⟨425⟩ IODOMETRIC ASSAY—ANTIBIOTICS

The following method is provided for the assay of most of the Pharmacopeial penicillin antibiotic drugs and their dosage forms, for which iodometric titration is particularly suitable.

ASSAY

Standard preparation: Dissolve in the solvent specified in the table of *Solvents and Final Concentrations* a suitable quantity of the USP Reference Standard specified in the individual monograph, previously dried under the conditions specified in the individual monograph and accurately weighed, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about that specified in the table. Pipet 2.0 mL of this solution into each of two 125-mL glass-stoppered conical flasks.

Solvents and Final Concentrations

Antibiotic	Solvent*	Final Concentration
Amoxicillin	Water	1.0 mg/mL
Ampicillin	Water	1.25 mg/mL
Ampicillin Sodium	*Buffer B.1*	1.25 mg/mL
Cloxacillin Sodium	Water	1.25 mg/mL
Cyclacillin	Water	1.0 mg/mL
Dicloxacillin Sodium	*Buffer B.1*	1.25 mg/mL
Methicillin Sodium	*Buffer B.1*	1.25 mg/mL
Nafcillin Sodium	*Buffer B.1*	1.25 mg/mL
Oxacillin Sodium	*Buffer B.1*	1.25 mg/mL
Penicillin G Potassium	*Buffer B.1*	2,000 units/mL
Penicillin G Sodium	*Buffer B.1*	2,000 units/mL
Penicillin V Potassium	*Buffer B.1*	2,000 units/mL
Phenethicillin Potassium	*Buffer B.1*	2,000 units/mL

* Unless otherwise noted, the *Buffers* are the potassium phosphate buffers defined in the section *Media and Diluents* under *Antibiotics—Microbial Assays* ⟨81⟩, except that sterilization is not required before use.

Assay preparation: Unless otherwise specified in the individual monograph, dissolve in the solvent specified in the table of *Solvents and Final Concentrations* a suitable quantity, accurately weighed, of the specimen under test, and dilute quantitatively with the same solvent to obtain a solution having a known final concentration of about that specified in the table. Pipet 2 mL of this solution into each of two 125-mL glass-stoppered conical flasks.

Procedure

Inactivation and titration: To 2.0 mL of the *Standard preparation* and of the *Assay preparation*, in respective flasks, add 2.0 mL of 1.0 N sodium hydroxide, mix by swirling, and allow to stand for 15 minutes. To each flask add 2.0 mL of 1.2 N hydrochloric acid, add 10.0 mL of 0.01 N iodine VS, immediately insert the stopper, and allow to stand for 15 minutes. Titrate with 0.01 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, and continue the titration to the discharge of the blue color.

Blank determination: To a flask containing 2.0 mL of the *Standard preparation* add 10.0 mL of 0.01 N iodine VS. If the *Standard Preparation* contains amoxicillin or ampicillin, immediately add 0.1 mL of 1.2 N hydrochloric acid. Immediately titrate with 0.01 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, and continue the titration to the discharge of the blue color. Similarly treat a flask containing 2.0 mL of the *Assay preparation.*

Calculations

Calculate the microgram (or unit) equivalent (F) of each mL of 0.01 N sodium thiosulfate consumed by the *Standard preparation* by the formula:

$$\text{Result} = (2CP)/(B - I)$$

C = concentration of Reference Standard in the *Standard preparation* (mg/mL)
P = potency of the Reference Standard (µg/mg or units/mg)
B = volume of 0.01 N sodium thiosulfate consumed in the *Blank determination* (mL)
I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and titration* (mL)

Calculate the potency of the specimen under test by the formula given in the individual monograph.