Quantitation of Carbenicillin Disodium, Cefazolin Sodium, Cephalothin Sodium, Nafcillin Sodium, and Ticarcillin Disodium by High-Pressure Liquid Chromatography

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Abstract □ High-pressure liquid chromatographic (HPLC) methods for the quantitation of carbenicillin, cefazolin, cephalothin, nafcillin, and ticarcillin were developed. The stability of 2% solutions of the antibiotics in normal saline and in 5% dextrose in water were studied at 24 and 5°. The assays were conducted using a previously reported colorimetric method, and some assays also were performed using HPLC. For discolored solutions of cephalothin, the colorimetric method was not stability indicating. The percent relative standard deviations by HPLC based on six injections were 1.69, 0.94, 1.30, 1.59, and 1.6 for carbenicillin, cefazolin, cephalothin, nafcillin, and ticarcillin, respectively. Both carbenicillin and ticarcillin apparently may be mixtures of two isomers at equilibrium with each other. The shelflives recommended by the manufacturers at 5° may be too conservative.

Keyphrases □ Carbenicillin disodium—high-pressure liquid chromatographic analysis □ Cefazolin sodium—high-pressure liquid chromatographic analysis □ Cephalothin sodium—high-pressure liquid chromatographic analysis □ Nafcillin sodium—high-pressure liquid chromatographic analysis □ Ticarcillin disodium—high-pressure liquid chromatographic analysis □ Antibiotics—carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium, high-pressure liquid chromatographic analysis

The preparation of intravenous admixtures is a common service provided by hospital pharmacies in the United States. Most pharmacists are concerned about the stability of the solutions after mixing. Some of these solutions contain antibiotics such as carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium in normal saline or in 5% dextrose in water. The stability studies must be conducted using a stability-indicating assay.

Pharmaceutical manufacturers have begun to report limited information on the stability of antibiotics in intravenous admixtures. For example, the following information has been reported in the package inserts. Intravenous solutions of carbenicillin disodium (1), cefazolin sodium (2), cephalothin sodium (3), and ticarcillin disodium (4) are stable for 1 day at room temperature and for 3, 4, 4, and 3 days, respectively, when stored in a refrigerator. The intravenous solution of nafcillin sodium has been reported to be stable for 3 days at room temperature and for 7 days when stored in a refrigerator (5).

BACKGROUND

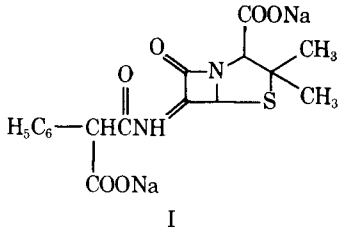
Brief studies on the stability of frozen and refrigerated solutions of cefazolin sodium (6, 7) and cephalothin sodium (8, 9) have been reported. These reports did not describe the methodology in detail, especially the analytical techniques. One study (9) recommended expiration dates of 2 days at 5° and of 6–24 hr at 25° for a 1% solution of cephalothin sodium in normal saline or in 5% dextrose in water. Other reports (6–8) recommended an expiration date of 4 days for both cefazolin sodium and cephalothin sodium when stored in a refrigerator. Longer expiration dates were recommended for frozen solutions.

Zia and Zargarbashi (10) reported the kinetics of carbenicillin sodium in aqueous solution at 35°. The degradation products of cephalothin and cefazolin in alkaline and neutral aqueous solutions at 35° were reported also (11).

Several analytical techniques have been used for the stability studies. A fluorometric method for cephalothin and cefazolin was reported (12). Zia and Zargarbashi (10) preferred the iodometric method, and Yamana and Tsuji (11) used anion-exchange chromatography for cephalothin and high-pressure liquid chromatography (HPLC) for cefazolin. One method for the quantitation of cefazolin was based on a reaction with hydroxylamine hydrochloride (13). This method was modified (14) and applied to other penicillins including carbenicillin and cephalothin.

The high experimental errors inherent in microbiological assays were reported (15), and the factors affecting the iodometric assay technique and its limitations were described (16, 17).

