Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

SPECIAL PHARMACEUTICAL CHEMISTRY

Pyrimidine-thiazole vitamins (B1 vitamins)

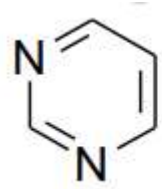
Lesson 15

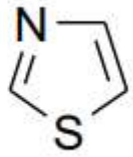
VII term

Volgograd, 2023

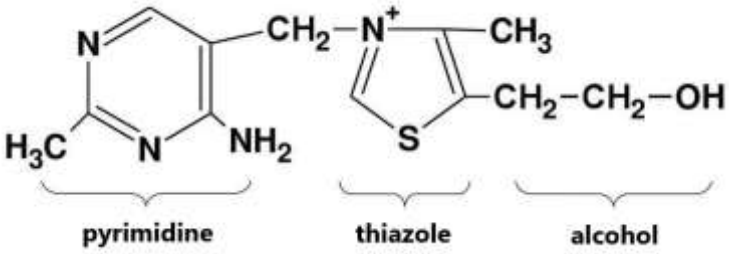
GENERAL CHARACTERISTIC

The chemical structure of thiamine is based on two heterocyclic cycles, the pyrimidine cycle and the thiazole cycle:





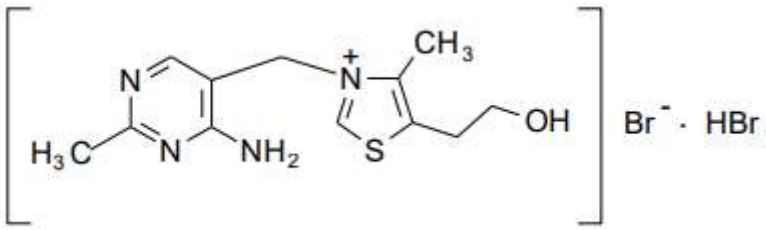
In the thiamine molecule, they are linked together by a methylene group, so thiamine is categorized as a pyrimidinothiazole or pyrimidylmethylthiazole vitamin.



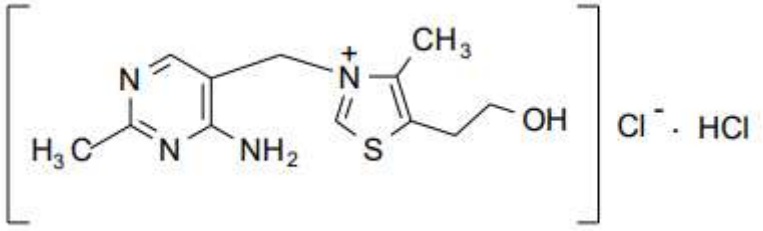
In medical practice, thiamine is used in the form of 4 preparations:

- benfotiamine
- phosphothiamine
- thiamine
- cocarboxylase (thiamine diphosphate)

THIAMINE BROMIDE AND THIAMINE CHLORIDE



Thiamine bromide



Thiamine chloride

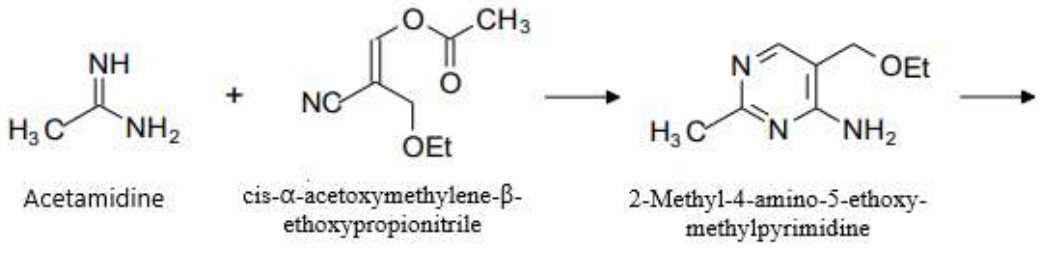
OBTAINING

Thiamine biosynthesis is carried out in plant cells. The human body is unable to synthesize thiamine. It comes with food, which may not always meet the body's needs. Thiamine is found in the yolks of chicken eggs, yeast, in the germ and seed coatings of cereal crops (wheat, oats, buckwheat, corn, etc.), as well as in nuts and peanuts. These products can serve as sources of thiamine. However, the process of vitamin extraction is complicated and the yield is very small. Thus, only 0.25 g of thiamine can be obtained from 1 ton of yeast. Therefore, a significant number of methods of thiamine synthesis have been developed.

Of the numerous methods of thiamine production, the most interesting is the method consisting of three steps:

- Synthesis of the pyrimidine part of the thiamine molecule.
- Synthesis of the thiazole cycle.
- Binding of the pyrimidine part of the thiamine molecule to the thiazole cycle.

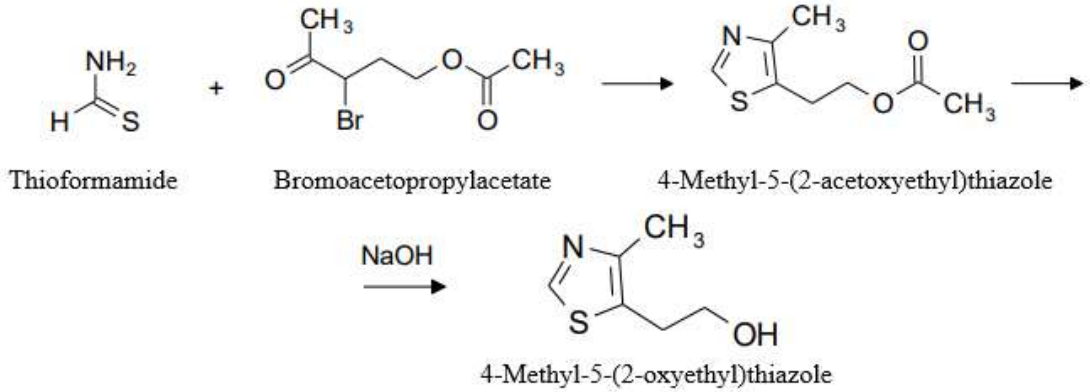
The synthesis of the pyrimidine cycle is based on the condensation of acetamidine and cis-α-acetoxymethylene-β-ethoxypropionitrile:



Acetamidine — cis-α-acetoxymethylene-β-ethoxypropionitrile — 2-Methyl-4-amino-5-ethoxy-methylpyrimidine

