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Research Article

STABILITY STUDY OF ANTIBIOTIC (CEFOTAXIME) IN PERITONEAL DIALYSIS SOLUTION WITH VALIDATION OF ANALYZING METHOD

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ABSTRACT

Objective: The objective of the present study is to check the stability of Cefotaxime in peritoneal dialysis (PD) solutions containing 1.5% and 2.3 % dextrose solutions stored at different temperature.

Method: Cefotaxime (CTX) 2gm vial were injected into 2 L bags of PD solution containing 1.5% and 2.3 % dextrose separately. Samples were withdrawn aseptically at different time intervals; Concentration was analyzed by a modified and validated spectrophotometeric method at λ 235nm. The linear relationship of modified and validated method was found in both 1.5% and 2.3 % dextrose PD solution with $R^2 = 0.992$ and $R^2 = 0.997$ respectively. Concentration of the admixture solution was taken as 100% at time Zero.

Result: At 37 °C the initial CTX concentration declined to 86.78% at 24 hrs in the 1.5 % PD solution while the mean percentage of remaining drug in 2.3 % dextrose were 88.87% at 24hr. At ambient room temperature, the concentration declined to 89.18% during 24hours in 1.5% dextrose solution, while in 2.3% dextrose solution to 89.4% at 24hours. Similarly at 40°C the concentration declined to 86.45% in 12 hours in 1.5% PD solution, and to 85.08% in12 hours in 2.3 % PD solution. Under refrigerator, a mean of 98.2% and 98.56% remained after 168 hours for 1.5% and 2.3% solution respectively.

Conclusion: The conclusion of the present study is that Cefotaxime 1g/L is stable in both 1.5% and 2.3 % peritoneal dialysis solutions for the respective periods of time necessary for dialysis. Thus this admixture can be used safely and effectively for the treatment.

Keywords: Cefotaxime, Peritoneal Dialysis Solution, Drug stability, Peritonitis, ESRD.

INTRODUCTION

End-stage renal disease (ESRD) is a major global health crisis and numbers of new cases are rising rapidly. The World Health Organization estimates that more than 180 million people around the world have diabetes and 10 to 20 percent of them will die of renal failure, as diabetes and hypertension are the leading causes of kidney failure worldwide.

Transplantation is the preferred mode of treatment for patients with ESRD, but transplantation usually does not take place because of limited number of donors. Patients with end-stage renal disease (ESRD) requires renal replacement therapy (RRT) thus maintained on dialysis, this mean dialysis is still the main treatment for most patients **[1].**

Pakistan is also encountering a rapid rise in kidney diseases. Unfortunately there is no comprehensive data available on the incidence and prevalence of end-stage renal disease (ESRD) in Pakistan. Most of the data available is hospital based.

Peritoneal dialysis has gained global acceptance as an alternative to Hemodialysis. The study conducted by Jain et al. [**2**] showed that the number of patient treated with PD increased worldwide from 1997-2008.

PD therapy is a very simple therapy, limited need for trained medical staff, and minimal requirement for technical support but in spite of these PD is not very common in Pakistan due to their high cost because it is not formulated (manufactured) locally. Secondly as per Dr Jafer Naqvi [**3**] greatest obstacles in the use of PD in Pakistan are the health profession themselves, who have personal and commercial interest as they have set up their own dialysis centre and Patients themselves who feel more safe and secure in the hospital environment in Pakistan.

Peritoneal Dialysis is often associated with the development of peritonitis due to organisms such as Staphylococcus sp., Streptacoccus sp., gram-negative and anaerobic organisms **[4].** It is usually treated with intraperitoneal antibiotic administration [**5]**. Most antibiotics have been administered through the intraperitoneal route; the usual method is to add drugs to each bag just before instillation. This method is inexpensive and more convenient to the patient, but it requires drug stability over at least 24 hour.

Stability is an essential quality attribute for drug products. It concerns with safety, efficacy, and quality of drug product. In the clinical setting of peritonitis, it is important to make it sure that the prescribed antibiotics are compatible with the PD solution and its container and should be clinically effective [**6]**. Cefotaxime is a third-generation, broad spectrum cephalosporin antibiotic. It is used for infections of the respiratory tract, skin, bones, joints, urogenital system, meninges, and useful in the treatment of peritonitis and other infections in patients on PD.

The aim of the present study was to analyze the chemical stability of Cefotaxime sodium in two commonly used peritoneal dialysis solution that contained dextrose 1.5% and 2.3%. During the study also targeting the effect of temperature on the stability of admixture and it was done at four different temperature i.e. refrigerator (4°C), body temperature (37°C), room temperature (Ambient condition) and at 40°C elevated temperature (climatic zone IV). As well as checked the effect of dextrose concentration (1.5 % and 2.3 %) on antibiotics (Cefotaxime).

