Effect of Reconstitution Solvents and Containers on Kinetics and Safety of Cephradine Neutralized with L-Arginine

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Abstract

The effect of reconstitution solvents such as water for injection, 0.5% metronidazole injection, 0.9% sodium chloride injection and 5% dextrose injection, has been investigated on the kinetics of degradation of cephradine neutralized with L-arginine contained in glass, polyvinylchloride (PVC) and polyethylene pthalate (PET) containers at 5, 15 and 30°C. The analytical method described in USP-31 for the analysis of cephradine injection was employed in this study after slight modification and validation in respect of specificity, linearity, accuracy and precision. The degradation of the compound showed first-order kinetics and the degradation rate constants 'k_{obs}' were found in the range of $1.84\text{-}3.07 \times 10^{-3}$ hr^{-1} (r^2=0.990-0.999) at 5°C, $2.3\text{-}4.2 \times 10^{-3}$ hr^{-1} (r^2=0.993-0.999) at 15°C and $1.18\text{-}9.97 \times 10^{-3}$ hr^{-1} (r^2=0.998-0.999) at 30°C, respectively. The compound showed maximum stability in dextrose solution followed by water for injection, sodium chloride injection and metronidazole injection; however, linear effect of containers on degradation rate could not be established. The extended degradation did not change the kinetics of the reaction. The abnormal toxicity/ safety test on mice for the admixtures in different containers at various temperatures showed no abnormal toxicity.

Introduction

Cephradine is chemically (7R)-7-(α-D-cyclohexa-1, 4-dienylglucylamino)-3-methyl-3-cephem-4-carboxylic acid [1]. It is a first generation cephalosporin antibiotic and is available in different dosage forms such as capsules, dry suspension and dry powder injections [2]. Alkaline substances such as sodium carbonate or arginine to provide sufficient solubility and physiological acceptability must neutralize injection of cephradine. The drug is usually prescribed in the treatment of infections caused by sensitive organisms such as upper respiratory tract infections e.g. pharyngitis, sinusitis, otitis media, tonsillitis, and larygotracheo-bronchitis. Lower respiratory tract infections e.g. acute and chronic bronchitis and bronchopneumonia; urinary tract infections e.g. cystitis, urethritis, pyelonephritis; skin and soft tissue infections e.g. abscess, cellulitis, furunculosis; gastrointestinal tract infections e.g. bacillary dysentery, enteritis, peritonitis as well as bone and joint infections.
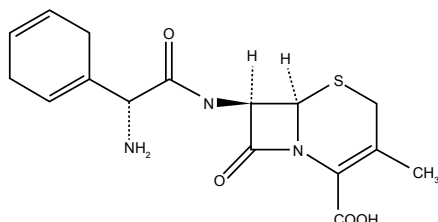
Cephradine

In clinical practice cephradine injection is usually admixed with IV solutions such as dextrose, normal saline and metronidazole solutions etc, contained in various containers. These IV solutions can affect the stability of cehpradine as shown in the studies reported earlier [3]. The stability of these admixtures can be affected by a number of factors such as storage temperature, the solvent admixed and the nature of the container etc [4-6]. The storage temperature not only influence the rate of degradation of the compound in the solvent but also affects the leaching process (migration of chemical from containers to the solvent kept inside it), and thereby rendering the solutions impure and unsafe [7,8]. The nature of the leachable is determined by the nature of the container [9-12].

Thorough review of the literature revealed that very little work has been reported for these aspects of cephradine injection. This fact motivated the present study in which focus has been made on the effect of various reconstitution solvents used commonly in clinical practice i.e. water for injection, 0.5% metronidazole, 0.9% sodium chloride and 5% dextrose solutions, on the kinetics of degradation of cephradine. Attempts have also been made to correlate the increase in temperature and reaction container with the rate of degradation of the compound. The effect of extended degradation on the kinetics of the reaction has also been evaluated. Experiments have also been performed to assess the abnormal toxicity of cephradine injection (due to degradation products of cephradine or any leachable from the container), when admixed with these IV solutions in containers made of different materials such as polyvincychloride (PVC), glass and polyethylene phthalate (PET) at 5, 15 and 30°C.