2-Methyl-4-amino-5-bromomethyl-pyrimidine hydrobromide

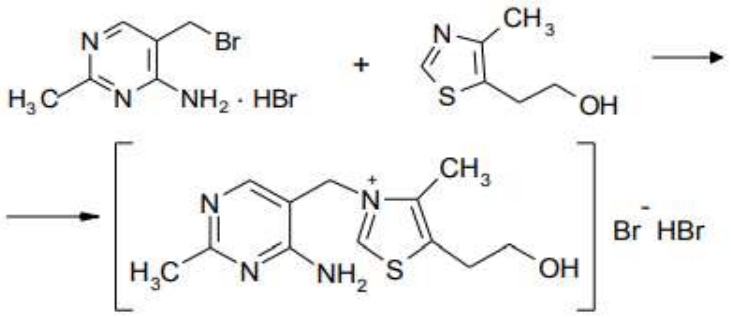
The thiazole cycle is synthesized by condensation of thioformamide and bromacetopropylacetate:



Thioformamide — Bromoacetopropylacetate — 4-Methyl-5-(2-acetoxyethyl)thiazole

4-Methyl-5-(2-oxyethyl)thiazole

The pyrimidine and thiazole parts are linked into one molecule by fusion of the obtained products at 100-120°C or by heating in an organic solvent, e.g., butyl alcohol:



Thiamine bromide hydrobromide

PHYSICAL PROPERTIES

The drugs are practically identical in terms of physical properties. They are white or yellowish tinged crystalline substances with a faint characteristic odor. Thiamine chloride is characterized by a slightly higher hygroscopicity.

Easily soluble in water and methyl alcohol, hardly soluble in ethyl alcohol, practically insoluble in ether, acetone, benzene, chloroform.

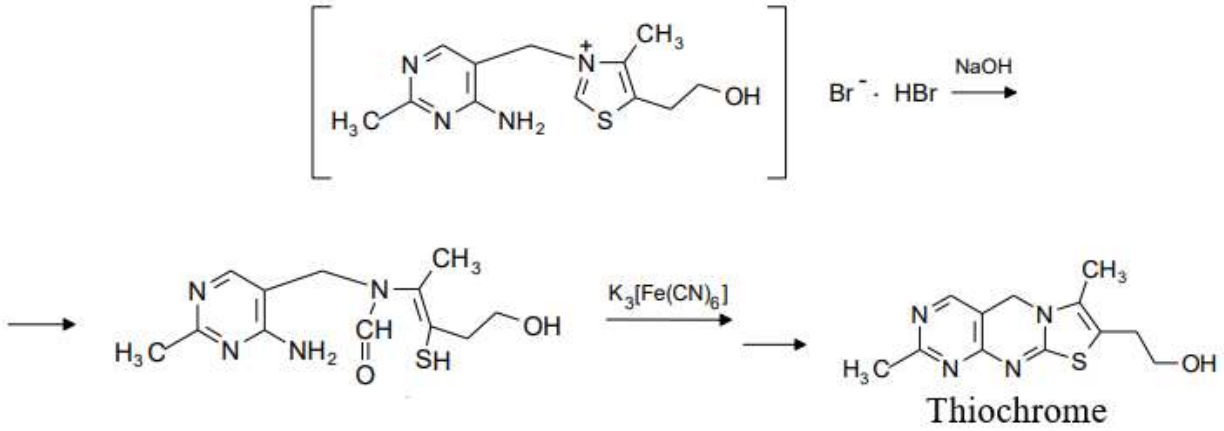
IDENTIFICATION

1. ***UV spectrometry***

   It can be confirmed by UV spectra. For example, a 0.0015 % solution of thiamine bromide in 0.1 M hydrochloric acid solution has an absorption maximum at 246 nm.1. UV spectrometry.

2. ***Thiochrome test***

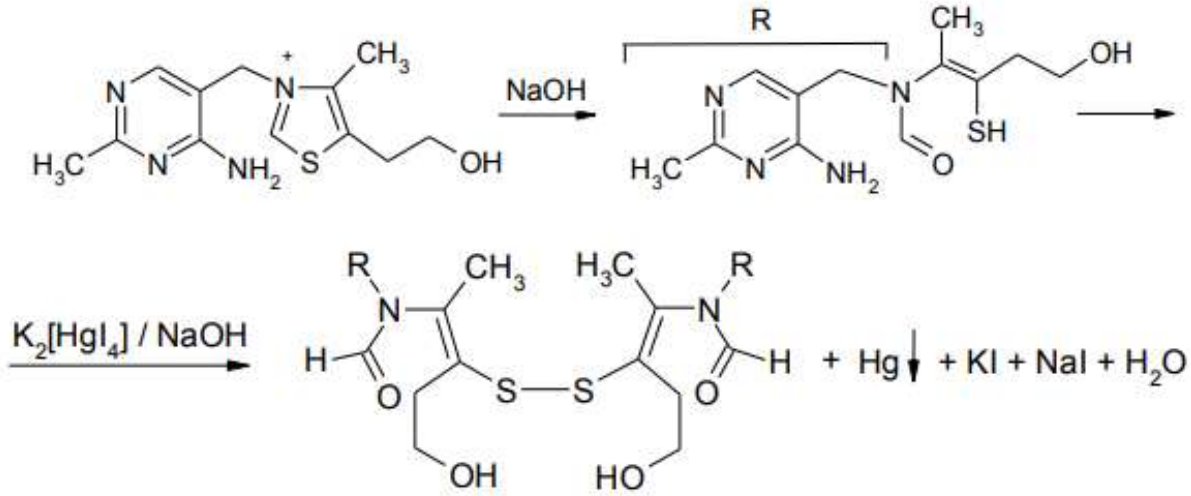
   Identify drugs using a reaction based on oxidation of thiamine in alkaline medium.



Thiochrome

Thiochrome is extracted from aqueous solutions with butyl or isoamyl alcohol. The obtained alcoholic solutions have a characteristic blue fluorescence under UV irradiation, which disappears upon acidification and reappears upon alkalinization.

