Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

GENERAL PHARMACEUTICAL CHEMISTRY

METHODS FOR THE DETERMINATION OF HEAVY METAL AND ARSENIC IMPURITIES IN MEDICINAL PRODUCTS

Lesson 4

V term

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METHODS FOR THE DETERMINATION OF HEAVY METAL IN MEDICINAL PRODUCTS

The definition of heavy metals

The concept of a “heavy metal” is not clearly scientifically defined.

Depending on the classification criteria (density, periodic number etc.), differing elements are sometimes classified here.

In technical terms, every metal with a density greater than 5 g/cm3 is considered to be a heavy metal.

In common parlance, “heavy metal” is typically understood to mean an element that is toxic. This is however only partially correct, as elements that are essential for humans in small quantities also come into this category.

The State Pharmacopoeia of the Russian Federation lists the following heavy metals: lead, mercury, bismuth, antimony, tin, cadmium, silver, copper, molybdenum, vanadium, ruthenium, platinum and palladium.

The origin of heavy metals in nature

Heavy metals are largely found in nature as minerals and ores. They get into the environment as a result of being extracted, from erosion or from volcanic activity. Heavy metals are used in a number of technical applications and processes and can get into the environment or into products unintentionally.

Heavy metals in medical plants

Intake of heavy metals occurs in the growth process of plants by absorption from water, from the ground and by aerosols from the ambient air. Contamination can also occur from the spillage of pesticides or sewage sludge containing heavy metal.

Heavy metals in finished products and raw materials

In the manufacturing of pharmaceutical products, catalysts containing heavy metals are often involved in the synthesis. Heavy metals can also transfer into the process by abrasion or by leaching (e.g. Fe, Ti, Cu, Cr and so on.) If they are not removed efficiently, then the tainted products could get into the market.

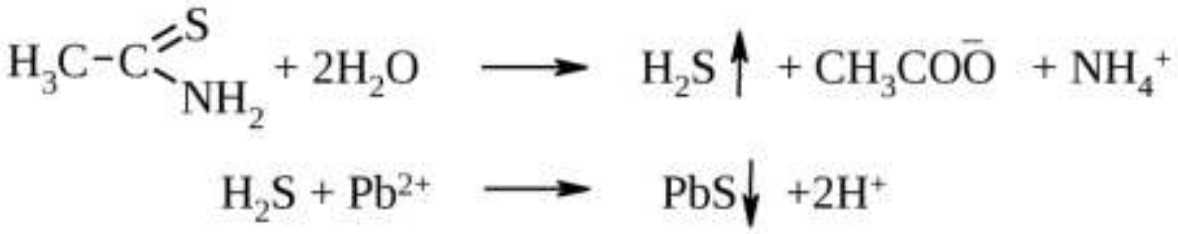
Legal regulations

For many years, it has been known that certain heavy metals exhibit toxic effects even at low concentrations. As a result, limit values for the protection of the

patients have been defined in the legislation and in the various pharmacopoeias (e.g. SPRF., Ph. Eur., USP, JP, BP).

Detection methods

If testing is not performed for a specific heavy metal, the most common source of evidence nowadays come from a limit test being carried out. After treatment, the heavy metal is complexed with thioacetamide (method 1)



or precipitated as a sulphide (method 2).

Then, one compares the resulting colouring of the sample solution against that from a reference lead solution. The color appearing in the sample solution should not exceed the color of the reference solution.

Test is considered accurate if the reference solution has a slight brownish colour compared to blank solution.

These limit tests still form the majority of testing for heavy metals in the current national and international Pharmacopoeias. Thereby, it is possible however to make only a semi-quantitative statement about the total contents of heavy metals in the sample, and in addition - and additionally, only for those heavy metals that actually form dark coloured complexes or sulphides.

Determination of heavy metal content in pharmaceutical products is possible for substances that form a clear, colourless solution and do not affect the interaction between metal ions with sulphide-ions due to complex-forming properties. In all other cases, determination is performed using sulphate ash or other mineralization technique of the test pharmaceutical product.

Maximum permissible content of heavy metals, testing method and conditions for preparation of the test sample must be indicated in the general monograph.

The following methods can be used for quantitative determination:

- atomic absorption spectrometry;
- atomic emission spectrometry with inductively coupled plasma;
- inductively coupled plasma mass spectrometry.

METHODS FOR THE DETERMINATION OF ARSENIC IMPURITIES IN MEDICINAL PRODUCTS

Arsenic

Arsenic (Latin Arsenicum, chemical symbol — As) — chemical element of the 15th group of the fourth period of the periodic system; has the atomic number 33.

The simple substance is a brittle steel-colored semi-metal with a greenish tinge (in a gray allotropic modification).

Poison and carcinogen. The lethal dose of arsenic for humans is 50-170 mg.

Methods for detecting arsenic impurities.

Very often arsenic is an undesirable impurity in medicines. It can get into medicines from the material of equipment used in the manufacture of drugs, or together with raw materials, solvents, most often with sulfuric acid, used in most pharmaceutical industries.

Due to the high toxicity of arsenic and often the inadmissibility of its impurities in medicines, special sensitive reactions are used to detect small amounts of arsenic, regardless of its valence. These include the of Bugo and Thiele, Gutzeit, Bettendorf and the Marsh method.

