Cefotetan Stability in Normal Saline and Five Percent Dextrose in Water

Scott E. Walker and John Iazzetta

ABSTRACT

The stability of cefotetan disodium solution (1 g and 2 g) was evaluated using a validated, stability-indicating liquid chromatographic method during 31 days storage in PVC minibags containing 50 mL of 0.9% normal saline or 5% dextrose in water at 4°C and room temperature (23°C). Complete ultra-violet and visible spectra, pH, and physical inspections were also completed on each of the 14 study days.

During the 31-day study period all solutions stored at 4°C and 23°C lost more than 10% of the initial cefotetan concentration. At 4°C 10% of the initial concentration was lost within 15 days, and at room temperature 10% was lost within 4 days. During the 31-day study period the pH increased in every solution. However, the increase was less than 0.5 of a pH unit. The colour gradually changed during the study period towards a yellow, and all solutions had a sulphurous odour. Solutions stored at room temperature changed colour faster and more rapidly developed the sulphurous odour. A small shift in the UV-spectra of these solutions was also observed during the 31-day study period.

We conclude that cefotetan solutions (1 g or 2 g/50 mL) stored at 4°C for 7 days followed by 12 hours storage at room temperature will retain more than 93% of the initial cefotetan concentration.

Keywords: *cefotetan, stability*

RÉSUMÉ

La stabilité d'une solution de cefotetan disodique (1 g et 2 g/50 mL) fut évaluée pendant une période de 31 jours de conservation dans des mini-sacs en chlorure de polyvinyle contenant soit 50 mL de solution de chlorure de sodium à 0,9%, soit 50 mL de solution aqueuse de dextrose à 5%, et cela à 4°C et à température ambiante (23°C), au moyen d'une méthode validée de chromatrographie en phase liquide indicatrice de la stabilité. Les échantillons ont été examinés 14 fois pendant la période d'évaluation, et lors de chaque analyse, on a enregistré les spectres ultraviolet et visible complets, mesuré le pH et procédé à un examen visuel.

Au cours des 31 jours de l'étude, la concentration en cefotetan de toutes les solutions conservées à 4°C et à 23°C a diminué de 10% par rapport à la concentration initiale. À 4°C, la concentration a baissé de plus de 10% en 15 jours, et à température ambiante, elle a baissé de 10% en 4 jours. Durant l'étude, le pH de toutes les solutions a augmenté. Cependant, l'augmentation était inférieure à 0,5 unité de pH. En outre, toutes les solutions ont dégagé une odeur sulfureuse et ont jauni progressivement. Ces derniers changements se sont manifestés plus rapidement pour les solutions conservées à température ambiante. On observe également un léger déplacement du spectre ultraviolet pour ces solutions.

Nous concluons que les solutions de cefotetan (1 g ou 2 g/50 mL) entreposées à 4°C pendant 7 jours, puis pendant 12 heures à température ambiante conservent plus de 93% de la concentration intiale de cefotetan.

Mots clés: *cefotetan, stabilité*

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INTRODUCTION

Cefotetan disodium is a beta-lactamase-resistant broad spectrum cephalosporin. At the time that this study was proposed only one study had reported the stability of cefotetan.[1] However, the analytical method used by Smith[1] was microbiologic and since this may not be stability-indicating[2,3], the accuracy of the results is questionable. More recently a study by Gupta et al[4] evaluated the stability of cefotetan using a stability indicating assay. These investigators found approximately 10% loss after 2 days storage at room temperature and only six percent loss after 41 days of storage at 5°C.

The purpose of this investigation was to evaluate the chemical stability of cefotetan disodium, 1 g and 2 g in 50 mL of normal saline (NS) or 5 percent dextrose in water (D5W), stored at 4°C or 23°C for 31 days using a validated stability-indicating analytical method.