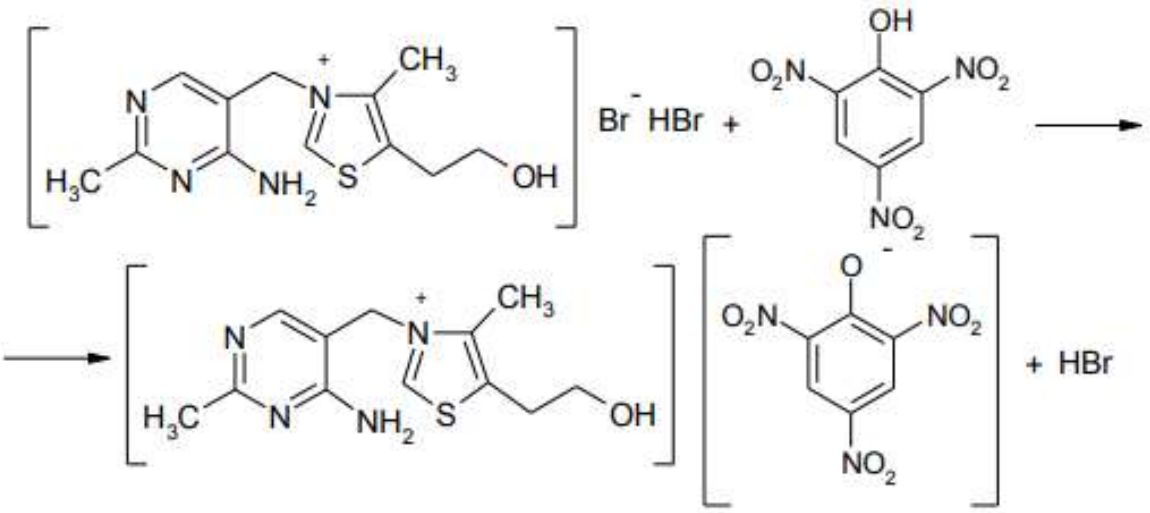
3. ***Oxidation with Nessler's reagent***.
   - When two drops of 15% sodium hydroxide solution are added to a 0.1% solution of the drug, a yellow coloration appears.
   - Upon further action with Nessler's reagent in alkaline medium, a yellow coloration appears, which changes to black due to reduction of metallic mercury:



4. Interaction with common alkaloid reagents

Thiamine can be detected by

- ✓ formation of a white precipitate with saturated mercuric (II) chloride solution;
- ✓ red-brown precipitate with 0.02 M iodine solution;
- ✓ yellow precipitate of picrate with a saturated solution of picric acid (melting point 206-208 °C).



5. Alloying with alkalis.

- When alloying with crystalline caustic alkalis, thiamine is destroyed with the formation of sulfides, which are easily detected with the help of sodium nitroprusside solution:

  To a drop of the solution obtained after dissolving the alloy of thiamine with alkali, add a drop of alkali and a drop of sodium nitroprusside solution. a red-violet coloration appears in the presence of sulfides:

$$R_2S \xrightarrow{NaOH,\ t^oC} Na_2S$$

$$Na_2S + Na_2[Fe(CN)_5NO] \longrightarrow Na_4[Fe(CN)_5NOS]$$

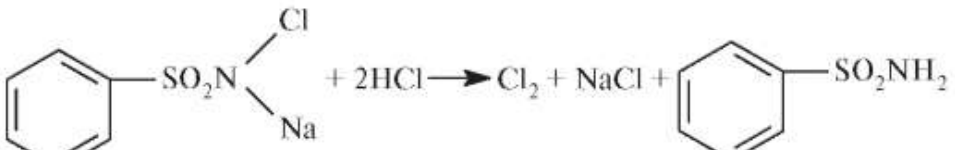
- Sulfides can be detected by adding lead acetate to the solution obtained after dissolution of thiamine alloy with alkali - a black precipitate is formed:

$$Na_2S + (CH_3COO)_2Pb \longrightarrow PbS\downarrow + CH_3COOH$$

6. Determination of halides

Thiamine bromide gives characteristic reactions to bromides and thiamine chloride to chlorides.

- **bromides**



$$2HBr + Cl_2 \longrightarrow Br_2 + 2HCl$$

The formed bromine stains the chloroform layer red-orange in color.

- **chlorides**

$$HCl + AgNO_3 = AgCl\downarrow + HNO_3$$

A white precipitate is formed.

QUANTIFICATION

1. *Gravimetry*

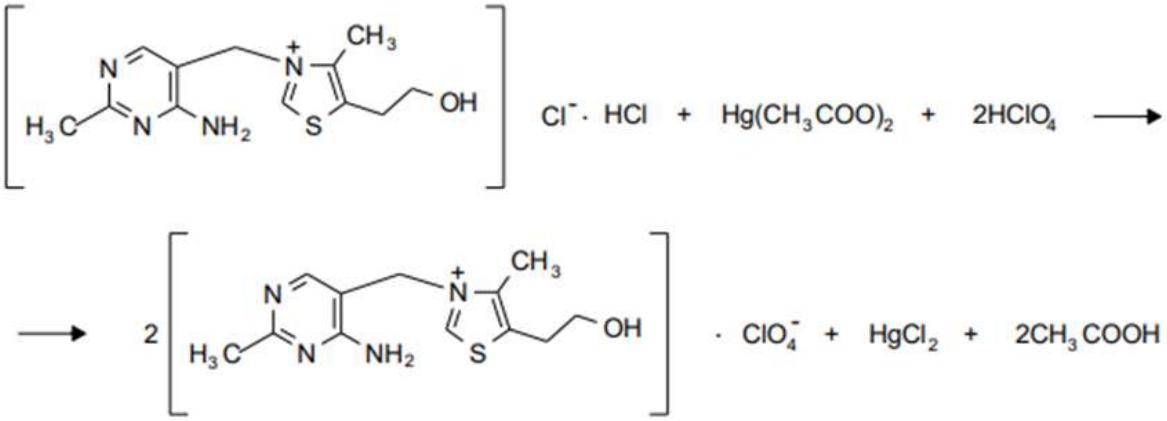
The essence of the quantitative gravimetric determination of thiamine bromide recommended by the pharmacopoeial article is to heat a mixture of an aqueous solution of the drug suspension, concentrated hydrochloric acid and 10% solution of silicon tungstic acid.

$$SiO_2\ 12WO_3\ 4\ H_2O + 4RN \longrightarrow 4RN\ SiO_2\ 12WO_3\ 4\ H_2O \downarrow$$

The formed precipitate is separated, washed on a filter successively with hot dilute hydrochloric acid, then with water and acetone. All operations are carried out on a funnel dried to a constant mass, which is dried together with the precipitate, cooled in the desiccator and weighed. The mass of the precipitate multiplied by a factor of 0.25 corresponds to the amount of thiamine bromide.

