Spectrophotometric Determination of Sodium 2,6-Dimethoxyphenyl Penicillin Monohydrate (Methicillin)

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Sodium 2,6-dimethoxyphenyl penicillin in pH 3.8 acetate buffer shows no absorbance in the region 290 mμ to 360 mμ. When heated in this buffer an absorption maximum appears at 330 mμ. The increase in absorption is directly proportional to the penicillin concentration. This procedure has been used to determine potencies in finished products. Results are compared with the iodimetric and microbiological methods.

HERRIOTT (1) investigated the degradation of salts of penicillin G, X, K, and F under controlled acidic conditions. Procedures are given for the determination of high and low potency material, in which solutions are diluted in 0.4 *M* acetic acid-sodium acetate buffer, pH 4.6, and heated for 15 minutes at 100°. The difference in absorbance at 322 mμ between heated and unheated buffered solutions provides a means of measuring total penicillin present. The results are within 5.0% of theoretical values. Herriott noted in his work that the change in absorbance did not increase linearly with concentration.

Stock (2) modified the method proposed by Herriott. His work demonstrates the importance of the presence of trace amounts of copper in the penicillin-penicillenic acid reaction. When copper is added to the buffer solution, reproducible results are obtained in the determination of benzylpenicillins in penicillin oral tablets. Stock demonstrated the effects of variable conditions such as time of immersion in water bath, volume of penicillin buffer mixture, and concentration of copper. By adding a small amount of copper, not only is the reproducibility improved, but the sensitivity is increased approximately ten per cent.

More recently Holbrook (3) investigated the experimental conditions and expanded the original method to include determination of various salts of benzylpenicillin in ointments, lozenges, oily injections, and suspensions. Penicillin potencies obtained by the spectrophotometric method are compared with those obtained by microbiological methods.

We have studied the application of these methods to one of the new synthetic penicillins, methicillin,[1] for the purpose of developing a routine analytical procedure. The main advantages of the proposed method are simplicity, speed, and sensitivity. The procedure has been successfully employed for analyzing large numbers of samples.

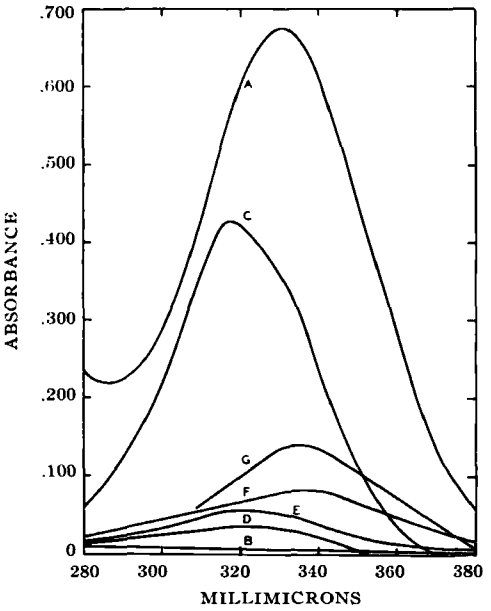

Fig. 1—Absorption spectra of penicillins: 4.76×10^{-5} moles per ml., 0.2 *M* buffer, pH 3.8, 30 min., 70°C. A, methicillin; B, 6-aminopenicillanic acid; C, benzylpenicillin potassium; D, potassium phenoxyethylpenicillin; E, potassium phenoxyethylpenicillin; F, D(-)α-aminobenzylpenicillin (free acid); and G, potassium methylphenylisoxazolylpenicillin.

EXPERIMENTAL

Reagents.—*Dilute Copper Sulfate Solution:* Dissolve 0.392 Gm. of copper sulfate pentahydrate in 100 ml. of distilled water. This solution contains 1 mg. of copper per ml.

Buffer Solution, pH 3.8: Mix 13 ml. of 2 *M* sodium acetate and 87 ml. of 2 *M* acetic acid. Add 0.5 ml. of the dilute copper sulfate solution and dilute to 1 L. with distilled water. The resulting solution is 0.2 *M* in respect to acetate and

Received April 13, 1962, from Bristol Laboratories, a division of Bristol-Myers Co., Syracuse, N. Y.
Accepted for publication July 5, 1962.
The authors are indebted to Mr. J. P. Messerly of Bristol Laboratories and Dr. W. D. Cooke of Cornell University for their suggestions and encouragement.

[1] Marketed as Staphcillin by Bristol-Myers Co.

contains 0.5 mcg. of copper per ml. Adjust the pH within the range 3.7–3.9 with sodium acetate or acetic acid, as required.