Materials and Methods

Materials

Cephradine (neutralized with L-arginine) samples and reference standard were kindly donated by M/S. GSK Pakistan (Pvt) Ltd Karachi. Samples of metronidazole (flagyl injection, Sanofi Aventis, Pakistan), sodium chloride injection and dextrose injection (Plasaline and

plades-5 injections, Outsuka, Pakistan) and water for injection (Tabros pharma, Pakistan) were purchased from authentic distributors. All the reagents and solvents, used in this study were of analytical and spectroscopic grades, respectively. Freshly prepared double distilled water was used throughout this work.

HPLC Apparatus and conditions

An HPLC system (Class 20A, Kyoto, Japan) provided with of an LC-20 AT pump with gradient mixer, an SPD-20A UV visible detector, a stainless steel column (C-18, 5 bµ, 4.6 × 150 mm id, Hypersil, Thermo Quest, USA) and an inbuilt CBM-20A lite communication bus module. The data collection and integration were obtained by using Schimadzu LC Solution Computer software version 1.2 (Kyoto, Japan). All separations were achieved isocratically at room temperature ($20 \pm 1°C$). The mobile phase was a degassed and filtered mixture of methanol: 0.5 M sodium acetate: 0.7 N glacial acetic acid: water (200:15:3: 782, v/v). The flow rate was maintained at 1.75 mL/min with detection at 254 nm.

pH Measurement

The pH measurements performed with a pH meter (Wertheim, Germany). Electrode of the pH meter standardized with buffer solutions (pH 2.0, 4.0 and 7.0, Merck) at 25°C.

Degradation studies of cephradine in admixture with intravenous solutions

An accurately weighed quantity of 10g of cephradine was taken in 1000ml volumetric flask. A volume of about 500ml of 0.9% sodium chloride solution, 5% Dextrose solution, 0.5% Metronidazole solution, or water for injection was added to the flask. The flask was kept in ultrasonic bath to promote dissolution of the drug in the solvent. After complete dissolution of the drug powder in the solvent, the volume was made upto the mark with additional volume of the respective solvent. Zero time samples were withdrawn for analysis while nine aliquots, each of 50 ml, of the remainder samples solution were withdrawn into three groups, each of PVC, Glass and PET containers. One sample from each group was placed at 5°C, the other at 15°C while the third at 30°C in refrigerator or oven for 24 hours. Samples were withdrawn at regular interval of 6 hours, diluted with the mobile phase (final concentration 100 µg/mL) and analyzed by HPLC. Quantification was made by comparing peak area or height of the sample to the peak area or height of the standard solution.

To determine the effect (if any) of extended degradation on the kinetics of degradation reaction of cephradine, the drug powder was dissolved in water for injection in glass container and kept at 50°C for two hours to produce sufficient amount of the degradation products. The degraded solution was analyzed by HPLC to estimate the extent of degradation. The degraded solution was kept further at 30°C in an oven and samples were withdrawn at regular time intervals. Any change in the kinetic behavior of cephradine was determined by comparing the kinetic data at 30°C of the pre-heated sample and the sample, which was not initially heated at 50°C for two hours.

Abnormal Toxicity/Safety Test

Eight groups (each of 5 healthy mice between 17-23g of weight for each container at each temperature) were selected for this study. First four groups were injected intravenously with 0.5cc of 10 mg/1cc solution of cephradine in water for injection, normal saline, 5% dextrose and 0.5% metronidazole injections, respectively. The time of the injection was kept about twenty to thirty seconds. To the rest of the

four groups 0.5cc of the same amount of diluents were administered over a similar period of 20-30 seconds. The criteria for pass and fail were kept as: None of the mice must die within twenty-four hours. If one of the animals dies within 24 hours, the test will be repeated. None of the animals in the second group must die within the 24 hours.

