Spectrophotometric Determination of Ampicillin in Presence of Hetacillin

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Abstract □ A procedure was developed for measuring small amounts of ampicillin in hetacillin bulk powders and formulations. The method depends on forming a UV-absorbing compound with Ni (II) in dimethyl sulfoxide. The response is linear over a 15-fold range and accuracy is generally better than ±5–10% except near the minimum sensitivity level. Procedural details and data for the effects of operating parameters are presented.

Keyphrases □ Ampicillin—spectrophotometric determination in presence of hetacillin □ Hetacillin, bulk powders and formulations—spectrophotometric determination of small amounts of ampicillin □ Nickel (II)-dimethyl sulfoxide reagent—used in spectrophotometric determination of ampicillin in hetacillin powders and formulations □ UV spectrophotometry—determination of small amounts of ampicillin in hetacillin powders and formulations

Hetacillin is a recently introduced broad-spectrum antibiotic. Its preparation and physicochemical properties (1) and pharmacological studies (2, 3) were reported. Hetacillin, which possesses an imidazolidinyl moiety, equilibrates with ampicillin, and this equilibrium was studied *in vitro* (4, 5). The superior solution stability of hetacillin compared to ampicillin was pointed out (5).

To take full advantage of the stability of hetacillin, it is important to manufacture a product having, at most, a low level of ampicillin and to monitor the ampicillin content analytically in the presence of bulk hetacillin.

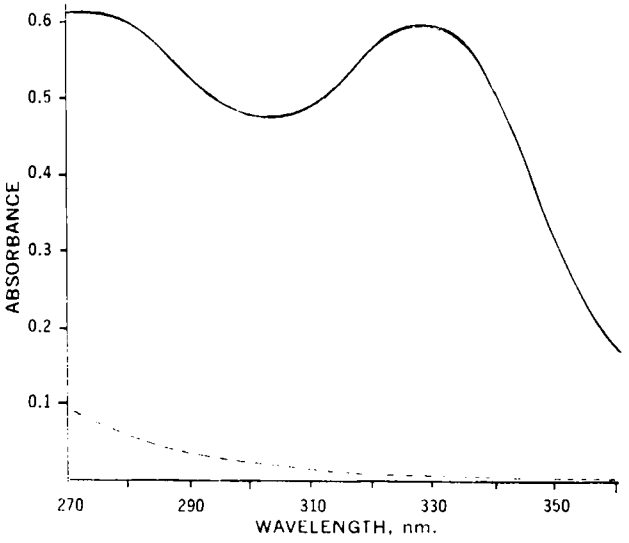


Figure 1—*Absorption spectra for ampicillin trihydrate with (——) and without (- - - -) Ni (II) in dimethyl sulfoxide. Ampicillin trihydrate concentration = 28 mcg./ml.*

Published methods for determining ampicillin depend on one of the following techniques: microbiological response (6), iodometry (6), hydroxamation (6), conversion to the penicillenic acid (7), or conversion to a fluorescing species (8). All of these approaches involve reaction and/or measurement in water. The rate of attainment of the hetacillin–ampicillin equilibrium in this medium, however, is too great to permit the selective determination of ampicillin. Both compounds are poorly soluble in nonpolar solvents, but they dissolve readily in the aprotic solvent dimethyl sulfoxide. The particular advantage of dimethyl sulfoxide, of course, is that the absence of water would be expected to inhibit the formation of ampicillin. By taking advantage of the presumed stability of hetacillin in dimethyl sulfoxide, the solubility of certain transition metal ions in the same solvent (9), and the tendency of amino compounds to form metal complexes, several organic compounds of interest were studied. This paper reports the use of Ni (II) in dimethyl sulfoxide to measure low levels of ampicillin in hetacillin and confirms the stability of the latter in this medium.

EXPERIMENTAL

Ni (II)–Dimethyl Sulfoxide Reagent—Stir 2.6 g. of finely powdered nickel sulfate ($NiSO_4 \cdot 6H_2O$) in 1 l. of dimethyl sulfoxide (ACS grade) for 5 hr. in a stoppered bottle. Filter through filter paper[1] to give a clear yellow solution containing about 0.5 mg. Ni (II)/ml., as determined by disodium edetate (EDTA) titration. The solution is stable indefinitely when protected from moisture.

