Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

GENERAL PHARMACEUTICAL CHEMISTRY

Determination of nitrogen in medicinal products.

Kjeldahl method.

Lesson 18

V term

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QUESTIONS FOR THE LESSON

1. Determination of nitrogen in organic pharmaceuticals.
2. Kjeldahl method.
3. Instrument for the determination of nitrogen by the Kjeldahl method.
4. Determination chemistry.
5. Determination techniquc.
6. Area of application.

DETERMINATION OF NITROGEN IN ORGANIC PHARMACEUTICALS

Nitrogen in organic compounds exists in the form of various functional groups: alkylamino, amino, azido, cyano, isocyano, azo, azoxy, diazo, hydrazo, urea, lactam, heterocyclic, hydrazine, semicarbazide, isocyanate, nitro, nitroso, N-oxide, ammonium cation.

When organic matter is completely broken down, nitrogen can be released as:

- ammonia,
- nitrogen oxides,
- dicyane,
- elemental nitrogen.

The formation of these substances depends on how the nitrogen-containing substances are broken down and on the nature of the nitrogen bond in the molecule. Different methods of breaking down nitrogen-containing substances are used: - reductive, - oxidative.

Three methods, based on different principles, are known for the quantitative determination of nitrogen in organic compounds:

1. The Dumas method (combustion of a substance with copper oxide. Elemental nitrogen is produced).
2. Kjeldahl method (decomposition of organic matter by boiling in concentrated sulphuric acid in the presence of catalysts and oxidants. Ammonia is formed).
3. ter-Meylen method (reductive method of hydrogenation. ammonia is produced).

KJELDAHL METHOD

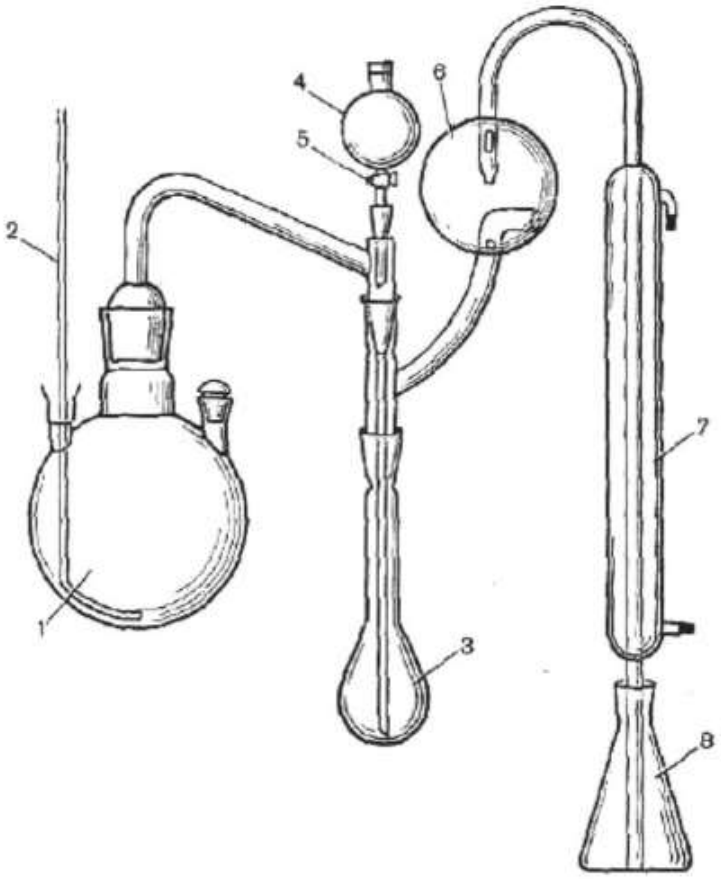
The Kjeldahl method is a pharmacopoeial method for the determination of nitrogen in organic drugs. It was proposed in 1883.

The method is based on the mineralisation of a drug by exposure to concentrated sulphuric acid when heated in the presence of catalysts.

A mixture of potassium sulphate, copper sulphate and/or selenium and/or titanium dioxide can be used as catalysts.

KJELDAHL NITROGEN DETERMINATION DEVICE

The Kjeldahl test is carried out in the special equipment shown in the picture



1. Steam generator - flask with a volume of 3 litres
2. Safety tube
3. Long-necked Kjeldahl exchange flasks for condensing water vapour and protecting against loss of substance,
4. Drop funnel
5. Clamp or tap for adding sodium hydroxide
6. Splashguard
7. Condenser
8. Trapping flask

CHEMISTRY OF DETERMINATION

The method involves several stages.

