THE SPECTROPHOTOMETRIC DETERMINATION OF 5-METHYL-3-(2,6-HALOGEN SUBSTITUTED PHENYL)-4-ISOXAZOLYL PENICILLINS (CLOXACILLN, DICLOXACILLIN AND FLUCLOXACILLIN)

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A method is described for the determination of 5-methyl-3-(2, 6-halogen substituted phenyl)-4-isoxazolyl penicillins (cloxacillin, dicloxacillin and flucloxacillin) by measuring the absorbance at 338~340 mμ of penicillenic acid formed on heating at 70°C in pH 2.0 buffer solution with copper as a catalyst. The results obtained with samples of cloxacillin, dicloxacillin and flucloxacillin are in good agreement with those obtained by biological assay.

In 1949 HERRIOTT[1] described a method for determination of penicillin by measuring the absorbance at 310~350 mμ of the penicillenic acid formed on heating in acidic buffer solution. It was reported by STOCK[2] that a trace of copper in the buffer solution plays an important role in the reaction, and that this addition of a trace amount of copper makes the assay sensitive and reproducible. Recently, it was reported that the potencies obtained by spectrophotometric determination of semi-synthetic penicillins, ampicillin[3] and methicillin[4] are in agreement with those obtained by biological methods.

We studied the formation conditions of penicillenic acids for cloxacillin, dicloxacillin and flucloxacillin, and found the optimal conditions for the spectrophotometric assay.

Although an assay method based on the same principle has been published in the British Pharmacopœia (1968) for sodium cloxacillin capsules, it is submitted that the new method described herein is more favorable than the B. P. method with respect to sensitivity and speed.

Materials and Methods

Copper sulfate solution: Dissolve 3.93 g copper sulfate in distilled water and dilute to 1,000 ml.

Buffer solution of pH 2.0 containing 40 mcg/ml Cu: Mix 53 ml of 0.2 N hydrochloric acid and 250 ml of 0.2 N potassium chloride, add 40 ml of the copper sulfate solution, and dilute to 1,000 ml with distilled water.

Apparatus: Absorbance was determined by using a Hitachi Perkins-Elmer Spectrophotometer (Model 139) or a Hitachi Recording Spectrophotometer (Model EPS-3T). pH was estimated by use of a Toa Electronics' pH-meter (Model HM-5A).

Procedure: Weigh accurately a portion of the powder corresponding to about 0.04 g of penicillin, add 15 ml of distilled water, shake for 15 minutes, and add distilled water to make exactly 20 ml and filter, if necessary (Test solution). Weigh accurately about 0.04 g

of the penicillin reference standard, and add distilled water to make exactly 20 ml (Standard solution).

Measure exactly 2.0 ml of each of these solutions, and add the buffer solution of pH 2.0 to make exactly 100 ml. Measure exactly 10 ml of the above solutions, transfer to 20-ml volumetric flasks, and allow to stand for 20 minutes at 70°C. Then, cool immediately with ice-cold water to room temperature, and add ethanol to make exactly 20 ml. Determine the absorbance of each solution, E_T and E_S, in a 10-mm cell at 338~340 mμ.

Unless otherwise stated, all experiments were carried out in aqueous solutions containing 40 mcg/ml of the penicillins.

Results

Wavelength of absorption maximum: Under the optimum conditions, the wavelengths of ultraviolet absorption maxima were 338, 339 and 400 mμ for cloxacillin, dicloxacillin and flucloxacillin, respectively (Fig. 1). In the experiments described below, the absorbance was measured at the absorption maximum of each penicillin.

Effect of pH: The penicillin solutions were adjusted to pH 1~6. In all the

Fig. 1. Ultraviolet absorption spectra of degraded penicillins. (pH 2.0, 70°C, 20 min., copper 40 mcg/ml)

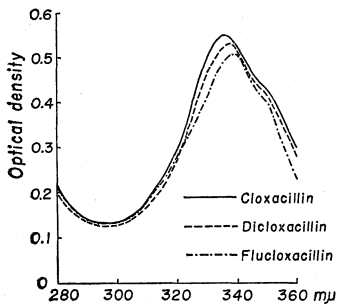

Fig. 2. Effect of pH. (70°C, 20 min., copper 40 mcg/ml)

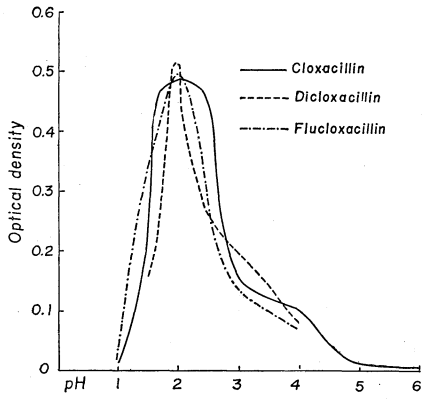

Fig. 3. Effect of copper concentration. (pH 2.0, 70°C, 20 min.)