The purposes of this investigation were to develop accurate and reproducible stability-indicating assays for the quantitation of carbenicillin disodium (I), cefazolin sodium (II), cephalothin sodium (III), nafcillin



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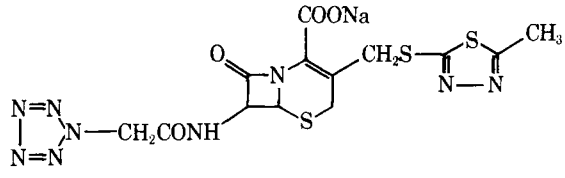

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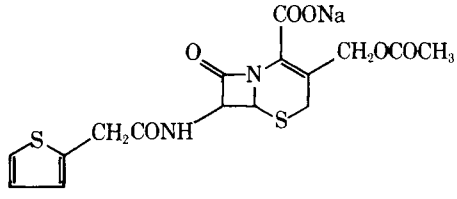

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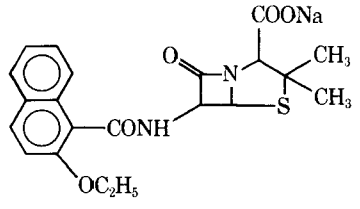

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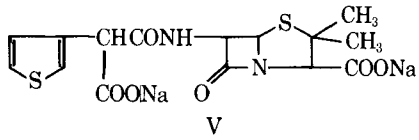

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Table I—Chromatographic Conditions

Antibiotic	Mobile Phase	Flow Rate, ml/min	Detector Setting, nm	Sensitivity Setting	Chart Speed, cm/hr	Concentration[a] of Standard Solution, μg/ml
Carbenicillin	0.01 *M* NH_4OOCCH_3 in water	1.6	245	0.04	30.5	600
Cefazolin	0.01 *M* NH_4OOCCH_3 in 30% methanol in water	1.3	254	0.1	30.5	60
Cephalothin	0.01 *M* NH_4OOCCH_3 in 30% methanol in water	2.2	254	0.04	30.5	50
Nafcillin	0.01 *M* NH_4OOCCH_3 in 50% methanol in water	2.0	280	0.04	30.5	80
Ticarcillin	0.01 *M* NH_4OOCCH_3 in water	1.6	245	0.1	30.5	600

[a] The concentrations reported are in terms of total sterile powder. The temperature was ambient.

Table II—Colorimetric Assay Results for Solutions (2 g/100 ml) Stored at Room Temperature (24 ± 1°)

		Percent of Potency Retained (Based on Label Claim) after						
Antibiotic	Solvent	1 day	4 days	5 days	5 days (HPLC)[a]	7 days	13 days (HPLC)[a]	15 days
Carbenicillin	NS[b]	96.8	89.7	86.5	87.4	—[c]	—[c]	60.1
Cefazolin	NS	98.0	95.4	93.9	93.0	90.9	—[c]	86.4
Cephalothin	NS	96.1	79.2[d]	78.0	62.2	—[c]	33.4	—[c]
Nafcillin	D5W[e]	97.9	93.6	91.6	92.0	89.5	—[c]	—[c,f]
Ticarcillin	NS	96.4	88.8	85.1	85.5	—[c]	—[c]	60.1

		Percent of Potency Retained (Based on Label Claim) after										
Antibiotic	Solvent	1 day	3 days	5 days	5 days (HPLC)[a]	6 days	6 days (HPLC)[a]	7 days	9 days	9 days (HPLC)[a]	11 days	11 days (HPLC)[a]
Carbenicillin	D5W	99.1	79.6	—[c]	—[c]	67.5	68.6	61.7	—[c]	—[c]	31.4	—[c]
Cefazolin	D5W	99.1	76.6	94.0	93.2	—[c]	—[c]	—[c]	89.4	88.5	86.1	—[c]
Cephalothin	D5W	95.8	88.4[d]	78.6	56.1	—[c]	—[c]	—[c]	72.1	50.5	60.6	40.9
Nafcillin	NS	100	94.3	—[c]	—[c]	87.5	86.5	85.1	—[c]	—[c]	—[c,f]	—[c]
Ticarcillin	D5W	99.2	81.4	—[c]	—[c]	45.0	43.5	43.3	—[c]	—[c]	14.3	—[c]

[a] By the HPLC method. [b] Normal saline. [c] Not assayed on this day. [d] Solution had discolored to light yellow. The intensity of the color increased with further storage. [e] Five percent dextrose in water. [f] There was precipitation in the bag (light yellow).

sodium (IV), and ticarcillin disodium (V) using HPLC and to study the stability of some intravenous admixtures containing these antibiotics. All of the antibiotics studied have a β-lactam ring.