1. Mineralisation (heating with concentrated H_2SO_4):

$$R{-}CH_2{-}NR_2 \xrightarrow{[O]} CO_2\uparrow + H_2O + (NH_4)HSO_4$$

2. Decomposition of $(NH_4)HSO_4$ with sodium hydroxide and distillation of the resulting ammonia into the trapping flask:

$$(NH_4)HSO_4 + 2OH^- \rightarrow NH_3\uparrow + 2H_2O + SO_4^{2-}$$

3. Interaction of NH_3 in a trapping flask with boric acid to form ammonium tetrahydroxiborate:

$$B(OH)_3 + H_2O \rightarrow H[B(OH)_4]$$

$$NH_3 + H[B(OH)_4] \rightarrow NH_4[B(OH)_4]$$

4. Titration of the distillation with 0.1M hydrochloric acid solution:

   the colour change of the mixed indicator from green to red-violet

$$NH_4[B(OH)_4] + HCl \rightarrow NH_4Cl + B(OH)_3 + H_2O$$

DETERMINATION TECHNIQUC

A Kjeldahl flask (3) of 200 to 300 mL capacity (other volumes of 50 to 500 mL should be specified in the pharmacopoeial article) should be filled with the precise weight (to be specified in the pharmacopoeial article) or the precise volume of the drug sample (0.5 to 10, 0 ml) with a nitrogen content of approximately 14 - 35 mg (if the sample preparation is required, it shall be described in the pharmacopoeial article), three glass beads for foaming and 1 g crumbled mixture of potassium sulphate and copper sulphate, taken in a ratio of 10 : 1 (other composition of the catalyst mixture should be described in the pharmacopoeial article). For difficult to burn substances, additionally 0.05 g selenium metal and/or 1 mL concentrated hydrogen peroxide solution are added to flask (3). Add 7 mL of concentrated sulfuric acid and carefully rotate the flask to drain the acid from the walls and mix it with the contents of the flask. Gradually heat the flask (3), closed with a glass funnel, with an electric heating device and then boil the contents for several hours until a light green solution is obtained. No charred material should remain on the sides of the flask. The boiling process is continued for another 30 minutes or more until the solution has cleared. If strong foaming occurs during boiling, it is recommended to remove the Kjeldahl flask from the heating device and allow the foam to settle, then continue heating again without allowing any foam to enter the neck of the flask. After the Kjeldahl flask has cooled down, 20 mL water is added carefully and the contents is stirred, the flask is cooled again and the flask is

connected to the assembled nitrogen determination apparatus (figure) previously washed by passing steam through it. Water, at least half full, acidified with 0.5 M or 0.05 M sulphuric acid solution by the methyl red indicator (2 - 3 drops) to a slightly pink colour, is poured into the steam generator to bind the ammonia which can come from the air. Glass beads are placed in the steamer to ensure even boiling of the water. Before starting the distillation, 20 mL of a 4% boric acid solution is poured into the receiver and 0.25 mL (5 drops) of mixed indicator is added. The lower end of the inner tube of the refrigerator has to be dipped into the solution in the receiver. After reassembling the apparatus, water is allowed to flow into the cooler and the water in the steam generator is brought to the boil. Then 40 mL of sodium hydroxide 30% solution are slowly added dropwise from a funnel into the flask (3), making sure that the solution in the flask (3) is stirred vigorously by the incoming steam. Some excess sodium hydroxide solution 30% should be left in the funnel to make the apparatus more airtight. Collect about 100 mL of the distillation (or the amount stated in the pharmacopoeial article). During the distillation the Kjeldahl flask is heated so that the volume of liquid in it remains constant. When the distillation is finished, the receiver is lowered so that the refrigerator tube is above the surface of the liquid in the receiver. The cooler tube is washed from the outside with water, continuing to supply steam into the flask (3) for 1 - 2 min; the washed water is collected in the same receiver. Afterwards, the heating of the steam generator is stopped and the Kjeldahl flask is immediately disconnected from the apparatus. After the distillate has been distilled off, titrate the distillate with 0.1 M hydrochloric acid or 0.05 M sulphuric acid (to be specified in the pharmacopoeia) until the colour of the mixed indicator changes from green to red-violet.

Carry out a control experiment in the same way and with the same reagents, but without the test sample; use the result to correct for the nitrogen content.

1 mL of 0.1 M hydrochloric acid or 0.05 M sulphuric acid corresponds to 1.401 mg nitrogen.

AREA OF APPLICATION

The area of application of the Kjeldahl method in pharmaceutical analysis is quite broad. The State Pharmacopoeia recommends it for the determination of urethanes (meprotane), amino acids (methionine, glutamic acid) and other nitrogen-containing drugs (benzohexonium, oxaphenamide, diprophylline).

A simplified version of the Kjeldahl method, which excludes the mineralisation stage, is used for certain drugs containing an amide group which is easily hydrolysed in an alkaline medium (salicylamide, nicotinic acid diethylamide, soluble saluside, proserine). The method of determination consists in destroying the drug with 30% sodium hydroxide solution in a Kjeldahl flask and distilling off the released ammonia (or dialkylamine) into a receiver.

The most significant disadvantage of the method is its labour-intensive nature.