# Chemical Stability of Cefotetan Disodium in 0.9% Sodium Chloride Sterile Saline Solutions and Storage in Polypropylene Syringe

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## الملخص

**الثباتية الكيميائية لمحلول السيفوتيتان ثنائي الصوديوم في المحلول المعقم 0.9% من كلورات الصوديوم و تخزينه في إبر البوليبروبيلين.**

**هدف البحث:** هدف هذه الدراسة هو تقدير الثباتية الكيميائية لمحلول السيفوتيتان ثنائي الصوديوم (60ملغم/ مل) وذلك بعد إعادة تحضيره في المحلول المعقم 0.9% من كلورات الصوديوم وتخزينه في إبر البوليبروبيلين على درجة حرارة الغرفة ( 25±1 درجة مئوية) وعلى 5±1 درجة مئوية وذلك باستخدام جهاز التحليل الكروماتوغرافي السائلي ذى القدرات العالية.

**الأسلوب:** تم تحضير محلول من السيفوتيتان ثنائي الصوديوم (60 ملغم/ 1مل) وذلك بتذويب حقنة 1 غم المتوفرة في صيدليات المستشفيات في المحلول المعقم 0.9% كلورات الصوديوم، تمت تعبئة المحلول في إبر 5 مل مصنوعة من مادة البوليبروبيلين حيث تم تخزين بعضها على درجة حرارة الغرفة و البعض الآخر على درجة حرارة 5 مئوية. تراكيز السيفوتيتان تم تقديرها بواسطة الفحص الكروماتوغرافي السائلي ذو القدرات العالية و ذلك في الأيام 0، 1، 2، 3، 6، 12، 18 ،24، و 30، تم حساب تركيز الدواء عن طريق مقارنة المساحة تحت المنحنى .تم حساب الانحراف المعياري المئوي على 5 تراكيز و كان 0.9%. كما أن درجة الحموضة بالإضافة إلى رائحة و لون المحلول تمت مقارنتهما.

**النتائج:** كان هناك وجود لمادة متفككة حيث تم فصلها عن المادة الأم. النقص في تركيز المحلول كان 9% بعد 6 أيام من التخزين عند درجة حرارة الغرفة بينما كان 2% في المحلول المخزن عند درجة حرارة 5 مئوية.

مقدار الحامضية زاد من 5.7 إلى 6 بعد 30 يوم من التخزين على درجة الحرارة الأخيرة.

لم يكن هناك ادمصاص على الإبر البلاستيكية. زادت شدة الإصفرار في المحلول خلال فترة التخزين عند درجة حرارة الغرفة لكنها لم تتغير بشكل واضح في المحلول المخزن عند درجة حرارة 5 مئوية.


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**الاستنتاج:** سيفوتيتان ثنائي الصوديوم 60 ملغم/ 1مل في محلول كلورات الصوديوم والمخزن في إبر البوليبروبيلين كان ثابتا" لمدة 6 أيام عند حفظه على درجة حرارة 25 مئوية ولمدة 24 يـــوم عند حفظه على درجة حرارة 5 مئوية.

# ABSTRACT

**Purpose:** The purpose of this study is to evaluate the stability of (60 mg/ml) Cefotetan disodium (CTT) in 0.9% sodium chloride sterile saline solution stored in polypropylene syringes at ambient temperature (25 ± 1°C) and 5 ± 1°C by the use of a stability indicating high performance liquid chromatographic assay method. **Methods:** Solutions of CTT (60 mg/ml) were prepared from commercially available 1 g lyophilized vials in normal saline. They were filled into 5-ml polypropylene syringes and stored at ambient and 5°C temperatures. The strengths of CTT were determined by high performance liquid chromatographic assay after 0, 1, 2, 3, 6, 12, 18, 24 and 30 days. The concentrations of the drug were directly related to the peak area, and the percent relative standard deviation based on 5 concentrations was 0.9. The initial and final pH values were recorded and compared, also the initial and final color of the solution were compared. **Results:** There was at least one decomposition product which was separated from the parent drug. The loss in potency was less than 9% after 6 days of storage at ambient temperature, while the loss of potency at 5°C was less than 2%. The pH value increased from 5.7 to 6 when the injection was stored at 5 °C for 30 days. The drug was not adsorbed onto the plastic syringes. The intensity of the light yellow color was increased during the storage period at ambient temperature but didn't change significantly with storage at 5 °C. **Conclusion:** Cefotetan 60 mg /ml in normal saline solution stored in polypropylene syringes was stable for 6 days at 25 °C and for 24 days when stored at 5 °C.

