Assay—[NOTE—Prepare solutions immediately before use, and protect from light.]

Buffer—Transfer 6.8 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve the contents in 900 mL of water. Adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, add 0.2 mL of triethylamine, and mix well.

Mobile phase—Prepare a mixture of *Buffer* and acetonitrile (84:16), and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Cabergoline RS in *Mobile phase* to obtain a solution having a known concentration of about 0.25 mg per mL. [NOTE—Sonication may be used to aid in the dissolution of cabergoline.]

Assay preparation—Grind not fewer than 20 tablets into a fine powder. Transfer an accurately weighed portion of the powder, equivalent to about 2.5 mg of cabergoline based on the label claim, to a 10-mL volumetric flask. Dilute with *Mobile phase* to volume, and sonicate until completely dissolved. This solution has a nominal concentration of about 0.25 mg per mL of cabergoline, based on the label claim. [NOTE—The *Assay preparation* may be passed through a PVDF type filter with a pore size of 0.45 µm prior to analysis.]

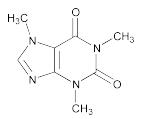
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 25-cm column that contains 10- µm packing L1. The flow rate is about 1.3 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of the label claim of $C_{26}H_{37}N_5O_2$ in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration of USP Cabergoline RS, in mg per mL, in the *Standard preparation;* C_U is the nominal concentration of carbergoline, in mg per mL, in the *Assay preparation,* based on the label claim; and r_U and r_S are the peak responses for cabergoline obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Caffeine



$C_8H_{10}N_4O_2 \cdot H_2O$ 212.21

$C_8H_{10}N_4O_2$ 194.19

1*H*-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-;
1,3,7-Trimethylxanthine [58-08-2].
Monohydrate [5743-12-4].

DEFINITION

Caffeine is anhydrous or contains one molecule of water of hydration. It contains NLT 98.5% and NMT 101.0% of $C_8H_{10}N_4O_2$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197M⟩**
- **B.** The retention time of the caffeine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.82 g/L of anhydrous sodium acetate
Mobile phase: Acetonitrile, tetrahydrofuran, and *Buffer* (25:20:955). Adjust with glacial acetic acid to a pH of 4.5.
System suitability solution: 0.02 mg/mL of theophylline in *Mobile phase*. Shake, and sonicate if necessary, to dissolve.
Standard solution: Transfer 5.0 mg of USP Caffeine RS to a 25-mL volumetric flask. Add 5.0 mL of the *System suitability solution* and 10 mL of *Mobile phase*. Shake, and sonicate if necessary. Dilute with *Mobile phase* to volume, and filter.
Sample solution: 0.2 mg/mL of Caffeine in *Mobile phase*. [NOTE—Shake, and sonicate if necessary, to dissolve.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for theophylline and caffeine are 0.69 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between theophylline and caffeine
Tailing factor: NMT 2.0 for theophylline and caffeine peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of caffeine ($C_8H_{10}N_4O_2$) in the portion of Caffeine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of caffeine from the *Sample solution*
r_S = peak response of caffeine from the *Standard solution*
C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Caffeine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm
- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Caffeine taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria
Individual impurities: NMT 0.1%
Total impurities: NMT 0.1%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method III* **⟨921⟩:** Dry a sample at 80° for 4 h: the anhydrous form loses NMT 0.5% of its weight, and the hydrous form loses NMT 8.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve hydrous Caffeine in tight containers. Preserve anhydrous Caffeine in well-closed containers.
- **LABELING:** Label it to indicate whether it is anhydrous or hydrous.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Caffeine RS

Caffeine Citrate Injection

» Caffeine Citrate Injection is a sterile solution containing Caffeine and citric acid in W ater for Injection. It contains not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of caffeine citrate ($C_{14}H_{18}N_4O_9$). It contains no bacteriostat or other preser vative.

Packaging and storage—Preserve in single-dose, tight containers of Type I glass, and store at a temperature between 15 ° and 30°.

USP Reference standards ⟨11⟩**—**
USP Caffeine RS
USP Endotoxin RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

B: It meets the requirements of the test for *Citrate* ⟨191⟩.

