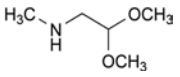
ASSAY

Dissolve 0.250 g in 75 mL of *water R*. Add 15.0 mL of *0.1 M sodium hydroxide*, mix and add with stirring, about 30 mL of *0.1 M silver nitrate*. Continue the titration with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

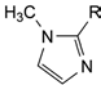
1 mL of *0.1 M sodium hydroxide* is equivalent to 11.42 mg of $C_4H_6N_2S$.

IMPURITIES

*Specified impurities: A, B, C.*



A. 2,2-dimethoxy-*N*-methylethanamine,



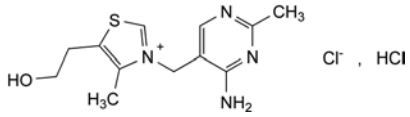
B. R = H: 1-methyl-1*H*-imidazole,

C. R = $SCH_3$: 1-methyl-2-(methylsulfanyl)-1*H*-imidazole.

**01/2008:0303**

# THIAMINE HYDROCHLORIDE

## Thiamini hydrochloridum



$C_{12}H_{18}Cl_2N_4OS$ $M_r$ 337.3

[67-03-8]

DEFINITION

3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride.

*Content*: 98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

*Appearance*: white or almost white, crystalline powder or colourless crystals.

*Solubility*: freely soluble in water, soluble in glycerol, slightly soluble in alcohol.

IDENTIFICATION

*First identification: A, C.*

*Second identification: B, C.*

A. Infrared absorption spectrophotometry (*2.2.24*).
*Comparison*: *thiamine hydrochloride CRS*.

B. Dissolve about 20 mg in 10 mL of *water R*, add 1 mL of *dilute acetic acid R* and 1.6 mL of *1 M sodium hydroxide*, heat on a water-bath for 30 min and allow to cool. Add 5 mL of *dilute sodium hydroxide solution R*, 10 mL of *potassium ferricyanide solution R* and 10 mL of *butanol R* and shake vigorously for 2 min. The upper alcoholic layer shows an intense light-blue fluorescence, especially in ultraviolet light at 365 nm. Repeat the test using 0.9 mL of *1 M sodium hydroxide* and 0.2 g of *sodium sulfite R* instead of 1.6 mL of *1 M sodium hydroxide*. Practically no fluorescence is seen.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

**Solution S**. Dissolve 2.5 g in *distilled water R* and dilute to 25 mL with the same solvent.

**Appearance of solution**. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution $Y_7$ or $GY_7$ (*2.2.2, Method II*).

Dilute 2.5 mL of solution S to 5 mL with *water R*.

**pH** (*2.2.3*): 2.7 to 3.3.

Dilute 2.5 mL of solution S to 10 mL with *water R*.

**Related substances**. Liquid chromatography (*2.2.29*).

*Solution A*. Add 5 volumes of *glacial acetic acid R* to 95 volumes of *water R* and mix.

*Test solution*. Dissolve 0.35 g of the substance to be examined in 15.0 mL of solution A and dilute to 100.0 mL with *water R*.

*Reference solution (a)*. Dissolve 5 mg of the substance to be examined and 5 mg of *thiamine impurity E CRS* in 4 mL of solution A and dilute to 25.0 mL with *water R*. Dilute 5.0 mL of the solution to 25.0 mL with *water R*.

*Reference solution (b)*. Dilute 1.0 mL of the test solution to 50.0 mL with *water R*. Dilute 5.0 mL of this solution to 25.0 mL with *water R*.

*Column*:
- *size*: *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 $m^2/g$ and a pore size of 10 nm,
- *temperature*: 45 °C.

*Mobile phase*:
- *mobile phase A*: 3.764 g/L solution of *sodium hexanesulfonate R* adjusted to pH 3.1 with *phosphoric acid R*,
- *mobile phase B*: *methanol R2*,

| Time (min) | Mobile phase A (per cent *V/V*) | Mobile phase B (per cent *V/V*) |
|---|---|---|
| 0 - 25 | 90 → 70 | 10 → 30 |
| 25 - 33 | 70 → 50 | 30 → 50 |
| 33 - 40 | 50 | 50 |
| 40 - 45 | 50 → 90 | 50 → 10 |

*Flow rate*: 1.0 mL/min.

*Detection*: spectrophotometer at 248 nm.

*Injection*: 25 µL.

*Relative retention* with reference to thiamine (retention time = about 30 min): impurity A = about 0.3; impurity B = about 0.9; impurity C = about 1.2.

*System suitability*: reference solution (a):
- *resolution*: minimum 1.6 between the peaks due to impurity E and to thiamine.

*Limits*:
- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent),
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
- *disregard limit*: 0.125 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Sulfates** (*2.4.13*): maximum 300 ppm.

