Spectrophotometric Determination of Pivampicillin Hydrochloride

E. MARCHI, G. MASCELLANI ˣ, and D. BOCCALI

Abstract □ A spectrophotometric method for the determination of pivampicillin hydrochloride is described based on the colorimetric reaction of chromotropic acid with formaldehyde, which is formed in a stoichiometric quantity on hydrolysis of pivampicillin hydrochloride. The analytical results are compared with those obtained by traditional methods.

Keyphrases □ Pivampicillin hydrochloride—spectrophotometric determination □ Colorimetry—spectrophotometric determination of pivampicillin hydrochloride □ Spectrophotometry—analysis, pivampicillin hydrochloride

Among the acyloxymethyl esters of ampicillin, interest has been aroused by pivampicillin (1), which can be administered orally with clinical and therapeutic advantages. With this antibiotic, because of its ready absorption and reasonable hydrolysis time, the concentrations of ampicillin reached in the blood in humans are about three times as high, and the quantities recovered from the urine at least twice as high, as those attainable by oral administration of a corresponding dose of ampicillin (1, 2).

It has been necessary to find a precise, accurate, and selective method of analysis for this series of products. Bricker and Johnson (3) adapted for spectrophotometry the well-known reaction between chromotropic acid and formaldehyde, which was proposed for quantitative use (4, 5). On the basis of these reports, a method was developed to obtain formaldehyde by acid hydrolysis of pivampicillin, as shown in Scheme I.

Subsequently, the quinonoid compound formed between chromotropic acid and formaldehyde (6) is determined.

EXPERIMENTAL[1]

Materials and Reagents—Pivampicillin hydrochloride was synthesized in these laboratories and purified to constant spectrophotometric values. The purity was also determined by TLC using 250-μm silica gel[2] GF_{254} plates and developed with methanol–isopropanol–water (50:50:10). Visualization was achieved by spraying with 0.3% ninhydrin in acetone and/or 1.5% sodium azide in 0.1 *N* iodine solution, giving R_f 0.65.

Concentrated sulfuric acid, 1.5 *N* hydrochloric acid, and a freshly prepared 4% aqueous solution of chromotropic acid[3] as the sodium salt were used.

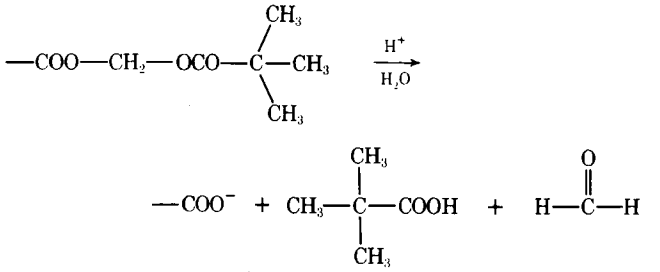
Absolutely aldehyde-free ethanol was prepared as follows. Five milliliters of 0.5% tetrazolium blue[4] in ethanol and 5 ml of 1% tetramethylammonium hydroxide[2] were added to 100 ml of alcohol. The solution was allowed to stand for 2 hr, and the ethanol was then distilled.

[1] A Perkin-Elmer twin-beam spectrophotometer (model 124) with a Perkin-Elmer recorder (model 165), a Beckman DU/2 single-beam spectrophotometer, a complete apparatus for TLC (Chemetron Milan), and a Beckman Expandomatic pH meter were used.

[2] Merck.

[3] Schilling.

[4] C. Erba.



Scheme I

The cupric chloride solution was prepared by dissolving 50 g of copper oxide in 500 ml of 3 *N* HCl in the cold and diluting to 1 liter. A Cu^{+2} concentration of 40 mg/ml was obtained.

Procedure—A sample of 40–80 mg of pivampicillin hydrochloride is accurately weighed and introduced into a 250-ml two-necked flask. A 100-ml aliquot of 1.5 *N* hydrochloric acid and 20 ml of cupric chloride solution are added. This is not necessary for pivampicillin hydrolysis, but cupric chloride keeps (according to experimental evidence) colored products otherwise easily distillable in the steam current. The flask is fitted with a condenser having an outlet adaptor dipping into a flask containing 6 ml of absolute ethanol and 15 ml of distilled water. A second flask containing water is connected in series with the first.

The solution in the distillation flask is heated to boiling (to effect the hydrolysis of the pivampicillin with consequent formation of formaldehyde) and slowly distilled in a gentle current of nitrogen. The distillation is continued until about 10 ml of liquid remains in the distillation flask. The distillate and the water used to wash the condenser, the outlet adaptor, and the receivers are combined and diluted to 200 ml in a graduated flask. The solution obtained in this way contains 0.3 μg/ml formaldehyde/mg pivampicillin hydrochloride in the weighed specimen.

The colorimetric reaction is then carried out in accordance with the details given by Mathers and Pro (7). One or more 0.2–1-ml samples are taken and transferred into an equal number of 25-ml graduated flasks. The volume of each sample is made to exactly 1 ml with 3% ethanol; a 0.5-ml aliquot of 4% chromotropic acid and 7.5 ml of concentrated sulfuric acid are then added to each flask