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, or ACS quality and were used without further purification. The sterile powders of carbenicillin disodium[1], cefazolin sodium[2], cephalothin sodium[2], nafcillin sodium[3], and ticarcillin disodium[4] were used as received. The powder of III contained sodium bicarbonate and the powder of IV contained sodium citrate as a buffering agent. The presence of buffering agents did not interfere in these investigations.

Apparatus—The high-pressure liquid chromatograph[5] was equipped with a multiple-wavelength detector[6], a recorder[7], and an integrator[8].

Column—A semipolar[9] column (30 cm long × 4 mm i.d.) was used.

Chromatographic Conditions—The chromatographic conditions are reported in Table I.

Preparation of Solutions—The standard solutions in water were prepared fresh. Their concentrations are reported in Table I. Solutions of other concentrations were prepared fresh as needed using a simple solution method.

Preparation of Antibiotic Solutions (Similar to Intravenous Admixtures)—Solutions of I–V (2.00%)[10] were prepared in either sterile normal saline[11] or in sterile 5% dextrose in water[11]. The final solutions were not sterile. All solutions were divided into two parts and transferred to the original containers[12]. One set of the solutions was stored at room temperature (24 ± 1°), and the other set was stored in a refrigerator (5 ± 1°). The solutions were assayed at appropriate intervals.

Assay Procedures—*Colorimetric Procedure*—The colorimetric procedure was reported previously (14). The method has been claimed to be stability indicating. The results are reported in Tables II and III.

HPLC Procedures—At appropriate intervals, solutions of antibiotics also were assayed using HPLC methods developed in this laboratory. Before analysis, all solutions were diluted with water to the appropriate concentrations (identical to the standard solutions; see Table I). A 20.0-μl aliquot of the assay solution was injected into the chromatograph using the described conditions (Table I). For comparison, an identical volume of the appropriate standard solution was injected after the assay solution eluted.

Calculations—Since preliminary investigations indicated that the peak areas (also peak heights) were related directly to the concentration (ranges tested were: I, 8–16 μg; II, 0.8–1.6 μg; III, 0.5–1.2 μg; IV, 1.3–2.0 μg; and V, 8–16 μg), the results were calculated using:

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

where $(Ph)_a$ is the peak height of the assay solution and $(Ph)_s$ is the peak height of the standard solution of identical concentration. For I and V, heights of the taller peaks (peak 1) were compared. The results are presented in Tables II and III and Figs. 1–3. The pH values are presented in Table IV.

RESULTS AND DISCUSSION

Carbenicillin—The results (Table II) indicate that the manufacturer-recommended expiration date of 1 day at room temperature is reasonable. At room temperature, a 2.00% solution of carbenicillin appears to have greater stability in normal saline (60.1% potency after 15

[1] Roerig, a Division of Pfizer, New York, NY 10017.
[2] Eli Lilly and Co., Indianapolis, IN 46206.
[3] Wyeth Laboratories, Philadelphia, PA 19101.
[4] Beecham Laboratories, Bristol, TN 37620.
[5] Model ALC 202 equipped with a U6K universal injector, Waters Associates, Milford, Mass.
[6] Spectroflow monitor SF770, Schoeffel Instrument Corp., Westwood, N.J.
[7] Omniscribe 5313-12, Houston Instruments, Austin, Tex.
[8] Autolab minigrator, Spectra-Physics, Santa Clara, Calif.
[9] Waters Associates μBondapak phenyl (Catalog No. 27198).
[10] The concentrations are in terms of total sterile powder.
[11] Travenol Laboratories, Deerfield, IL 60015.
[12] The original plastic Viaflex PL 146 bags from which either normal saline or 5% dextrose in water was withdrawn for making the solutions.

