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EFFECT OF pH, PHOSPHATE BUFFER CONCENTRATION AND THE IONIC STRENGTH ON THE STABILITY OF CEFOTETAN DISODIUM

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ABSTRACT

The stability of cefotetan disodium between the pH range 2-8 has been studied using a stability-indicating HPLC assay method. The optimum pH range of stability appears to be 3.6-6.4. At pH 2 or 8, the decomposition was approximately 2 times faster than between pH 3.6-6.4. The HPLC method used is accurate and precise with percent relative standard deviation of 1.0 based on 5 readings. The decomposition of cefotetan followed first-order law. The K_h and K_{oh} values were estimated to be 3.8 and 4.4×10^4 d^{-1}.

BACKGROUND

Cefotetan disodium is a semisynthetic broad spectrum, beta lactamase stable, cephalosporin (cephamycin) antibiotic for parenteral use. The plasma elimination half-life of cefotetan is 3 to 4.6 hours, allowing twice daily administration for the treatment of indicated infections[1]. In spite of its extensive use in medicine, no information is available about its degradation in the aqueous solutions. Gupta et al reported[2] the chemical stability of cefotetan disodium in 5% dextrose and 0.9% sodium chloride injections. This study was conducted using a stability-indicating HPLC assay method.

The purpose of these investigations was to study the effect of pH, phosphate buffer concentration and the ionic strength on the stability of cefotetan disodium.

MATERIALS AND METHODS

Materials: All the chemicals and reagents were USP, NF or ACS quality and used without further purification. Cefotetan disodium powder (Stuart Pharmaceuticals) was used as received. Cephalexin powder for the internal standard was generously supplied by E. Lilly & Co.

Equipment: A high-pressure liquid chromatograph (ALC 202, Waters Associates) equipped with an injector (Rheodyne Model 7125), a multiple wavelength detector (Schoeffel's SF770, Applied Biosystems) and a recorder (Omniscribe 5312-12, Houston Instruments) was used. A Med. Pharmex C_{18} column (Part No. MP 002, 25 cm x 4.6 mm i.d.) was used.

Chromatographic Conditions: The mobile phase contained 6% V/V of acetonitrile in 0.02M KH_2PO_4 aqueous buffer solution. The flow rate was 2.0 ml/min, the sensitivity was 0.04 AUFS at 262 nm, the chart speed was 30.5 cm/hr and the temperature was ambient.

Preparation of Cefotetan Disodium Solutions for Stability Studies: A 1% solution of cefotetan disodium in water was prepared as the stock solution. A 10.0 ml quantity of the solution was diluted with a phosphate buffer of an appropriate pH value, buffer concentration, and the ionic strength (Table 1) to 100.0 ml. After the initial data (physical appearances, pH values and the assays), the solutions were stored at

TABLE 1

LIST OF SOLUTIONS OF CEFOTETAN DISODIUM (1.0 mg/ml) PREPARED FOR STABILITY STUDIES

Solution No.	Final Concentration of Phosphate Buffer	Final Ionic Strength[a]	Final pH Value (± 0.05)
1	0.09	0.27	2.00
2	0.09	0.27	3.10
3	0.09	0.27	3.90
4	0.09	0.27	4.85
5	0.068	0.27	4.85
6	0.045	0.27	4.85
7	0.09	0.21	4.85
8	0.09	0.14	4.85
9	0.09	0.27	5.85
10	0.09	0.27	6.90
11	0.09	0.27	8.00

[a] All ionic strengths were adjusted with potassium chloride.

room temperature (25° ± 1°) in 4 ounce amber-colored bottles. The data were recorded again at the appropriate intervals.

Preparation of Stock and Standard Solutions for Analysis: A 0.1% stock solution each of cefotetan disodium and cephalexin in water were prepared fresh daily. A 1.5 ml quantity of the stock solution of cefotetan disodium was mixed with 2.0 ml quantity of the stock solution of cephalexin (the internal standard) and the mixture brought to volume (50 ml) with the mobile phase.

Preparation of Assay Solutions: A 1.5 ml quantity of the assay sample was mixed with 2.0 ml quantity of the stock solution of cephalexin and the mixture brought to volume (50 ml) with the mobile phase.

Assay Procedure: A 30 µl quantity of the assay solution was injected into the chromatograph using the conditions described above. For purpose of comparison, a similar volume of the standard solution was injected after the assay sample eluted. The standard solution contained identical concentrations of cefotetan disodium (based on the label claim) and the internal standard.

Calculations: Preliminary investigations indicated that the ratio of the peak heights (drug/internal standard) were directly related to the concentrations of cefotetan disodium (range tested ± 50% of the standard concentration), therefore, the results were calculated using a simple equation:

$$\frac{(R_{ph})a}{(R_{ph})s} \times 100 = \text{Percentage of the label claim found}$$

where $(R_{ph})a$ is the peak height ratio of the assay sample and $(R_{ph})s$ that of the standard solution.

