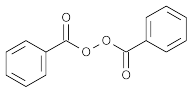
utes, accurately timed, then add 1.0 mL of 3.5 N hydrochloric acid, mix, add 1.0 mL of ferric chloride solution (8 in 100), and mix. Allow to stand for 30 minutes, accurately timed. Gently swirl the tubes for 1 minute to remove any gas bubbles present, then concomitantly determine the absorbances of the solutions in 1-cm cells, at the wavelength of maximum absorbance at about 500 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of benzonatate ($C_{30}H_{53}NO_{11}$ av.) in the number of Capsules taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Benzonatate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Hydrous Benzoyl Peroxide



$C_{14}H_{10}O_4$ (anhydrous) 242.23
Peroxide, dibenzoyl;
Benzoyl peroxide [94-36-0].

DEFINITION

Hydrous Benzoyl Peroxide contains NLT 90.0% and NMT 110.0% of the labeled amount of $C_{14}H_{10}O_4$. It contains a minimum of 20% of water for the purpose of reducing flammability and shock sensitivity.

[**CAUTION—**Hydrous Benzoyl Peroxide may explode at temperatures higher than 60° or cause fires in the presence of reducing substances. Store it in the original container, treated to reduce static charges.]

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
  Standard solution: 10 mg/mL of Hydrous Benzoyl Peroxide, previously subjected to the *Assay*, in methanol
  Sample solution: 10 mg/mL of benzoyl peroxide in methanol
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Toluene, dichloromethane, and glacial acetic acid (50:2:1)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Place the plate in a developing chamber containing and equilibrated with the *Developing solvent system*. Develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.
  Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.
- **B.** The *Sample solution* in the test for *Organic Impurities* exhibits a major peak for benzoyl peroxide, the retention time of which corresponds to that exhibited by the *Standard solution*.

ASSAY

- **PROCEDURE**
  Sample: 300 mg of previously mixed Hydrous Benzoyl Peroxide in a conical flask fitted with a ground-glass stopper. Weigh again to obtain the weight of the *Sample*.
  Analysis: Add 30 mL of glacial acetic acid, previously sparged with carbon dioxide for NLT 2 min just before use, and swirl the flask gently to dissolve. Add 5 mL of potassium iodide solution (1 in 2), and mix. Allow the solution to stand for 1 min. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, or equivalent, and continue the titration to the discharge of the blue color. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium thiosulfate is equivalent to 12.11 mg of $C_{14}H_{10}O_4$.
  Acceptance criteria: 90.0%–110.0% of the labeled amount

IMPURITIES

Organic Impurities

- **PROCEDURE**
  Solution A: Prepare a mixture of acetonitrile and glacial acetic acid (1000:1).
  Solution B: Prepare a mixture of water and glacial acetic acid (1000:1).
  Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

  System suitability solution: 100 µg/mL of benzoic acid and 60 µg/mL of methylparaben in acetonitrile
  Standard solution: Dissolve a quantity of Hydrous Benzoyl Peroxide, previously subjected to the *Assay*, in acetonitrile to obtain a solution containing 0.32 mg/mL.
  Sample solution: 0.32 mg/mL of benzoyl peroxide in acetonitrile
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 235 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 1.2 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *System suitability solution*
  Suitability requirements
  Resolution: NLT 2.0 between benzoic acid and methylparaben
  Tailing factors: NMT 2.0 for the benzoic acid and methylparaben peaks
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the area, as a percentage, of each peak in the chromatogram of the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times 100$$

  r_U = peak response for any individual peak other than the principal peak in the *Sample solution*
  r_T = sum of the peak responses of all the individual peaks including the principal peak in the *Sample solution*
  Acceptance criteria: The area of any individual peak other than the principal peak is NMT 1.5% of the total area. The sum of the areas of all peaks other than the principal peak is NMT 2.0% of the total area.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in the original container, at room temperature. [NOTE—Do not transfer Hydrous Benzoyl Peroxide to metal or glass containers fitted with friction tops. Do not return unused material to its original container, but destroy it by treatment with sodium hydroxide solution (1 in 10) until addition of a crystal of potassium iodide results in no release of free iodine.]

Benzoyl Peroxide Gel

» Benzoyl Peroxide Gel is benzoyl peroxide in a suitable gel base. It contains not less than 90.0 percent and not more than 125.0 per cent of the labeled amount of benzoyl peroxide ($C_{14}H_{10}O_4$).

Packaging and storage—Preserve in tight containers.

Identification—The retention time of the major peak for benzoyl peroxide in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

pH ⟨791⟩**:** between 2.8 and 6.6.

Related compounds—

Solution A—Prepare a filtered and degassed mixture of acetonitrile and glacial acetic acid (1000:1).

