Journal of the Pharmaceutical Society of Korea
18, 190-196 (1974)

Studies on the Colorimetric Determination of Drugs with Phosphomolybdic Acid. I.

Colorimetric Determination of Ampicillin with Phosphomolybdic acid

Wang-Kyu Lee, Byung-Tai Yoo and Gil-Jong Kang

(Received June 19, 1974)

Abstract — A new colorimetric method established for the determination of ampicillin was based on the reduction of phosphomolybdic acid by ampicillin to be resulted in a molybdenum blue which showed a maximal absorbance at 770 nm. The pH effects on a molybdenum blue, the reaction time and the stability of a molybdenum blue and the ef-fect of various substances were studied. The color of a molybdenum blue solution was dependent significantly on temperature, but the wavelength shift was not effected by the molybdenum blue.

Ampicillin (the D-isomer of α-aminobenzyl penicillin) has been a broad-spectrum penicillin for a good sensitivity against gram-negative bacteria since it was semi-synthesized in 1961. For this reason, many analytical methods for ampicillin have been reported. The mixture of ampicillin and its derivatives was examined by thin layer chromatography[1-4], ultra-violet by sodium hydroxide[5], infra-red by β-lactam ring[6], microbiological assay[7,8], and spectrophotometric method by Cu-complex formation[9,10] or by the benzoylation of a side chain α-amino group[1] have been applied for ampicillin analysis.

In this study we describe an analytical procedure for ampicillin which is based on the reduction of phosphomolybdic acid by ampicillin to form molybdenum blue[12, 13] in a buffer solution (pH 7.1), and which has a maximal absorbance at 770 nm (20°). Ampicillin can be quantitated to 1×10^{-5} M by the colorization of phosphomolybdic acid. The reaction mixture was very stable under the isothermal condition.

The procedure was very simple and also was used for the analysis of an ampicillin

From the Department of Pharmaceutical Analytical Chemistry, College of Pharmacy, Seoul, Korea

mixture so far as reducing materials were not contained.

EXPERIMENTAL

Preparation of a Test Solution — A standard solution was prepared by dissolving ampicillin • 3 H_2O (40.35 mg, Chong-Keun-Dang Corp., Seoul) into distilled water (100 ml). Phosphomolybdic acid ($P_2O_5 \cdot 24MoO_3 \cdot nH_2O$, Wako, E.P.) was dissolved into distilled water before use. The phosphate buffer solution (0.15 M) was prepared adjusting its pH 7.1 by potassium monobasic phosphate (KH_2PO_4) and sodium dibasic phosphate 12 hydrate ($Na_2HPO_4 \cdot 12H_2O$)

Apparatus — Measurements were made with a Hitachi recording spectrophotometer (Hitachi, Ltd. Tokyo Japan) and a Beckman DU type spectrophotometer (Beckman Instruments, Inc. Fullerton, California 92634). The pH mesurement was made with a Horiba M-7 (Horiba, Ltd. Minami-ku, Kyoto, Japan).

Procedure — In a 30-ml plugged tube, the ampicillin standard solution ($1\times10^{-5}\sim1\times10^{-3}$ M, 3 ml), phosphomolybdic acid (15% solution, 3 ml), and buffer solution (pH 7.1 ml) were added, and mixed by shaking. The mixture was heated in a water bath for 50 min. and cooled by tap-water and centrifuged for a min. at the rate of 3,000 rpm. After 40 min. the absorbance was measured at 770 nm (20°), against an appropriate blank.

RESULT and DISCUSSION

Absorption Spectrum — An absorption experiment with the ampicillin standard solution (3 ml) was performed as described above. The absorbance of a colored solution was estimated in the range of 400~800 nm and had a maximal absorbance at 770 nm as shown in Fig. 1.

Effect of pH — To the ampicillin standard (10^{-3} M, 3 ml) and phosphomolybdic acid solution (4 %, 3 ml), a serial buffer solution (pH 1 to 12, 4 ml) was placed. After reaction in boiling water for fixed time, the absorbance of blank and test solutions were measured. Below pH 7, this procedure can not be used because of precipitation. However, at pH 7.0 the color was most intense. As the precitation was formed just below pH 7, the measurement was done with pH 7.1 (Fig. 2).

Stability of the Molybdenum Blue Color (Time of Standing) — A stability test with the ampicilin standard solution (5×10^{-5} M, 3 ml) was performed as described above. As

shown in Table I, the absorbance was nearly uniform, especially after 40 min. The absorbance of a colored solution was measured daily for 5 days and shown as a constant value. It was assumed that the colored solution of molybdenum blue had very high stability.