The Gutzeit, Bugo and Thiele methods, which are recommended by the State Pharmacopoeia for the determination of arsenic impurities in medicinal preparations, have the greatest practical application in pharmaceutical analysis

Gutzeit method

Main stages:

1. Reduction of arsenic compounds to arsin AsH_3. The reduction is carried out using zinc in an acidic medium.

$$2H_3AsO_3 + 6Zn + 12HCl \longrightarrow 2AsH_3 + 6\,ZnCl_2 + 6\,H_2O$$

2. The interaction of arsin with a solution of silver nitrate, which is soaked with filter paper. The filter paper covers the vessel where the reaction takes place. As a result of the reaction, a complex compound of yellow color is formed.

$$AsH_3 + 6AgNO_3 \longrightarrow Ag_6[As(NO_3)_3] + 3\,HNO_3$$

3. Under the influence of moisture, the complex is destroyed with the release of metallic silver, which forms a dark spot on the filter paper.

$$Ag_6[As(NO_3)_3] + 3\,H_2O \longrightarrow H_3AsO_3 + 6\,Ag + 3\,HNO_3$$

Disadvantages of the Gutzeit method:

- ✓ Arsenic can be determined by the Gutzeit reaction if it is known that there are no antimony or phosphorus impurities in the samples under study. Since under these conditions of reduction of arsenic compounds by zinc, SbH_3 and PH_3 can be formed from these impurities, which will mask the main reaction to arsenic.
- ✓ It is impossible to open an impurity of arsenic in the presence of mercury or silver salts, since they are also restored and mask the discovery of arsenic.
- ✓ Oxidizing agents, compounds forming chlorine, bromine, iodine, hydrogen sulfide, and sulfur dioxide in an acidic environment, which have high volatility and can react with $AgNO_3$ solution, interfere with the reaction.

Due to the low stability of the $AgNO_3$ solution with respect to light and moisture, the Gutzeit reaction was subjected to various modifications aimed at replacing $AgNO_3$ with other AsH_3 fixators

The State Pharmacopoeia recommends a modified version of the Gutzeit reaction. It uses filter paper soaked with an alcoholic solution of sulema (mercury dichloride) $HgCl_2$ as an AsH_3 fixative.

At the same time, an orange-red spot appears on the paper due to the substitution of hydrogen in AsH_3 and the reduction of mercury (II) to mercury (I):

The resulting product is unstable in the presence of moisture and decomposes to form arsenic and mercury chloride (I). The release of free arsenic causes the orange-red color of the filter paper to change to brown.

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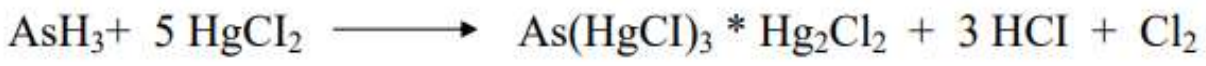


$$AsH_3 + 5\,HgCl_2 \longrightarrow As(HgCl)_3 * Hg_2Cl_2 + 3\,HCl + Cl_2$$

3. Decomposes to form arsenic and mercury chloride (I):

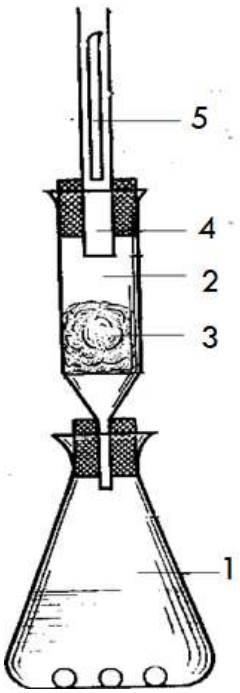
$$2\ As(HgCl)_3 * Hg_2Cl_2 \longrightarrow 2As + 5\ Hg_2Cl_2$$

Usually, a cotton swab soaked with lead acetate is placed in the test tube where the reaction takes place. It captures hydrogen sulfide H_2S, which is formed if there are sulfur compounds in the test sample.

$$Pb(CH_3COO)_2 + H_2S \longrightarrow PbS + 2\ CH_3COOH$$

The method of obtaining coloring on paper moistened with $HgCl_2$ solution is considered the best and makes it possible to open thousandths of a milligram of arsenic in the test sample.

The device for the determination of arsenic by this method consists of



1- flask;

2- glass tube;

3-cotton swab soaked in lead acetate;

4-glass tube;

5-a strip of paper soaked in a solution of mercury dichloride.

The Bugo and Thiele method

Under the action of sodium hypophosphite in an acidic medium, when heated, arsenic compounds are reduced by phosphoric acid to elemental arsenic. Depending on the concentration of arsenic, a brown precipitate or dark brown staining of the liquid is observed.

$$NaH_2PO_2 + HCl \longrightarrow NaCl + H_3PO_2$$

$$3\,H_3PO_2 + 2\,H_3AsO_3 \longrightarrow 2\,As + 3\,H_3PO_3 + 3\,H_2O$$

$$As_2O_3 + 3H_3PO_2 \longrightarrow 2As\downarrow + H_3PO_3$$

$$As_2O_5 + 5H_3PO_2 \longrightarrow 2As\downarrow + 5H_3PO_3$$

There are two options for testing:

method A (without using a standard solution) - by browning the solution or the formation of a brown precipitate;

method B - by comparing the intensity of the resulting color with the color of the reference solution.

In the case of browning or the formation of a brown precipitate, water, ether are added to the test tube after cooling, and shaken thoroughly. In the presence of arsenic, a brown film forms at the interface of liquids.

The disadvantage of the method:

The Bugo and Thiele method, although less sensitive than the Gutzeit method.

The advantage of the method:

It can be used for testing for arsenic in the presence of antimony compounds, phosphorus, lead, sulfides that are not reduced by phosphoric acid.