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

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Abstract: The effect of reconstitution solvents such as water, 0.5% metronidazole solution, 0.9% sodium chloride and 5% dextrose injections, have been investigated on the kinetics of degradation of cephradine neutralised 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 and validation in respect of specificity, linearity, accuracy and precision was observed. The degradation of the compound showed first-order kinetics and the degradation rate constants 'k_{obs}' were found in the range of 1.84-3.07 × 10^{-3}/h (r^2 = 0.990-0.999) at 5 °C, 2.3-4.2 × 10^{-3}/h (r^2 = 0.993-0.999) at 15 °C and 7.18-9.97 × 10^{-3}/h (r^2 = 0.998-0.999) at 30 °C, respectively. Cephradine showed maximum stability in dextrose solution followed by water, sodium chloride and metronidazole injections, 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.

Keywords. cephradine, degradation kinetics, polyvinyl chloride, polyethylene phthalate, abnormal toxicity

Introduction

Cephradine is chemically (7R)-7-(α-D-cyclohexa-1, 4-dienylglucylamino)-3-methyl-3-cephem-4-carboxylic acid (USP, 2008). It is a first generation cephalosporin antibiotic and is available in different dosage forms such as capsules, dry suspension and dry powder injections (BP, 2009). Injection of cephradine must be neutralised by alkaline substances such as sodium carbonate or arginine to provide sufficient solubility and physiological acceptability. 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, 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 (Wisebritish, 1987).

In clinical practice cephradine injection is usually admixed with intravenous (IV) injection solutions such as dextrose, normal saline and metronidazole injection solutions contained in various containers. These IV solutions can affect the stability of cehpradine as shown in the studies reported earlier (Wang and Monkhouse, 1983). The stability of these admixtures can be affected by a number of factors such as storage temperature, reconstitution solvent and the nature of the container (Conine *et al.,* 1978; Florey, 1976; Yamana and Tsnji, 1976). The storage temperature not only influence the rate of degradation of the compound in the solvent but also affects the extraction of chemical compounds form containers to the injection solution migration of chemical from containers to the solvent kept

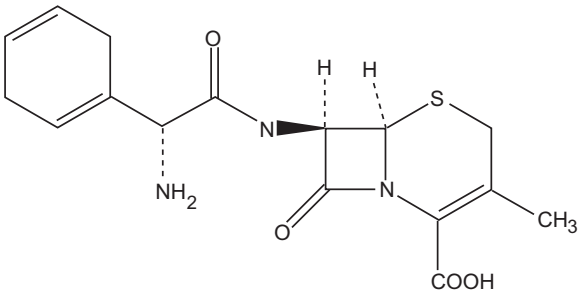

Fig. 1. Chemical structure of Cephradine.

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inside it, and thereby rendering the solutions impure and unsafe (G I, 1999; Autian, 1963). The nature of the leachable is determined by the nature of the container (Danielson, *et al.,* 1983; Ching *et al.,* 1981; Corley *et al.,* 1977; Jaeger and Rubin, 1972).

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 injection solution, 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 the type of the container in which reconstitution is made 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 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 ºC.

Materials and Methods

Materials. Cephradine (neutralised with L-arginine) samples and reference standard were 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. A high performance liquid chromatographic system (Class 20A, Kyoto, Japan) provided with a LC-20 AT pump with gradient mixer, an SPD-20A UV visible detector, a stainless steel column (C-18, 5µ 4.6×150 mm id, Hypersil, Thermo Quest, USA) and an inbuilt CBM-20A lite communication bus module was used in this study. The data collection and integration were obtained by using SHIMADZU LC solution computer software version 1.2 (Kyoto, Japan). All separations were achieved isocratically at room temperature (20±1 ºC). The mobile phase was degassed and filtered mixture of methanol: 0.5M sodium acetate: 0.7N 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. Injection volume was 20 µL.

pH measurement. The pH measurements were performed with a pH meter (Wertheim, Germany). Electrode of the pH meter was standardised with buffer solutions (pH 2.0, 4.0 and 7.0, Merck) at 25 ºC.