Penicillins—Standard reference grade ampicillin trihydrate (99.2% purity) and hetacillin (97.0% purity) were used throughout.

Microbiological Analyses—The disk-plate method[2] (10), using a medium prepared by overlaying BBL Base Agar 10944 with BBL Seed Agar AA#1 and inoculated with *Sarcina lutea* (ATCC 9341), was used.

Spectrophotometry—Readings were taken on two spectrophotometers[3] in 1-cm. cells.

Analytical Procedure for Ampicillin in Hetacillin—*Standard Curve*—Dissolve 100 mg. ampicillin trihydrate in 100 ml. dimethyl sulfoxide. Dilute 5.00 ml. of this solution to 100 ml. with dimethyl sulfoxide. To five 25-ml. volumetric flasks, transfer respectively 0, 5.00, 10.00, 15.00, and 20.00 ml. of the previous solution, add 5.00 ml. of Ni (II)–dimethyl sulfoxide reagent to each, and dilute to volume with dimethyl sulfoxide. Stopper, heat exactly 10 min. at 50° in a

[1] Whatman No. 2.
[2] Paper disks used were S&S No. 740-E.
[3] Cary 14 and Beckman Acta III.

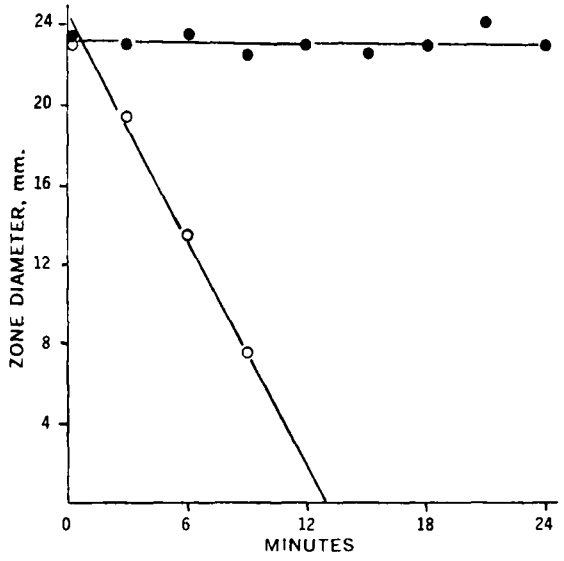


Figure 2—*Comparison of the reactivity of hetacillin (●) to ampicillin (○) when heated with Ni (II)-dimethyl sulfoxide at 50° for various times and assayed by the disk-plate microbiological method. Initial solutions had a potency of 20 mcg./ml.*

water bath, cool to room temperature, and measure the absorbance at 330 nm. against the standard containing no ampicillin as a blank. Plot absorbance *versus* concentration to obtain the standard curve.

Samples—Dissolve in dimethyl sulfoxide to contain 200 mcg. ampicillin/ml. Dilute a 10.00-ml. aliquot to 100 ml. with dimethyl sulfoxide. Pipet 20.00 ml. into a 25-ml. volumetric flask, add 5.00 ml. of Ni (II)-dimethyl sulfoxide reagent, and proceed in the same way as for standards.

EFFECTS OF ANALYTICAL VARIABLES

Figure 1 is the absorption spectrum for the product of the Ni (II)-dimethyl sulfoxide reaction with ampicillin trihydrate. Also shown is the absorption spectrum obtained when the same amount of ampicillin trihydrate is heated for 10 min. in dimethyl sulfoxide at 50° without the addition of Ni (II)-dimethyl sulfoxide reagent. Hetacillin, when treated with Ni (II)-dimethyl sulfoxide reagent under the same conditions as the ampicillin trihydrate, gave no significant absorbance at 330 nm.

