zation of 1,2-diethyl-4-pyrazolidinethiol was not depressed. The IR spectra of the two picrates were superimposable.

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Colorimetric Determination of Penicillins and Related Compounds in Intravenous Solutions by Nickel(II)-Catalyzed Hydroxamic Acid Formation

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Abstract □ Solutions of ampicillin, carbenicillin, methicillin, oxacillin, penicillin G, and cephalothin in 5% dextrose were analyzed by nickel(II)-catalyzed hydroxylaminolysis. The reactions of these antibiotics were complete within 20 min at room temperature. Under the analytical conditions, molar absorptivities of the ferric–hydroxamate complexes ranged from 830 to 1005 liters/mole/cm. Coefficients of variation for the analysis of these antibiotics in 5% dextrose were typically <3% at concentrations of 1 mg/ml. Oxacillin was analyzed by the same method in normal saline and/or lactated Ringer solutions. The method also was applied to the analysis of chloramphenicol in aqueous solutions. Only ampicillin showed a significant decrease in concentration in 48 hr.

Keyphrases □ Penicillins—analysis, colorimetry, intravenous solutions, dextrose, nickel(II)-catalyzed hydroxamic acid formation □ Antibiotics—analysis, colorimetry, ampicillin, carbenicillin, methicillin, oxacillin, penicillin G, cephalothin, intravenous solutions, dextrose □ Colorimetry—analysis, penicillins in intravenous solutions, dextrose, nickel(II)-catalyzed hydroxamic acid formation

Hydroxamic acid formation has been employed in the analysis of various carboxylic acid derivatives. Typically, the substrate is reacted with an alkaline hydroxylamine solution, acidified, and then complexed with ferric iron to form a reddish-violet complex. This complex absorbs in the 515–545-nm range with molar absorptivities near 10^3 liters/mole/cm. Esters (1–3), amides (4–6), acid chlorides (7), acid anhydrides (8), lactones (9), and imides (10) have been determined by the alkaline hydroxylaminolysis reaction.

Compounds containing the β-lactam moiety, *e.g.*, penicillins, also have been analyzed by the alkaline reaction (11). However, these compounds also react satisfactorily with hydroxylamine at neutral pH (12, 13). Nickel(II)-catalyzed hydroxylaminolysis has been applied to the analysis of carboxylic acids and acid hydrazides (14, 15). This method also has been applied to cephalosporins that contain the β-lactam group (16).

Table I—Stability of the Ferric–Hydroxamate Complex in Methicillin–Dextrose 5% Solutions

	Neutral Hydroxylamine Method (12)		Nickel(II)-Hydroxylamine Method	
Time[a]	In Water, absorbance[b]	In 5% Dextrose, absorbance[b]	In Water, absorbance[c]	In 5% Dextrose, absorbance[c]
2	0.465	0.455	0.485	0.475
5	0.450	0.400	0.480	0.475
10	0.440	0.330	0.477	0.475
15	0.425	0.165	0.470	0.455
30	—	—	0.463	0.455

[a] Time after mixing ferric iron reagent with the reaction mixture. [b] Absorbance at 490 nm in 1-cm cell. [c] Absorbance at 520 nm in 1-cm cell.

In this study, the stability of some penicillins and cephalothin was examined in solutions for intravenous administration. In the presence of relatively high dextrose concentrations (5% w/v), the neutral hydroxylamine method produced low color yields and color instability. Since the drugs were present in low concentrations, it was not feasible to dilute the samples to reduce the dextrose concentration. Consequently, the nickel(II)-catalyzed hydroxylaminolysis method was applied to these systems.

This paper reports the results on the application of the nickel(II)-catalyzed method to the analysis of ampicillin, carbenicillin, methicillin, oxacillin, penicillin G, and

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Table II—Analysis of Penicillins in 5% Dextrose Solutions (n = 4)

Drug	Molar Absorptivity		Concentration Added, mg/ml	Concentration Found, mg/ml	CV, %
	In Water[a]	In 5% Dextrose[a]			
Methicillin sodium	860	831	1.00	1.05	2.3
Carbenicillin disodium	798	791	1.00	1.01	1.1
Oxacillin sodium	1005	911	0.84	0.87	2.9
Penicillin G potassium	934	894	0.60	0.59	1.4
Ampicillin trihydrate	916	849	1.28	1.32	1.9

[a] Calculated on the basis of the concentration of the sample in the final solution. Units are liters per mole per centimeter at 520 nm.

Table III—Analysis of Oxacillin Sodium in Other Intravenous Fluids by Nickel(II)-Catalyzed Hydroxylaminolysis

Intravenous Fluid	Apparent Molar Absorptivity[a], M^{-1} cm^{-1}	Concentration Added, mg/ml	Concentration Found, mg/ml	CV, %	n
Normal saline	837	1.00	0.95	1.6	3
5% Dextrose in normal saline	770	0.998	1.00	2.4	4
Lactated Ringer	778	0.998	0.98	2.2	4
5% Dextrose in lactated Ringer	801	0.998	0.94	3.6	3

[a] At 520 nm.

cephalothin in 5% dextrose solutions. Data also are presented on oxacillin analysis in normal saline solution, lactated Ringer solution, and mixtures of these solutions with 5% dextrose. Finally, data are presented on the application of this method to chloramphenicol analysis.

