

If volatile oils are present in small proportions only, and a cloudy distillate is obtained, the solvent hexane treatment not having been employed, the distillate may be clarified and rendered suitable for the specific gravity determination by shaking it with about one-fifth its volume of solvent hexane, or by filtering it through a thin layer of talc.

Method II—Gas Chromatographic Method

Use *Method IIa* when *Method II* is specified in the individual monograph. For a discussion of the principles upon which it is based, see *Gas Chromatography* under *Chromatography* (621).

USP Reference Standards—USP Alcohol Determination—Acetonitrile RS. USP Alcohol Determination—Alcohol RS.

Method IIa

Apparatus—Under typical conditions, use a gas chromatograph equipped with a flame-ionization detector and a 4-mm × 1.8-m glass column packed with 100- to 120-mesh chromatographic column packing support S3, using nitrogen or helium as the carrier. Prior to use, condition the column overnight at 235° with a slow flow of carrier gas. The column temperature is maintained at 120°, and the injection port and detector temperatures are maintained at 210°. Adjust the carrier flow and temperature so that acetonitrile, the internal standard, elutes in 5 to 10 minutes.

Solutions—

Test Stock Preparation—Dilute the specimen under examination stepwise with water to obtain a solution containing approximately 2% (v/v) of alcohol.

Test Preparation—Pipet 5 mL each of the *Test Stock Preparation* and the USP Alcohol Determination—Acetonitrile RS [NOTE—Alternatively, a 2% aqueous solution of acetonitrile of suitable quality may be used as the internal standard solution] into a 50-mL volumetric flask, dilute with water to volume, and mix.

Standard Preparation—Pipet 5 mL each of the USP Alcohol Determination—Alcohol RS and the USP Alcohol Determination—Acetonitrile RS [NOTE—Alternatively, a 2% aqueous solution of acetonitrile of suitable quality may be used as the internal standard solution] into a 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Inject about 5 µL each of the *Test Preparation* and the *Standard Preparation*, in duplicate, into the gas chromatograph, record the chromatograms, and determine the peak response ratios. Calculate the percentage of alcohol (v/v) in the specimen under test according to the formula:

$$CD(R_U/R_S)$$

in which C is the labeled concentration of USP Alcohol Determination—Alcohol RS; D is the dilution factor (the ratio of the volume of the *Test Stock Preparation* to the volume of the specimen taken); and R_U and R_S are the peak response ratios obtained from the *Test Preparation* and the *Standard Preparation*, respectively.

System Suitability Test—In a suitable chromatogram, the resolution factor, R , is not less than 2; the tailing factor of the alcohol peak is not greater than 2.0; and six replicate injections of the *Standard Preparation* show a relative standard deviation of not more than 2.0% in the ratio of the peak of alcohol to the peak of the internal standard.

Method IIb

Apparatus—The gas chromatograph is equipped with a split injection port with a split ratio of 5:1, a flame-ioniza-

tion detector, and a 0.53-mm × 30-m capillary column coated with a 3.0-µm film of phase G43. Helium is used as the carrier gas at a linear velocity of 34.0 cm per second. The chromatograph is programmed to maintain the column temperature at 50° for 5 minutes, then to increase the temperature at a rate of 10° per minute to 200°, and maintain at this temperature for 4 minutes. The injection port temperature is maintained at 210° and the detector temperature at 280°.

Solutions—

Test Stock Preparation—Dilute the specimen under examination stepwise with water to obtain a solution containing approximately 2% (v/v) of alcohol.

Test Preparation—Pipet 5 mL each of the *Test Stock Preparation* and the USP Alcohol Determination—Acetonitrile RS [NOTE—Alternatively, a 2% aqueous solution of acetonitrile of suitable quality may be used as the internal standard solution] into a 25-mL volumetric flask, dilute with water to volume, and mix.

Standard Preparation—Pipet 5 mL each of the USP Alcohol Determination—Alcohol RS and the USP Alcohol Determination—Acetonitrile RS [NOTE—Alternatively, a 2% aqueous solution of acetonitrile of suitable quality may be used as the internal standard solution] into a 25-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Inject about 0.2 to 0.5 µL each of the *Test Preparation* and the *Standard Preparation*, in duplicate, into the gas chromatograph, record the chromatograms, and determine the peak response ratios. Calculate the percentage of alcohol (v/v) in the specimen under test according to the formula:

$$CD(R_U/R_S)$$

in which C is the labeled concentration of USP Alcohol Determination—Alcohol RS; D is the dilution factor (the ratio of the volume of the *Test Stock Preparation* to the volume of the specimen taken); and R_U and R_S are the peak response ratios obtained from the *Test Preparation* and the *Standard Preparation*, respectively.

