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

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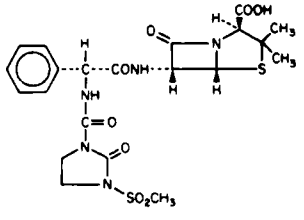
Abstract □ The stability of mezlocillin sodium solutions in water with either phosphate buffers or other ingredients used in intravenous admixtures (dextrose, fructose, and sodium chloride) has been studied using a stability-indicating high-performance liquid chromatographic method. This assay shows a relative standard deviation of 1.42% based on six injections. The optimum stability was shown at an approximate pH of 4.8, and solutions in dextrose (5%) and sodium chloride (0.9%) were stable for up to 4 days at 25°, 36 days at 5°, and for 60 days at −10°. When refrigerated, the solutions in 5% fructose and 10% dextrose were as stable as those in 5% dextrose.

Keyphrases □ Mezlocillin sodium—stability, determined by high-performance liquid chromatography □ Stability—mezlocillin sodium, determined by high-performance liquid chromatography □ High-performance liquid chromatography—stability of mezlocillin sodium

Mezlocillin, a new semisynthetic broad-spectrum penicillin antibiotic, commonly administered by intravenous admixture, is effective against a wide variety of microorganisms. Limited information on the stability is available (1): solutions for intravenous use appear to be stable from 24 to 72 hr at controlled room temperature, from 24 to 168 hr under refrigeration, and for 28 days at −12° (1). This investigation studies the stability of mezlocillin sodium at varying pH in some commonly used vehicles for intravenous administration. A stability-indicating high-performance liquid chromatographic (HPLC) method was developed.



Mezlocillin

EXPERIMENTAL

Materials—All chemicals and reagents were USP, NF, or ACS quality and were used without further purification. Mezlocillin sodium[1] powder was used as received. The 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 contained 0.02 *M* ammonium acetate and 42% (v/v) methanol in water. The flow rate was 2.0 ml/min, the sensitivity was 0.2 AUFS (230 nm), and the chart speed 30.5 cm/hr. The temperature was ambient.

Methods—A standard solution was prepared daily by dissolving 100 mg of mezlocillin sodium in enough water to make 100 ml of the solution. All of the antibiotic solutions were prepared as indicated in Table I using a simple solution method. After the initial data were obtained (physical appearance, pH values[7], and assays), the solutions were stored either at room temperature (25 ± 1°), under refrigeration (5 ± 1°), in the freezer (−10 ± 1°), or at all three temperatures.

Table I—Mezlocillin Solutions Prepared for Stability Studies

Solution	Mezlocillin Sodium, mg/ml	pH (Initial)	Other Ingredient(s)	Ionic Strength[a]
1[b]	10.0	4.8	5% dextrose	—[d]
2[b]	10.0	4.8	0.9% NaCl	—[d]
3[c]	10.0	4.8	5% dextrose	—[d]
4[c]	10.0	4.4	10% dextrose	—[d]
5[c]	10.0	5.2	5% fructose	—[d]
6[c]	1.0	2.9	phosphate buffer, 0.05 *M*	0.2
7[c]	1.0	4.0	phosphate buffer, 0.05 *M*	0.2
8[c]	1.0	4.8	phosphate buffer, 0.05 *M*	0.2
9[c]	1.0	4.8	phosphate buffer, 0.1 *M*	0.2
10[c]	1.0	5.8	phosphate buffer, 0.05 *M*	0.2
11[c]	1.0	6.9	phosphate buffer, 0.05 *M*	0.2
12[c]	1.0	7.8	phosphate buffer, 0.05 *M*	0.2

[a] Adjusted with KCl. [b] Stored in plastic bag; the original plastic Viaflex PL 146 bags from which either 0.9% NaCl or 5% dextrose injection in water was withdrawn for making the solutions. [c] Stored in bottle; sixty-milliliter amber-colored glass bottle; Brockway Glass Co., Brockway, Pa. [d] Ionic strength of this solution was not adjusted.

At appropriate intervals, solutions of mezlocillin were assayed using HPLC. Before analysis, all solutions were diluted with water to an appropriate concentration (identical to the standard solution based on the label claim). Before being diluted, the solutions were brought to room temperature by putting the bags/bottles in tap water. One set of 60-day-old frozen samples was thawed using a microwave oven[8]; the frozen solutions were exposed to microwaves for 3 min.