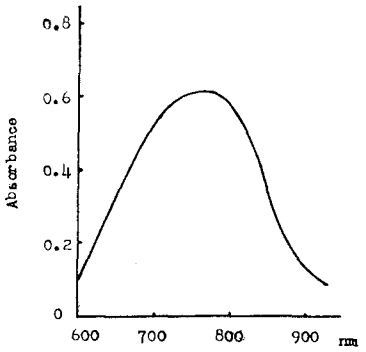

Fig. 1 — Spectrum of molybdenum blue.

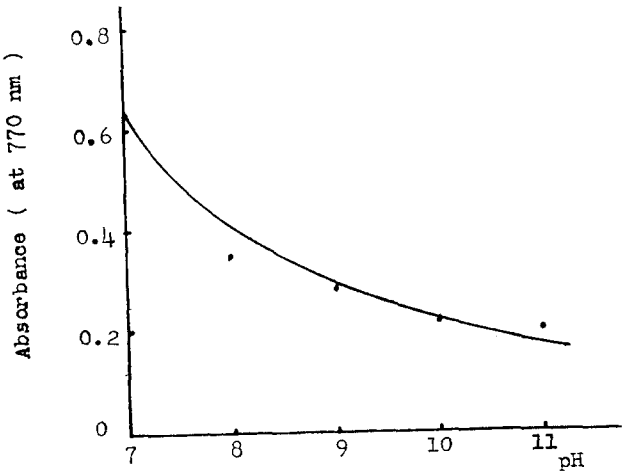

Fig. 2 — Effect of pH.

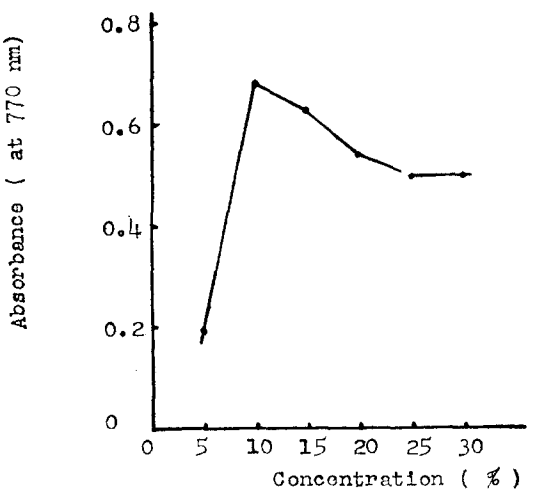

Fig. 3 — Effect of P.M.A* concentration.
* Phosphomolybdic acid.

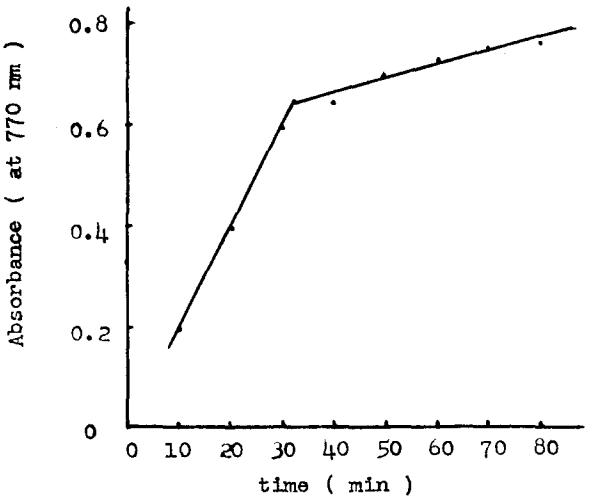

Fig. 4 — Effect of reaction time (10% P.M.A).

Effect of Phosphomolybdic Acid Concentration — To the ampicillin standard solution (3 ml), a phosphomolybdic acid solution ranging from 5 to 30 % was added. The effect depending on phosphomolybdic acid concentrations was examined as described above. The

absorbance for blanks are shown in Fig. 3. When the concentration of phosphomolybdic acid was reached to 10 % as shown in Fig. 4, the reaction was taken too much time. Therefore, a 15 % -phosphomolybdic acid solution could be recommended.

Effect of Reaction Time — To the 3-ml ampicillin standard solution (5×10^{-5} M), the reaction time ranging from 10 to 70 min. was studied. The absorbance (770 nm) for blank was shown in Fig. 5. The most suitable reaction time was 50 min.