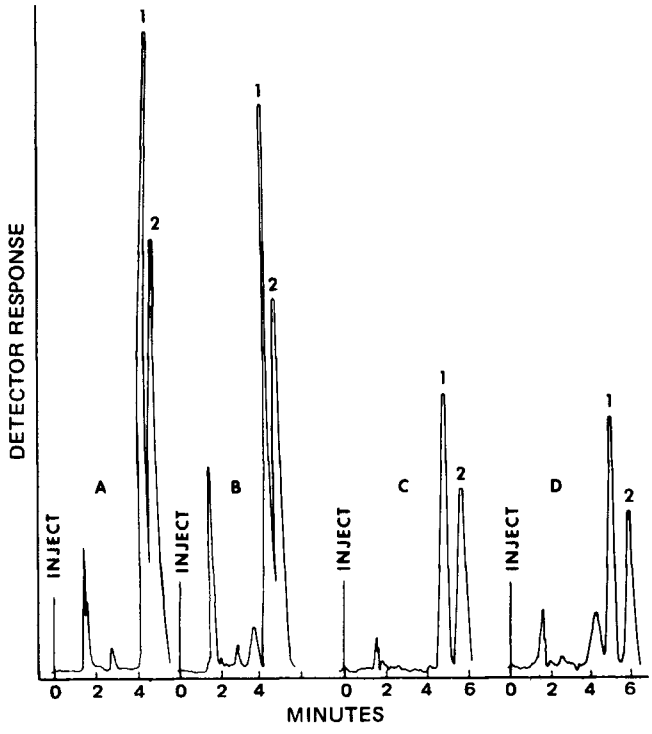

Figure 1—*Sample chromatograms. Peaks 1 and 2 in chromatograms A and B are from ticarcillin (V); in chromatograms C and D, they are from carbenicillin (I). Key: A and C, standard solutions of V and I, respectively; and B and D, 5-day-old solutions of V and I, respectively, in normal saline (storage at 24°). The chromatographic conditions are given in Table I.*

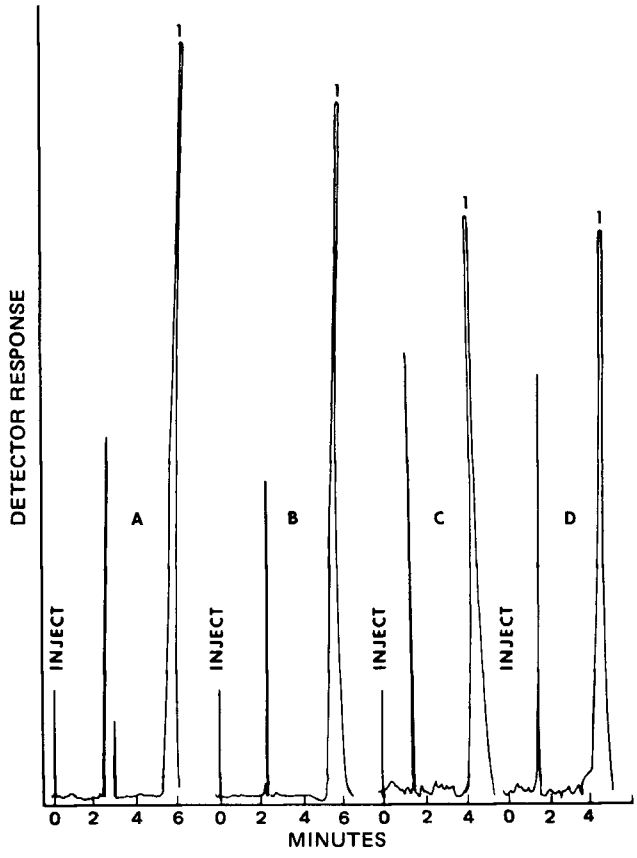

Figure 2—*Sample chromatograms. Peak 1 in chromatograms A and B is from cefazolin (II); in chromatograms C and D, it is from nafcillin (IV). Key: A and C, standard solutions of II and IV, respectively; B, 5-day-old solution of II in normal saline (storage at 24°); and D, 15-day-old solution of IV in normal saline (storage at 5°). The chromatographic conditions are given in Table I.*

days of storage) *versus* 31.4% (after only 11 days of storage) in 5% dextrose in water. After 7 days, the potency retained in dextrose solution was about the same as that after 15 days in normal saline.