System Suitability Test—In a suitable chromatogram, the resolution factor, R , between alcohol and the internal standard is not less than 4; the tailing factor of the alcohol peak is not greater than 2.0; and six replicate injections of the *Standard Preparation* show a relative standard deviation of not more than 4.0% in the ratio of the peak of alcohol to the peak of the internal standard.

<616> BULK DENSITY AND TAPPED DENSITY OF POWDERS

BULK DENSITY

This general chapter has been harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. *The portion that is not harmonized is marked with symbols (✦) to specify this fact.✦

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per mL (g/mL) although the international unit is kilograms per cubic meter

(1 g/mL = 1000 kg/m³) because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm³). The bulking properties of a powder are dependent upon the preparation, treatment, and storage of the sample, i.e., how it was handled. The particles can be packed to have a range of bulk densities; however, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made. The bulk density of a powder is determined by measuring the volume of a known weight of powder sample, that may have been passed through a screen, into a graduated cylinder (*Method I*), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (*Method II*) or a measuring vessel (*Method III*).

Method I and *Method III* are favored.

Method I—Measurement in a Graduated Cylinder

Procedure—Pass a quantity of material sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated 250-mL cylinder (readable to 2 mL) introduce, without compacting, approximately 100 g of test sample, M , weighed with 0.1% accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (V_0) to the nearest graduated unit. Calculate the bulk density in g/mL by the formula m/V_0 . Generally, replicate determinations are desirable for the determination of this property. If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as the test sample, such that its untapped apparent volume is 150 mL to 250 mL (apparent volume greater than or equal to 60% of the total volume of the cylinder); the weight of the test sample is specified in the expression of results. For test samples having an apparent volume between 50 mL and 100 mL, a 100-mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

Method II—Measurement in a Volumeter

Apparatus—The apparatus (*Figure 1*) consists of a top funnel fitted with a 1.0-mm screen.¹ The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup of specified capacity mounted directly below it. The cup may be cylindrical (25.00 ± 0.05 mL volume with an inside diameter of 30.00 ± 2.00 mm) or a square (16.39 ± 0.2 mL volume with inside dimensions of 25.4 ± 0.076 mm).

¹The apparatus (the Scott Volumeter) conforms to the dimensions in ASTM 329 90.

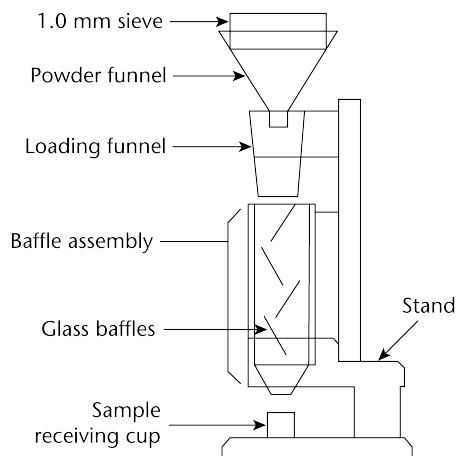


Figure 1.

Procedure—Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of 25 cm³ of powder with the square cup and 35 cm³ of powder with the cylindrical cup. Carefully scrape excess powder from the top of the cup by smoothly moving the edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular to prevent packing or removal of powder from the cup. Remove any material from the sides of the cup, and determine the weight, M , of the powder to the nearest 0.1%. Calculate the bulk density, in g/mL, by the formula:

$$(M)/(V_0)$$

in which V_0 is the volume, in mL, of the cup. Record the average of three determinations using three different powder samples.

Method III—Measurement in a Vessel

Apparatus—The apparatus consists of a 100-mL cylindrical vessel of stainless steel with dimensions as specified in *Figure 2*.

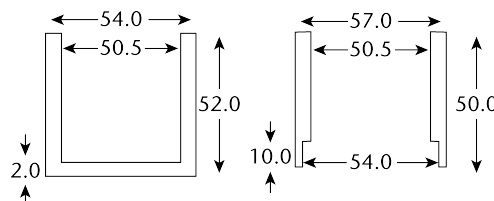


Figure 2.

Procedure—Pass a quantity of powder sufficient to complete the test through a 1.0-mm sieve, if necessary, to break up agglomerates that may have formed during storage, and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for *Method II*. Determine the weight (M_0) of the powder to the nearest 0.1% by subtraction of the previously determined mass of the empty measuring vessel. Calculate the bulk density (g/mL) by the formula $M_0/100$, and record the average of three determinations using three different powder samples.