2. *Non-aqueous titration*

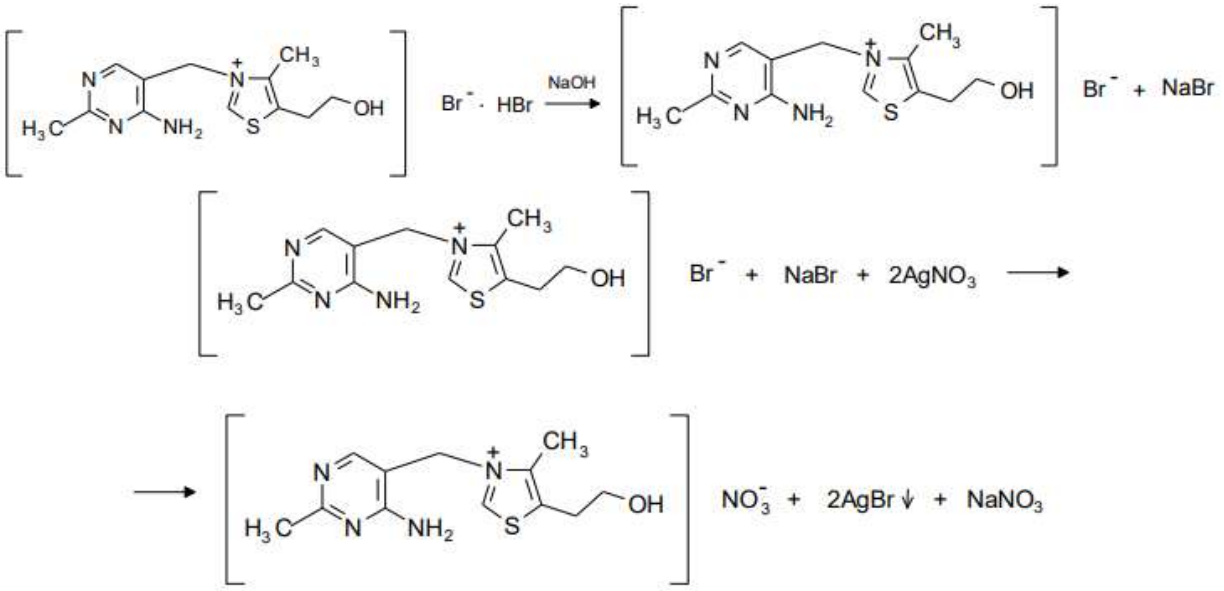
Thiamine chloride is quantified by the non-aqueous titration method. Anhydrous acetic acid is used as a solvent, 0.1 M chloric acid solution serves as a titrant, and crystal violet is the indicator:



Titrate until the color changes from violet through blue to bluish-green.

3. *Argentometry. Volgard method*

Thiamine bromide is quantified by a method based on neutralization of the hydrobromide and subsequent. Argentometric titration of the sum of bromide ions (indicator iron-ammonium alum):

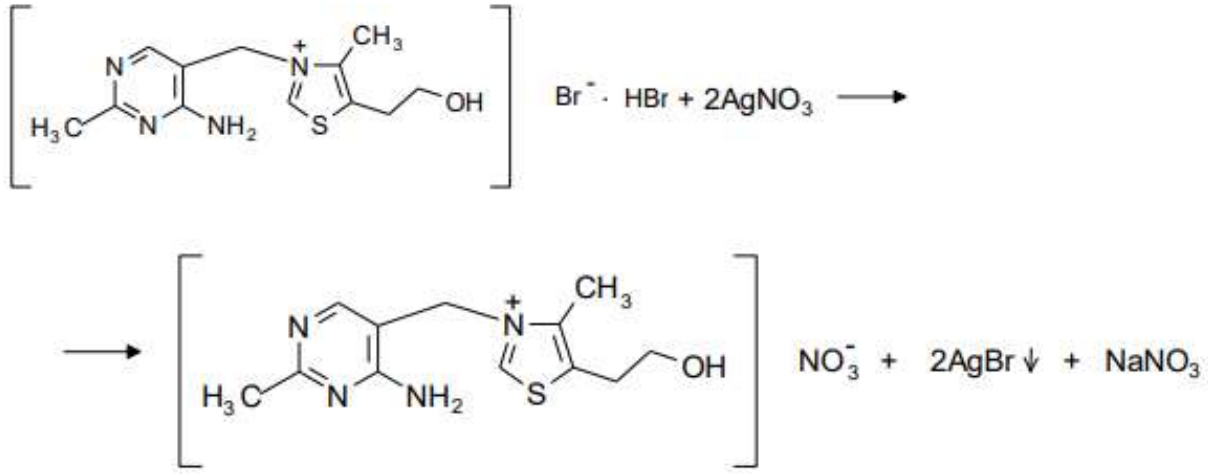


$$AgNO_3 + NH_4SCN \longrightarrow AgSCN + NH_4NO_3$$

$$FeNH_4(SO_4)_2 + 3NH_4SCN \longrightarrow Fe(SCN)_3 + 2(NH_4)_2SO_4$$

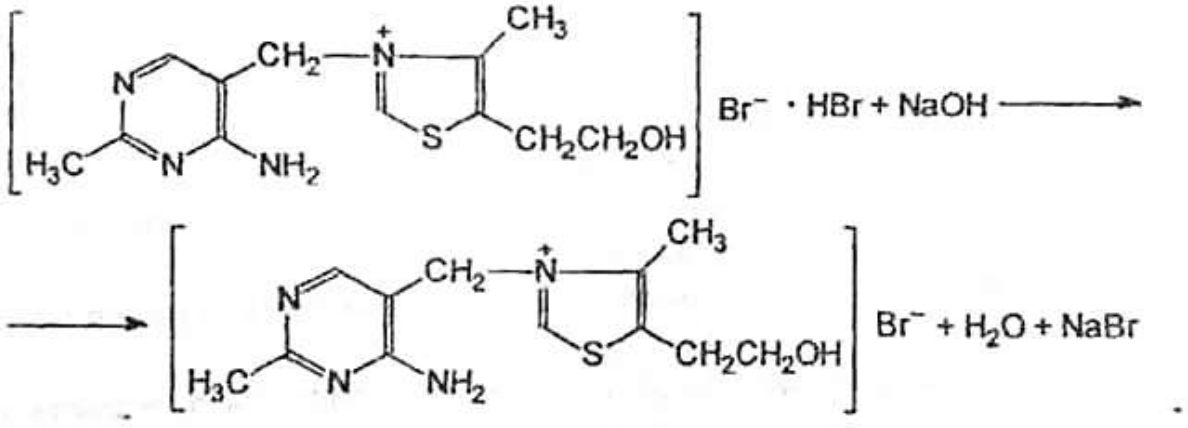
4. Argentometry. Fayans method.

Thiamine preparations can be determined by chloride and bromide ion argentometrically by the Fayans method using bromophenol blue as an indicator in the presence of dilute acetic acid, which is added to create the required pH.



