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Papain

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CAS RN®: 9001-73-4; UNII: A236A06Y32.

DEFINITION

Papain is a purified proteolytic substance derived from *Carica papaya* Linné (Fam. Caricaceae). Papain, prepared as directed in the *Assay*, contains NLT 6000 Units/mg. Papain of a higher digestive power may be reduced to the official standard by admixture with papain of lower activity, lactose, or other suitable diluents.

One USP Unit of Papain activity is that which releases the equivalent of 1 µg of tyrosine from a specified casein substrate under the conditions of the *Assay*, using the enzyme concentration that liberates 40 µg of tyrosine per mL of *Sample solution*.

ASSAY

• **Casein Digestive Power**

Dibasic sodium phosphate solution: Dissolve 7.1 g of anhydrous dibasic sodium phosphate in water to make 1 L. Add 1 drop of toluene as a preservative.

Citric acid solution: Dissolve 10.5 g of citric acid monohydrate in water to make 1 L. Add 1 drop of toluene as a preservative.

Casein substrate: Disperse 1g of Hammersten-type casein in 50 mL of *Dibasic sodium phosphate solution*. Place in a boiling water bath for 30 min with occasional stirring. Cool to room temperature, and add *Citric acid solution* to adjust to a pH of 6.0 ± 0.1. Stir the solution rapidly and continuously during the addition of the *Citric acid solution* to prevent precipitation of the casein. Dilute with water to 100 mL. Prepare fresh daily.

Buffer solution: Dissolve 3.55 g of anhydrous dibasic sodium phosphate in 400 mL of water in a 500-mL volumetric flask. Add 7.0 g of disodium edetate and 3.05 g of cysteine hydrochloride monohydrate. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 6.0 ± 0.1, dilute with water to volume, and mix. Prepare fresh daily.

Trichloroacetic acid solution: 300 mg/mL of reagent-grade trichloroacetic acid. This solution may be stored at room temperature.

Standard solution: Weigh accurately 100 mg of USP Papain RS in a 100-mL volumetric flask, and add *Buffer solution* to dissolve. Dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Use within 30 min after preparation.

Sample solution: Weigh accurately an amount of Papain equivalent to about 100 mg of USP Papain RS in a 100-mL volumetric flask, and add *Buffer solution* to dissolve. Dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Use within 30 min after preparation.

Analysis: Into each of 12 test tubes (18 × 150 mm) pipet 5.0 mL of *Casein substrate.* Place in a water bath at 40°, and allow 10 min to reach bath temperature. The tests are run in duplicate except for the blanks. Into each of two of the tubes labeled S_1 pipet 1.0 mL of the *Standard solution* and 1.0 mL of *Buffer solution*. Mix by swirling, note zero time, insert the stopper, and replace in the bath. Into each of two tubes labeled S_2 pipet 1.5 mL of the *Standard solution* and 0.5 mL of *Buffer solution*, and proceed as before. Repeat this procedure for two more tubes labeled S_3 to which 2.0 mL of the *Standard solution* is added, and for two tubes labeled U_2 to which 1.5 mL of the *Sample solution* and 0.5 mL of *Buffer solution* are added. After 60 min, accurately timed, add to all 12 tubes 3.0 mL of *Trichloroacetic acid solution,* and shake vigorously. With the four tubes to which neither the *Standard solution* nor the *Sample solution* was added, prepare blanks by pipeting, respectively, 1.0 mL of the *Standard solution* and 1.0 mL of *Buffer solution*; 1.5 mL of the *Standard solution* and 0.5 mL of *Buffer solution*; 2.0 mL of the *Standard solution*; and 1.5 mL of the *Sample solution* and 0.5 mL of *Buffer solution*. Replace all tubes in the 40° water bath for 30–40 min to allow the precipitated protein to coagulate fully. Pass through filter paper of medium pore size, discarding the first 3 mL of the filtrate (filtrates used are clear). Read the absorbances, at 280 nm, of the filtrates of all solutions against their respective blanks. Plot the absorbance readings for S_1, S_2, and S_3 against the enzyme concentration of each corresponding level.

By interpolation from this curve, taking into consideration dilution factors, calculate the potency in Units in the weight of Papain taken:

$$\text{Result} = A \times C \times F$$

A = activity of the USP Papain RS (Units/mg)

C = concentration from the standard curve (mg/mL)

F = 100 × (50/2) × (10/1.5), dilution factor

Acceptance criteria: NLT 6000 Units/mg

SPECIFIC TESTS

- **pH** ⟨791⟩

  Sample solution: 1 in 50

  Acceptance criteria: 4.8–6.2

- **Loss on Drying** ⟨731⟩

  Analysis: Dry under vacuum at 60° for 4 h.

  Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store in a cool place.
- **USP Reference Standards** ⟨11⟩

  USP Papain RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
PAPAIN	Maria Monagas Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	NBDS2020 Non-botanical Dietary Supplements

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