METHOD

Assay Validation

Following set-up of the liquid chromatographic (LC) system for cefotetan, the suitability of this system for use as a stability-indicating assay method was tested by accelerating the degradation of cefotetan. Cefotetan® disodium 26 mg (Cefotan®, Ayerst Laboratories, lot #1CDN-1D, potency 909 mg/g) was dissolved in 25 mL of water acidified with 1N hydrochloric acid. The final pH of this solution was 1.3 and the concentration after correction for potency was 0.945 mg/mL. This solution was placed in a 30 mL multidose vial (Bencard; Division of Beecham Laboratories) incubated in a water bath at 80°C and protected from light for 174 minutes. Samples were drawn just prior to incubation and at 10, 23, 31, 43, 58, 111, 148 and 174 minutes. Chromatograms were inspected for the appearance of additional peaks and the cefotetan peak was compared between samples for changes in concentration, retention time and peak shape. To further test if the chromatographic system could separate cefotetan from degradation products, the purity of the peak referred to in chromatograms as cefotetan was tested using a photodiode array detector (Waters, 990+). Complete UV-VIS spectra (200-800 nm, slit width 0.25 nm, resolution 1.4 nm, deuterium lamp) from the leading edge, middle and tail of the cefotetan peak in a degraded sample were compared to UV-VIS spectra observed for the time zero sample.

Following this first phase of evaluation and validation, the accuracy and reproducibility of standard curves were tested over a 5-day period and system suitability criteria developed. On each day 400 mg of cefotetan disodium powder (Cefotan®, Ayerst Laboratories, lot #1CDN-1D) was dissolved in 10 mL of water. Samples of this stock solution were diluted to 2 mL to obtain standards with final concentrations of 5, 10, 15, 20, and 30 mg/mL. These standards served to construct a standard curve. Each sample was chromatographed in duplicate. To test accuracy, a 32 mg/mL sample of cefotetan disodium (Cefotan®, Ayerst Laboratories, lot #1CDN-1D), prepared each day was chromatographed and its concentration determined and compared to its known concentration. Inter- and intra-day reproducibility were assessed using the coefficient of variation of the peak area for samples determined in duplicate. System suitability criteria (theoretical plates, tailing and retention time) were also established to ensure consistency between study days.

Stability Study

On study day zero, 48 - 1 g vials of cefotetan disodium (Cefotan®, Ayerst Laboratories, lot #1CDN-ID) were each reconstituted with 10 mL of sterile water for injection. Ten mL (1 g) was added to each of eight 50 mL PVC bags of normal saline and eight 50 mL bags of D5W. Four normal saline bags and four D5W bags were stored at 4°C and the other four of each solution were stored at room temperature.

Two vials (2g) were added to each of eight 50 mL PVC bags of normal saline and eight 50 mL bags of D5W. Four normal saline bags and four D5W bags were stored at 4°C and the other four of each solution were stored at room temperature (23°C). Each bag was sampled on each of the 14 study days (0, 1, 2, 4, 7, 9, 11, 15, 17, 21, 23, 25, 28, and 31).

Cefotetan LC Analysis

Using a 1-mL 27-gauge tuberculin syringe, samples of 0.5 mL were drawn from each of three containers on each study day. Three microlitres of each of these samples was directly chromatographed in duplicate.

Standards were prepared by dissolving 400 mg of cefotetan disodium powder (Cefotan®, Ayerst Laboratories, lot #1AVW-1C, potency 910 mg/g) in 10 mL of distilled water. Samples of this stock solution were then diluted to 2 mL to obtain standards with final concentrations of 5, 10, 15, 20 and 30 mg/mL. Three microlitres of each of these standards and a blank were directly chromatographed in duplicate, and used to construct a standard curve. An additional standard of 32 mg/mL prepared and chromatographed each day was used to calculate accuracy.

The chromatographic system consisted of an isocratic solvent delivery pump (Spectra Physics, Model 4200) which pumped a mixture of acetonitrile (Fisher, cat. #A998) and 0.025 molar potassium phosphate monobasic (Fisher, cat. #P286) through a 25 cm x 4.2 mm reversed-phase C-18, 5 μm column (Beckman, Ultrasphere ODS, cat. #235329) at 2 mL/min. The ratio of acetonitrile to phosphate buffer was 5:95 and was held constant during a chromatographic run. On each day the strength of the mobile phase was titrated to achieve a retention time for cefotetan between 340 and 425 seconds. Samples were introduced into the LC system using an autoinjector (Waters, WISP 715).