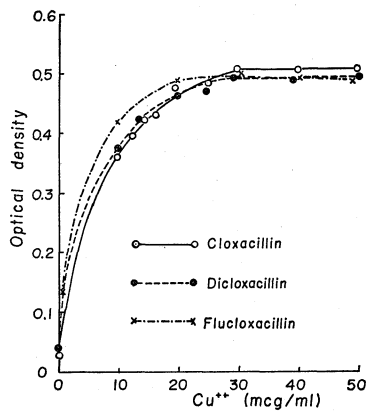

Fig. 4. Time of heating. (70°C, pH 2.0, copper 40 mcg/ml)

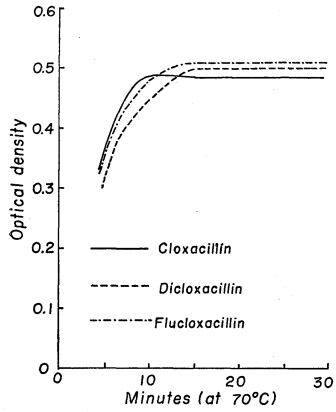

Fig. 5. Effect of temperature. (20 min., pH 2.0, copper 40 mcg/ml)

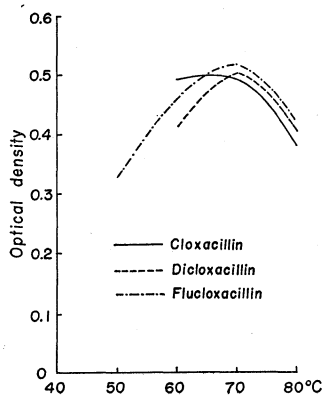

Fig. 6. BEER's law (pH 2.0, 70°C, 20 min., copper 40 mcg/ml)

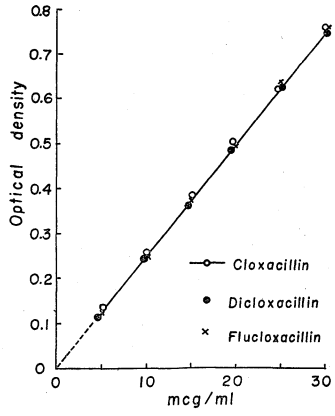

Table 1. Precision of spectrophotometric method.

Sample	Replication	Coefficient of variation
Cloxacillin	5	0.7 %
Flucloxacillin	7	0.5 %

Table 2. Comparison of spectrophotometric assay and biological assay.

Sample	Sample No.	Spctrophotometric assay potency (mcg/mg)	Biological assay potency (mcg/mg)
Cloxacillin	1	882	888
〃	2	437	445
〃	3	133	123
Cloxacillin capsules*	4	882	888
〃 〃	5	862	864
〃 〃	6	848	848
〃 〃	7	848	842
〃 〃	8	840	848
Dicloxacillin	9	891	890
〃	10	801	818
〃	11	649	658
〃	12	578	578
Flucloxacillin	13	924	921
〃	14	890	872
〃	15	765	748
〃	16	694	703
〃	17	552	534
Flucloxacillin capsules	18	671	685

* Capsules were prepared with a suitable inert diluent.

Table 3. Comparision of British Pharmacopœia method and new method.

Condition for determination	B. P. method	New method
Copper concentration	1.2 mcg/ml	40 mcg/ml
Buffer solution	pH 3.2	pH 2.0
Temperature	75°C	70°C
Heating time	50 min.	20 min.
Measuring concentration	150 mcg/ml	20 mcg/ml
Absorbance at 338 mμ	about 0.360	about 0.500
Solvent of formed penicillenic acid	water	ethanol-water

penicillins tested, the highest absorbance was observed at pH 2.0 as shown in Fig. 2.

Effect of copper concentration: The effect of copper ion concentrations is shown in Fig. 3. A copper ion concentration of 40 mcg/ml in the buffer solution was selected.

Effect of heating time: Under the conditions of pH 2.0 and Cu^{++} 40 mcg/ml, the effect of heating time for 5~30 minutes was examined. As shown in Fig. 4, the absorbance increased with the increase in heating time until after 15 minutes at 70°C.

Effect of temperature: Under the conditions of pH 2.0, Cu^{++} 40 mcg/ml, and heating for 20 minutes, the effect of temperature was examined. From the results shown in Fig. 5, 70°C was selected as optimal.

BEER's law: Under the optimal conditions described above, solutions of the penicillins at 5~30 mcg/ml were assayed spectrophotometrically. As shown in Fig. 6, BEER's law was observed between the concentrations of the penicillins and the absorbance. The penicillin solutions showed the same absorbance for 5 hours after they were prepared.

Precision: The precision was studied by measuring the same sample several times. The results are shown in Table 1.

Comparison with biological assay: Using 18 different samples of cloxacillin, dicloxacillin and flucloxacillin, the potencies obtained by the spectrophotometric method were compared with those obtained by a biological method. By both methods, practically the same potency was assayed as shown in Table 2.

Discussion

It was shown that the potencies of cloxacillin, dicloxacillin and flucloxacillin can be estimated by the same spectrophotometric method. The method is simple and accurate.

Many samples can be assayed simultaneously, because the solutions are stable at room temperature. A comparison of the British Pharmacopœia method and our new method is given in Table 3.

The new method has the following characteristic features: the copper concentration is considerably higher, the heating time is less than a half, and the sensitivity is approximately 10-times higher than the B. P. method. Ethanol is added to dissove penicillenic acid formed. The new method gives satisfactorily reproducible results which are in agreement with those obtained by a biological method.

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