## INTRODUCTION:

Cefotetan disodium (CTT) is a sterile, semisynthetic, broad spectrum, betalactamase resistant, cephalosporin (Cephamycin) antibiotic used for ministration (Figure 1). Its solution varies from colorless to yellow depending on the concentration. The pH of the solution is usually in the range of 4.5 to 6.5 depending on the concentration and the excipients used in the formulation of the lyophilized powder [1, 2]. CTT is used for the treatment of infections caused by certain bacteria in many different parts of the body, including the abdomen, chest and women's reproductive organs. It may also be given to prevent infections before, during and after surgery [3]. In a clinical study made in pediatric field, CTT showed a remarkable therapeutic effect on cystitis and pyelonephritis. Neither adverse clinical reactions nor abnormal laboratory findings were signed. Basing on this finding CTT is considered to be appropriate and useful in the treatment of bacterial infections especially urinary tract infections in children [4]. The favorable clinical response could be achieved by the use of doses of 30mg /Kg of body weight being given twice daily at intervals of 12 hours [5]. It is well known that the most important issue of cephalosporin's parenteral solutions is related to their chemical stability after their reconstitution and storage in polyvinyl chloride mini bags and polypropylene syringes. Therefore the stability of many parenteral cephalosporins like Cefazoline sodium, Cefuroxime, Ceftazidime and other injectable drugs was evaluated after their reconstitution and stoage in polyvinyl chloride bags [6,7,8,9]. Moreover, other researchers studied the stability of Cefazoline sodium, Cefotaxime and other cephalosporins after their reconstitution and storing in polypropylene syringes for pediatric use [10,11]. S.E Walker & J. Iazzetta [12] studied the stability of CTT in 0.9% sodium chloride injection and 5% dextrose injection at concentrations of 20 mg/ml and 40 mg/ml. The resulting injections were stored in polyvinylchloride mini bags at 4°C and at (23 ±1°C). The loss in potency was less than 10% of the initial concentration after 4 days of storing at 23°C and after 15 days of storage at 4°C. In another similar study, V. Das Gupta et al [13] studied the chemical stability of CTT in 5% dextrose and 0.9% sodium chloride injections. In this study the drug appears to be relatively unstable at 25°C (expiry time 2 days), compared with at least 41 days at 5°C. The manufacturer recommends that after the reconstitution of CTT vials, (vial contents are dissolved in 0.9% sodium chloride injection to a concentration of 60 mg/ml) the resulting admixture should be used within 12 hours when stored at ambient temperature and within 7 days when stored in the refrigerator at 5

°C [14]. Therefore this study was conducted to evaluate the stability of CTT injection (60 mg/ml) in 0.9% sodium chloride after storage in polypropylene syringes at 25 ±1°C (ambient temperature) and at 5 ±1°C and to investigate adsorption of the drug onto the plastic syringes or not.

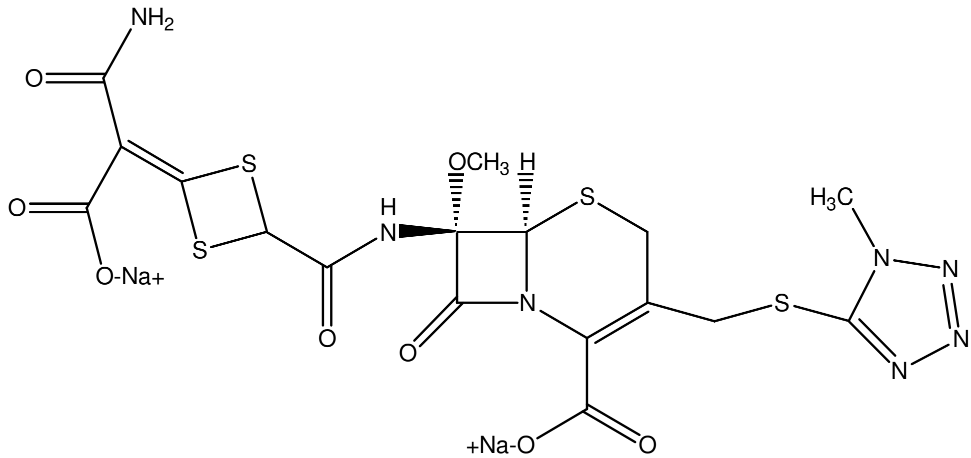

Figure 1. Chemical structure of Cefotetan disodium (CTT).