Both solutions were stable (based on 90% potency) for 15 days at 5°. Therefore, the manufacturer-recommended expiration date of 3 days (1) is too conservative.

The colorimetric assay reported previously (14) appears to be stability indicating. The results by the literature method and by the HPLC method were almost the same (Table II), considering experimental errors. Nevertheless, the HPLC method gave more information than the assay results alone. For example, it appears that carbenicillin is a mixture of two isomers (peaks 1 and 2 in Fig. 1C), and the percent decomposition in both isomers was the same. The isomers apparently are at equilibrium with each other, which is normal.

For the HPLC method, the percent relative standard deviation based on six injections was 1.69.

Cefazolin—The results (Table II) indicate that the cefazolin solutions, both in normal saline and in dextrose, were stable for at least 5 days (based on 90% potency) at room temperature and for 15 days at 5°. Therefore, the recommended (2) expiration dates of 1 and 4 days at room temperature and at refrigerator temperature, respectively, are very conservative.

Of the five antibiotics studied, cefazolin appears to have the greatest stability (Tables II and III). This result may have been due to the fact that the pH values of cefazolin solutions increased during storage (Table IV), while the pH values of all of the other solutions decreased (Table IV).

Table III—Colorimetric Assay Results for Solutions (2 g/100 ml) Stored at 4 ± 1°

Antibiotic	Solvent	Percent of Potency Retained (Based on Label Claim) after 4 days	7 days	15 days
Carbenicillin	NS[a]	98.4	97.5	94.0
Cefazolin	NS	99.5	94.7	94.7
Cephalothin	NS	96.5	96.3	95.0
Nafcillin	D5W[b]	97.4	96.0	91.8
Ticarcillin	NS	97.9	94.6	91.0

Antibiotic	Solvent	Percent of Potency Retained (Based on Label Claim) after 3 days	4 days	7 days	8 days	9 days	9 days (HPLC)[c]	15 days	15 days (HPLC)[c]	24 days
Carbenicillin	D5W	100.0	—[d]	99.6	—[d]	99.2	99.7	90.9	—[d]	—[d]
Cefazolin	D5W	—[d]	99.7	—[d]	97.1	96.5	95.2	95.0	—[d]	95.1
Cephalothin	D5W	—[d]	100.0	—[d]	96.4	96.8	96.5	95.0	93.5	—[d]
Nafcillin	NS	99.8	—[d]	98.3	—[d]	—[d]	94.9	95.5	95.4	94.1
Ticarcillin	D5W	99.3	—[d]	98.4	—[d]	—[d]	96.6	97.6	93.3	—[d]

[a] Normal saline. [b] Five percent dextrose in water. [c] By the HPLC method. [d] Not assayed on this day.

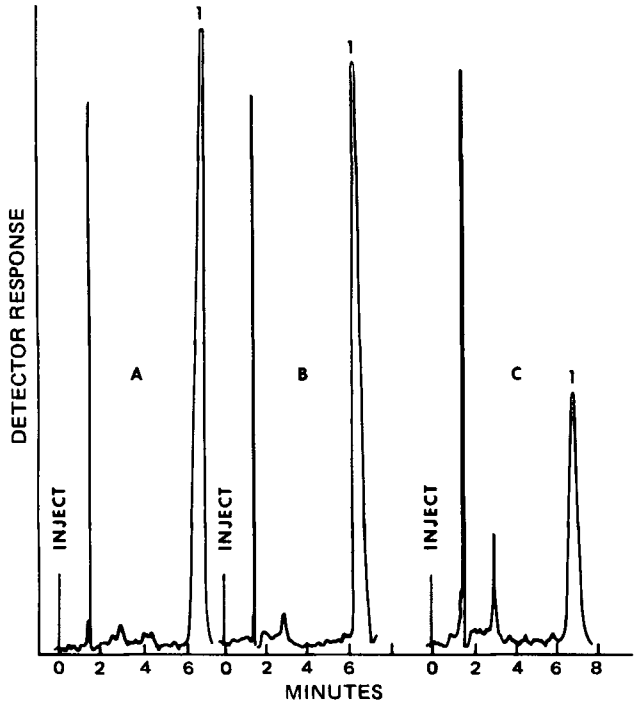

Figure 3—*Sample chromatograms. Peak 1 is from cephalothin (III). Key: A, standard solution; B, 15-day-old solution of III in 5% dextrose in water (storage at 5°); and C, 11-day-old solution of III in 5% dextrose in water (storage at 24°). The chromatographic conditions are given in Table I.*

The results using the HPLC method (Table II) were similar to those obtained using the literature method (14). Since these solutions did not decompose much, there was no significant difference between the chromatogram of a standard solution (Fig. 2A) and a chromatogram from a 5-day-old solution in normal saline when stored at room temperature (Fig. 2B).