Table 1: standard composition of Peritoneal Dialysis Solution

Electrolyte	Standard Solution
Sodium	132
Potassium	0
Calcium	2.5,3.5
Magnesium	0.5,1.5
Chloride	96-102
Lactate	35-40
Glucose	1.5,2.3,4.25
pH	5.2-5.5

MATERIALS AND METHOD

Instrument

All absorbance measurements were performed using a SHIMADZU UV Spectrophotometer Model UV 1800 attached with PC- IV loaded with UV Probe version 2.3 software, pH meter JENCO Model # 6173 (USA), Stability Chamber NUAIRE (Ardco Temper Gard, USA), Laminar Flow Hood (GELAIRE FLOW LAB 09922 model HF72, Germany) and Incubator (HERAEUS KENDRO LABORATORIES Product D-63450 Type B6, Germany) Cefotaxime Sodium (Reference standard) was a kind gift from Aventis Pharmaceutical Pakistan Pvt.).Claforan® batch number Wh012 (1gram IV vials were purchased from local market), 1.5 % Dextrose PD fluid (Fresenius Medical Care CAPD/DPCA 2 batch no. PKB 312C and 325), 2.3 % Dextrose PD Fluid (Fresenius Medical Care CAPD/DPCA 4 batch no. QBB 124Band 043)

The study was conducted to assess the chemical stability of cefotaxime sodium (CTX) in peritoneal dialysis solution containing 1.5 % and 2.3% dextrose, stored at room temperature(ambient), Body temperature (37°C), refrigerator(4°C) and 40°C, due to the climatic condition as Pakistan comes under zone IV.

Dialysis Solution: CAPD/DPCA ANDY disc is clear sterile water based solution. It is available in a number of different strength in various calcium ranges. Potassium is omitted from all Strengths **[7].**

The two selected strengths are

A*) Fresenius Peritoneal dialysis solution with 1.5% dextrose CAPD/DPCA ANDY disc 2

B*) Fresenius Peritoneal dialysis solution with 2.3% dextrose CAPD/DPCA ANDY disc 4

Table 2: Label information of 1.5 % and 2.3 % dextrose solution.

Composition/1000ml	A*	B*
Sodium Chloride	5.786/L	5.786/L
Sodium Lactate	3.925/L	3.925/L
Calcium Chloride ($2H_2O$)	0.257/L	0.257/L
Magnesium Chloride ($6H_2O$)	0.1017/L	0.1017/L
Glucose Monohydrate	16.5/L	25g/L
Glucose Anhydrous	15g/L	22.73/L
Theoretical Osmolarity	401mosm/L	401mosm/L
pH	5.5	5.5
Ionic Concentration mmol/L		
Na^+	134 mmol/L	134 mmol/L
Ca^{++}	1.75mmol/L	1.75mmol/L
Mg^{++}	1 mmol/L	1 mmol/L
Cl^-	102 mmol/L	102 mmol/L
Lactate	35 mmol/L	35 mmol/L
Glucose Anhydrous	1.5%	2.3%

Sample Preparation

Cefotaxime-peritoneal dialysis samples were prepared by adding 2 gm of CTX to 2L bags of commercially available peritoneal dialysis bags containing 1.5% and 2.3% dextrose separately. The final concentration was according to the recommended treatment dose that was 1mg/ml [**4].** Samples were prepared by transferring CTX after reconstitution in each of three 2-liter bags of 1.5 % dextrose PD solution under aseptic technique. The same procedure was repeated for the 2.3 % dextrose dialysis solution. All 24 bags had a theoretical initial Cefotaxime concentration of 1000 µg /ml. The bags were gently shaken to ensure the proper mixing of the drug and measured at λ 235 nm .After the initial sampling at time 0 min, the bags were placed into room temperature, 37°C, 4°C and 40°C. Sampling was accomplished by withdrawing 40 ml of admixed solution from each bag into two 20ml sterile syringe under aseptic condition.

Room Temperature (Ambient condition): Samples were drawn aseptically at 0, 2, 6, 12, hour and at 1, 2, days, measure antibiotic concentration and physical parameters.

Body Temperature: Dialysis bags stored at 37°C were kept in incubator and samples were drawn from each at 0, 2, 4,6,8,12,24 and 48 hours, measure antibiotic concentration and physical parameters.

Refrigeration: The bags were placed at 4 °C. Sequential sample were withdrawn at 0, 12hr and on day 1, 2 and 7 days, measure antibiotic concentration and physical parameters.