5 mL of solution S diluted to 15 mL with *distilled water R* complies with the limit test for sulfates.

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

12 mL of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

**Water** (*2.5.12*): maximum 5.0 per cent, determined on 0.40 g.

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

ASSAY

Dissolve 0.110 g in 5 mL of *anhydrous formic acid R* and add 50 mL of *acetic anhydride R*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*) and carrying out the titration within 2 min. Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 16.86 mg of $C_{12}H_{18}Cl_2N_4OS$.

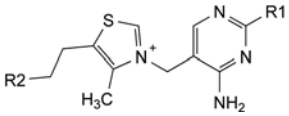
STORAGE

In a non-metallic container, protected from light.

IMPURITIES

*Specified impurities: A, B, C.*

*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*): *D, E, F, G, H.*



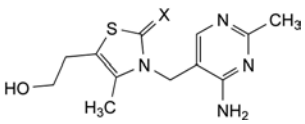

A. R1 = $CH_3$, R2 = O-$SO_3^-$: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-5-[2-(sulfonatooxy)ethyl]thiazolium (thiamine sulfate ester),

B. R1 = H, R2 = OH: 3-[(4-aminopyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium (desmethylthiamine),

C. R1 = $CH_3$, R2 = Cl: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-chloroethyl)-4-methylthiazolium (chlorothiamine),

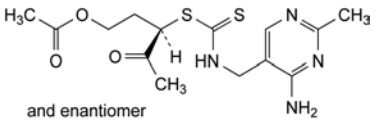
F. R1 = $C_2H_5$, R2 = OH: 3-[(4-amino-2-ethylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium (ethylthiamine),

G. R1 = $CH_3$, R2 = O-CO-$CH_3$: 5-[2-(acetyloxy)ethyl]-3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methylthiazolium (acetylthiamine),




D. X = O: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazol-2(3*H*)-one (oxothiamine),

E. X = S: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazol-2(3*H*)-thione (thioxothiamine),

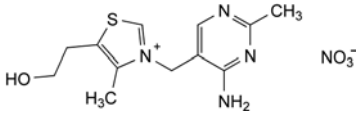



H. (3*RS*)-3-[[[(4-amino-2-methylpyrimidin-5-yl)methyl]thiocarbamoyl]sulfanyl]-4-oxopentyl acetate (ketodithiocarbamate).

**01/2008:0531**
**corrected 6.0**

# THIAMINE NITRATE

## Thiamini nitras




$C_{12}H_{17}N_5O_4S$ $M_r$ 327.4
[532-43-4]

DEFINITION

3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium nitrate.

*Content*: 98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

*Appearance*: white or almost white, crystalline powder or small, colourless crystals.

*Solubility*: sparingly soluble in water, freely soluble in boiling water, slightly soluble in alcohol and in methanol.

IDENTIFICATION

*First identification: A, C.*

*Second identification: B, C.*

A. Infrared absorption spectrophotometry (*2.2.24*).
*Comparison*: *Ph. Eur. reference spectrum of thiamine nitrate.*

B. Dissolve about 20 mg in 10 mL of *water R*, add 1 mL of *dilute acetic acid R* and 1.6 mL of *1 M sodium hydroxide*, heat on a water-bath for 30 min and allow to cool. Add 5 mL of *dilute sodium hydroxide solution R*, 10 mL of *potassium ferricyanide solution R* and 10 mL of *butanol R* and shake vigorously for 2 min. The upper alcoholic layer shows an intense light-blue fluorescence, especially in ultraviolet light at 365 nm. Repeat the test using 0.9 mL of *1 M sodium hydroxide* and 0.2 g of *sodium sulfite R* instead of 1.6 mL of *1 M sodium hydroxide*. Practically no fluorescence is produced.

C. About 5 mg gives the reaction of nitrates (*2.3.1*).

TESTS

**Solution S**. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

**Appearance of solution**. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution $Y_7$ (*2.2.2, Method II*).

**pH** (*2.2.3*): 6.8 to 7.6 for solution S.

**Related substances**. Liquid chromatography (*2.2.29*).

*Solution A*. Add 5 volumes of *glacial acetic acid R* to 95 volumes of *water R* and mix.

*Test solution*. Dissolve 0.35 g of the substance to be examined in 15.0 mL of solution A and dilute to 100.0 mL with *water R*.

*Reference solution (a)*. Dissolve 5 mg of the substance to be examined and 5 mg of *thiamine impurity E CRS* in 4 mL of solution A and dilute to 25.0 mL with *water R*. Dilute 5.0 mL of the solution to 25.0 mL with *water R*.

*Reference solution (b)*. Dilute 1.0 mL of the test solution to 100.0 mL with *water R*.

*Column*:
- *size*: *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (4 µm) with a specific surface area of 350 $m^2/g$ and a pore size of 10 nm,
- *temperature*: 45 °C.