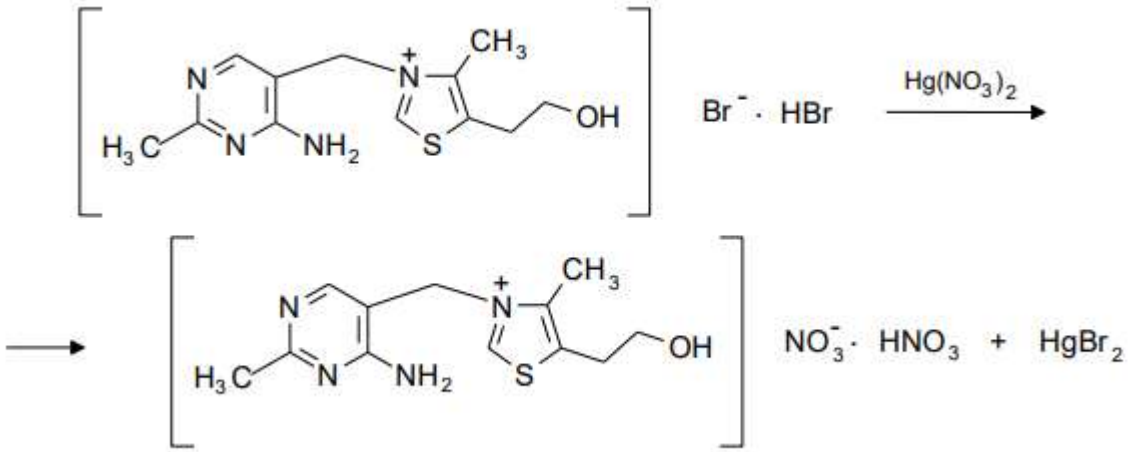
5. Alkalimetric titration

The most widely used alkalimetric method for the determination of thiamine chloride and thiamine bromide using the indicators bromthymol blue or phenolphthalein. Titrant 0.1 M sodium hydroxide solution.

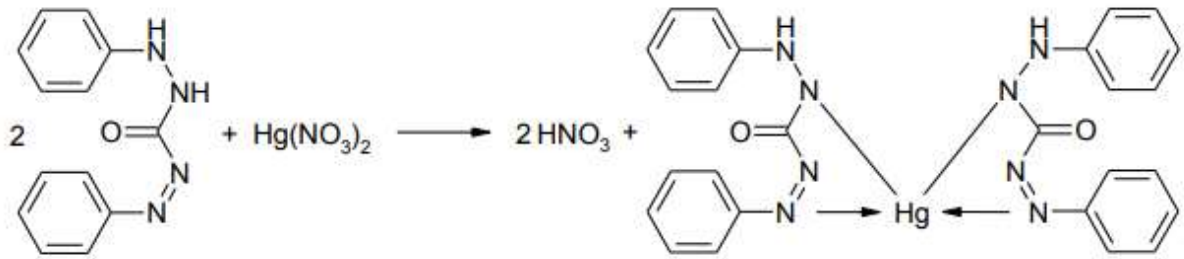


6. *Mercurimetry*

The mercurimetric method is known for the determination of thiamine preparations in nitric acid medium with the indicator diphenylcarbazide or diphenylcarbazone. The titrant is 0.1 M solution of mercury (II) nitrate:



When titrated with mercury nitrate, a low-dissociation mercury bromide molecule is formed. At the equivalence point, an extra drop of working solution of mercury (II) nitrate forms a strong complex with diphenylcorbazone, and the color of the solution changes from pale pink (almost colorless) to blue:



STORAGE

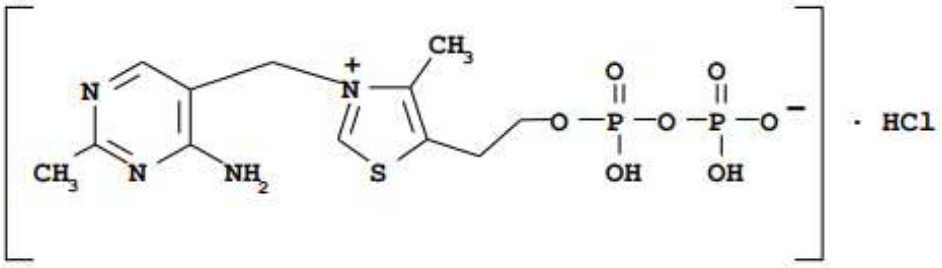
Thiamine preparations are stored in hermetically sealed containers, protected from light and avoid contact with metals. Keep away from children.

MEDICAL USE

Thiamine - vitamin B1 - is a water-soluble vitamin. It is a coenzyme of enzymes responsible for the regulation of protein and carbohydrate metabolism. Thiamine ensures the conduction of nerve impulses in synapses (the point of contact between two neurons). It also has an antioxidant effect and increases the protection of cell membranes from the toxic effects of peroxidation products.

Thiamine preparations are prescribed for nervous system disorders.

COCARBOXYLASE HYDROCHLORIDE

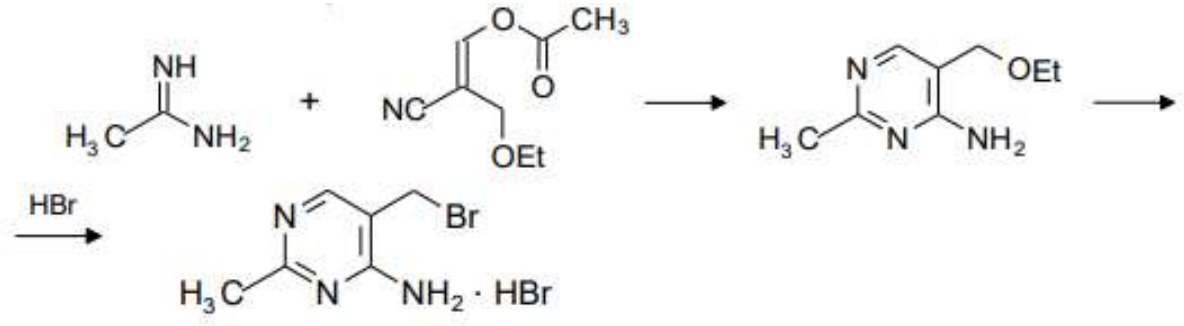


OBTAINING

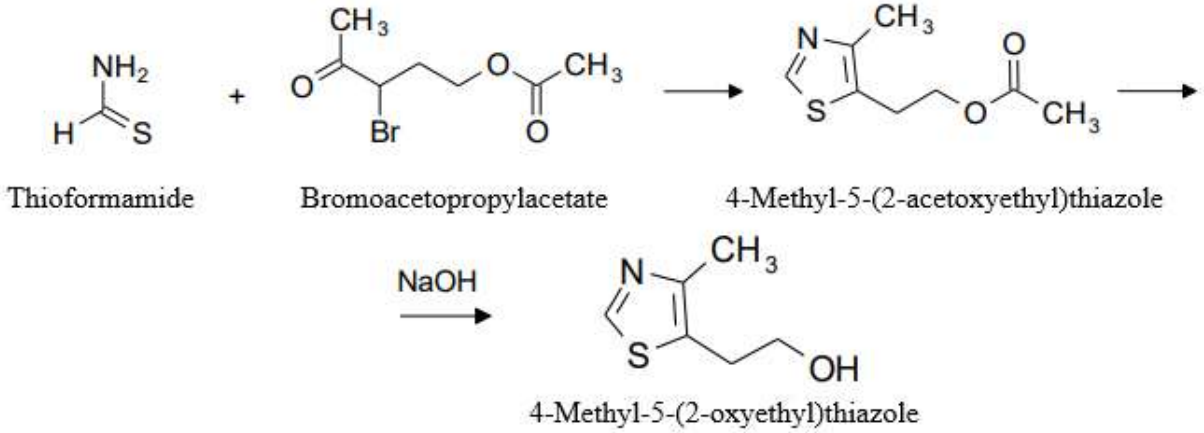
The presence of an alcoholic hydroxyl in the thiamine molecule allowed the synthesis of its mono-, di-, and triphosphoric esters. Some of these esters, such as thiamine diphosphate (cocarboxylase), were isolated from yeast in 1937.