The refrigerated bags were removed from 4°C on the indicated assay day only for the several minutes required for sampling.

Elevated temperature (40°C): The bags were kept at 40°C in stability chamber and samples were withdrawn at 1,2,4,6,8,12 and 24 hr.

Visual Inspection: All samples were visually inspected for precipitation, cloudiness and discoloration throughout the study period. Temperature and pH were also monitored throughout the study period.

Validation of method

Spectrophotometeric technique provides practical and significant economic advantages over other method therefore; they are the frequent choice for pharmaceutical analysis. As an alternative to existing method first select the method then validated to determine CTX on UV detector [**8**][**9**]. The method was validated as per ICH guidelines [**10**]

Preparation of stock solution

Stock solution of CTX reference standard (100ug/ml) was prepared by dissolving 10mg of CTX in 100ml of peritoneal dialysis solution of 1.5% dextrose. Same procedure was repeated for 2.3% dextrose solution.

Working solution

Suitable volume of stock solution was pipette out in 50 ml of volumetric flask and diluted with PD solution (1.5 %) to make dilution series of 0.625-40 µg/ml. The same procedure was repeated for 2.3 % PD solution.

Calibration curve

For the calibration of curve, the series of sample dilution (0.312-20 µg/ml) were analyzed at 235 nm and then standard curve of peak area ratio were plotted versus their respective concentrations. The values of regression coefficient was more close to 1 (r^2 = 0.992 and 0.997 respectively for 1.5% dextrose and 2.3% dextrose solution. The result based on the triplicate absorbance.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. The values of regression coefficient, r^2 = 0.992 and 0.997 respectively, indicate the linearity of the proposed method (Fig 1)

Table 3: Linearity of the standard solution with LOD and LOQ

Parameter	Conc. value in 1.5% dextrose Solution	Conc. value in 2.3% dextrose Solution
Concentration range (ug/ml)	0.312-20	0.312-20
Correlation coefficient	0.992	0.997
Slope	0.05	0.048
y-intercept	0.016	0.007
LOD	0.312	0.312
LOQ	0.625	0.625

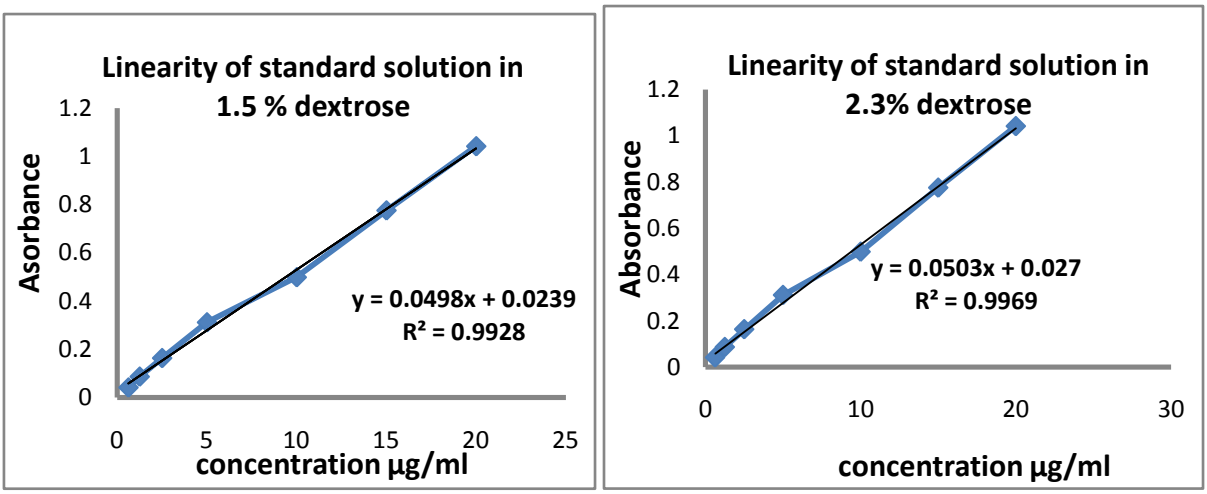


Fig. 1: Calibration Curve in 1.5% and 2.3% dextrose solution

Table 4: Stability Evaluation of CTX in 1.5 % and 2.3 % Dextrose Solution at 4°C, 24°C, 37°C 40^{0}C

