Stability of Cefotaxime Sodium as Determined by High-Performance Liquid Chromatography

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Abstract □ The stability of cefotaxime sodium in water (with either hydrochloric acid, phosphate buffers, or other ingredients) dextrose, and sodium chloride has been studied using a stability-indicating high-performance liquid chromatographic method with a relative standard deviation of 1.9% based on six injections. The optimum pH range of stability was determined to be ~4.3-6.2. In this pH range, the decomposition process was catalyzed only by the solvent. At pH ≤3.4, the hydrogen ion also catalyzed the reaction while at pH >6.2, the hydroxyl ion hastened the process of decomposition. The solutions in 5% dextrose and 0.9% NaCl injections were stable for at least 1, 22, and 112 d at 24°C, 4°C, and −10°C, respectively. For both solutions, the loss in potency was <5% at −10°C in 224 d, <9% at 4°C in 42 d, and <3.1% at 24°C in 1 d.

Keyphrases □ Cefotaxime sodium—stability as determined by high-performance liquid chromatography □ Stability—cefotaxime sodium, determined by high-performance liquid chromatography

Cefotaxime sodium, a new semisynthetic cephalosporin antibiotic, is effective against a wide variety of microorganisms when administered intravenously. Stability studies (1) indicate that solutions for intravenous use are stable for 24 h at 25°C, 10 d under refrigeration (<5°C) and for at least 13 weeks in the frozen state. It also appears that cefotaxime solutions exhibit maximum stability in the pH range of 5–7 and that it is not advisable to prepare solutions with diluents having a pH >7.5 (1). In general, cephalosporins have been reported to be stable (2).

Elrod *et al.* (3) reported the various methods available for the quantitation of β-lactam antibiotics, including microbiological (4), iodometric (5), colorimetric (6–8), and high-performance liquid chromatographic (HPLC) (9–12) assays. The two official procedures often specified for cephalosporins are the microbiological agar diffusion (13) and hydroxylamine (14) methods. An HPLC procedure for the quantitation of cefotaxime in serum (15) has been reported.

The purpose of this investigation was to study the stability of cefotaxime sodium at various pH values and in intravenous admixtures. A stability-indicating HPLC method is described which is rapid, accurate, and sensitive.

EXPERIMENTAL

Materials—All chemicals and reagents were either USP, NF, or ACS quality and were used without further purification. Cefotaxime sodium[1] powder was used as received. The high-pressure liquid chromatograph[2] was equipped with a multiple-wavelength detector[3], a recorder[4], and an integrator[5]. A semipolar[6] column (30 cm long × 4 mm i.d.) was used.

The mobile phase for method A contained 0.02 M ammonium acetate and 18% v/v of methanol in water. The flow rate was 2.5 mL/min, the sensitivity was 0.1 AUFS (254 nm), and the chart speed 30.5 cm/h. The temperature was ambient. Chromatographic conditions were the same for method B except that the mobile phase contained 9% v/v of acetonitrile instead of methanol and the sensitivity was 0.04 AUFS.

Methods—A stock solution was prepared fresh daily by dissolving 100 mg of cefotaxime sodium in enough water to make 100 mL of the solution. The stock solution was diluted further with water as needed. The standard solution contained 100 μg/mL of cefotaxime sodium. All the antibiotic solutions were prepared using a simple solution method (Table I). After the initial data (physical appearance, pH values[7], and assays) were obtained, the solutions were stored either at room temperature (24 ± 1°C), under refrigeration (4 ± 1°C), in the freezer (−10 ± 1°C), or at all the three temperatures.

At appropriate intervals, solutions of cefotaxime were assayed using the HPLC method. Before analysis, all solutions were diluted with water to an appropriate concentration (identical to the standard solution based on the label claim). Before dilution, the solutions were always brought to room temperature by putting the bags/bottles in tap water.

A 20-μL aliquot of the assay solution was injected into the chromatograph using the described conditions. For comparison, an identical volume of the standard solution was injected after the assay solution eluted. The concentration of cefotaxime sodium in the standard solution was identical to label claim of the assay solution.

Since preliminary investigations indicated that the peak heights were related directly to the concentrations (range tested was 0.3–2.4 μg), the results were calculated using:

$$\frac{(Ph)_a}{(Ph)_s} \times 100 = \text{percent of label claim} \qquad \text{(Eq. 1)}$$

where $(Ph)_a$ is the peak height of the assay solution and $(Ph)_s$ is the peak height of the standard solution of an identical concentration based on the label claim.