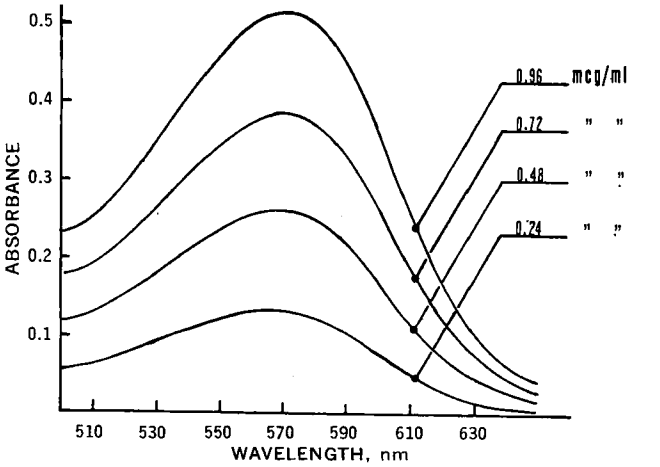


Figure 1—*Absorption spectra of the quinonoid compound of formaldehyde and chromotropic acid at various formaldehyde concentrations (temperature, 65°; heating time, 30 min; and chromotropic acid, 800 μg/ml).*

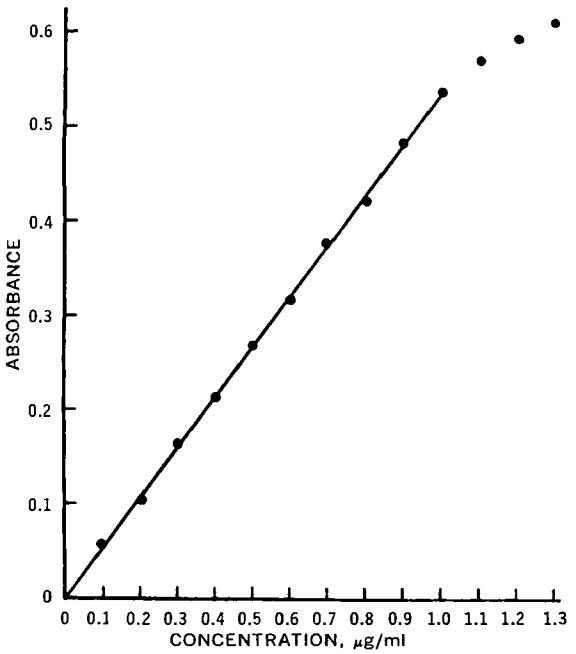


Figure 2—*Calibration curve of the formaldehyde at 570 nm (temperature, 65°; heating time, 30 min; and chromotropic acid final concentration, 800 μg/ml).*

with agitation in a bath of cold water. The solutions obtained are heated on a water bath at 65° for 30 min, immediately cooled, and then diluted to 25 ml with distilled water.

The absorbance of each solution is measured at 570 nm against a blank prepared in the same way but with an identical volume of 3% ethanol instead of the sample. The concentration of formaldehyde in the solution is determined from the absorbance with the aid of a suitable calibration curve, and the concentration of pivampicillin hydrochloride is then found by means of the factor 16.685.

The calibration curve can be constructed with substances that produce formaldehyde, such as methenamine or paraformaldehyde that has been previously standardized by the method described in the British Pharmacopoeia (8) or in the British Pharmaceutical Codex (9), respectively. The two substances, when treated as described, including the distillation, give identical and perfectly reproducible results.

Figure 1 shows some absorbance curves for the quinonoid compound. Figure 2 shows the calibration curve. The calibration curve, obtained by plotting at least 12 average values of the absorbance at 570 nm as functions of the concentrations, shows that the Beer-Lambert law is perfectly satisfied up to a concentration of 1 μg/ml.

According to the experimental results, the molar absorptivity of formaldehyde is $\epsilon = 1.61 \times 10^4$.

The experimental conditions described were chosen on the basis of preliminary investigations.

In agreement with other authors (7, 10), maximum absorbance values are obtained after heating for 30 min at 65°, with other conditions remaining constant. When the reaction is complete, the solution can be kept at ambient temperature for long periods with no appreciable decrease in the absorbance (decrease < 0.1%/min). According to the experiments, the concentration of chromotropic acid should not fall below 4%.

RESULTS AND DISCUSSION

The results obtained by the proposed method were compared with those obtained by two other procedures (Table I) and are the average ($\bar{X}$) and the standard deviation (SD) of six different determinations carried out on five samples of pivampicillin hydrochloride.

The results show that the proposed method is precise and accurate. It is a useful alternative for the determination of pivampicillin in pharmaceutical preparations together with excipients and/or other antibiotics that are not acyloxymethyl esters.

Table I—Comparison of Results Obtained with Different Methods

Pivampicillin Sample	Proposed Method		Ampicillin Method[a]		Microbiological Method[b],
	$\bar{X}$, %	SD, %	$\bar{X}$, %	SD, %	$\bar{X}$, %
1	97.1	1.09	96.2	1.25	101
2	98.6	0.87	98.4	0.83	100
3	99.6	0.92	99.0	0.91	102
4	96.8	1.15	95.3	1.12	99
5	97.7	1.17	99.2	1.05	104

[a] Adapted for pivampicillin hydrochloride (11), from the method proposed by Smith *et al.* (12) for ampicillin. This method has been approved for some time (13–15) and has been extended to other semisynthetic penicillins (16). [b] *Sarcina lutea* ATCC 9341 was used as the test microorganism, according to the general conditions described by Grove and Randall (17).

The formaldehyde–chromotropic acid colorimetric method can also be used for the analysis of all acyloxymethyl derivatives of well-known antibiotics, such as the cephalosporins including pivaloyloxymethyl cephaloglycin (18).

Chromotropic acid, which is mentioned in the British Pharmacopoeia (19) in the test for the identification of penicillins, may also be used under suitable conditions for the quantitative determination of compounds whose molecules contain groups of the type $-N{=}CH_2$ or $>N-CH_2-N<$ that yield formaldehyde on hydrolysis.

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