EXPERIMENTAL

Materials—Ampicillin trihydrate[1], carbenicillin disodium[2], methicillin sodium[3], oxacillin sodium[4], penicillin G potassium[4], cephalothin sodium[5], and chloramphenicol[6] were used as received. Hydroxylamine hydrochloride, nickel(II) chloride, sodium hydroxide, hydrochloric acid, and ferric chloride hexahydrate were reagent grade. Dextrose solutions (5% w/v) were obtained commercially[7]. All water was double distilled in an all-glass system.

The nickel–hydroxylamine reagent was prepared by dissolving 6.95 g of hydroxylamine hydrochloride and 2.38 g of nickel chloride in 30 ml of distilled water. Sodium hydroxide, 10 N, was added (~7.5 ml) to adjust the solution to pH 6.2 using a silver–silver chloride reference electrode. This solution was then quantitatively diluted with water to 50.0 ml.

The ferric chloride reagent (0.35 M) was prepared by dissolving 94.6 g of ferric chloride hexahydrate in 400 ml of 0.1 N HCl. This solution was quantitatively transferred to a 1-liter volumetric flask with the aid of 0.1 N HCl and was diluted to exactly 1 liter with 0.1 N HCl. The final solution was stored in the dark to prevent photoreduction of the ferric ion.

Assay—Samples from the intravenous solutions containing the drug (2.0 or 3.0 ml) were transferred to a test tube, and sufficient water was added to make exactly 4.0 ml. Exactly 2.0 ml of the nickel–hydroxylamine reagent was added to the samples, which were allowed to stand for 20 min at room temperature. Exactly 1.0 ml of 4 N HCl and 5.0 ml of the ferric chloride solution were then added. After 5 min, the absorbance was measured in 1-cm cells on a recording spectrophotometer at the appropriate wavelength. An appropriate blank was prepared for all analyses. Concentrations were determined from calibration curves obtained from the analysis of standard solutions prepared in 5% dextrose solutions.

RESULTS AND DISCUSSION

The effect of dextrose on the stability of the ferric–hydroxamate complex generated by the neutral hydroxylaminolysis reaction (12, 13) and the nickel(II)-catalyzed method on methicillin is shown in Table I. With the neutral reaction, absorbance decreased with time even without dextrose, but the decrease was far more dramatic in the presence of 5% dextrose. A spectral shift toward higher wavelengths also was observed with time in the dextrose-containing samples.

As shown in Table I, the color instability was less with the nickel(II)-catalyzed method. Minimal absorbance changes occurred within the first 15 min after mixing. The 520-nm wavelength was chosen to minimize the blank absorbance values while maximizing the analytical sample absorbance. In some cases, a gradual increase in absorbance by the blank was observed with time. The use of freshly prepared blanks avoided this problem. No shifts in wavelength maxima were observed with this method. The greater stability in the nickel(II)-catalyzed method was probably due to nickel complexation with hydroxylamine, which lessened its tendency to reduce ferric iron to ferrous iron.

The nickel(II)-catalyzed method was applied to the analysis of methicillin, carbenicillin, oxacillin, penicillin G, and ampicillin in intravenous solutions containing 5% dextrose. In all cases, the absorbance of the resultant ferric–hydroxamate complex was linearly related to the drug concentration in the reaction mixture. A typical calibration curve for methicillin sodium can be described by:

$$\text{absorbance (520 nm)} = 0.00194\ \text{concentration (mg/ml)} + 0.015 \quad \text{(Eq. 1)}$$

In the 80–400-μg/ml range, the correlation coefficient for this line was 0.9993.

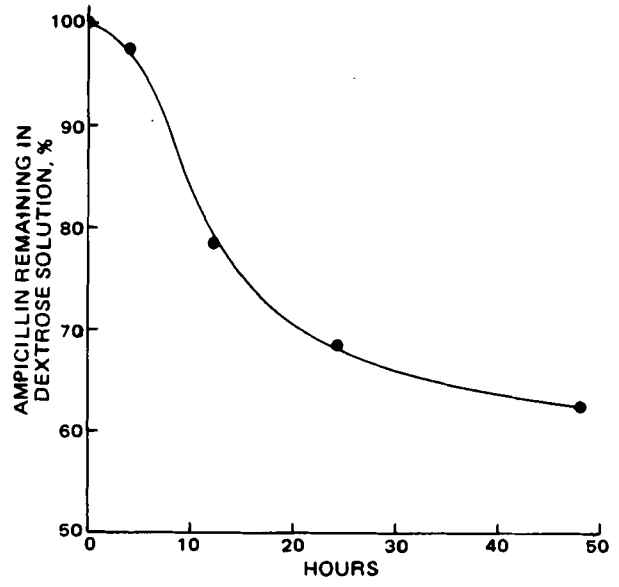

Figure 1—*Ampicillin in 5% dextrose as a function of time as determined by the nickel(II)-catalyzed hydroxylamine method.*

[1] Bristol Laboratories, Syracuse, N.Y.
[2] Pfizer Laboratories, New York, N.Y.
[3] Beecham Laboratories, Piscataway, N.J.
[4] E. R. Squibb, Princeton, N.J.
[5] USP reference standard, Rockville, Md.
[6] Parke-Davis, Detroit, Mich.
[7] McGaw Laboratories, Irvine, Calif.