The column effluent was monitored with a variable wavelength ultra-violet detector (Applied Biosystems, Model 759A) at 290 nm. A signal from the detector was integrated and recorded with a chromatographic integrator (Spectra Physics, Model 4240). The area under the cefotetan peak at 290 nm was reported and used to calculate the cefotetan concentration.

Standard curves were prepared daily, as previously described. The area under the cefotetan peak was subjected to least squares regres-

sion and the actual cefotetan concentration of each solution was interpolated from these curves and recorded to the nearest 0.01 mg/mL.

Physical Evaluation

On each of the 14 study days the pH of one container of each solution stored at each concentration and temperature was measured and recorded to the nearest 0.001 of a pH unit. The pH meter (Fisher, Accumet, model 925) was fitted with a microprobe glass body electrode (Fisher: cat #13-639-280) and was standardized each day with two commercially available buffer solutions.

On each of the 14 study days, each container was inspected visually for colour and clarity. Visual particulate matter inspection was performed against a black and white background.

UV-Visible Absorption Spectra

On each study day a complete UV-VIS spectrum (200 nm to 800 nm) was recorded using a photodiode array detector (Waters, 990+, resolution 1.4 nm, band width 1 nm). One microlitre of each solution was injected directly into the flow cell of the photodiode array detector (PDA) and the UV-VIS spectrum was recorded and archived on computer diskette. This is a more extensive test than measuring the absorbance at one discrete wavelength, since the entire spectrum is evaluated. Whole spectra from each sample were then compared for maxima, minima and overall similarity using PDA 990+ software (Waters). Identical spectra have a 100% match +/- 1%. Spectra from each study day were matched against a standard, and the match (expressed as a percentage) was recorded.

Data Reduction and Statistical Analysis

Means (+/- standard deviation) were calculated for analyses completed in duplicate or triplicate. Reproducibility was assessed by coefficient of variation (CV). Mean results from different days of an identical test were compared statistically by least squares linear regression to determine if an association existed between the observed result and time. Log-linear and linear-linear fits for the data from the accelerated degradation study (80°C) were compared for goodness of fit by the Maximum Likelihood Method of Box and Cox.[5,6] Analysis of variance and the least significant difference multiple range test or Student's t test (where appropriate) were used to compare differences between temperature, and/or solutions for similar analytical tests. The five percent level was used as the apriori cut-off for significance.

Cefotetan concentrations were considered "acceptable" or "within acceptable limits" if the concentration on any day of analysis was not less than 90% of the initial (day-zero) concentration.

RESULTS

Accelerated Degradation and Assay Validation

When dissolved in water acidified to a pH of 1.3 and heated to 80°C, cefotetan degraded rapidly. The rate of degradation was described better by a first-order (log-linear) degradation process in which the coefficient of determination (r^2) was 0.9983 rather than a zero order rate in which the coefficient of determination was 0.8217. The half-life of degradation was 25 minutes under these conditions and so after 111 minutes less than 5% of the initial cefotetan concentration remained and at least one major degradation product was observed (Figure 1). This study was terminated at 174 minutes since no cefotetan could be detected at this time. The peak corresponding to cefotetan in each chromatogram was evaluated for UV-VIS spectrum similarity. UV-VIS spectra from 200-800 nm observed in the leading edge, middle, and tail of cefotetan peaks from each sample