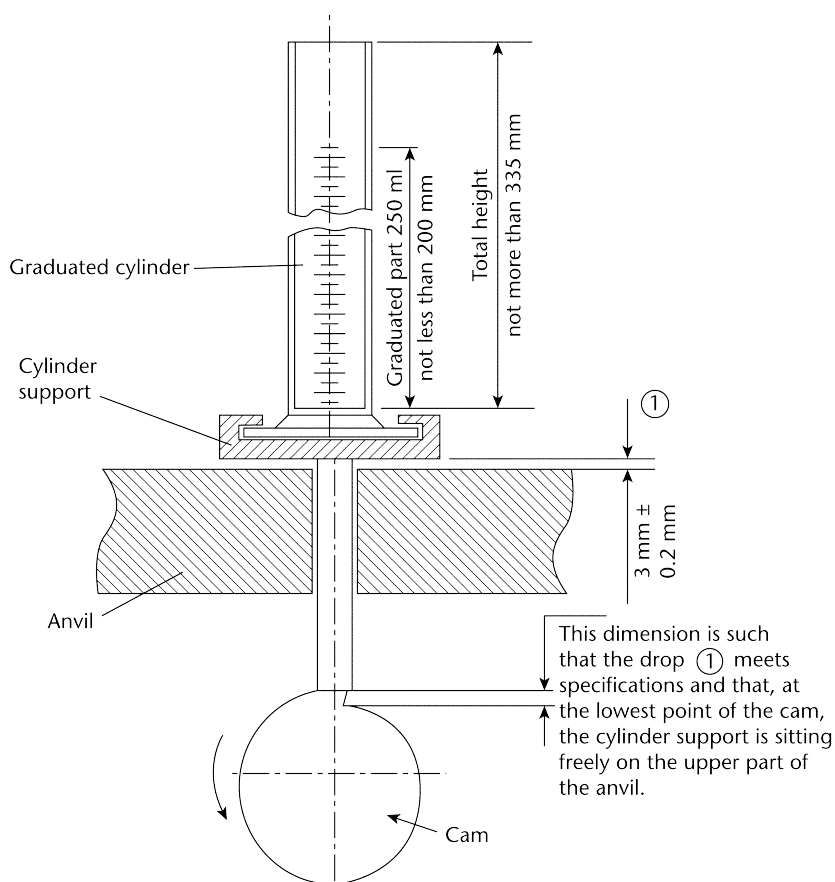


Figure 3.

TAPPED DENSITY

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. Tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing a powder sample. After observing the initial powder volume or weight, the measuring cylinder or vessel is mechanically tapped, and volume or weight readings are taken until little further volume or weight change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop under its own weight a specified distance by either of three methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

Method I

Apparatus—The apparatus (*Figure 3*) consists of the following:

- A 250-mL graduated cylinder (readable to 2 mL with a mass of 220 ± 44 g)
- A settling apparatus capable of producing, in 1 minute, either nominally 250 ± 15 taps from a height of 3 ± 0.2 mm, or nominally 300 ± 15 taps from a height of 14 ± 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 ± 10 g.

Procedure—Proceed as described above for the determination of the bulk volume (V_0). Secure the cylinder in the holder. Carry out 10, 500, and 1250 taps on the same powder sample and read the corresponding volumes V_{10} , V_{500} , and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than 2 mL, V_{1250} is the

tapped volume. If the difference between V_{500} and V_{1250} exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula m/V_f in which V_f is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results. If it is not possible to use a 100-g test sample, use a reduced amount and a suitable 100-mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. The modified test conditions are specified in the expression of the results.

Method II

Apparatus and Procedure—Proceed as directed under *Method I* except that the mechanical tester provides a fixed drop of 3 ± 0.2 mm at a nominal rate of 250 taps per minute.

Method III

Apparatus and Procedure—Proceed as directed in *Method III—Measurement in a Vessel* for measuring bulk density using the measuring vessel equipped with the cap shown in *Figure 2*. The measuring vessel with the cap is lifted 50–60 times per minute by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap, and carefully scrape excess powder from the top of the measuring vessel as described in *Method III—Measurement in a Vessel* for measuring the bulk density. Repeat the procedure using 400 taps. If the difference between the two

masses obtained after 200 and 400 taps exceeds 2%, carry out a test using 200 additional taps until the difference between succeeding measurements is less than 2%. Calculate the tapped density (g/mL) using the formula $M_f/100$ where M_f is the mass of powder in the measuring vessel. Record the average of three determinations using three different powder samples. The test conditions including tapping height are specified in the expression of the results.