Degradation studies of cephradine in admixture with intravenous solutions. An accurately weighed quantity of 10 g of cephradine was taken in 1000 mL volumetric flask. A volume of about 500 mL of 0.9% sodium chloride solution or 5% dextrose solution or 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 up to 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 h. Samples were withdrawn at regular interval of 6 h, diluted with the mobile phase (final concentration 100 µg/mL) and analysed 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 2 h to produce sufficient amount of the degradation products. The degraded solution was analysed 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 behaviour 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 4 groups were injected intravenously with 0.5 mL of 10 mg/1 mL solution of cephradine in water for injection, 0.9% sodium chloride solution, 5% dextrose and 0.5% metronidazole injections, respectively. The time of the injection was kept 20-30 sec. To the rest of the four groups 0.5 mL of the same amount of diluents were administered over a similar period of 20-30 sec. The

criteria for pass and fail was kept as: None of the mice must die within 24 h. If one of the animals dies within 24 h, the test will be repeated. None of the animals in the second group must die within the 24 h.

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 (t ½ = log (2)/k.) Data was expressed as the mean of replicate determinations (n = 3). Statistical analyses were achieved using statistical package for social sciences (SPSS, version 15).

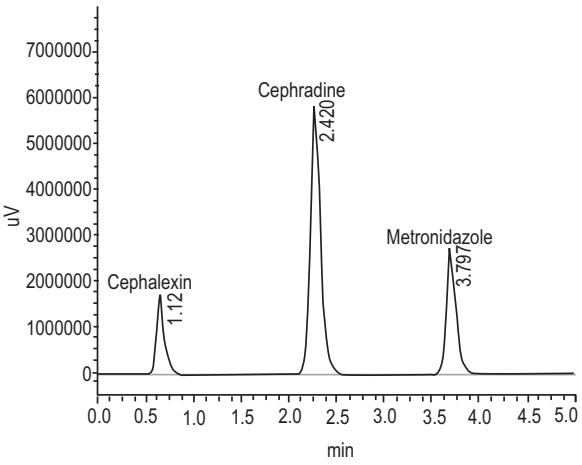

Fig. 2. HPLC chromatogram of cephradine solution spiked with the excipiant metronidazole and degradation product cephalexin.

Results and Discussion

Validation of analytical method. The USP-31 method for analysis of cephradine injection (USP, 2008) was slightly modified (changing flow rate from 1mL per minute to 1.75 mL per minute and the C-18 column id from 4.6 × 250 mm to 4.6 × 150 mm) and used in this study. The method was 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 5-125µg/mL (Table 1).

The reconstitution solvents and the degradation products did not interfere with the peak of the compound (Fig. 2). 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 (100µg/mL). The inter-day and intra-day precision of the method were also found within limits i.e. % RSD below 2% (Table 2).

Kinetics of degradation of cephradine in IV 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 in agreement with the previous studies (Yamana and Tsnji, 1976). 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 $1.84\text{-}9.97\times10^{-3}$/h with square of a correlation ranging from 0.9905-0.9999 (Table 3-5). 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 ºC and 30 ºC as compared to 5 ºC.

The increase in degradation rate of the drug with increase in temperature has also been evidenced by earlier

Table 1. Linearity and accuracy data of cephradine in IV solutions

Vehicle	Slope	Y-Intercept	Correlation coefficient (r^2)	% Recovery (Means of % amount found from 50%, 100% &150% amount of cephradine added)
Water for injection	0.4365	-0.0145	0.9996	99.93, 99.78, 99.80
5% dextrose	0.8344	-0.0313	0.9995	99.63, 100.16, 100.06
0.9% NaCl solution	0.28267	-0.02324	0.9998	100.10, 99.96, 100.19
0.5% Metronidazole solution	0.4480	-0.0211	0.9997	99.93, 99.85, 99.95

Table 2. Intra-day and Inter-day repeatability results of cephradine in various IV solutions

Sample no.	% Cephradine in water for injection		% Cephradine in 5% dextrose solution		% Cephradine in 0.9% NaCl solution		% Cephradine in 5% metronidazole solution	
	Day-I	Day-II	Day-I	Day-II	Day-I	Day-II	Day-I	Day-II
1st analyst								
1	101.6	100.9	100.8	101.0	100.8	101.0	100.6	100.4
2	100.3	99.7	100.8	101.0	100.7	100.9	100.5	100.5
3	100.2	100.1	100.6	100.9	100.7	100.6	100.5	101.0
4	100.6	101.0	100.3	100.2	100.3	100.6	99.7	101.0
5	99.9	100.7	100.1	100.6	100.6	99.8	100.3	100.9
Mean	100.52	100.48	100.52	100.74	100.62	100.46	100.32	100.76
% RSD	0.65	0.56	0.31	0.34	0.19	0.49	0.36	0.28
2nd analyst								
1	101.2	100.3	101.1	100.7	100.7	100.2	100.5	100.9
2	101.4	100.2	101.0	100.5	101.0	100.2	100.5	100.2
3	100.9	100.5	99.9	100.5	101.0	100.2	100.9	100.2
4	100.8	101.2	100.8	100.8	99.8	100.8	100.7	99.8
5	100.1	100.2	100.4	101.0	100.3	100.6	101.0	99.7
Mean	100.88	100.48	100.64	100.7	100.56	100.4	100.72	100.16
% RSD	0.44	0.53	0.49	0.21	0.51	0.28	0.23	0.22