The analyses of the penicillins in 5% dextrose solutions are summarized in Table II.

In most cases, the apparent molar absorptivities of the ferric–hydroxamate complexes were slightly lower in the 5% dextrose samples. Replicate determinations of 1-mg of methicillin sodium/ml samples gave good precision and accuracy.

Only ampicillin in 5% dextrose showed any appreciable change in concentration over 48 hr at room temperature (Fig. 1). Samples were obtained at zero time and at 48 hr and were analyzed by TLC. A large spot at R_f 0.65, indicative of ampicillin degradation, appeared in the 48-hr sample.

Although cephalothin was previously determined by this method (16), a study was undertaken to determine if the method is applicable to cephalothin analysis in dextrose solutions. Replicate analyses of a solution containing cephalothin sodium (1.00 mg/ml) gave an average value of 1.04 mg/ml with a coefficient of variation of 2.7%. In this study, the absorbance was measured at 490 nm and the complex was stable for at least 15 min.

Oxacillin was analyzed in several other intravenous fluids with good precision and accuracy (Table III).

The nickel-catalyzed method also was applied to a chloramphenicol succinate sodium solution. In this case, it was necessary to heat the reaction for 2 hr at 80° to obtain an adequate color yield. Under these conditions, as little as 25 μg/ml could be detected. A typical calibration curve can be described by:

$$\text{absorbance} = 0.00273 \text{ concentration } (\mu g/ml) + 0.00 \quad \text{(Eq. 2)}$$

In the 25–250-μg/ml range, correlation coefficients for this equation were typically 0.999.

Considering the extensive use of β-lactam antibiotics in intravenous admixtures, the nickel(II)-catalyzed hydroxylaminolysis method offers a rapid and reliable means of assessing antibiotic stability in numerous intravenous fluids.

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COMMUNICATIONS

Identification of Griseofulvic Acid as a Urine Metabolite of Griseofulvin in Humans

Keyphrases □ Griseofulvin—human urinary metabolite, identification, griseofulvic acid □ Antifungal agents—griseofulvin, human urinary metabolite, identification, griseofulvic acid □ Metabolite—of griseofulvin, human urine, identification, griseofulvic acid □ Griseofulvic acid—metabolite of griseofulvin, human urine, identification

To the Editor:

Griseofulvin was introduced in 1959 as an effective oral antifungal agent for humans and animals. Many studies of griseofulvin metabolism have been reported since then. Investigators of griseofulvin metabolism in humans reported that almost all of a daily griseofulvin dose was excreted as 6-desmethylgriseofulvin in the urine in 24 hr (1). Studies using ^{14}C-griseofulvin and GLC showed that the major metabolite was 6-desmethylgriseofulvin but that the drug also was excreted partially as 4-desmethylgriseofulvin and as several other unidentified metabolites (2). Although these unidentified compounds represent only a small portion of the total drug, their identities are important in understanding griseofulvin metabolism.

A simple and rapid determination of griseofulvin and its derivatives using reversed-phase high-pressure liquid chromatography was developed in this laboratory. Analysis of human urine samples by this method revealed that, in addition to 6-desmethylgriseofulvin, griseofulvic acid (7-chloro-4,6-dimethoxy-6′-methylgrisan-3,2′,4′-trione) also is a urinary griseofulvin metabolite. However, the reported 4-desmethylgriseofulvin was not observed in this study. The urinary concentration ratio of griseofulvic acid to 6-desmethylgriseofulvin was ~1:15.

Figure 1 shows chromatograms of urine samples obtained from a normal subject 6 hr after a 250-mg oral griseofulvin dose. The griseofulvin metabolites in the urine sample were extracted into cyclohexane–ethylene dichloride (1:1) (3), and the mixture then was evaporated to dryness and reconstituted with the chromatographic mobile phase solvent. Figure 1A represents the direct extraction of the urine sample. Figure 1B represents the same sample after incubation overnight at 37° with β-glucuronidase to hydrolyze the glucuronide conjugate prior to the extraction.

The chromatograms were obtained with a reversed-phase column[1] at a 1.0-ml/min flow rate. The mobile phase was 45% acetonitrile in water (v/v), with the pH adjusted to 3.4 using acetic acid. The upper chromatograms represent the response of a fluorescence detector excited at 280 nm. The lower chromatograms represent the response of a UV detector at 290 nm. The advantage of using both

[1] μBondapak C_{18}, Waters Associates, Milford, Mass.

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