A 20.0-μl aliquot of the assay solution was injected into the chromatograph using the described conditions. 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 heights were related directly to the concentrations (range tested: 0.2–1.2 mg/ml), the results were calculated using:

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

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

RESULTS AND DISCUSSION

HPLC Assay Method—The method developed is reproducible with a relative standard deviation of 1.42% based on six injections, and separates a number of decomposition products (Fig. 1B and C) from the intact drug (peak 1 in Fig. 1).

It is well documented (2) that at lower pH (~3), the β-lactam moiety undergoes hydrolysis and at higher pH (~8), the side chain undergoes hydrolysis. In Fig. 1B (as compared with Fig. 1A) the additional unidentified peaks are from the hydrolysis of the 3-(methylsulfonyl)-2-oxo-1-imidazolidine ring (see structure of mezlocillin) since the pH of the assay solution was 7.8. In Fig. 1C, the additional peaks are from the hydrolysis of the β-lactam ring. The decomposition of mezlocillin in this

[1] Miles Pharmaceuticals, West Haven, Conn.
[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, Tex.
[5] Autolab minigrator; Spectra-Physics, Santa Clara, Calif.
[6] Waters Associates μBondapak phenyl (Catalog No. 27198).
[7] All pH values were measured using Beckman Zeromatic SS-3, pH meter.
[8] Amana's Radarrange, Model MR-3.

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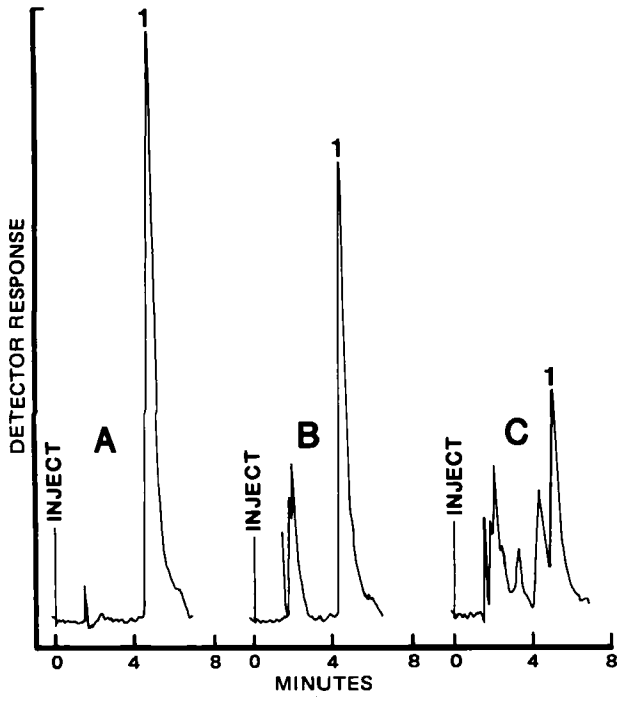

Figure 1—*Typical chromatograms. Peak 1 is from mezlocillin and all others are unidentified. Key: (A) standard solution; (B, C) 4-day old solutions (solutions 12 and 6, respectively) when stored at room temperature.*

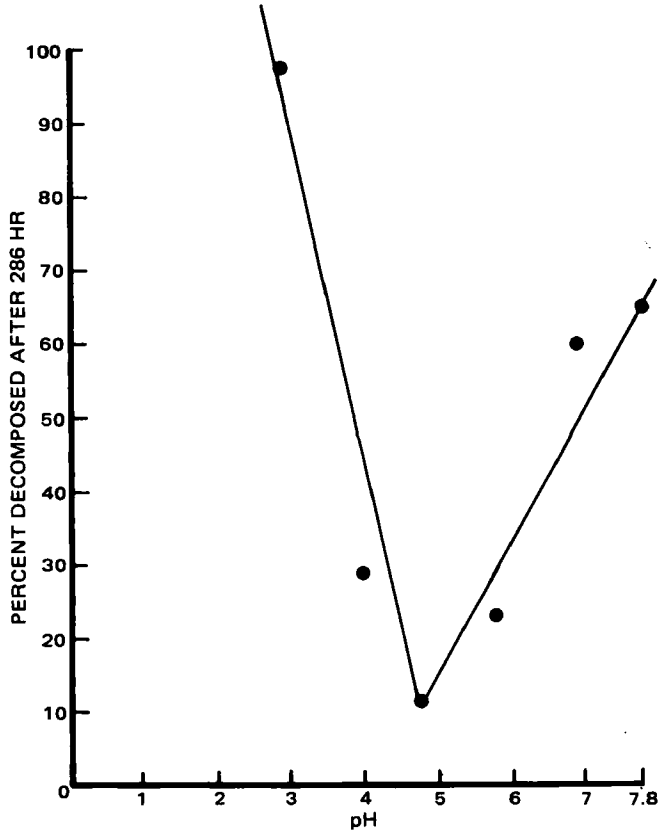

Figure 2—*A pH–rate profile curve from data of solutions 6–12 after 286 hr (~12 days) of storage at room temperature.*

assay solution (pH 2.9) was very fast. It is obvious that decomposition products from the side chain elute immediately after the solvent, while products from β-lactam ring hydrolysis elute immediately before the intact drug (peak 1 in Fig. 1).

