**Chemical-toxicological analysis plan. Analytical screening, classification and general characteristics of methods. Common principles for assessing the results of forensic chemical research**

Chemical-toxicological analysis involves solving two major problems:

1. The isolation of toxic substances from the object of study (isolation);
2. The determination the content of these substances in the isolated phase

The choice of isolation methods is determined by the circumstances of the case, the nature of the object, results of preliminary tests. If there are no precise indications of the presence of a particular substance in the objects of study, then a general isolation scheme is used, which makes it possible to extract substances exhibiting the properties of bases or acids.

Special methods are used to extract certain groups of substances. The choice of isolation and cleaning method is determined by the following factors:

1. A specific practical task, i.e. nature of the object, metrological parameters of the technique.
2. Background of the object (preliminary research, circumstances of poisoning, indication of origin, etc.).
3. The compatibility of the selected isolation and cleaning method with the subsequent method detection and determination of toxic substances in the extract.

**Chemical-toxicological analysis plan**

The need to draw up an analysis plan is determined by the fact that the objects of study cannot be duplicated. In case of acute poisoning with toxic substances, blood, urine and other fluids of the human body change rapidly, and their repeated analysis will give completely different results. In case of fatal poisoning, physical evidence cannot be provided a second time.

Drawing up a plan for a chemical-toxicological analysis depends on the nature and nature of the research object, the questions posed to the expert, the content of the accompanying documents and the results of the external examination of the object.

The chemical-toxicological analysis plan should be structured in such a way as to most rationally and with little time spent solving the main problem - to detect and quantify toxic substances and (or) their metabolites in the studied objects.

The plan is drawn up in accordance with Order Ministry of Health of the Russian Federation No. 289 dated October 5, 1989 and Order Ministry of Health of the Russian Federation No. 161 dated April 24, 2003 in the following order.

1. **Inspection of an object sent for analysis**

***External inspection of the facility***. Objects are subjected to detailed inspection and comparison with a description in the accompanying document. Pay special attention to the packaging features of the object, the inscriptions on jars, flasks, bags, boxes, boxes, their contents, imprint, and seal integrity. Once you are convinced of complete compliance, proceed to opening the package, which is done carefully to prevent getting into the object of printing or packaging parts.

***Inspection of the object after opening the package.*** Site inspection data allows assume what caused the poisoning and include it in the analysis plan first testing for suspected substances.

***Determining the nature, character and smell of an object.*** After opening the package, it is important to establish which organs or parts there of were delivered for examination and in what condition they are - whether there are signs of putrefactive decomposition. Specific smell of the object (especially the contents of the stomach) can be detected in the absence of sharp signs of rotting, since hydrogen sulfide and ammonia formed in this case can mask the odor foreign compounds. Many “volatile” substances can give a characteristic odor to an object poisons. For example, the smell of bitter almonds indicates possible cyanide poisoning, the smell of pyridine bases indicates possible poisoning with denatured alcohol. You can smell the characteristic odor of phenol, fusel oil, chloroform, acetone, formaldehyde, ethyl alcohol and other odorous substances.

***Determination of the presence of foreign inclusions***. The object is examined first visually and then using a magnifying glass. Foreign inclusions may be found in the stomach contents. These are parts of plants, seeds, crystals of salts of alkaloids, metals, undisintegrated tablets, powders, etc.

***Determining the color of an object.*** Coloring the object (mainly the contents) stomach) may also indicate possible poisoning by certain poisonous substances. For example, a yellow color indicates possible poisoning with chromates, nitrogen acid, some aniline dyes, picric acid, quinine. Biological fluids (blood, urine) may also have an unusual color when poisoned by certain toxic substances. For example, when phenol enters the body urine is usually olive or green in color due to phenol oxidation products.

1. **Preliminary tests with the object**

Preliminary tests are aimed at reducing the time of object research, which is especially important for non-directional analysis. Such tests help narrow down the substances in the final test and determine the direction of its main investigation. Usually, group reactions are selected for preliminary tests, having high sensitivity. With their help, you can detect not only toxic, but also therapeutic doses of substances taken, and sometimes naturally contained in the connection object.

A positive result of preliminary tests indicates that one substance or a group of substances can be found in the test object that give the same reactions. In this case, the analysis plan includes the main study on this group of compounds using special techniques, methods and confirmatory reactions.

A negative result of preliminary tests indicates the absence of the corresponding substances in the test object, and this substance (or group of substances) excluded from the analysis plan, after the end of the examination a conclusion is made about it (or their) non-detection.

* **Preliminary tests with stomach contents**

***Determination of the pH*** of the medium is carried out in the contents of the stomach and is important for a preliminary decision on the issue of substances that could be the cause of poisoning. For this purpose, indicator papers impregnated with phenolphthalein, litmus, Congo red and a universal indicator are used. Small amount of object crush, add purified water and shake. In the resulting aqueous extract determine the reaction of the medium by applying it with a glass rod to a strip of indicator paper.