Preparation of Standard Curve.—Prepare an aqueous solution containing 500 mcg. of standard methicillin per ml. Transfer a 2.0-ml. aliquot to each of two 100-ml. volumetric flasks. Dilute the contents of one flask to volume with the buffer solution. Dilute the other to volume with distilled water. Transfer 10 ml. of the buffer dilution to a 16 mm. × 150 mm. test tube and place in a 70° water bath for 30 minutes. Remove and cool immediately in an ice bath to quench the reaction. Determine the absorbance within 1 hour in a 1-cm. cell at 330 mμ, using the water dilution as reference. Establish additional points on the calibration curve by using 3.0- and 4.0-ml. aliquots of the methicillin standard solution. Plot absorbance *versus* mcg. per ml.

Procedure.—Prepare an aqueous solution containing approximately 500 mcg. methicillin per ml. Dilute 3.0 ml. of the solution to 100 ml. with the buffer solution. Prepare a similar 3.0 to 100 dilution with water, which serves as the reference solution. Proceed as directed in preparation of the standard curve, and determine the concentration from the prepared graph.

RESULTS AND DISCUSSION

Figure 1-A illustrates the change that occurs when methicillin is heated under optimum conditions of pH, time, and temperature. Equivalent amounts of other natural and synthetic penicillins heated under these conditions produce absorption spectra shown in Fig. 1, B-G. Note that methicillin develops an absorption maximum at 330 mμ, whereas maxima of the other penicillins appear at different wavelengths in the 310–340 mμ range. Table I contains the absorptivities at the absorption maximum for each penicillin.

TABLE I.—ABSORPTIVITIES AND ABSORPTION MAXIMA OF VARIOUS PENICILLINS IN 0.2 *M* ACETATE BUFFER, pH 3.8, AFTER HEATING FOR 30 MIN. AT 70°C.

	Wavelengths at Absorption Maximum in 310–340 mμ Range	Absorptivities at λ Max.
2,6-Dimethoxyphenylpenicillin, sodium salt	330 mμ	330
6-Aminopenicillanic acid	None	...
Benzylpenicillin, potassium salt	317 mμ	240
Phenoxymethylpenicillin, potassium salt	320 mμ	11
Phenoxyethylpenicillin, potassium salt	320 mμ	21
D(−) α-Aminobenzylpenicillin (free acid)	335 mμ	44
Methylphenylisoxazolylpenicillin, potassium salt	334 mμ	64

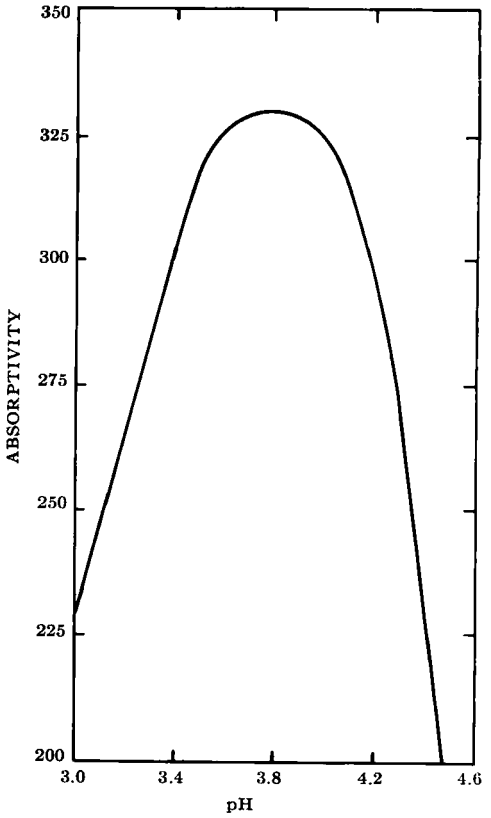

Fig. 2.—Effect of pH: 0.2 *M* buffer, 30 min., 70°C.

The conditions necessary to produce maximum sensitivity and reproducibility for methicillin were found to be 30 minutes heating at 70° in 0.2 *M* acetate buffer, pH 3.8, containing 0.5 mcg. copper

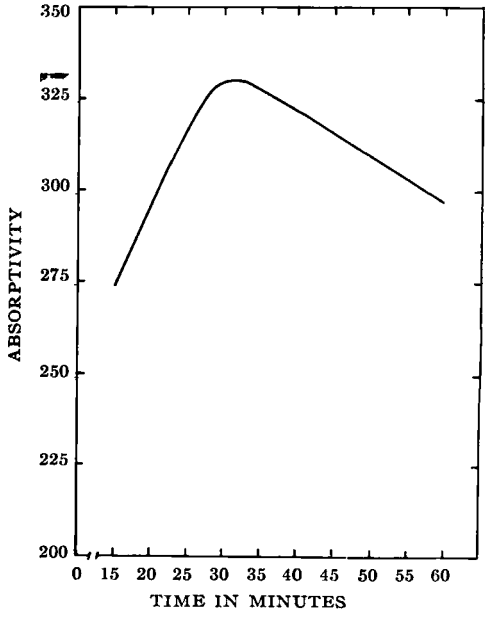

Fig. 3.—Effect of heating time: 0.2 *M* buffer pH 3.8, 70°C.