The results indicate (Table II, solutions 1 and 2) that the manufacturer's recommended expiration of 48 hr (1) for intravenous solution prepared in 5% dextrose–0.9% NaCl can be extended to 96 hr with a loss in potency of <5%. Under refrigeration, the expiration date may be ex-

Table II—Assay Results and pH Values of Mezlocillin Sodium Solutions at Room Temperature

	Results at 25 ± 1°									
	Day 0		Day 1		Day 4		Day 7		Day 12 (286 hr)	
Solution[a]	pH	Found, %	pH	Found, %	pH	Found, %	pH	Found, %	pH	Found[b], %
1	4.8	100.5	4.7	100.3	4.7	96.3	4.7	90.5	—[c]	—[c]
2	4.8	99.2	4.7	98.8	4.5	96.9	4.5	90.5	—[c]	—[c]
6	2.9	100.6	2.9	80.6	2.9	34.6	2.9	14.0	2.9	2.5
7	4.0	101.1	4.0	100.5	4.0	91.8	4.0	83.3	4.0	71.6
8	4.8	100.9	4.8	100.7	4.8	97.7	4.8	97.2	4.8	89.1
9	4.8	100.6	4.8	101.2	4.8	98.1	4.8	96.9	4.8	87.6
10	5.8	101.3	5.8	101.3	5.8	96.2	5.8	88.6	5.8	77.0
11	6.9	100.6	6.9	100.3	6.9	84.8	6.9	68.3	6.9	40.2
12	7.8	101.0	7.8	100.6	7.8	83.5	7.8	65.2	7.7	35.4

[a] For composition of solution, see Table I. [b] All solutions were clear to the last day of testing. [c] Not determined on this day.

Table III—Assay Results and pH Values of Mezlocillin Sodium Solutions at 5° and −10°

	Results at 5 ± 1°													
	Day 0		Day 1		Day 4		Day 7		Day 14		Day 28		Day 36	
Solution[a]	pH	Found, %	pH	Found, %	pH	Found, %	pH	Found, %	pH	Found, %	pH	Found, %	pH	Found[b], %
1	4.8	100.5	—[c]	—[c]	—[c]	—[c]	4.7	100.1	4.7	100.0	4.7	98.9	4.6	97.8
2	4.8	99.2	—[c]	—[c]	—[c]	—[c]	4.7	99.4	4.7	99.2	4.5	98.3	4.4	96.9
3	4.8	97.8	4.7	97.5	4.7	97.3	—[c]	—[c]	4.7	97.0				
4	4.4	98.0	4.4	97.8	4.4	98.0	—[c]	—[c]	4.4	94.3				
5	5.2	98.0	5.2	98.0	5.2	97.7	—[c]	—[c]	5.1	95.1				

	Results at −10 ± 1°							
	Day 0		Day 28		Day 60		Day 60[d]	
Solution[a]	pH	Found, %	pH	Found, %	pH	Found, %	pH	Found[b], %
1	4.8	100.5	4.8	100.2	4.8	93.5	4.8	93.3
2	4.8	99.2	4.8	99.4	4.7	98.3	4.7	98.3

[a] For composition of solution, see Table I. [b] All solutions were clear to the last day of testing. [c] Not determined on this day. [d] These results are of solutions which were thawed using a microwave oven. For details, see text.

tended (Table III, solutions 1 and 2) to 36 days *versus* 7 days as recommended (1). The loss in potency after a 36-day storage was <3.2%, and the samples were clear. The expiration date for the frozen samples may be extended to 60 days (solutions 1 and 2, Table III) *versus* 28 days as recommended (1) by the manufacturer. There was a loss of <2 and 7% in potency in 60 days for solutions in 0.9% NaCl and 5% dextrose, respectively. There was no significant change in pH values, and the solutions were clear. Furthermore, the frozen samples can be thawed in <4 min using a microwave oven, without any observable decomposition.