Effect of Temperature for the Absorbance — In observing the absorbance of a reacted solution, the wavelength of the maximal absorbance of a molybdenum blue solution was not dependent up on temperature. But the absorbance was varied sensitively from the temperature. To the ampicillin standard solutions (2×10^{-5}, 4×10^{-5}, 6×10^{-5}, 8×10^{-5} and 10×10^{-5} M), experiments were proceeded as described above, and for each sample reacted, the absorbance varying its temperature of a solution (5° to 30°C) was observed. The result showed that the absorbance was inversely proportional to the temperature (Fig. 6). To each reacted solution, the regression equation was derived from that plotted the absorbance against the temperature (Table II).

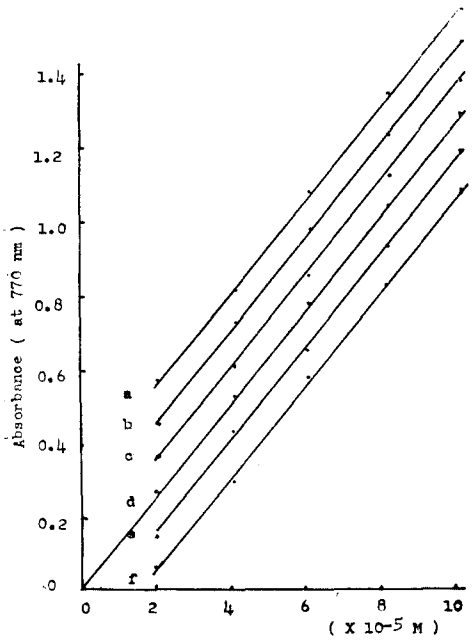

Fig. 5 — Effect of reaction time (15% P.M.A).

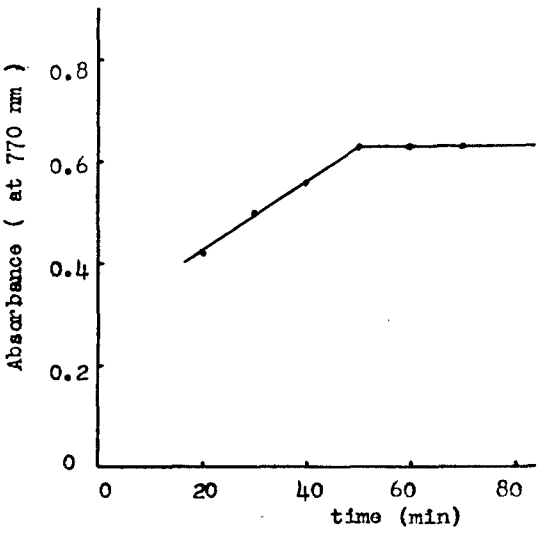

Fig. 6 — Regression line of molybdenum blue at various concentrations.
a ; 5°, b ; 10°, c ; 15°, d ; 20°, e ; 25°, f ; 30°

Table I — Stability of molybdenum blue.

Time (min)	15	20	25	30	35	40	45	50	55	65	75	85	95	125	155	185	1 day
Absorbance (at 770 nm)	0.64	0.64	0.63	0.63	0.62	0.62	0.62	0.62	0.62	0.61	0.60	0.61	0.61	0.60	0.61	0.61	0.62

Table II — Regression line of molybdenum blue at various concentration.

Concentration (M)	Equation*
2×10^{-5}	$Y=-0.02X+0.64$
4×10^{-5}	$Y=-0.02X+0.89$
6×10^{-5}	$Y=-0.02X+1.14$
8×10^{-5}	$Y=-0.02X+1.39$
10×10^{-5}	$Y=-0.02X+1.64$

* X : temperature (5°~30°C) Y : absorbance

Table III — The stability of ampicillin standard solution*.

Day	1	2	3	4—8
Absorbance** (at 263 nm)	a	a	a+0.005	a+0.015

* Concentration : 1×10^{-5} M ** a : initial absorbance

If the ampicillin standard solution was reacted completely with phosphomolybdic acid in a buffer solution, penicillamine disulfide and molybdenum blue should be formed. Since penicillamine disulfide has no absorption in the visible region, the regression equation could be derived from the change of the molybdenum blue.

The equation $Y = aX + b$ could be formulated, where *a* is a characteristic absorbance coefficient of a molybdenum blue solution; b (intercept on the Y axis), a factor related to concentration of ampicillin ; Y, absorbance ; and X, temperature. This relationship is shown in Fig. 6 and can be used for the estimation of ampicillin.

Calibration Curve — Experiments with the ampicillin solution ranging from 1×10^{-5}M to 1×10^{-4} M were performed as described above. The absorbance was observed at 770 nm (20°) against its appropriate blank. A linear relationship can be observed between the absorbance and concentration from 1×10^{-5} M to 1×10^{-3} M.