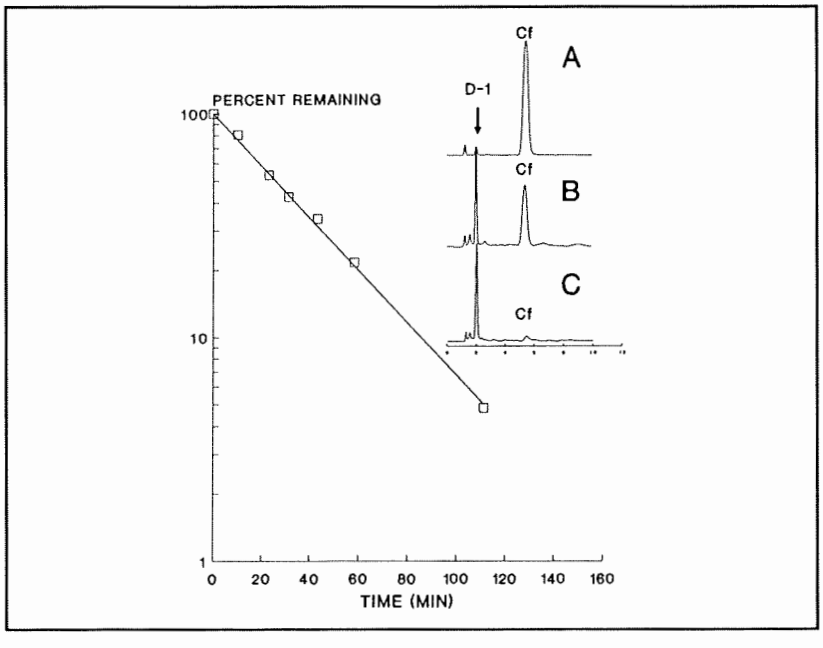

Figure 1. Rate of loss of cefotetan during the accelerated study at 80 C at pH = 1.3. As the cefotetan concentration declined, a degradation product (D-1) could be observed in chromatograms (Inset panels B and C, represent chromatograms of samples taken at 31 and 111 minutes respectively compared to time zero — Panel A). Cf represents cefotetan.

in the accelerated study and a reference standard were identical with respect to maxima, minima and overall shape.

These results (predictable degradation, and observed separation of degradation products and cefotetan), indicated that this analytical method was stability-indicating.

Accuracy, and reproducibility were then evaluated. Cefotetan (400 mg) was added to 10 mL of water and the concentration of this sample was estimated. The results of this investigation indicated that the cefotetan concentration was measured accurately (recovery, based on the mean of duplicate determinations was within 97%-103%). Intra-day reproducibly was acceptable (CV determined on six replicates averaged 1%) as was the inter-day reproducibility (CV of samples tested on different days averaged 3.5%). This indicates that differences of 8% or more can be confidently detected with acceptable error rates.[7,8] System suitability criteria were developed based on this data (theoretical plates, tailing, retention time and accuracy) and were used to ensure continued chromatographic performance during the study period.

Table I: Mean* Cefotetan Concentrations (mg/mL) in Normal Saline

STUDY DAY	2 g/50 mL @ RT	1 g/50 mL @ RT	2 g/50 mL @ 4°C	1 g/50 mL @ 4°C
0	30.06 ± 3.56	17.73 ± 1.96	30.84 ± 1.23	17.73 ± 4.55
1	27.99 ± 2.12	16.01 ± 1.42	28.18 ± 0.75	16.82 ± 4.14
2	28.00 ± 1.94	16.51 ± 3.90	29.35 ± 0.28	17.37 ± 4.05
4	28.00 ± 3.16	15.15 ± 1.91	29.10 ± 1.23	16.65 ± 5.19
7	20.65 ± 1.47	12.88 ± 4.53	24.46 ± 1.73	15.30 ± 7.04
9	22.54 ± 2.41	12.94 ± 2.86	27.21 ± 0.45	16.11 ± 4.53
11	21.52 ± 4.14	12.20 ± 2.24	26.43 ± 0.00	15.69 ± 4.27
15	19.68 ± 1.71	11.58 ± 2.76	26.20 ± 0.51	15.74 ± 4.53
17	18.36 ± 3.09	10.47 ± 3.47	25.53 ± 0.64	15.27 ± 4.30
21	16.75 ± 4.98	9.20 ± 2.79	24.94 ± 0.64	14.63 ± 5.13
23	16.14 ± 3.41	9.17 ± 2.91	25.14 ± 0.69	15.08 ± 3.98
25	14.79 ± 3.13	8.19 ± 3.17	24.56 ± 1.56	14.35 ± 4.18
28	13.30 ± 3.08	7.41 ± 1.48	23.32 ± 0.39	N/A
31	12.35 ± 3.27	6.78 ± 2.36	23.37 ± 0.47	13.66 ± 4.56
% remaining (Day 31/Day 0)	41.08%	38.24%	75.77%	77.04%
First Order Rate Constant (day^{-1})	0.02727	0.02907	0.00727	0.00709
r^2 Log-linear	0.9706	0.9875	0.7785	0.8840
T_{90} (days)	3.85 d	3.61 d	14.44 d	14.81 d

* Concentrations are reported as the mean (± standard deviation) of three PVC minibags, each determined in duplicate on each study day.