# MATERIALS AND METHODS:

## Chemicals & Reagents

All chemicals and reagents used in this study were of United State Pharmacopoeia/ National Formulary (USP/NF), American Chemical Society (ACS) and Merck grades and they were used without further purification. The CTT powder for injection was from a commercial lot ( Zeneca, S. P. A., Milan, Italy).

## **Equipment**:

A high performance liquid chromatographic apparatus (HPLC) (Par-2000 and UV 2070 multiple wavelength detector, Jasco, UK) provided with an injector (model 7125, Rheodyne, Cotati, California) and recorder (Omniscribe, Houston Instruments,) were used. A reverse phase hypersil BDS C-18 column of 15 cm dimensions, 4.6 mm ID, 5µm, (Supleco Company, Bellefonte) was used. All pH values were measured with a pH 211 microprocessor pH meter (Hanna Instruments, Woonsocket, Rhode Island).

## Chromatographic Conditions:

The mobile phase is composed of 15 volumes of acetonitrile and 85 volumes of a 17 mM dipotassium phosphate buffer solution at pH 3. The wavelength of the detection was at 287 nm. The injection volume of each solution was 80µl. The flow rate was 0.8 ml /minute at pump pressure of 2500 psi. The sensitivity was 0.9 AUC at 287 nm, and the temperature was ambient.

## Preparation of injection for stability study

CTT (1g per vial of free Cefotetan di acid) was used to prepare the injection solutions for stability assessment, 9 grams CTT powder for injection was dissolved in 0.9% w/v sodium chloride solution for injection to bring it to 150 ml (60 mg of CTT per milliliter of injection). The solution was immediately filled into 5 ml polypropylene syringes. The syringes were divided into two groups (12 syringes/group); one group was stored at 25 ± 1°C and another group was stored at 5 ± 1°C. The solution was assayed and the pH was measured immediately in order to be used as a reference value for the study. The content and pH of the solution in the syringes were tested again at the following time intervals: 1, 2, 3, 6, 12, 18, 24 and 30 days. The concentration and pH values were recorded and the color of the solution was detected. A volume of 30 ml of the injection solution was also stored at 25 ± 1°C in a 50 ml glass volumetric flask in order to investigate if the drug was adsorbed into the polypropylene syringes, or not.

## Preparation of standard solutions

A quantity equivalent to 100 mg of CTT powder for injection was accurately weighed (107.64 mg is equivalent to 100 mg of Cefotetan diacid) and dissolved in sufficient distilled water to give 100 ml of the solution. The stock solution was used to prepare solutions of lower strength. The internal standard in this study was not used due to low value of standard deviation (0.9 as a result of the use of 80 µl for each injection). The most commonly used standard solution of the drug (100µg/ml) was prepared by diluting 2.5 ml of the stock solution to 25 ml with water. This concentration was adopted in order to minimize the interference of the solvent peaks with the drug chromatogram (Figure 2).


Figure 2. A Chromatogram of Cefotetan Disodium Standard Solution

## Preparation of assay solution:

A volume of 2 ml of the tested solution was diluted to 100 ml with water and 2.5 ml of this solution was taken and transferred into a 25 ml glass volumetric flask and water was added up to volume.

## Decomposition of Cefotetan disodium:

The capacity of the stability indicating assay method was also evaluated. Decomposition under basic conditions was accelerated by heating the CTT stock solution in 0.01M NaOH at 50 °C for 1.5 hours (higher temperature caused complete decomposition of CTT in very short time). The solution was cooled, and the pH was neutralized with 0.01 M HCl solutions. Samples were assayed after dilution to a concentration of 100 μg /ml (Figure 3).


Figure 3. A Chromatogram of Cefotetan disodium solution that was decomposed by use of heat and 0.01M NaOH.

### Assay Procedure and Calculations

A volume equal to 80 µl of the assay solution was injected under the conditions described. To carry out the comparison another equal volume of the standard solution, which contains the same concentration of the drug was also injected. Because peak area of the drug was directly related to its concentration (25 – 125 µg/ml), the results were evaluated by the use of the simple equation $[(AUC)_a/(AUC)_s].100$ = Percentage of label claim amount, where $(AUC)_a$ and $(AUC)_s$ are the area under the curve of the drug in the assay solution and area under the curve of the drug in the standard solution respectively.

