthetic appeal (odor, taste, color and texture) properties.

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Conflict of interest

The authors declare that no conflict of interest.

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