Table II. Mean* Cefotetan Concentrations (mg/mL) in 5% Dextrose in Water

STUDY DAY	2 g/50 mL @ RT	1 g/50 mL @ RT	2 g/50 mL @ 4°C	1 g/50 mL @ 4°C
0	29.24 ± 4.74	16.98 ± 3.83	28.04 ± 1.44	16.77 ± 3.54
1	28.59 ± 1.97	17.00 ± 4.22	27.69 ± 1.46	16.71 ± 4.77
2	28.34 ± 3.40	16.02 ± 3.66	28.25 ± 1.79	16.35 ± 4.49
4	28.59 ± 5.19	14.66 ± 5.07	28.55 ± 1.96	14.85 ± 6.94
7	23.10 ± 4.79	11.81 ± 2.29	24.52 ± 4.09	14.30 ± 4.45
9	22.66 ± 3.62	13.29 ± 2.63	26.42 ± 1.11	15.81 ± 4.79
11	20.55 ± 5.26	11.56 ± 3.63	24.23 ± 1.17	N/A
15	19.33 ± 3.52	11.36 ± 3.49	25.04 ± 0.87	15.26 ± 4.87
17	18.06 ± 3.86	10.50 ± 3.46	24.35 ± 1.07	14.63 ± 4.53
21	15.41 ± 3.92	8.59 ± 3.26	22.86 ± 1.09	13.47 ± 4.18
23	15.79 ± 4.03	9.09 ± 2.42	24.00 ± 1.24	14.58 ± 4.39
25	14.44 ± 4.25	8.04 ± 3.94	23.48 ± 0.50	13.77 ± 4.62
28	12.78 ± 5.06	7.16 ± 2.89	21.94 ± 1.02	13.06 ± 4.49
31	12.09 ± 4.52	6.64 ± 3.41	22.20 ± 0.89	13.22 ± 5.17
% remaining (Day 31/Day 0)	41.35%	39.10%	79.17%	78.83%
First Order (k) ($days^{-1}$)	0.02883	0.02942	0.00792	0.00693
r^2 Log-linear	0.9932	0.9704	0.8500	0.7668
T_{90} (days)	3.64 d	3.569 d	13.26 d	15.14 d

* Concentrations are reported as the mean (± standard deviation) of three PVC minibags, each determined in duplicate on each study day.

Cefotetan Stability Study

Over the 31 day study period there was a significant trend for cefotetan concentrations to decrease in solutions containing normal saline (Table I) and D5W (Table II) at 4°C and 23°C. After 31 days of storage, saline and D5W solutions stored at 4°C retained between 75 and 80% of the initial cefotetan concentration and solutions stored at room temperature retained between 38 and 42% (Tables I and II). More than 10% of the initial cefotetan concentration was lost after 4 days storage at room temperature and 15 days storage at 4°C (Table III).

During the study, relative to a known concentration of 32 mg/mL, accuracy averaged 101.5% ± 1.1% and was always within 3% of the known concentration (range 98.0 - 102.9%). Additional peaks, similar to those observed during accelerated degradation studies, increased in relative size during the 31-day study period. Although the apparent concentration of these degradation products increased during the study as cefotetan concentrations declined, the concentration of these products was not quantified.

No significant difference in degradation rate was apparent between concentrations of 2 g/50 mL and 1 g/50 mL, nor was there any significant difference in degrada-

Table III. Degradation Rate Calculations

Mean Degradation Rate, Combined Data.