## RESULTS & DISCUSSION:

### Assay Method:

The assay method used for this study was developed by Marini D. et al[15], who found it precise and accurate, with intra-day variability being 0.1% to 1.13% and the inter-day variability being 1.6%; the percent standard deviation (SD) was 0.9, based on five readings. In fact the large volume of the injected sample (80 µl) produced accurate and precise peaks without the use of an internal standard; also this volume minimized the interference between the solvent and the peaks of the drug. The concentration of the drug was directly proportional to the area under the curve (range tested from 25 to125 µg/ml) demonstrating a linearity of the method (Table 1). The volume of the injected sample (80 µl) must remain constant to ensure the linearity between the concentrations of the drug and the peak areas. The standard solution, which was decomposed by heat, showed another additional peak from the products of decomposition. The peak of decomposition eluted after the drug peak without interfering with the peak of CTT; thus, the method can be considered specific for CTT. The potency of CTT decreased to 99,3% (Table 2) after 1 day of storage at 25 °C, and the pH was increased from 5.5 to 5.7. After 6 days storage at 25 °C, the potency was 91.01%. After 12 days of storage the potency decreased to 84.4%.

**Table 1. Relation between the concentration and the area under the curve of five Cefotetan disodium concentrations.**

| Concentration of drug (µg/ml) | Area Under the Curve (AUC) |
|---|---|
| 25 | 89.95 |
| 50 | 119.50 |
| 75 | 145.49 |
| 100 | 181.44 |
| 125 | 212.43 |

**Table 2. Effect of storage temperatures on decomposition of Cefotetan disodium injection (60 mg /ml) in polypropylene syringes.**

| Time (Days) | Percentage of remaining concentration of drug at: | |
|---|---|---|
| | 25 °C | 5 °C |
| 0 | 100 | 100 |
| 1 | 99.31 | 99.8 |
| 2 | 98.60 | 99.3 |
| 3 | 96.21 | 98.6 |
| 6 | 91.01 | 98.02 |
| 12 | 84.40 | 96.10 |
| 18 | a | 94.3 |
| 24 | a | 91.80 |
| 30 | a | 89.92 |
| a: analysis was not carried out at this interval | | |

During the 30 days of the study period the pH increased in all solutions (due to the alkaline character of the product of decomposition). However, the increase was less than 0.4 of a pH unit. The color was gradually changed during the study period at room temperature towards yellow but no detectable change in the color of the solution stored at 5 °C. Regarding the odor of the solutions, all samples had sulphurous odor, but the intensity of this odor was increased faster at room temperature than at 5 °C. At 25 °C a beyond – used date (BUD) of 6 days is the maximum. Since the potency of CTT decreased to 91.01 % after storage for 6 days, a BUD of 6 days is accepted by the united states pharmacopoeia [8, 16]. At 5 °C, the potency after 24 days of storage at 25 °C was 91.8% and after 30 days the loss of potency was about 10 % (which is accepted by the united states pharmacopoeia) and the pH value of the injection had increased up to 0.4. Therefore at 5 °C, a BUD of at least 24 days is appropriate. Obviously, there was no adsorption of the drug on the plastic syringes, since the injection that was stored in the glass volumetric flask for 1 day gave the same results as the sample stored in the polypropylene syringes (Table 3).

**Table 3. Percentage of remaining concentrations and the correspondent AUC of Cefotetan disodium stored in a polypropylene syringe and in a glass volumetric flask after 24 hours.**

| Type of container | Drug Concentration (μ/ml) | AUC |
|---|---|---|
| Polypropylene syringes | 99.33 | 180.16 |
| Glass bottle | 99.32 | 180.15 |

## CONCLUSION:

The results obtained in this study represent a practical value in clinical pharmacy, since they demonstrate that reconstituted formulation CTT (60 mg/ml) in 0.9% sodium chloride injection stored in 5-ml polypropylene syringes was stable for 6 days at room temperature and for at least 24 days at 5 °C. Precisely this last data suggests that the storage of the formulation at 5 °C prolongs its shelf – life stability to 24 days. This increase of BUD improves the work in hospital pharmacy with increase of economical benefits due to the reduction of the cost of preparation.

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