1. Synthesis of the pyrimidine portion of the thiamine molecule.
2. Synthesis of the thiazole cycle.
3. Binding of the pyrimidine portion of the thiamine molecule to the thiazole cycle.
4. Obtaining the phosphoric ester.

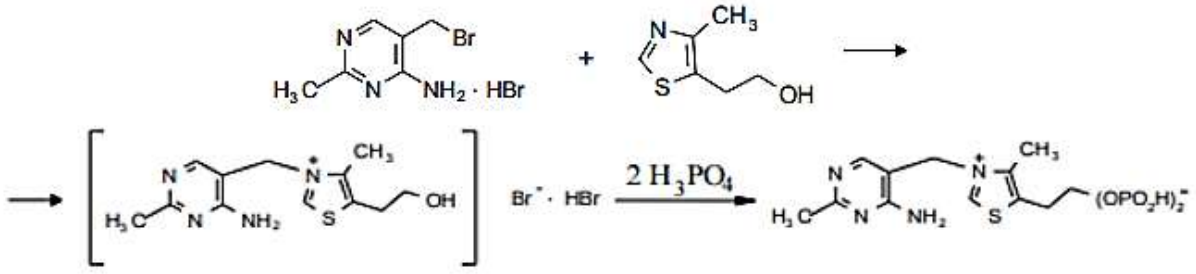
Synthesis of the pyrimidine cycle is based on condensation of acetamidine and cis-α-acetoxy-simethylene-β-ethoxypropionitrile:



The thiazole cycle is synthesized by condensation of thioformamide and bromacetopropylacetate:



The pyrimidine and thiazole parts are linked into one molecule by fusion of the resulting products at 100-120 °C, or by heating in an organic solvent, such as butyl alcohol, and then treated with two moles of phosphoric acid:



PHYSICAL PROPERTIES

Cocarboxylase is characterized by high hygroscopicity. The drug is easily soluble in water, insoluble in ethyl alcohol and practically insoluble in other organic solvents. Aqueous solutions (5-6%) of the drug have pH 2.7 - 3.4.

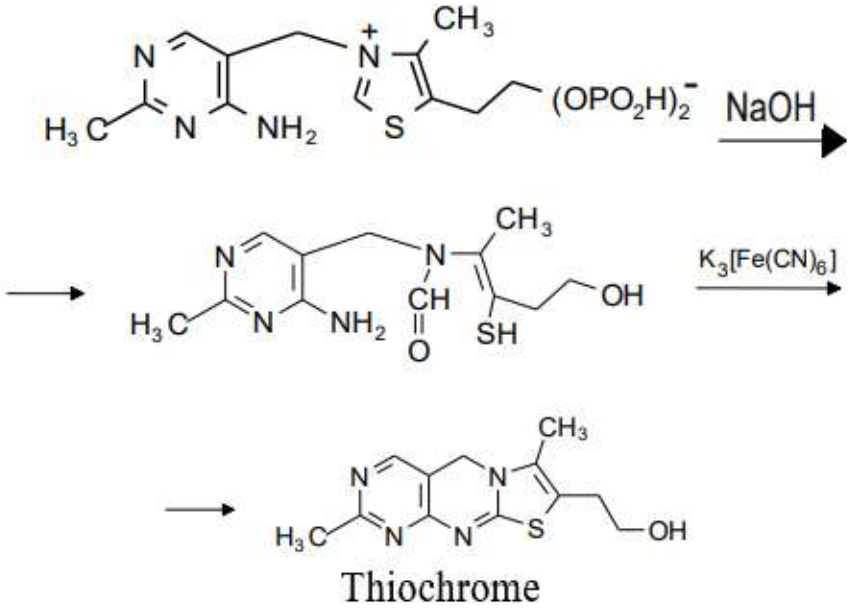
IDENTIFICATION

1. ***Infrared spectroscopy.***

   Thiamine phosphate esters have their clear characteristic bands.

2. ***Thiochrome test.***

The reaction based on the oxidation of thiamine in an alkaline medium:



Thiochrome

Thiochrome is extracted from aqueous solutions with butyl or isoamyl alcohol. The resulting alcoholic solution has a characteristic blue fluorescence under UV irradiation, which disappears upon acidification and reappears upon alkalinization.

3. ***Reaction for phosphate ions after dissolving the drug in dilute nitric acid.***

Cocarboxylase hydrochloride for injection is preliminarily destroyed by boiling for 5 min in concentrated nitric acid to form phosphate ions. Ammonium molybdate solution is used as a reagent for phosphate ions, with which a yellow crystalline precipitate is formed:

$$H_3PO_4 + 12(NH_4)_2MoO_4 + 21HNO_3 = (NH_4)_3PO_4 \cdot MoO_3\downarrow + 21NH_4NO_3 + 12H_2O$$

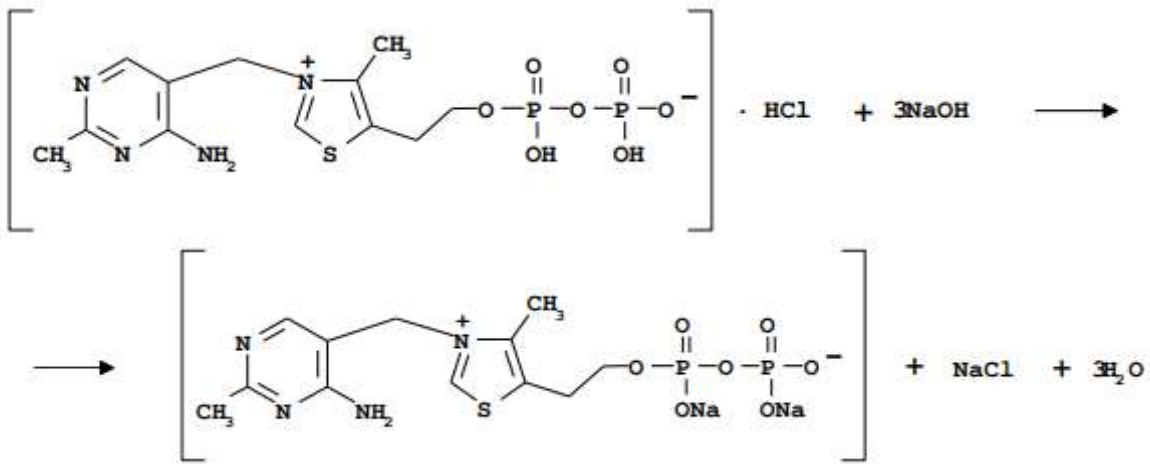
4. ***Chloride detection reaction.***

$$HCl + AgNO_3 = AgCl\downarrow + HNO_3$$

QUANTIFICATION

1. ***Neutralization method.***

The content of cocarboxylase hydrochloride is determined by neutralization of the drug suspension with sodium hydroxide solution using thymolphthalein as indicator. The titration process is based on the following reaction:



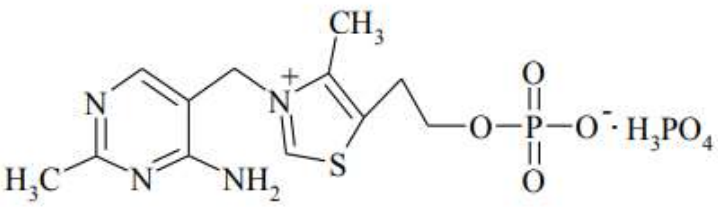
STORAGE

Store in a dry place protected from light at room temperature.

MEDICAL USE

Cocarboxylase hydrochloride for injection is administered intramuscularly and intravenously for disorders of cardiovascular system and coronary circulation.

PHOSPHOTHIAMINE



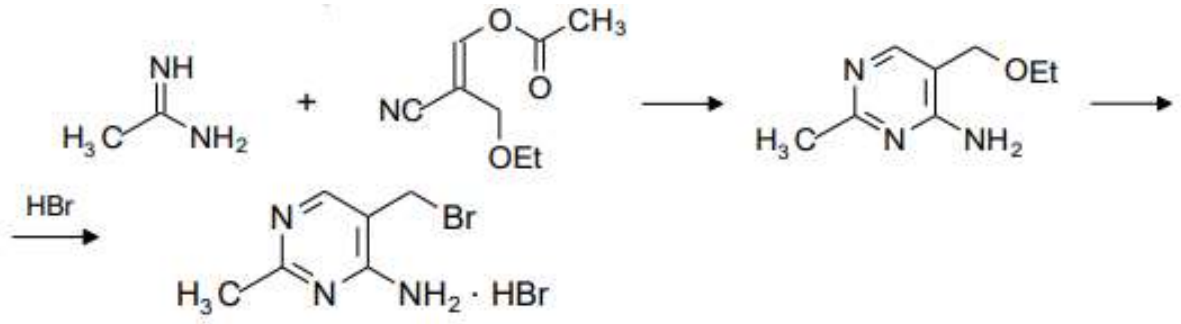
PHYSICAL PROPERTIES

Crystalline powder of sour taste-white color with weak characteristic odor. Phosphothiamine is easily soluble in water, insoluble in ethyl alcohol and practically insoluble in other organic solvents. Aqueous solutions of the drug are slightly acidic.

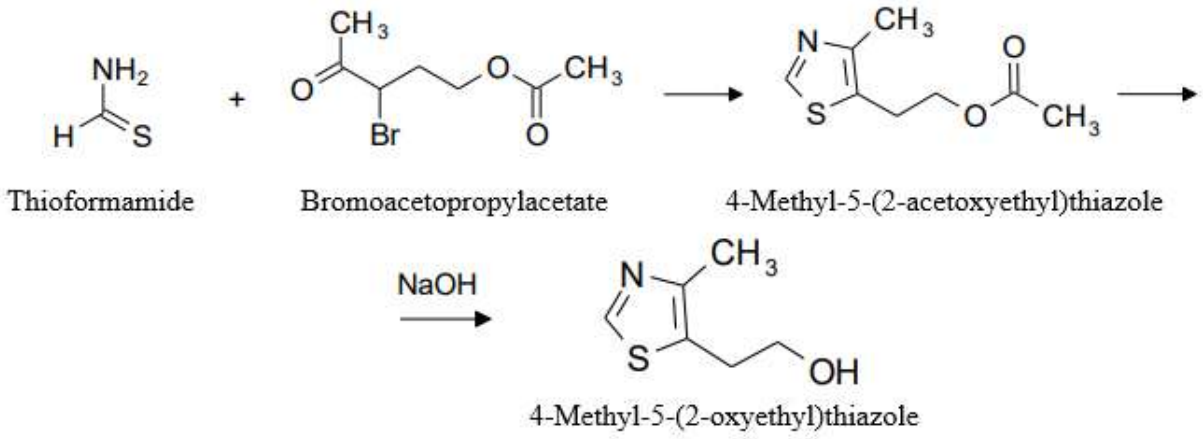
OBTAINING

1. Synthesis of the pyrimidine portion of the thiamine molecule.
2. Synthesis of the thiazole cycle.
3. Binding of the pyrimidine portion of the thiamine molecule to the thiazole cycle.
4. Preparation of phosphoric ester.

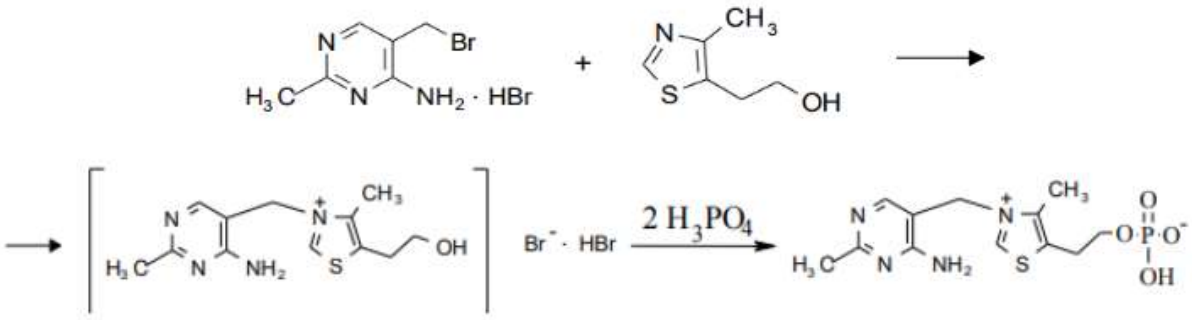
Synthesis of the pyrimidine cycle is based on condensation of acetamidine and cis-α-acetoxy-simethylene-β-ethoxypropionitrile:



