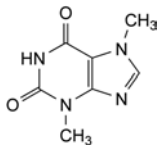
01/2008:0298
corrected 6.0

THEOBROMINE

Theobrominum



$C_7H_8N_4O_2$ M_r 180.2
[83-67-0]

DEFINITION

Theobromine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, very slightly soluble in water and in ethanol, slightly soluble in ammonia. It dissolves in dilute solutions of alkali hydroxides and in mineral acids.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *theobromine CRS*.

B. Dissolve about 20 mg in 2 mL of *dilute ammonia R1*, warming slightly, and cool. Add 2 mL of *silver nitrate solution R2*. The solution remains clear. Boil the solution for a few minutes. A white, crystalline precipitate is formed.

C. It gives the reaction of xanthines (*2.3.1*).

TESTS

Acidity. To 0.4 g add 20 mL of boiling *water R* and boil for 1 min. Allow to cool and filter. Add 0.05 mL of *bromothymol blue solution R1*. The solution is yellow or yellowish-green. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution. To 0.2 g of the finely powdered substance to be examined add 10 mL of a mixture of 4 volumes of *methanol R* and 6 volumes of *chloroform R*. Heat under a reflux condenser on a water-bath for 15 min, shaking occasionally. Cool and filter.

Reference solution. Dissolve 5 mg of *theobromine CRS* in a mixture of 4 volumes of *methanol R* and 6 volumes of *chloroform R* and dilute to 50 mL with the same mixture of solvents.

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R*, 30 volumes of *acetone R*, 30 volumes of *chloroform R* and 40 volumes of *butanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals (*2.4.8*). 1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 mL of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 125 mL of boiling *water R*, cool to 50 °C to 60 °C and add 25 mL of *0.1 M silver nitrate*. Using 1 mL of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 mL of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

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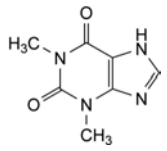
corrected 6.0

THEOPHYLLINE

Theophyllinum



$C_7H_8N_4O_2$ M_r 180.2
[58-55-9]

DEFINITION

1,3-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, sparingly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 270 °C to 274 °C, determined after drying at 100-105 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of theophylline.*

C. Heat 10 mg with 1.0 mL of a 360 g/L solution of *potassium hydroxide R* in a water-bath at 90 °C for 3 min, then add 1.0 mL of *diazotised sulfanilic acid solution R*. A red colour slowly develops. Carry out a blank test.

D. Loss on drying (see Tests).

E. It gives the reaction of xanthines (*2.3.1*).

TESTS

Solution S. Dissolve 0.5 g with heating in *carbon dioxide-free water R*, cool and dilute to 75 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity. To 50 mL of solution S add 0.1 mL of *methyl red solution R*. The solution is red. Not more than 1.0 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 10 mg of *theobromine R* in the mobile phase, add 5 mL of the test solution and dilute to 100 mL with the mobile phase. Dilute 5 mL of this solution to 50 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase: mix 7 volumes of *acetonitrile for chromatography R* and 93 volumes of a 1.36 g/L solution of *sodium acetate R* containing 5.0 mL/L of *glacial acetic acid R*.

Flow rate: 2.0 mL/min.

Detection: spectrophotometer at 272 nm.

Injection: 20 µL.

Run time: 3.5 times the retention time of theophylline.

Relative retention with reference to theophylline (retention time = about 6 min): impurity C = about 0.3; impurity B = about 0.4; impurity D = about 0.5; impurity A = about 2.5.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to theobromine and theophylline.

Limits:

– *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (*2.4.8*): maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 mL of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

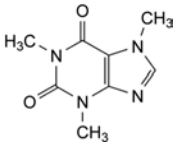
Dissolve 0.150 g in 100 mL of *water R*, add 20 mL of *0.1 M silver nitrate* and shake. Add 1 mL of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide*.

1 mL of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

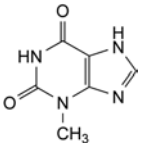
IMPURITIES

Specified impurities: A, B, C, D.

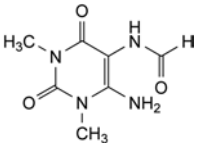
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, F.*



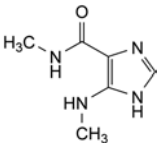
A. 1,3,7-trimethyl-3,7-dihydro-1*H*-purine-2,6-dione (caffeine),



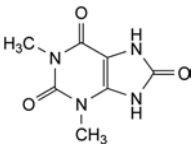
B. 3-methyl-3,7-dihydro-1*H*-purine-2,6-dione,



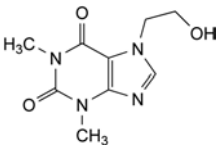
C. *N*-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide,



D. *N*-methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide (theophyllidine),



E. 1,3-dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione,

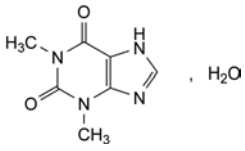


F. 7-(2-hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (etofylline).

01/2008:0302
corrected 6.0

THEOPHYLLINE MONOHYDRATE

Theophyllinum monohydricum



$C_7H_8N_4O_2,H_2O$ M_r 198.2
[5967-84-0]

DEFINITION

1,3-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione monohydrate.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, sparingly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 270 °C to 274 °C, determined after drying at 100-105 °C.