Solution B—Prepare a filtered and degassed mixture of water and glacial acetic acid (1000:1).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system* (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution in acetonitrile containing 100 µg of benzoic acid and 60 µg of methylparaben per mL.

Test preparation—Transfer an accurately weighed quantity of Gel, equivalent to about 100 mg of benzoyl peroxide, to a 50-mL volumetric flask, add 25 mL of acetonitrile, shake vigorously to disperse the specimen, sonicate for 5 minutes, dilute with acetonitrile to volume, mix, and filter.

Standard preparation A—Prepare a solution of benzoic acid in acetonitrile containing 500 µg per mL.

Standard preparation B—Prepare a solution of ethyl benzoate in acetonitrile containing 20 µg per mL.

Standard preparation C—Prepare a solution of benzaldehyde in acetonitrile containing 20 µg per mL.

Standard preparation D—Prepare a solution of hydrous benzoyl peroxide, previously subjected to the *Assay* under *Hydrous Benzoyl Peroxide*, in acetonitrile containing the equivalent of 40 µg of anhydrous benzoyl peroxide per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm × 25-cm column containing packing L1. The flow rate is about 1.2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	18	82	equilibration
0–20	18→60	82→40	linear gradient
20–30	60	40	isocratic

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between benzoic acid and methylparaben is not less than 2.0; and the tailing factors for the benzoic acid and methylparaben peaks are not more than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparations* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. The responses of any peaks obtained from the *Test preparation* corresponding to benzoic acid, ethyl benzoate, and benzaldehyde are not greater than those of the main peaks obtained from *Standard preparation A* (25%), *Standard preparation B* (1%), and *Standard preparation C* (1%), respectively; the response of any other impurity peak obtained from the *Test preparation,* other than the main benzoyl peroxide peak, any benzoic acid, ethyl benzoate, benzaldehyde, methylparaben, or propylparaben peak, and any solvent peak, is not more than that obtained from *Standard preparation D* (2%); and the sum of the responses of all the impurity peaks, other than those of benzoic acid, ethyl benzoate, and benzaldehyde is not more than that obtained from *Standard preparation D* (2%).

Assay—

Mobile phase—Prepare a solution of acetonitrile in water (about 5 in 10) such that the retention times for ethyl benzoate and benzoyl peroxide are about 7 and 14 minutes, respectively.

Internal standard solution—Dissolve ethyl benzoate in acetonitrile to obtain a solution having a concentration of about 3.6 mg per mL.

Standard preparation—Place a suitable quantity of hydrous benzoyl peroxide, recently subjected to the *Assay* under *Hydrous Benzoyl Peroxide,* in an accurately weighed conical flask fitted with a glass stopper, weigh again to obtain the weight of the specimen, and quantitatively dissolve in acetonitrile to obtain a solution containing a known concentration of about 0.8 mg of benzoyl peroxide per mL. Pipet 10 mL of this solution and 5 mL of *Internal standard solution* into a 25-mL volumetric flask, dilute with acetonitrile to volume, and mix. This *Standard preparation* contains about 0.32 mg of benzoyl peroxide per mL.

Assay preparation—Transfer an accurately weighed quantity of Gel, equivalent to about 40 mg of benzoyl peroxide, to a 50-mL volumetric flask. Add 40 mL of acetonitrile, and shake until the material is thoroughly dispersed. Sonicate the mixture for 5 minutes, dilute with acetonitrile to volume, mix, and filter. Pipet 10 mL of the filtrate and 5 mL of *Internal standard solution* into a 25-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1, and is operated at room temperature. The flow rate is about 1 mL per minute. Chromatograph three replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the lowest and highest peak response ratios (R_S) agree within 2.0%; the resolution, *R,* between ethyl benzoate and benzoyl peroxide is not less than 2.0; and the tailing factors for the ethyl benzoate and benzoyl peroxide peaks are not more than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation,* record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of benzoyl peroxide ($C_{14}H_{10}O_4$) in the portion of Gel taken by the formula:

$$125C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of benzoyl peroxide in the *Standard preparation;* and R_U and R_S are the peak response ratios of benzoyl peroxide to ethyl benzoate obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Benzoyl Peroxide Lotion

» Benzoyl Peroxide Lotion is benzoyl peroxide in a suitable lotion base. It contains not less than 90.0 percent and not more than 110.0 per cent of the labeled amount of $C_{14}H_{10}O_4$.

Packaging and storage—Preserve in tight containers.

Identification—

A: Dilute a quantity of Lotion with acetone to obtain a solution having a concentration of benzoyl peroxide equivalent to 10 mg per mL, and proceed with the solution so obtained as directed in the *Identification* test *A* under *Hydrous Benzoyl Peroxide*, beginning with "Apply 5 µL of this solution." The solution responds to the test.