The thiazole cycle is synthesized by condensation of thioformamide and bromoacetopropylacetate:



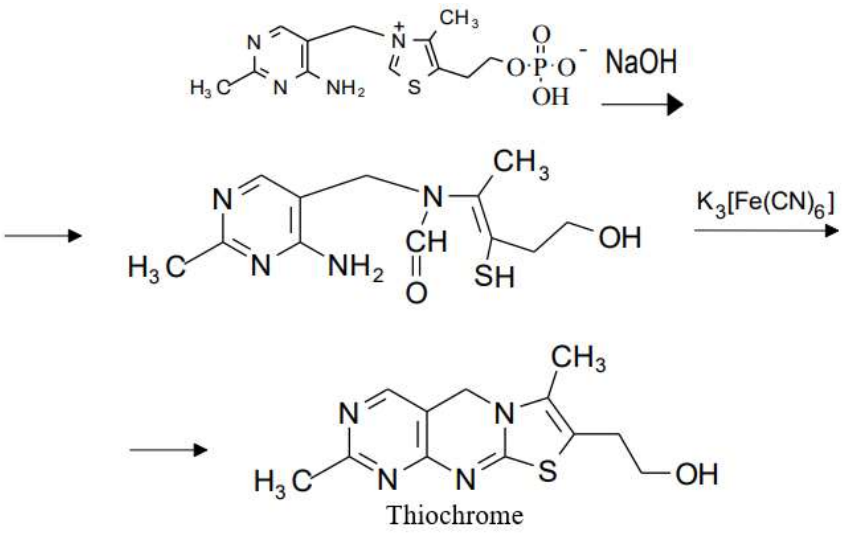
The pyrimidine and thiazole parts are linked into one molecule by fusion of the resulting products at 100-120 oC, or by heating in an organic solvent, such as butyl alcohol, and then treated with two moles of phosphoric acid:



IDENTIFICATION

1. ***Infrared spectroscopy***
2. ***Thiochrome test***

   Thiochrome is extracted from aqueous solutions with butyl or isoamyl alcohol. The obtained alcoholic solutions under UV-irradiation have a characteristic blue fluorescence, which disappears upon acidification and reappears upon alkalinization.



3. *Determination of phosphorus*

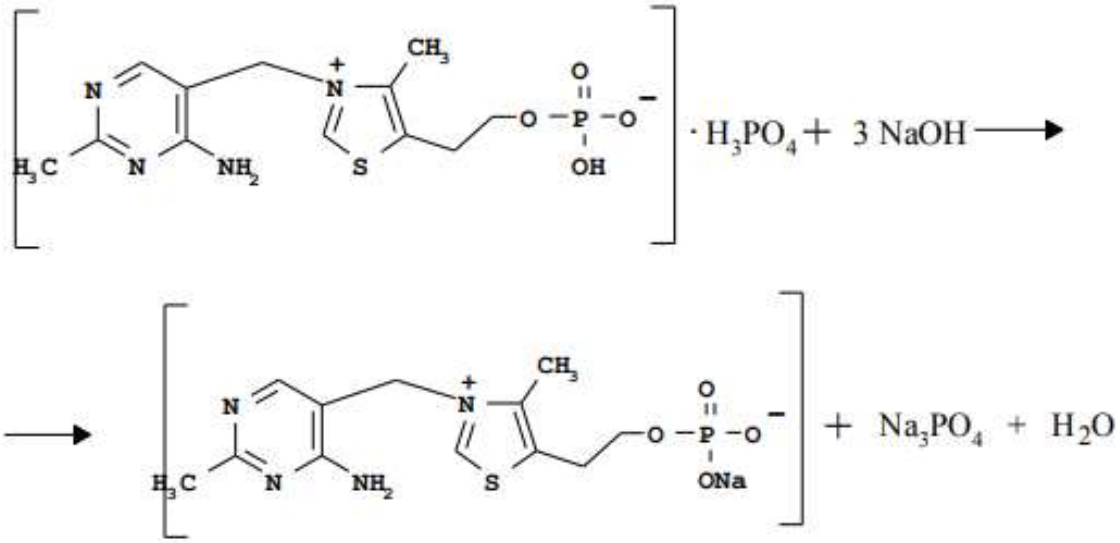
Phosphothiamine gives a positive reaction to phosphate ions after dissolving the preparation in dilute nitric acid. It is preliminarily destroyed by boiling for 5 min in concentrated nitric acid to form phosphate ions. Ammonium molybdate solution is used as a reagent for phosphat ions, with which a yellow crystalline precipitate is formed:

$$H_3PO_4 + 12(NH_4)_2MoO_4 + 21HNO_3 = (NH_4)_3PO_4 \cdot MoO_3\downarrow + 21NH_4NO_3 + 12H_2O$$

QUANTIFICATION

1. *Neutralization method.*

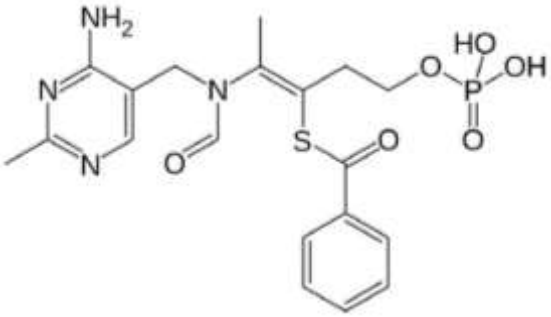
The phosphothiamine content is determined by neutralization of the drug suspension with sodium hydroxide solution using thymolphthalein as an indicator. The titration process is based on the following reaction:



STORAGE
Store in a dry place protected from light at room temperature.

MEDICAL USE
Compared to thiamine chloride and thiamine bromide, phosphothiamine is more deposited in the tissues of the body, less destroyed by the enzyme thiaminase, more easily converted to the active form - cocarboxylase, less toxic.
Used in neuritis, polyneuritis, as an additional remedy for chronic circulatory failure, chronic gastritis, accompanied by impaired motor and secretory (secretion of digestive juices) functions of the stomach.

BENFOTIAMINE



PHYSICAL PROPERTIES

White crystalline powder with weak characteristic odor. Benfotiamine is practically insoluble in water and ethanol, but soluble in 1% sodium hydroxide solution.

IDENTIFICATION

1. ***IR spectroscopy.***
2. ***UV spectrometry***
3. ***Thiochrome test.***

   The reaction is performed after preheating the solution for 20 min on a boiling water bath.
4. ***Determination of phosphate after hydrolysis***

QUANTIFICIFICATION

1. ***Spectrophotometry***

STORAGE

Benfotiamine is stored in a dry place protected from light at room temperature.

MEDICAL USE

Benfotiamine is used as an analog of thiamine, more resistant than it to the action of thiaminase. It is available in the form of tablets.