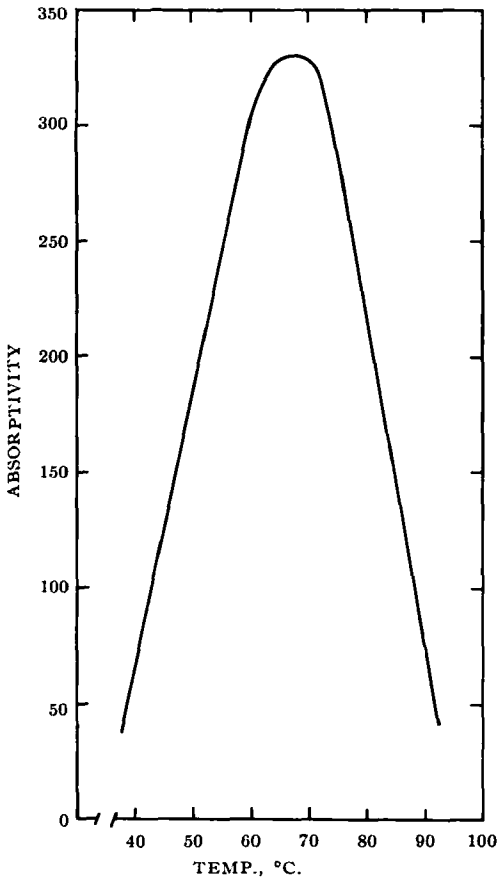

Fig. 4.—Effect of temperature: 0.2 *M* buffer, pH 3.8, 30 min.

per ml. The effect of acetate buffers over a pH range of 3.0 to 4.6 was studied, the results being shown in Fig. 2. Figure 3 shows the effect of time of heating at 70° in 0.2 *M* acetate buffer, pH 3.8. The maximum absorptivity develops after 30 minutes. Between 28 and 40 minutes the range of variation in the absorptivity is approximately 1.3%. Figure 4 indicates the effect on the absorptivity at 330 mμ when methicillin is heated in pH 3.8 buffer at various temperatures for 30 minutes. Heating at 25°, 50°, and 100° at other pH levels produces lower absorptivities.

The effect on absorptivity using buffers ranging from 0.1 to 1.0 molar is illustrated in Fig. 5.

The method was also applied to buffered methicillin containing 5% sodium citrate. The results are compared with iodimetric and biological assays

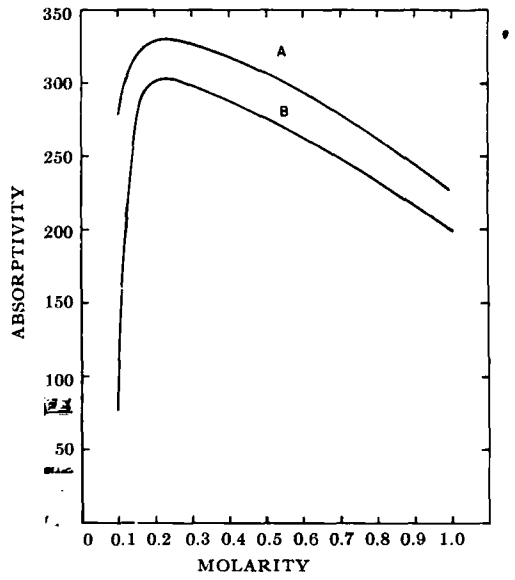

Fig. 5.—Effect of buffer molarity: pH 3.8, 30 min., 70°C. *A*, 0.5 mcg. copper per ml. added; *B*, without copper.

in Table II. It can be seen that sodium citrate causes no interference.

TABLE II.—COMPARISON OF THE RESULTS BY THE SPECTROPHOTOMETRIC METHOD WITH THOSE BY IODIMETRIC AND BIOASSAY

Sample	Spectrophotometric, mcg./mg.	Iodimetric, mcg./mg.	Bioassay, mcg./mg.
Buffered methicillin	850	854	850
Buffered methicillin	834	830	860
Buffered methicillin	847	837	830
Unbuffered methicillin[a]	877	880	870

[a] Theoretical potency of methicillin (sodium salt, monohydrate) is 905 mcg./mg.

Precision.—A statistical study of the recommended procedure was made by assaying eight samples ranging from 10 to 20 mcg. methicillin per ml. The apparent relative standard deviation(s) calculated as percentage of average absorptivity is ±1.8%.

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(2) Stock, F. G., *Analyst*, **79**, 662(1954).
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