Temperature	Time (in hrs)	%initial conc. Remaining for 1.5 % solution (Mean ± SD)	% initial conc. Remaining for 2.3% solution (Mean ± SD)
4^{o}C	0	100	100
	6	99.31 ± 0.2	100 ± 0.1
	12	99.52 ± 0.6	99.92 ± 0.15
	24	100.04 ± 0.1	99.85 ± 0.25
	48	99.45 ± 0.1	99.78 ± 0.15
	168	98.2 ± 0.1	98.56 ± 0.3
24 ^{0}C	0	100	100
	2	99.1 ± 0.4	99.50 ± 0.2
	6	97.72 ± 0.9	97.79 ± 0.2
	12	94.39 ± 0.7	95.66 ± 0.28
	24	89.18 ± 0.2	89.41 ± 0.43
37 ^{0}C	0	100	100
	2	99.3 ± 0.4	99.63 ± 0.15
	4	96.78 ± 0.7	98.16 ± 0.57
	6	95.22 ± 2.4	96.85 ± 0.56
	8	93.56 ± 0.2	95.31 ± 0.7
	12	90.98 ± 0.1	93.11 ± 0.15
	24	86.78 ± 0.05	88.87 ± 0.3
	0	100	100
40 ^{0}C	1	97.52 ± 0.74	98.47 ± 0.34
	2	98.44 ± 0.47	97.67 ± 0.25
	4	95.64 ± 0.17	95.19 ± 0.36
	6	91.17 ± 1.79	92.13 ± 0.85
	8	90.30 ± 1.25	90.39 ± 0.2
	12	86.45 ± 1.04	85.08 ± 0.2
	24	63.79 ± 1.59	57.93 ± 0.15

Precision

Precision was calculated by performing Interday (between days) and intraday (within days) analysis of the specific dilution (0.312-20 µg/ml) that covered the range of studies.

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitaion (LOQ) revealing the sensitivity of the method was calculated by the equation given in ICH guideline [**8**]. In the purposed method the detection limit (LOD) is 0.312µg/ml and Limit of Quantitation (LOQ) is 0.625 µg/ml.

Table 5: pH measurement of the Admixed Solution

Temperature	pH (CTX +1.5% soln)	pH (CTX+2.3%Soln)
4°C	5.18-5.18	5.25-5.2
37° C	5.04 - 4.9	5.06-4.8
Room Temp.(Ambient)	5.15-5.08	5.05-5.06
40 °C	5.08-4.36	5.04-4.39

RESULT AND DISCUSSION

Cefotaxime concentrations from each sample were analyzed. Mean and standard deviation of cefotaxime was calculated for each triplicate sample taken from each of the 24 bags. For easier comparison of all results, the concentration at time 0 min was taken as 100% and that in all other samples was expressed as percentages of the initial concentration. Statistical significance was set at the 0.05 level of significance. Microsoft office excels (2007) spreadsheet was chosen to store, display raw data and to perform statistical calculation with a built in statistical function.

Peritoneal dialysis solutions contain various concentrations of electrolytes (calcium, magnesium, sodium, chloride) osmotic agents i.e. glucose, icodextrin, amino acid. Buffers i.e. bicarbonate, lactate / bicarbonate and different container (PVC, polyolefin) and an acidic pH of approximately 5.5 antibiotics are commonly added in the PD bags for the prophylaxis of infection and for the treatment of peritonitis. Therefore there is a need of assessment of the stability of antibiotics when mixed into PD bags. Chemical and physical

instabilities should be determined for commercially available bags prior to administering medications intraperitoneally **[6].**

During the present study, a range of temperature was chosen as 4°C, 24°C, 37°C and 40^{0}C to investigate the effect of temperature on stability. Because PD bags are commonly stored at room temperature for practical reasons, but refrigeration provides a longer shelf life and decreased microbial growth for the solution. While determination at body temperature is necessary because PD solutions are pre-warm before each exchange in order to increase comfort.