The lack of reactivity of hetacillin to the Ni (II)-dimethyl sulfoxide reagent was also demonstrated by a microbiological method. In Fig. 2 the diameters of the zones of inhibition are plotted *versus*

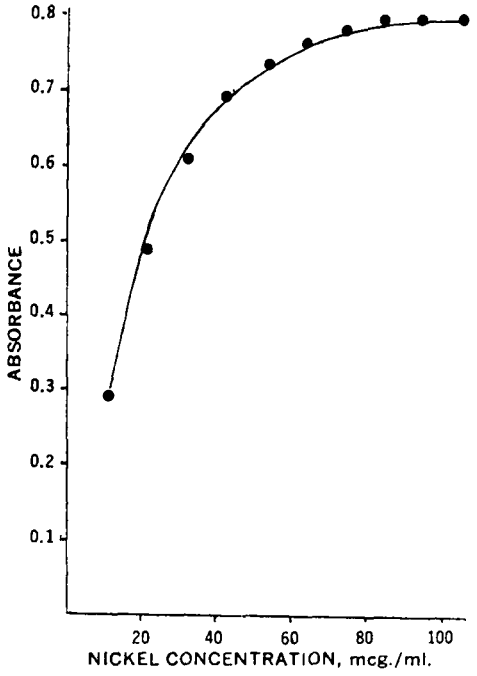


Figure 4—*Effect of Ni (II) concentration on the reaction of ampicillin with Ni (II) in dimethyl sulfoxide. Ampicillin trihydrate concentration = 38 mcg./ml.*

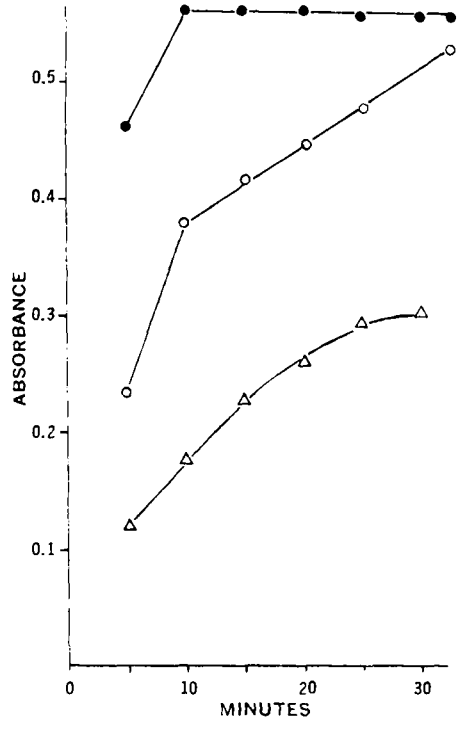


Figure 3—*Effect of temperature on the rate of reaction of ampicillin in Ni (II)-dimethyl sulfoxide reagent. Key: ●, 50°; ○, 40°; and △, 30°. Ampicillin trihydrate concentration = 28 mcg./ml.*

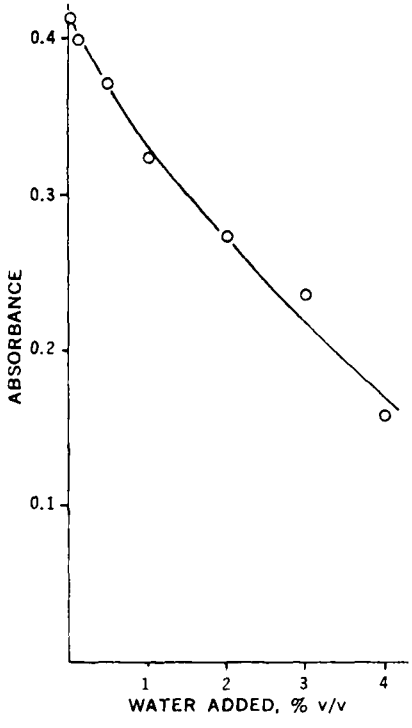


Figure 5—*Effect of added water on the reaction of ampicillin with Ni (II) in dimethyl sulfoxide. Ampicillin trihydrate concentration = 24 mcg./ml.*