RESULTS AND DISCUSSION

HPLC Assay Method - The method used was developed earlier[2] and is stability-indicating. The method developed used benzoic acid as the internal standard whose peak was very broad because of a higher retention time[2]. In these studies, a new internal standard, cephalexin, was developed which gave a sharp peak (peak 2, Figure 1) and had the advantage of being a compound similar to the drug. In earlier studies, a wavelength of 238 nm was used to increase the absorption of benzoic acid (the internal standard). In these studies, a wavelength of 262 nm was selected because the absorptions of both the drug and internal

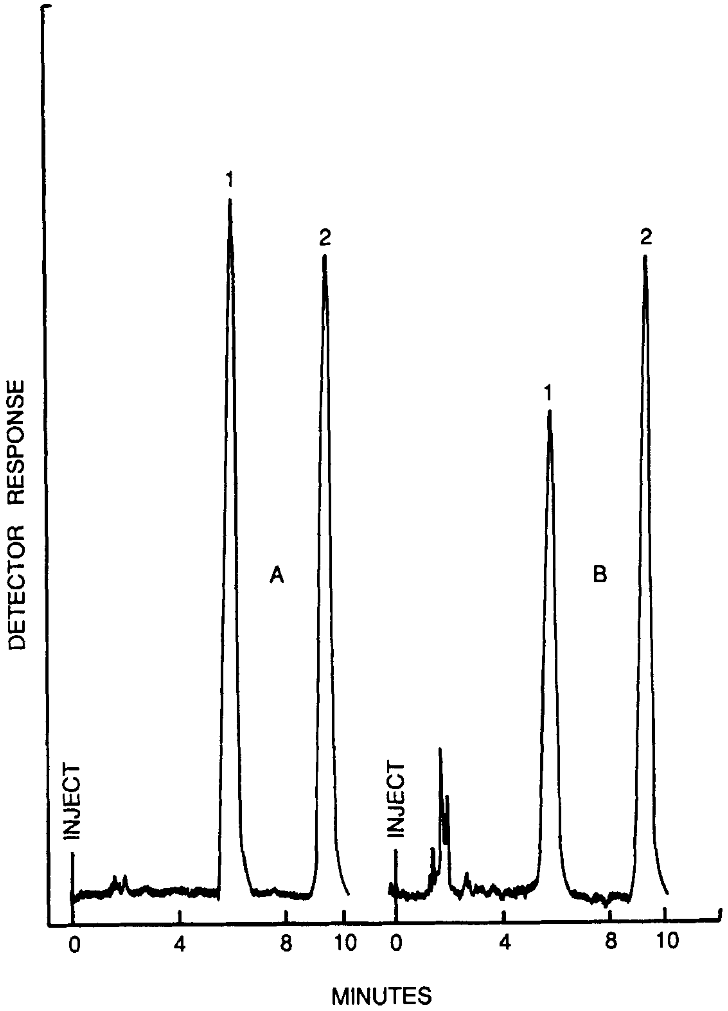


Figure 1 Sample chromatograms. Peaks 1-2 are from cefotetan and cephalexin (the internal standard), respectively. Chromatogram A is from a standard solution and B from a 9 day old, pH 4.85 solution (solution 4, Table 1) when stored at 25°. For chromatographic conditions, see text.