The Stability of Ampicillin Standard Solution — The ampicillin standard solution (1×10^{-3} M) was prepared and identified at 257, 263 and 268 nm which were identical to the peaks of penicillin[14]. The ultra-violet spectrum of the ampicillin standard solution (1×10^{-3}M) is shown in Table III. The result showed that the ampicillin standard solution

was not changed at least for two days.

The activity of a sodium ampicillin solution (250 mg) was reported to be decreased by the ratio of 10 %/hr. and the activity of an ampicillin solution (30 mg/ml) not to be changed within 8 hrs, but to be resulted in ampicillin-polymer formation after 8 hrs. [15,16]. The maximal concentration of ampicillin · $3H_2O$ used in this study was 0.4035mg/ml. However, in small quantities of ampicillin, the polymer formation time was expected to be more than 8 hrs.

Effect of Various Substance — An oxidation-reduction was involved in the reaction between phosphomolybdic acid and ampicillin. The effect of various substances against ampicillin is shown in Table IV.

Table IV — The effect of various substances.

Substance	Amounts added (p.p.m.)	Absorbance
Magnesium-stearate	20	0.60
Formic acid	20	0.61
Lactic acid	20	0.60
Oxalic acid	20	0.60
Soluble starch	20	0.60
Lidocaine base	20	0.60
Propyl gallate	20	1.70
Ascorbic acid	20	0.98

The concentration of ampicillin·$3H_2O$: 20 p.p.m.

Table V — Standard deviation.

Sample No.	Ampicillin found (mg)	Deviation	%
1	48.0	−2.0	96.0
2	48.5	−1.5	97.0
3	50.2	0.2	100.4
4	50.0	0.0	100.0
5	50.1	+0.1	100.2

Standard deviation : 2.06.

CONCLUSION

In this study, the analytical procedure was very simple as compared to others and ampicillin can be analysed up to 10^{-5} M. An oxidation-reduction was involved in the reaction between ampicillin and phosphomolybdic acid. Therefore, ampicillin mixture can be estimated quantitatively when reducing materials are not present in ampicillin preparations.

If reducing materials are present, ampicillin preparations can be analysed after removed the reducing material. Magnesium stearate, an additive, present in a commercial ampicillin capsule did not show a color reaction with phosphomolybdic acid.

REFERENCES

1. I. J. McGilvery and R. D. Strickland, *J. Pharm. Sci.* **56**, 77 (1967) [C.A. 66, 40746 s(1967)]
2. B. G. Luigi, Barbaro, A. M., Gamba, M. F. and Guerra, M. C., *J. Chromatogr.* **41**, 371 (1969) (Eng) [C.A. **71**, 42212 s (1969)]
3. Zuidweg, M. H. J., Oostendorp, J. G. and Bos, C. J. K., *J. Chromatogr.* **42**, 552 (1969) [C.A. **71**, 74072u(1969)]
4. Wang, J. T., Chon, H. H., Lin, C. H. and Ch'en, K. S., *Tai-Wan Ko Hsueh*, **24**, 19 (1970) [C.A. **74**, 115942 d (1971)]
5. Buryak, V. P. and Kurinnaya, N.V., *Farm. Zh.* (*Kiev*) **25**, 42(1970) [C.A. 74, 6412k(1971)]
6. Coclers, L., Delahaut, R. and Versolato, A., *J. Pharm. Bleg.* **24**, 475(1969) (*Fr*) [C.A. **73**, 7285 (1970)]
7. L. D. Sabath, V. J. Estey and M. Finland, *Appl. Microbial.* **15**, 468 (1967)
8. Grimshaw, J.J. and Jones, A., *Analyst.*, **95**, 466 (1970)
9. J. W. G. Smith, G. E. de Grey and V. J. Patel, *Analyst*, **92**, 247(1967)
10. Doadrio, Antonio and Garcia-Mirasierra Gomez, M., *An. Ral Aecad. Farm.*, **35**, 115 (1969)
11. D. E. Tutt and M. A. Schwartz, *Anal. Chem*, **43**, 338 (1971)
12. F. Feigel, "*Spot test in Organic Analysis*", **129**, 575
13. E.P. Abraham, Echain, W. Baker and R. Robinson, *Nature*, **151**, 107 (1943)
14. 分析化學便覽, **1971**, p-1411.
15. Edward J. and Levy, Gerard N., *J. Pharm. Sci.*, **61**, 727(1972)
16. Schwartz, Michael A. and Hayton, William L., *J. Pharm. Sci.*, **61**, 906(1972)