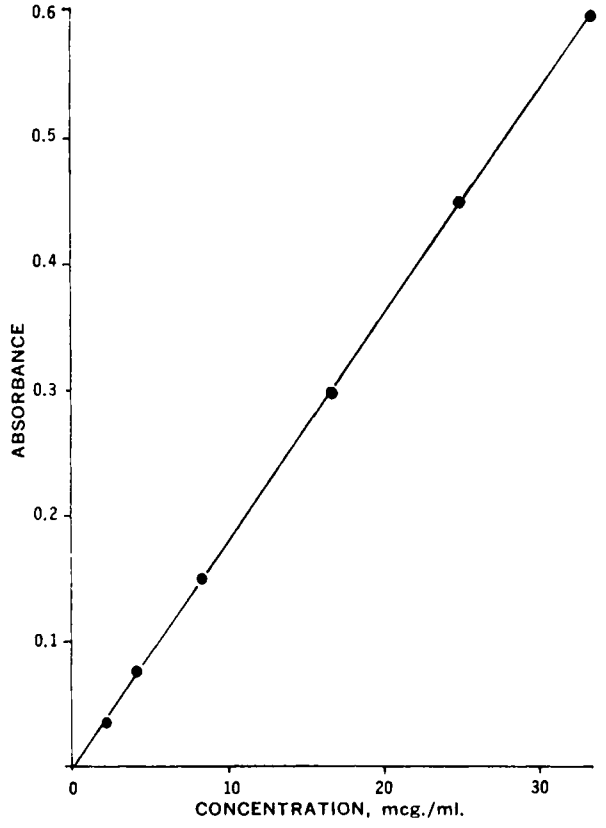


Figure 6—*Calibration curve for ampicillin trihydrate as determined by the Ni (II)–dimethyl sulfoxide reaction.*

reaction time at 50° for ampicillin and hetacillin. The size of the zone of inhibition produced by hetacillin is virtually unchanged over 24 min., whereas the last detectable bioactivity for ampicillin is obtained at 9 min. In addition to showing the lack of reactivity of hetacillin to Ni (II)–dimethyl sulfoxide, these data establish that ampicillin is converted to a nonantibiotic by reacting with Ni (II)–dimethyl sulfoxide reagent.

Table I—Recovery of Ampicillin Trihydrate Added to Potassium Hetacillin

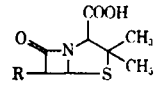
Added[a], %	Found[a], %	Recovered, %
0.75	1.0	130
1.0	1.0	100
1.5	1.7	113
2.0	2.1	105
2.5	2.7	104
3.5	3.8	109
4.9	4.5	92
5.8	5.4	93
7.4	7.8	105
9.8	9.4	96
17	16	95
27	26	97
41	40	99

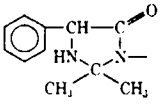
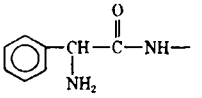
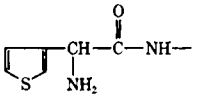
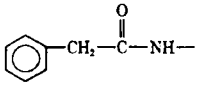
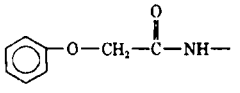
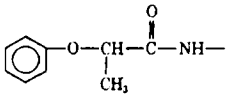
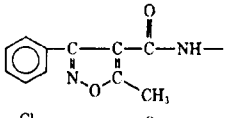
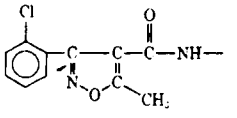
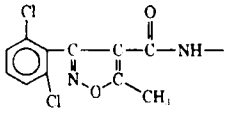
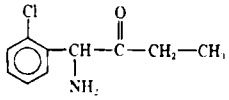
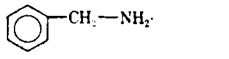
[a] This sample of hetacillin, without added ampicillin, was found to contain 1.65% as the trihydrate. The data reported were corrected for this original content.