MEASURES OF POWDER COMPRESSIBILITY

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the *Compressibility Index* or the *Hausner Ratio* as described below.

The *Compressibility Index* and *Hausner Ratio* are measures of the propensity of a powder to be compressed as described above. As such, they are measures of the powder's ability to settle, and they permit an assessment of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the *Compressibility Index* and the *Hausner Ratio*.

Compressibility Index—Calculate by the formula:

$$100(V_0 - V_f)/V_0$$

V_0 = unsettled apparent volume

V_f = final tapped volume

Hausner Ratio—

$$V_0/V_f$$

Depending on the material, the compressibility index can be determined using V_{10} instead of V_0 . [NOTE—If V_{10} is used, it will be clearly stated in the results.]

(621) CHROMATOGRAPHY

INTRODUCTION

Chromatographic separation techniques are multistage separation methods in which the components of a sample are distributed between two phases, of which one is stationary and the other mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, distributed as a film, or applied by other techniques. The mobile phase may be gaseous or liquid or supercritical fluid. The separation may be based on adsorption, mass distribution (partition), or ion exchange; or it may be based on differences among the physicochemical properties of the molecules, such as size, mass, and volume. This chapter contains general procedures, definitions, and calculations of common parameters and describes general requirements for system suitability. The types of chromatography useful in qualitative and quantitative analysis employed in USP proce-

dures are column, gas, paper, thin-layer (including high-performance thin-layer chromatography), and pressurized liquid chromatography (commonly called high-pressure or high-performance liquid chromatography).

GENERAL PROCEDURES

This section describes the basic procedures used when a chromatographic method is described in a monograph. The following procedures are followed unless otherwise indicated in the individual monograph.

Paper Chromatography

Stationary Phase: The stationary phase is a sheet of paper of suitable texture and thickness. Development may be ascending, in which the solvent is carried up the paper by capillary forces, or descending, in which the solvent flow is also assisted by gravitational force. The orientation of paper grain with respect to solvent flow is to be kept constant in a series of chromatograms. (The machine direction is usually designated by the manufacturer.)

Apparatus: The essential equipment for paper chromatography consists of a vapor-tight chamber with inlets for addition of solvent and a rack of corrosion-resistant material about 5 cm shorter than the inside height of the chamber. The rack serves as a support for solvent troughs and for antisiphon rods that, in turn, hold up the chromatographic sheets. The bottom of the chamber is covered with the prescribed solvent system or mobile phase. Saturation of the chamber with solvent vapor is facilitated by lining the inside walls with paper wetted with the prescribed solvent system.

Spotting: The substance or substances analyzed are dissolved in a suitable solvent. Convenient volumes, delivered from suitable micropipets, of the resulting solution, normally containing 1–20 µg of the compound, are placed in 6- to 10-mm spots not less than 3 cm apart.

Descending Paper Chromatography Procedure

- (1) A spotted chromatographic sheet is suspended in the apparatus, using the antisiphon rod to hold the upper end of the sheet in the solvent trough. [NOTE—Ensure that the portion of the sheet hanging below the rods is freely suspended in the chamber without touching the rack, the chamber walls, or the fluid in the chamber.]
- (2) The chamber is sealed to allow equilibration (saturation) of the chamber and the paper with the solvent vapor. Any excess pressure is released as necessary.
- (3) After equilibration of the chamber, the prepared mobile phase is introduced into the trough through the inlet.
- (4) The inlet is closed, and the mobile solvent phase is allowed to travel the desired distance down the paper.
- (5) The sheet is removed from the chamber.
- (6) The location of the solvent front is quickly marked, and the sheet is dried.
- (7) The chromatogram is observed and measured directly or after suitable development to reveal the location of the spots of the isolated drug or drugs.

Ascending Paper Chromatography Procedure

- (1) The mobile phase is added to the bottom of the chamber.
- (2) The chamber is sealed to allow equilibration (saturation) of the chamber and the paper with the solvent vapor. Any excess pressure is released as necessary.
- (3) The lower edge of the stationary phase is dipped into the mobile phase to permit the mobile phase to rise on the chromatographic sheet by capillary action.
- (4) When the solvent front has reached the desired height, the chamber is opened, the sheet is removed, the location of the solvent front is quickly marked, and the sheet is dried.