It is interesting to point out that the manufacturer has recommended (1) an expiration of 24 hr under refrigeration for solutions in 5% fructose and 10% dextrose *versus* 7 days for solution in 5% dextrose. In our investigations (Table III, solutions 3–5), there was no difference in the stability of these three solutions. No decomposition was found in any sample for up to 4 days under refrigeration, which was also evident from the absence of any additional peak(s) in the chromatograms. After 14 days of storage there was only slightly more decomposition of drug in solutions containing either 10% dextrose or 5% fructose *versus* 5% dextrose (Table III, solutions 3–5). There were no significant changes in pH values, and all solutions were clear.

The optimum pH of stability (Fig. 2) appears to be ~4.8. The phosphate buffer did not catalyze the reaction (solutions 8 and 9, Table III). Trials to treat the data mathematically using first-order equations were not successful, which may be due to the complexity of the reaction as evidenced by a number of new peaks (Fig. 1C) in the chromatogram. The general information about the degradation of penicillins is available in the literature (2, 3).

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COMMUNICATIONS

Buccal Absorption of Protirelin: An Effective Way to Stimulate Thyrotropin and Prolactin

Keyphrases □ Protirelin—buccal absorption, stimulation of thyrotropin and prolactin, use as a diagnostic tool □ Buccal absorption—evaluation of protirelin, use as a diagnostic tool, measurement of thyrotropin and prolactin stimulation □ Thyrotropin and prolactin stimulation—buccal absorption of protirelin, use as a diagnostic tool

To the Editor:

Most of the biologically active oligopeptides are almost, or completely, inactive if administered perorally. This can be partly attributed to low chemical stability in the course of intestinal passage, and partly to low invasion rates along with rapid plasma degradation. For the same reasons some peptides, like thyrotropin-releasing hormone (TRH) (protirelin), need extremely high peroral doses in order to stimulate biological response. Therefore, intravenous injection is the most common form of peptide administration. However, few studies on nasal (1) and rectal (2) administration have been reported. The purpose of this investigation was to evaluate buccal absorption of protirelin as a model peptide. The major objective was to set the groundwork for future research on buccal peptide delivery and absorption. This study also evaluated buccal protirelin as a diagnostic tool.

Thyroid gland diagnostics by protirelin is mainly a domain of the intravenous test, although some doubts have emerged due to serious side effects (3–5). On the other hand, the peroral protirelin test exhibits only slow response at high doses, and usually needs a 3-hr period to attain maximum stimulation. This is often considered inconvenient for routine clinical diagnostics. Therefore, buccal protirelin could become an appropriate supplement with both intravenous and peroral protirelin, if absorbed properly. This study will show the overall feasibility of buccal protirelin for use in thyroid diagnostics. Buccal absorption will be followed by monitoring thyrotropin and prolactin stimulation.

Ten clinically healthy volunteers, five males and five females, took part in the study. The age range was 23–35 years. For all volunteers an euthyroid state was certified by determinations of triiodothyronine, thyroxine, and thyroxine-binding globulin. The body weights of all volunteers were within normal limits of the ideal body weight according to Broca. All tests were performed beginning at 2 p.m.

For the intravenous test, 200 μg of protirelin[1] was injected in the antecubital vein. Blood samples were taken immediately before and 30 min after injection. For buccal application a polytef disk was prepared with a diameter of ~3.5 cm, corresponding to an area of ~10 cm^2 and a height of 1 cm. The disk had a central circular depression depth of 4 mm, leaving an elevated rim. A previously water-soaked filter paper disk was placed into the depression, and 20 mg of crystalline protirelin[2] was spread onto the filter paper. The protirelin dissolved immediately. Subsequently, the device was put into contact with the buccal mucosa. After 30 min the device was removed, and the mouth was thoroughly washed with tap water. Blood samples were taken at 0, 30, 60, 120, and 180 min *via* a cannula placed into the antecubital vein.

After centrifugation the plasma was separated from the blood and stored at −20° until analysis of thyrotropin and prolactin.

Measurements of thyrotropin[3], prolactin[4], thyroxine-binding globulin[5], triiodothyronine[6], and thyroxine[6] were performed in duplicate using commercially available radioimmunoassays. Standard errors of the radioimmunoassays were from 6 to 8% within kits and from 3 to 9% between kits. A second, but slightly modified, test was per-

[1] Antepan, Henning, D-Berlin.
[2] Hoechst, D-Frankfurt.
[3] TSHK-PR, CIS-CEA-Sorin, I-Saluggia.
[4] PROLK-PR, CIS-CEA-Sorin, I-Saluggia.
[5] RIA-gnost TBG-kit, Behringwerke, D-Marburg.
[6] ARIA II, Becton and Dickinson, Paramus N.J.

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