Table 3. Apparent first-order rate consents (k_{obs}) of cephradine in various solvents in various containers at 5° C.

Solvents	Glass containers			PET containers			PVC containers		
	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)
5% dextrose solution	2.68	0.9994	10.77	2.68	0.9997	10.77	3.07	0.9972	9.4
Water for injection	2.68	0.9994	10.77	2.0	0.9996	12.55	2.3	0.9991	12.55
0.9% NaCl solution	1.92	0.6688	15.03	1.91	0.9905	15.11	1.91	0.9996	15.11
0.5% Metronidazole solution	1.84	0.9905	15.69	2.3	0.9999	12.55	1.91	0.9995	15.11

Table 4. Apparent first-order rate consents (k_{obs}) of cephradine in various solvents in various containers at 15 °C.

Solvents	Glass containers			PET containers			PVC containers		
	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)
5% dextrose solution	3.83	0.9939	7.53	4.2	0.9996	6.87	4.2	0.9996	6.87
Water for injection	3.45	0.9999	8.36	3.45	0.9998	8.36	3.45	0.9997	8.36
0.9 NaCl solution	3.07	0.9999	9.40	2.3	0.9999	12.55	3.07	0.9999	9.40
0.5% Metronidazole solution	2.3	0.9997	12.55	2.68	0.9981	10.77	2.3	0.9994	12.55

Table 5. Apparent first-order rate consents (k_{obs}) cephradine in various solvents in various containers at 30 °C.

Solvents	Glass containers			PET containers			PVC containers		
	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)	$k_{obs} \times 10^3$/h	r^2	Half-life ($t_{1/2}$) (day)
5% dextrose solution	9.21	0.9996	3.13	9.97	0.9997	2.89	11.8	0.9996	2.4
Water for injection	9.21	0.9995	3.13	9.59	0.9993	3.01	9.21	0.9989	3.13
0.9% NaCl solution	6.52	0.9995	4.42	6.14	0.9995	4.70	7.67	0.9995	3.76
0.5% Metronidazole solution	6.14	0.9996	4.70	6.52	0.9996	4.42	6.14	0.9993	4.70

Table 6. Abnormal toxicity/safety test for cephradine injection admixed with various IV solutions in different containers at 5, 15 and 30 °C.

Injection composition	Observations	
	Immediate after injection	24 h after injection
Cephradine admixed with water for injection	No signs of any untoward reaction	No signs of any untoward reaction
Cephradine admixed with 5% dextrose solution	No signs of any untoward reaction	No signs of any untoward reaction
Cephradine admixed with 0.9 Nacl solution	No signs of any untoward reaction	No signs of any untoward reaction
Cephradine admixed with 0.5% metronidazole solution	No signs of any untoward reaction	No signs of any untoward reaction
Water for injection	No signs of any untoward reaction	No signs of any untoward reaction
5% dextrose solution	No signs of any untoward reaction	No signs of any untoward reaction
0.9 % NaCl solution	No signs of any untoward reaction	No signs of any untoward reaction
0.5 % Metronidazole solution	No signs of any untoward reaction	No signs of any untoward reaction

investigations (Conine *et al.,* 1978). The reaction container also influenced the rate of degradation. In metronidazole injection, and in water for 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 PVC while the lowest in glass 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 (Viaene *et al.,* 2002). Extended degradation lowers the concentration of the solute in the solution which has been shown in some earlier studies to change the kinetics of degradation reaction (Ito *et al.,* 2005; Meakin *et al.,* 1978), but in the present of case, the comparison of kinetics of the sample at 30 °C after being degraded at 50 °C for 2 h 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 this 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 6).

Conclusion

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 are 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.

Acknowledgement

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