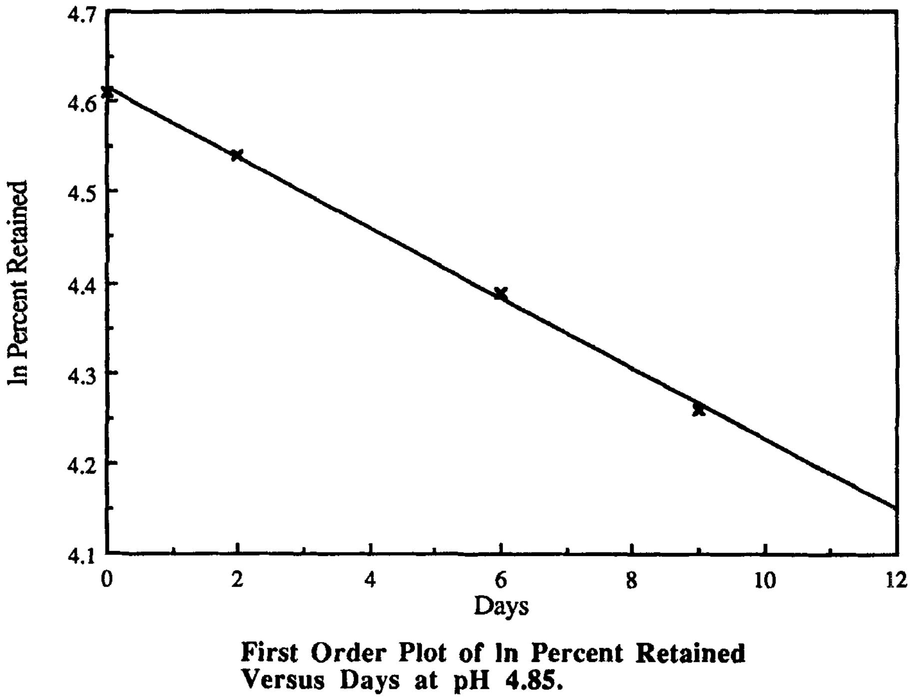
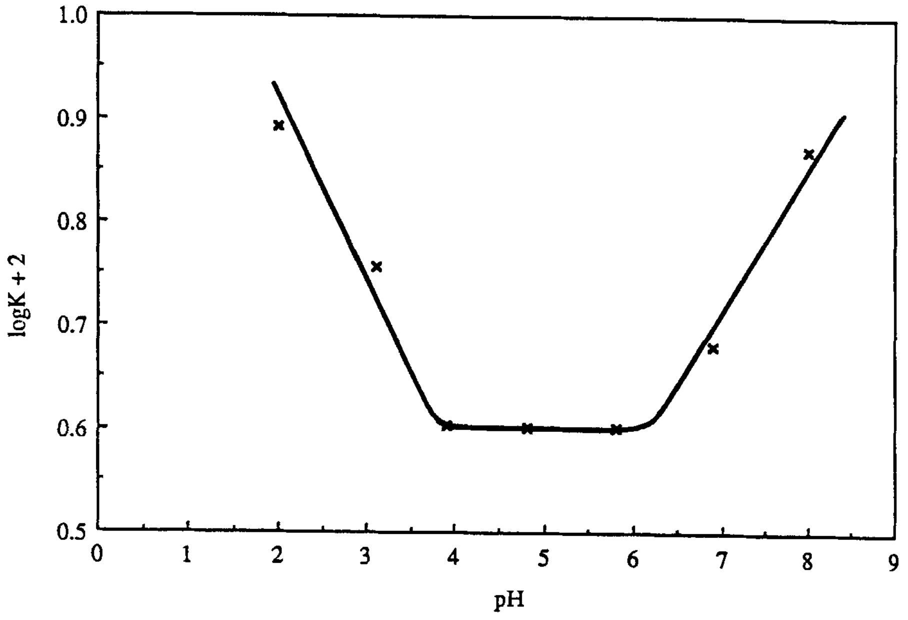


Figure 2 First-order plot of ln percent retained versus days at pH 4.85.

standard were very good at this wavelength and the background noise was less than at 238 nm. Furthermore, with cephalexin as the internal standard, the percent relative standard deviation based on 5 readings was 1.0 versus 1.7 with benzoic acid based on 6 readings.

Stability Studies, Effect of pH - The decomposition of cefotetan followed first-order law (Figure 2) at all pH values. The pH-rate curve indicated (Figure 3) that cefotetan is stable between a pH range of about 3.6 to 6.4. This is true of many other cephalosporins. The decomposition of cefotetan at the pH values of 2 or 8 was approximately 2 times faster than the decomposition within the pH range of maximum stability (3.6-6.4). A similar pH change accelerated the



pH - Rate Profile Curve of Cefotetan.

Figure 3 pH-Rate profile curve of cefotetan.

TABLE 2

ASSAY RESULTS (BASED ON 100% ON 0 DAY) OF SOLUTIONS 4-8 (TABLE 1) OF pH 4.85 (± 0.05).

Solution No. (See Table 1)	Assay Results[a] After (Days) 2	6	9
4	93.8	81.0	70.6
5	92.6	80.4	70.6
6	93.2	79.9	71.2
7	93.4	80.1	70.0
8	93.2	81.4	71.1

[a] All the solutions were clear throughout the study period and the pH values did not change.

decomposition of cefotaxime[3] by almost 10 times. Using the approximations similar to those reported earlier[3], the K_h and K_{oh} values were estimated to be 3.8 and 4.4 x 10^4 day^{-1}. All the solutions were clear (except that pH 2 solution was slightly cloudy) throughout the study period and the pH values did not change.

Effect of Buffer Concentration and Ionic Strength - The phosphate buffer concentration and ionic strength did not affect the K_{obs} value of cefotetan (Table 2). This is true of most of the cephalosporins.

Pathways of Decomposition - The decomposition of cefotetan is considered to be similar to other cephalosporins and has been reported in the literature[4]. Briefly, at lower pH values, the β-lactam moiety undergoes hydrolysis and at higher pH values (above 6.5), the side chain undergoes hydrolysis.

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