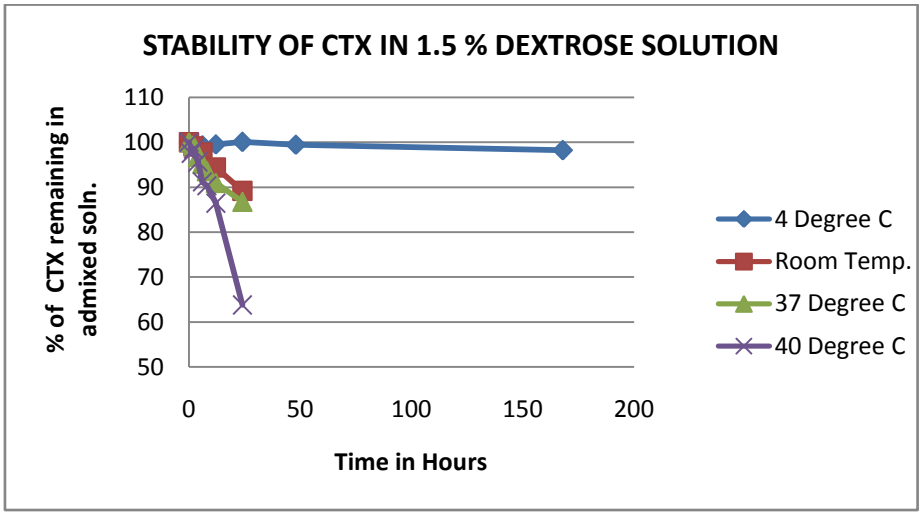
Validation of the methods was done by studying various parameters. Linearity was studied by analyzing 0.312 - 20 µg /ml (triplicate) concentrations of the drug prepared in the1.5 % and 2.3 % PD Solution separately (Table 3) and fitting the data into best-fitted curve (Fig 1)with r^2 = 0.992 and 0.997 respectively.

The Precision of the method was verified by Intraday and interday studies .No significant changes found in the pH of both admixed solution of 1.5% and 2.3 %.(Table 5).The stability data of cefotaxime in PD solution are shown in (Table 4), a decreasing concentration of drug were seen at each temperature for both 1.5 % and 2.3 % PD solutions (Fig 2).

Appearance of yellow color in dialysis solution was also observed. It was observed that color appeared in all samples except in the sample bags kept in refrigerator. The coloration started at 6hr in bags kept at 40°C, 8hrs at 37°C and 12 hrs at room temperature. This is due to the hydrolysis of the A 3-cephem ring occurs in cephalosporin in aqueous solution, but is obviously not sufficient to explain all these observations. On the other hand, purpose data agreed with the well-known yellowish coloration of cephalosporin during ageing as illustrated in the literature [11]. At the same time pH measurement showed a decrease of pH-units but it was not significant.

The results of present study support the potential clinical use of cefotaxime in PD solution.

When store in refrigerator, the admixed solution is stable for a week, 12 hrs at room temperature, up to 6 hrs at body temperature. Based on the literature, it is recommended that dialysis bags should be used immediately after warming to avoid possible degradation of drug prior to administration. Furthermore, any admixtures should be stored in refrigerator until ready for use then warmed and instilled as soon as possible. Finally, the practice of injecting the additive to many bags or several days' supply of bags at one time should be avoided.



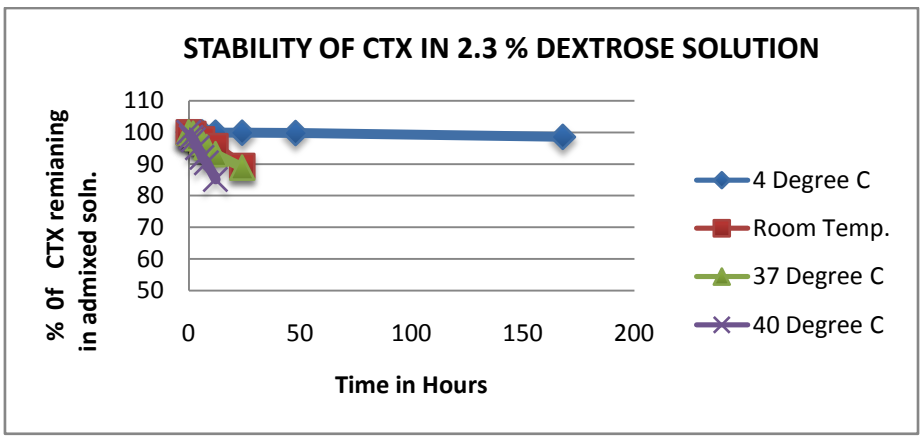


Fig. 2: Stability study of CTX in 1.5% and 2.3% dextrose solution.

Limitations of study

Several limitations exist in this stability analysis although the drug appears to be stable; the sterility of dialysis solutions over extended periods of time cannot be guaranteed and were not determined in this study. Despite the use of aseptic technique contamination may occur. For this reason, it is suggested that solutions should be refrigerated whenever possible. Further study may be warranted in order to determine the time by which microbial growth occurs.

CONCLUSION

The result indicates that Cefotaxime is stable in peritoneal dialysis solution at dextrose concentration of 1.5 % and 2.3 %, however it was found that CTX was influenced by higher storage temperature it was not stable for extended period of time which require precautionary measures and better storage conditions for climatic zone IV. Refrigeration of the sample provides long shelf life. Stability information of CTX added to dialysis bags may be useful in intraperitoneal use of this medication.

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