Table II—Ampicillin in Nonhetacillin Preparations

Ampicillin	Second Component	Ampicillin [Ni (II)–Dimethyl Sulfoxide]
250 mg. (bioassay)	Oxacillin	245 mg.
520 mg. (bioassay)	Oxacillin	513 mg.
250 mg. (label claim)	Dicloxacillin, 125 mg.	260 mg.
28% (synthetic mixture)	Lactose	27%
42% (synthetic mixture)	Lactose	40%

Table III—Compounds Tested for Reactivity with Ni (II)–Dimethyl Sulfoxide



Compound	R	Reaction to Produce Chromophore
A. Penicillin		
Hetacillin		No
Ampicillin		Yes
		Yes
Penicillin G		No
Phenoxymethyl penicillin		No
Phenethicillin		No
Oxacillin		No
Cloxacillin		No
Dicloxacillin		No
B. Compounds related to penicillins		
6-Aminopenicillanic acid		No
Penicilloic acid of phenoxymethyl penicillin		No
Benzylpenillic acid		No
Benzylpenilloic acid		No
C. Other compounds		
		Yes
		No
NH_2CH_2COOH		No
$NH_2CH_2CO-NH-CH_2COOH$		No

The effects of temperature, time, and Ni (II) concentration on the rate of reaction of ampicillin are shown in Figs. 3 and 4. These data led to the adoption of the standard reaction conditions of 50° heating for 10 min. with a Ni (II) concentration of approximately 100 mcg./ml.

The effect of water on the sensitivity of the Ni (II)–dimethyl sulfoxide reaction for ampicillin is demonstrated in Fig. 5. The addition of 0.5% water to the reaction medium decreased the absorbance by approximately 10%. A very similar effect was obtained when methanol was added. The addition of acetonitrile or tetrahydrofuran, on the other hand, had no significant effect on absorbance. Consideration of these data led to the conclusion that alcohol and excessive water must be excluded from the reaction medium to obtain maximum sensitivity. Water is naturally allowed from three sources; it is calculated that the contribution of water from the ampicillin trihydrate will not be greater than 0.001%, the contribution from nickel sulfate will be less than 0.004%, and the contribution from dimethyl sulfoxide itself will be 0.02%. The water from the last two sources is maintained constant. The data shown in Fig. 5 are for water above that contributed by these three sources and show that water from sources such as wet glassware or aqueous samples may significantly affect sensitivity. The water naturally present in powdered samples should not be significant.

Figure 6 is a calibration curve for ampicillin trihydrate measured as the product of the Ni (II)–dimethyl sulfoxide reaction. A linear relationship exists between absorbance and concentration from at least 2 to 30 mcg./ml.

RESULTS

The described procedure was used to determine ampicillin in bulk hetacillin. Table I lists recovery data for bulk to which was added 0.75–41% ampicillin trihydrate. The original hetacillin was found to contain 1.65% ampicillin trihydrate. The same procedure appears to be satisfactory in the presence of compounds other than hetacillin (Table II). Except for the recovery at the lowest level, accuracy appears to be generally better than ±5–10% of the amount of ampicillin present.

DISCUSSION

Table III lists compounds tested for reaction with the nickel reagent and the results obtained. The nature of the response is consistent with the required presence of an α-aminoaryl acetyl group, although additional model compounds should be considered before the aryl group is determined as necessary.

The coincidence of the absorbance maximum with that shown by penicillenic acids suggests that the latter may be involved. This is almost certainly not the case, however, based on the following facts: (*a*) a number of the penicillins (Part A of Table III) have been shown to form penicillenic acids (11, 12), but only ampicillin produces the chromophore under these conditions; (*b*) at least one compound (Part C of Table III) is not capable of forming a penicillenic acid but gives a positive response to this procedure; and (*c*) the substitution of Ni (II) for the Cu (II) used in catalyzing the formation of penicillenic acids from intact penicillins leads to failure of that method.

The reactivity is assumed to be due to the ampicillin side chain alone, and Ni (II) presumably either complexes or catalyzes a reaction to form a chromophore in dimethyl sulfoxide. Since the application of Job's (13) method of continuous variation failed to support a simple molar relationship between ampicillin and nickel ion, there is no evidence that the chromophore is a complex. Complexation, of course, may be a step in the mechanism of reaction. Studies with additional model compounds are continuing.

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