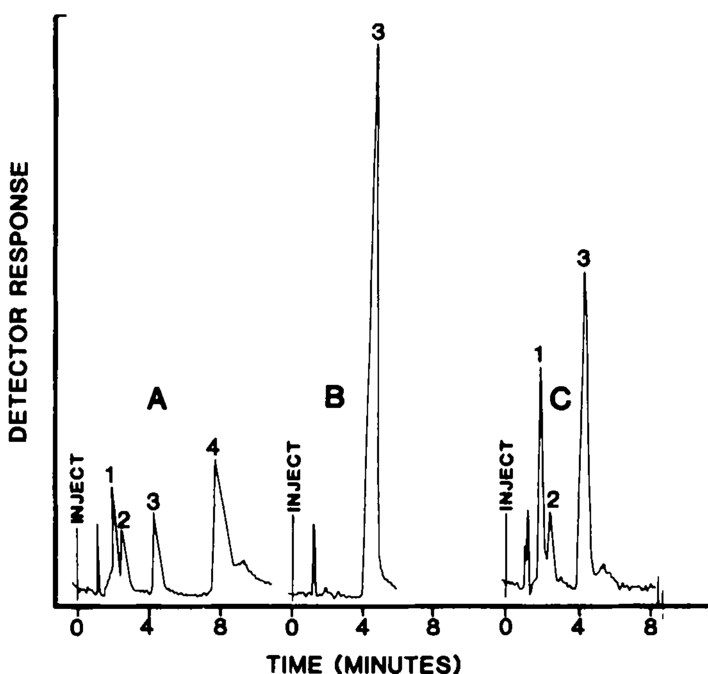

Figure 1—*Typical chromatograms; peak 3 is from cefotaxime and all others are from the decomposition products. Chromatograms A–C are from a 3-d old solution (pH 1.5), a standard solution, and 3-d old solution (pH 8.5), respectively. Chromatographic method A was used (see text).*

[1] Hoechst-Roussel Pharmaceuticals, Somerville, N.J.
[2] Model ALC 202 equipped with U6K universal injector; Waters Associates, Milford, Mass.
[3] Spectroflow monitor SF770; Schoeffel Instrument Corp., Westwood, N.J.
[4] Omniscribe 5313-12; Houston Instruments, Austin, Tx.
[5] Autolab minigrator; Spectra-Physics, Santa Clara, Calif.
[6] μBondapak phenyl (Catalog No. 27198); Waters Associates.
[7] All pH values were measured using Beckman Zeromatic SS-3, pH meter.

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Table I—Cefotaxime Solutions Prepared for the Stability Studies

Solution	Conc. of Cefotaxime Sodium, mg/mL	pH, Initial	Other Ingredients	Ionic Strength[a]	Storage Container
1	10.0	4.7	5% Dextrose	[b]	Plastic Bag[c]
2	10.0	4.6	0.9% NaCl	[b]	Plastic Bag[c]
3	1.0	1.5	0.1 M HCl	0.3	Bottle[d]
4	1.0	2.8	0.1 M Phosphate	0.3	Bottle
5	1.0	3.4	0.1 M Phosphate	0.3	Bottle
6	1.0	4.3	0.1 M Phosphate	0.3	Bottle
7	1.0	4.3	0.2 M Phosphate	0.3	Bottle
8	1.0	4.3	0.1 M Phosphate	0.6	Bottle
9	1.0	5.4	0.1 M Phosphate	0.3	Bottle
10	1.0	6.2	0.1 M Phosphate	0.3	Bottle
11	1.0	7.5	0.1 M Phosphate	0.3	Bottle
12	1.0	8.5	0.1 M Phosphate	0.3	Bottle

[a] Adjusted with KCl. [b] Ionic strength of this solution was not adjusted. [c] The original plastic Viaflex PL 146 bags (Travenol Laboratories), from which either 5% dextrose or 0.9% NaCl injection in water was withdrawn to make the solutions. [d] All bottles are 60-mL amber colored glass; Brockway Glass Co., Brockway, Pa.

RESULTS AND DISCUSSION

Assay Method—The method developed is reproducible with a relative standard deviation of 1.9% based on six injections. The developed procedure separated a number of decomposition products (Fig. 1A–C) from the intact drug (peak 3 in Fig. 1).

The HPLC methods developed are stability indicating (Fig. 1). In a solution of pH 1.5, there was no intact drug present after 7 d of storage. Instead there were four new peaks from the decomposition products. The author feels that the HPLC method may be better, since the results are more accurate and precise compared with the microbiological assay method. Moreover, the decomposition products, especially when only the 3-acetoxy group may have been hydrolyzed, could interfere with the microbiological assay technique, since the intact β-lactam moiety is expected to be active against the bacteria. With the HPLC procedure, this interference is not expected, due to separation of the decomposition products.