Statistical Analysis

The orders of the degradation reactions were determined graphically using the half-life methods. The observed degradation rate constants (k_{obs}) were estimated from the slope of the log-linear phase of declining cephradine concentration versus time plots. All first-order plots reported in this study were linear with the square of correlation coefficient (r^2) greater than 0.990. The half-lives were calculated using the half-life equation. Data was expressed as the mean of replicate determinations (n=3). Statistical analyses were achieved using Statistical package for social sciences (SPSS, version 15).

Results and Discussion

Validation of analytical method

The USP-31, 2008 (USP,2008) method for analysis of cephradine injection was slightly modified and partially validated by including parameters like specificity, linearity, accuracy and precision. A linear response (r^2=0.9995-0.9998) was shown by the compound in all solvents when measured by both peak area and height within the concentration range of 5-125 µg/mL (Table 1).

The reconstitution solvents did not interfere with the peak of the compound. The method was also found accurate as overall mean of the recoveries of the method was found within 99-101% of the 50-150% range of the nominal content (100 µg/mL). The inter-day and intra-day precision of the method were also found within limits i.e. RSD below 2% (Data not shown).

Kinetics of degradation of Cephradine in I.V Solutions

The kinetic treatment of the data on degradation of cephradine in the solvents studied showed that degradation of the drug follows first-order kinetics. This observation is an agreement with the previous studies [4]. The observed rate constants; k_{obs}, for the degradation of the drug in the solvents stored in glass, polyethylene phthalate and polyvinylchloride containers at 5, 15 and 30°C, are in the range of 1.84-$9.97 \times 10^{-3} hr^{-1}$ with square of a correlation ranging from 0.9905-0.9999 (Tables 2-4). The half-lives of the reactions were found to be in the range of 2.40-15.69 days. The highest rate of degradation of the drug was found in 5% dextrose injection and water for injection followed by 0.9% sodium chloride injection and metronidazole solution, respectively. The rate of degradation was also found to accelerate with increase in temperature by 1.4 and 3.2 folds, respectively, at 15°C and 30°C as compared to at 5°C.

The increase in degradation rate of the drug with increase in temperature has also been evidenced by earlier investigations. The reaction container also influenced the rate of degradation. In metronidazole injection, the highest rate was noted in PET containers and the lowest in glass and PVC containers. In dextrose, injection the highest degradation rate was seen in PET while the lowest in PVC containers. In sodium chloride solution the highest rate of degradation was noted in PVC containers while the lowest in PET containers. The variable degradation rate in different containers clearly indicates the role of containers on the degradation.

Change in pH and kinetics of the reaction

A slight increase in pH of the admixtures was also observed as seen with other cephalosporins [13]. Extended degradation lowers the concentration of the solute in the solution, which has been shown in some studies to change the kinetics of degradation reaction [14-15]. But in the present case the comparison of kinetics of the sample at 30°C after being degraded at 50°C for 2 hours with the sample degraded at 30°C (Not treated initially) did not show any difference.

Abnormal Toxicity

The identification and quantification of any leachable could not be made in the study however any toxicity associated with such phenomenon was evaluated by conducting abnormal toxicity tests of the admixtures stored in glass, PVC and PET containers at 5, 15 and 30°C. Results of these experiments showed no evidence of any abnormal toxicity of the admixtures under the conditions studied (Table 5).

Conclusion

The thermal degradation of cephradine in admixture with 5% dextrose injection, 0.9% sodium chloride injection or 0.5% metronidazole injection follows first-order kinetics. The kinetics of degradation of the compound is influenced by temperature, solvent and container used however, the abnormal toxicity test is not influenced. The results of these studies necessitate that appropriate storage conditions and containers must be ensured while storing solutions of the product. Delayed injectability of the product while admixed with intravenous solutions should also be avoided in clinical practice.

Acknowledgment

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