	Degradation Rate (days $^{-1}$)	T_{90} (days)	Percent Remaining After 12 hours	24 hours	7 days
at 4°C	0.00730	14.38	99.64	99.27	95.02
at 23°C	0.02864	3.67	98.58	97.18	81.83

Estimated percent remaining after 7 days at 4°C and 12 hours at 23°C
% remaining = (.9502 x .9858) x 100 = 93.67%

Fastest Degradation Rate

	Degradation Rate (days $^{-1}$)	T_{90} (days)	Percent Remaining After 12 hours	24 hours	7 days
at 4C	0.00792	13.26	99.60	99.21	94.61
at 23°C	0.02942	3.57	98.54	97.10	81.39

Estimated percent remaining after 7 days at 4°C and 12 hours at 23°C
% remaining = (.9461 x .9854) x 100 = 93.23%

tion rate between solutions (Tables I and II). However, temperature (4°C vs 23°C) did have a significant effect on degradation rate, such that a greater rate of loss of cefotetan concentration was observed at room temperature (Table III).

During the 31 day study period pH decreased in all solutions stored at 4°C and 23°C. However, the pH remained between 4.17 and 4.74 and all changes were less than 0.5 of a pH unit and there was no apparent dependency on solution type, cefotetan concentration or storage temperature.

The colour gradually changed during the study period towards a yellow, and all solutions had a sulphurous odour. Solutions stored at room temperature changed colour faster and more rapidly developed the sulphurous odour. UV-VIS absorbance spectra determined for solutions stored at 4°C and 23°C on each day demonstrated a shift in maxima and minima during the study period. Spectra determined on study day zero were not superimposable on spectra from study day 31. For solutions stored at room temperature, the degree of similarity was reduced from 100% on study day zero to 94.7% and 95.3% by day 31 for D5W and normal saline solutions, respectively. Solutions stored at 4°C also demonstrated a shift in maxima and minima (97.9% and 97.8% for D5W and normal saline solutions), respectively).

DISCUSSION

Statistical analysis of the cefotetan concentration time data in this study was limited to least squares log-linear regression, because demonstration of a trend for the concentration to decrease was considered more important than demonstrating a statistical difference in concentration between any two days. In fact, the random fluctuations in concentration around a line of 'best fit' are not of practical importance and should be considered 'noise' or experimental error.

Least squares log-linear regression indicated that a 10% loss in the initial cefotetan concentration was observed after less than 4 days storage at room temperature and within 15 days storage at 4°C (Table III). However, a recommended expiry date must consider that a prepared product will be stored for a period of time at both 4°C and room temperature. Using the first order degradation rates observed in this study (Table III) it is estimated that greater than 93% of the initial cefotetan concentration would remain after 7 days storage at 4°C followed by an additional 12 hours storage at room temperature.

Two other studies have evaluated the stability of cefotetan. The study reported by Smith[1] was completed using a microbiologic assay which may not have been stability indicating.[2,3] This study found no change from the initial concentration and no difference in percent remaining between solutions stored for 14 days at 4°C and room temperature. The study by Gupta et al[4] used a stability indicating assay and found approximately 10% loss after 2 days storage at room temperature. This is similar to the results observed in our study. However, after 41 days storage at 5°C, Gutpa et al[4] reported that approximately 94% of the initial cefotetan concentration remained. This must be compared to the observation of 75% to 80% remaining (Tables I and II) after 31 days storage at 4°C in our study. This difference in results is significant and cannot be easily reconciled since both studies used a stability indicating liquid chromatographic method and prepared standards each day from cefotetan disodium powder.

We conclude that freshly reconstituted cefotetan powder used to prepare solutions (2 g/50 mL and 1 g/50 mL in NS or D5W) retain more than 90% of the initial cefotetan content when stored for less than 4 days at 23°C or for less than 15 days when stored at 4°C. However, we recommend that after consideration of sterility and the contamination rate in an IV admixture program that an expiry date not exceeding seven days storage at 4°C be established. With such an expiry date (which allows for up to an additional 12 hours of storage at room temperature) it is estimated that more than 93% of the initial cefotetan concentration will remain.

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