Effect of pH and Order of Reaction—The decomposition was first order (Fig. 2). The optimum pH range of stability (Fig. 3) was in the 4.3–6.2 range. At pH values of 3.4 and 7.5, the decomposition was ~10% faster than in the 4.3–6.2 pH range. At pH 1.5, the degradation was the fastest (~11 times faster than in the optimum range), and at pH 8.5 the K_{obs} was ~3 times faster than the K_{obs} between pH 4.3 and pH 6.2. The increase in buffer concentration (phosphate) and the ionic strength (solutions 6–8 in Table I) did not affect the decomposition constant. Since pH had very little effect on K_{obs} the pH range of 4.2–6.2 and reaction was catalyzed only by water, it can be assumed that K_w in Eq. 2 is 0.056 d^{-1} (K_{obs} in the pH range of 4.3–6.2):

$$K_{obs} = K_H(H^+) + K_w + K_{OH}(OH^-) \quad \text{(Eq. 2)}$$

Neglecting the effect of OH^- at pH 1.5 and that of H^+ at pH 8.5, the K_H and K_{OH} values were estimated to be 18.0 and 3.3×10^4 M^{-1} · d^{-1}, respectively.

Decomposition of Cefotaxime—It is well documented (2) that at lower pH, the β-lactam moiety undergoes hydrolysis (K_1 step in Scheme I, *cf.* Ref. 2), and at higher pH (~8), the side chain undergoes hydrolysis (K_2 step). In Fig. 1A (as compared with Fig. 1B) the additional unidentified peaks are from the hydrolysis of side chain (peaks 1–2) and lactonization (peaks 4–5) of the β-lactam ring. In Fig. 1C, the additional peaks (peaks 1 and 2) are from the hydrolysis of the 3-acetoxy group (side chain). The decomposition of cefotaxime in this assay solution of pH 1.5 was fast. It is obvious that decomposition products from the side chain elute immediately after the solvent, while those from the β-lactam lactonization elute after the intact drug (Fig. 1A). This was expected, since the hydrolysis of the 3-acetoxy group produced more polar product(s) while lactonization produced less polar product(s). Lactonization predominates only in the acidic medium (2). In these investigations, peaks 3–4 were small in solutions of pH 2.8 and 3.4 and absent in others (pH 4.3–8.5). The absence of lactonization in neutral and basic solutions was expected. Obviously, lactonization does not proceed even under weakly acidic conditions. Peaks 1–2 were larger in pH 8.5 solution, since hydrolysis of the 3-acetoxy group was fastest at this pH (highest in the series of solutions studied).

Stability of Intravenous Solutions—The testing results of intravenous solutions indicate (Table II) that the manufacturer-recommended (1) expiration date of 24 h at room temperature is reasonable. However, cefotaxime sodium solutions were stable for 22 d at 4°C *versus* the manufacturer-recommended expiration date of 10 d. Even in 35 d, the loss in potency was <5%. The frozen

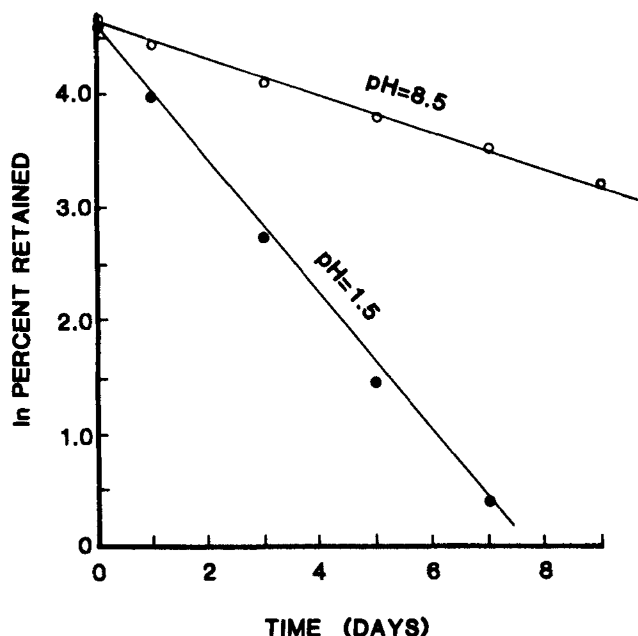


Figure 2—*First-order plots of the degradation of cefotaxime in solutions of pH 1.5 and 8.5.*