For the HPLC method, the percent relative standard deviation based on six injections was 0.94.

Cephalothin—The results at room temperature (Table II) indicate that the manufacturer-recommended (3) expiration date of 1 day is reasonable. However, if stored in a refrigerator, the solutions were stable for at least 15 days (Table II), compared to the manufacturer-recommended (3) shelflife of 4 days. Moreover, the solutions that were stored in a refrigerator remained colorless during the investigation. However, at room temperature, the solutions started discoloring to a light-yellow color within 48–72 hr. The intensity of this color increased on further storage.

Once the solutions became discolored, the colorimetric analysis gave misleading results (Table II). For example, after 5 days of storage at 24°, cephalothin in normal saline and in dextrose solutions indicated a potency of ~78% (Table II). When assayed using the HPLC method (Fig. 3), the potencies were 62.2 and 56.1% for solutions in normal saline and dextrose, respectively (Table II). After 11 days, the solution with dextrose indicated 60.6% potency by the colorimetric method compared to 40.9% by the HPLC method. Therefore, the literature method (14) probably is unreliable for stability studies of III once the solutions become discolored.

For the HPLC method, the percent relative standard deviation based on six injections was 1.3.

Nafcillin—The results at room temperature (Table II) indicate that the expiration date of 3 days recommended by the manufacturer (5) is reasonable. However, when stored in a refrigerator, the solutions were stable for at least 15 days (Table III). The manufacturer recommends (5) an expiration date of 7 days.

The results with the HPLC method were similar (Table II) to those with the colorimetric method (14). For the HPLC method, the percent relative standard deviation based on six injections was 1.59.

Ticarcillin—The results at room temperature (Table II) indicate that the manufacturer-recommended shelflife (4) of 1 day is reasonable. However, when stored in a refrigerator, the solutions were stable for at least 15 days (Table III). Therefore, the recommended shelflife of 3 days is too conservative.

Table IV—Change in pH of the Antibiotic Solutions (2 g/100 ml) with Various Storage Times and Temperatures

Antibiotic	Solvent	pH Value (±0.1) after Initial	1 day at 24°	7 days at 24°	7 days at 5°
Carbenicillin	NS[a]	6.5	6.0	5.6	6.4
Cefazolin	NS	4.4	5.8	6.4	5.2
Cephalothin	NS	7.5	6.4	4.6[b]	7.3
Nafcillin	D5W[c]	6.6	6.4	5.6	6.4
Ticarcillin	NS	6.1	5.8	5.6	5.9

Antibiotic	Solvent	pH Value (±0.1) after Initial	1 day at 24°	15 days at 24°	15 days at 5°
Carbenicillin	D5W	6.5	5.9	5.1	5.9
Cefazolin	D5W	4.6	5.6	6.2	5.6
Cephalothin	D5W	7.6	6.3	4.6[b]	6.9
Nafcillin	NS	6.6	6.4	4.6[d]	6.1
Ticarcillin	D5W	6.2	5.7	5.1	5.6

[a] Normal saline. [b] Solution had discolored to yellow. [c] Five percent dextrose in water. [d] Precipitation occurred (light yellow).

The colorimetric analysis appears to be stability indicating, but the HPLC method gave some new information. Ticarcillin appears to be a mixture of two isomers (Figs. 1A and 1B). These isomers are at equilibrium, as explained for carbenicillin, since the percent decomposition in both isomers was similar.

For the HPLC method, the percent relative standard deviation based on six injections was 1.6.

The data could not be treated mathematically since the pH values of the solutions were not constant (Table IV). The degradation products of cefazolin and cephalothin were reported previously (11). General information about the degradation of carbenicillin (10) and other penicillins (18) is available in the literature.

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