C: Transfer about 4 g of potassium iodide to a 100-mL volumetric flask. Add 10 mL of water, and shake until the potassium iodide is dissolved. Transfer 2 g of iodine to the volumetric flask, and shake until dissolved. Dilute with water to volume, and mix. Transfer 5 drops of the solution so obtained to a 25-mL centrifuge tube containing 5.0 mL of the Injection, and mix. Add 0.5 mL of 2.0 M hydrochloric acid solution, and mix: a brown precipitate that dissolves on neutralization with 0.5 mL of sodium hydroxide TS is produced.

Color and clarity—Transfer a suitable portion of the Injection to a clear glass test tube, and visually examine the solution in a well-lighted area: the solution is colorless and free of haze, obvious turbidity, and precipitate.

Bacterial endotoxins ⟨85⟩**:** not more than 0.25 USP Endotoxin Unit per mg of caffeine.

Sterility ⟨71⟩**—**It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩**:** between 4.2 and 5.2.

Particulate matter ⟨788⟩**:** not more than 150 particles are equal to or greater than 10 µm, and not more than 25 particles are equal to or greater than 25 µm.

Related compounds—

Mobile phase and *Theophylline solution*—Proceed as directed in the *Assay.*

Standard solution—Use the *Standard preparation,* prepared as directed in the *Assay.*

System sensitivity solution—Transfer 2.5 mL of the *Standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Use the *Assay preparation,* prepared as directed in the *Assay.*

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* Chromatograph the *System sensitivity solution,* and record the peak responses as directed for *Procedure:* the theophylline peak produces a discernible peak response at its retention time.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the per centage of any related compound in the portion of Injection taken by the formula:

$$100F(386.31/194.19)(C_S / C_W)(r_i / r_S)$$

in which F is the relative response factor and is equal to 0.878 for theobromine at a relative retention time of about 0.4, equal to 1.10 for paraxanthine at a relative retention time of about 0.6, equal to 0.905 for theophylline at a relative retention time of about 0.7, and equal to 1.0 for any other related compound; 386.31 and 194.19 are the molecular weights of caffeine citrate and caffeine, respectively; C_S is the concentration, in mg per mL, of USP Caffeine RS in the *Standard solution;* C_W is the caffeine citrate concentration, in mg per mL, in the *Test solution,* as obtained in the *Assay;* r_i is the individual peak response for each related compound obtained from the *Test solution;* and r_S is the caffeine peak response obtained from the *Standard solution:* not more than 0.10% of any individual related compound is found; and not more than 0.1% of total impurities is found.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Mobile phase—Prepare a mixture of 0.01 M sodium acetate, acetonitrile, and tetrahydrofuran (191:5:4). Adjust with glacial acetic acid to a pH of 4.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Theophylline solution—Dissolve an accurately weighed quantity of theophylline in water, and dilute quantitatively, and stepwise if necessar y, with water, to obtain a solution having a concentration of about 0.02 mg per mL.

Standard preparation—Transfer about 5 mg of USP Caffeine RS, accurately weighed, to a 25-mL volumetric flask. Add 5 mL of *Theophylline solution,* dissolve in and dilute with water to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of caffeine, to a 250-mL volumetric flask. Dilute with water to volume, mix, and pass through a polyvinylidene difluoride or equivalent membrane having a 0.45-µm porosity.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 150-cm column that contains 5- µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for theophylline and 1.0 for caffeine; the resolution, R, between theophylline and caffeine is not less than 6.0; the tailing factor, determined from the theophylline and caffeine peaks, is not more than 2.0; and the relative standard deviation for replicate injections, determined from the caffeine peaks, is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the caffeine peak responses. Calculate the quantity, in mg, of caffeine citrate ($C_{14}H_{18}N_4O_9$) in the volume of Injection taken by the formula:

$$250(386.31/194.19)C(r_U / r_S)$$

in which 386.31 and 194.19 are the molecular weights of caffeine citrate and caffeine, respectively; C is the concentration, in mg per mL, of USP Caffeine RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.