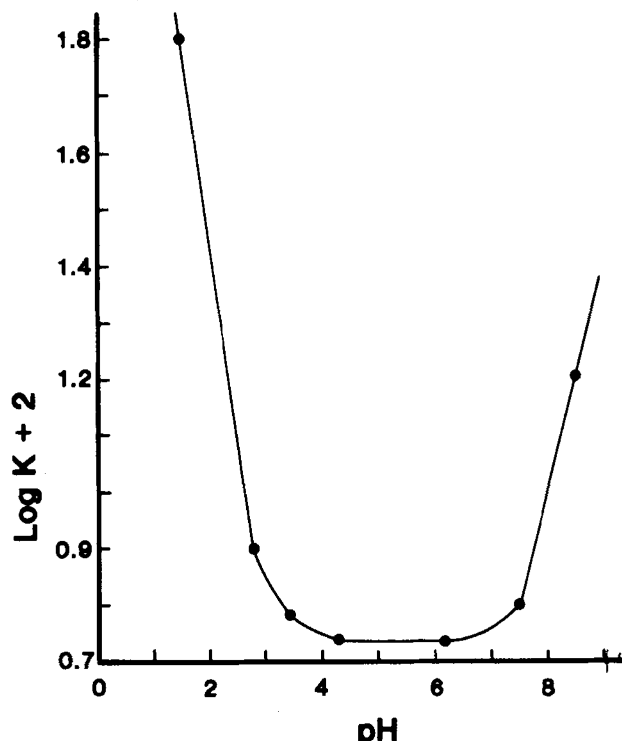
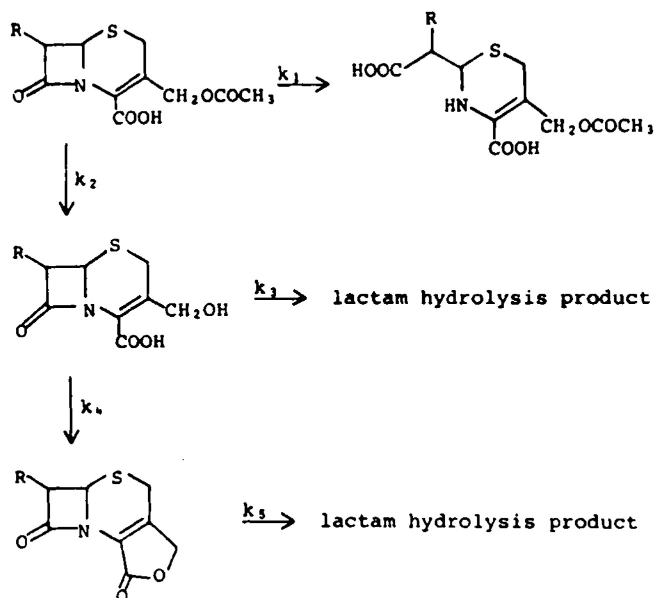


Figure 3—*A pH-rate profile curve from data of solutions 3–12 (Table I).*

Table II—Assay Results and pH Values of the Solutions at Various Temperatures [a]

Vehicle	Amount Retained[b] Based on the Label Claim, %					
	24 ± 1°C					
	0 d	1 d	3 d	7 d		
5% Dextrose	100.2	97.0	75.9	60.4		
	(4.7)	(4.5)	(4.5)	(4.4)		
0.9% NaCl	100.8	98.0	76.7	59.0		
	(4.6)	(4.4)	(4.4)	(4.2)		
	4 ± 1°C					
	0 d	11 d	17 d	22 d	35 d	42 d
5% Dextrose	100.2	101.2	101.2	100.8	96.1	92.5
	(4.7)	(4.7)	(4.7)	(4.7)	(4.5)	(4.7)
0.9% NaCl	100.8	99.6	100.2	99.2	95.2	91.7
	(4.6)	(4.6)	(4.6)	(4.6)	(4.5)	(4.6)
	−10 ± 1°C					
	0 d	28 d	63 d	112 d	224 d	
5% Dextrose	100.2	100.2	100.4	99.8	95.3	
	(4.7)	(4.7)	(4.7)	(4.7)	(4.6)	
0.9% NaCl	100.8	100.8	100.2	99.6	95.6	
	(4.6)	(4.6)	(4.6)	(4.6)	(4.5)	

[a] Results (using chromatographic method A) of buffered solutions are presented in Figs. 2 and 3. The increase in buffer concentration and the ionic strength (solutions 6-8, Table I) did not affect the K_{obs} value. All solutions containing 1.0 mg/mL of cefotaxime sodium were clear and pH values were constant to the last day of these investigations. The solutions containing 1% cefotaxime sodium were very slightly yellow in color on day 0. The intensity of color increased after 1, 25, and 112 d of storage at 24°C, 4°C, and −10°C, respectively. [b] These were determined using chromatographic method B. The results obtained on selected samples with chromatographic method A were similar. The pH value is in parentheses.



Scheme I—Decomposition of Cefotaxime. R represents the amide side chain.

solutions were stable for at least 16 weeks. In 32 weeks, the loss in potency was <5%, and the change in pH was negligible. However, an additional important factor must be considered, *e.g.*, a poor mixing technique could contaminate the admixture and make it unsuitable for use.

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