* **Preliminary tests of urine for some toxic substances**

Foreign compounds are excreted from the human body with urine, both in the form of metabolites and in an unchanged state. The following tests may be performed:

* Test for ethyl and methyl alcohols.
* Alkylhalide test
* Acetone test
* Test for barbiturates
* Test for phenothiazine derivatives
* Salicylic acid test
* Test for pyrazole derivatives
* Quinine test
* Test for chlordiazepoxide
* Test for amphetamine

If a positive result of a preliminary test with an object is obtained, the detected substance is included in the main study plan.

If a negative result of a preliminary test with an object for a certain substance (or group of substances) is received, the main study is not carried out, and a conclusion is made that this substance or group of substances has not been detected in the object.

**Analytical screening**

**Analytical screening** is a system of methodological techniques that allows during research operations, exclude (“weed out”) or identify groups substances (individual compounds) at the stage of preliminary research.

Analytical screening is effective if it meets certain requirements:

* specificity (usually group);
* high sensitivity;
* expressiveness;
* accuracy and reproducibility;
* possibility of combination with other methods of analysis.

**Screening** is a step-by-step movement towards identifying an individual substance by sequentially eliminating groups of toxic substances and then sifting out substances in a detected group until a specific compound is identified.

Modern physicochemical methods of analysis include:

1. Chromatographic;
2. Spectroscopic;
3. Immunochemical

When using these methods, it is necessary to carefully clean the extracts from endogenous compounds that can distort the analysis results and clog chromatograph columns.

Chromatographic methods:

1. *TLC (Thin Layer Chromatography)* screening in normal phase version.

The method is used in chemical-toxicological analysis when studying substances

isolated from the object by extraction and sorbtion (medicines, narcotic substances, pesticides).

1. *TLC screening in the “Toxi-Lab AB” version.* In this version, the classical method of thin layer chromatography is modified. This a specially developed analytical system that is designed to study one sample of an object (urine) for the presence of narcotic drugs, psychotropic and potent substances. This system provides the sample preparation stage, extraction, concentration, separation of substances and their detection.

* “Toxi-Lab A” is used to detect basic, neutral substances (opiates, methadone, amphetamines, etc.);
* “Toxi-Lab B” is used to detect acidic and neutral substances character (barbiturates, organic acids, etc.);
* "Toxi-Lab Cannabinoid" is used to detect THC and its derivatives

1. *Reverse phase TLC screening (RPTLC)*
2. *In-group TLC screening in private systems solvents*
3. *Gas-liquid chromatography (GLC)* - the main condition for using this method is volatility connections at evaporator temperature. The basis for identifying substances using GLC is a comparison of the retention index of an unknown substance with the retention index of a known compound.

Immunochemical methods:

Are based on the interaction of specific protein antibodies (antisera) with the analyte acting as antigen (hapten). The greater the concentration of the antigen substance in the object, the greater an antigen-antibody complex is formed. To detect the result obtained, one of the reaction components - a hapten or antibody - is labeled with a special label.

1. *Homogeneous enzyme immunoassay (ELISA)*

In homogeneous ELISA, oxidases are used as labels. Among them, lysozyme, glucose-6-phosphate dehydrogenase, and malate dehydrogenase are most often used. These enzymes are capable of oxidizing a chromogenic substrate (a special chemical such as chloronaphthol) to form a colored compound.

1. *Polarization fluoroimmunoassay (PFIA)*

This method is used to analyze the level of drugs and narcotic substances in biological fluids. As a labeled drug, a molecule of a suspected substance with a group attached to it is used, giving the labeled molecule ability to fluoresce. Fluorescein is used as a label. The method is based on a shift in the angle of the emitted beam of light from a fluorescent sample upon irradiation its monochromatic polarized light.

1. *Heterogeneous immunoassay*

The following enzymes are used as labels in this version of the immunochemical method: peroxidase, β-galactosidase, phosphatase, etc. The method is highly sensitive and allows the determination of substances at a concentration of 10-6-10-8 g/l. The analysis time is 2-4 hours. Diagnostic kits are produced for the detection of opium alkaloids, derivatives of barbituric acid, ephedrone, cannabinoids, etc.

1. *Radioimmune method*

This method uses a drug (or a similar modification thereof) labeled with a radioactive isotope. A radiolabeled drug (R) is added to the specific antibody (A), and then the test

solution (urine, blood plasma) containing the test substance (S), which displaces the labeled drug from the complex.

The method is given value if the result is negative. If the analysis gives a positive result, the data is confirmed by other methods and reactions.

Analytical screening using chemical reactions

At the first stage of analysis, it is necessary to select reactions that make it possible to establish or exclude the presence of certain groups of chemical compounds or individual substances. At this stage of chemical toxicological analysis, the most sensitive reactions are used. As a rule, these reactions are nonspecific for individual substances. In the absence of analytical effect, the entire group or specific substance is excluded from the analysis.