STUDY OF MEDICINAL POISONS IN FORENSIC CHEMISTRY

Elena Welchinska

Bogomolet's National Medical University of Ministry of Health of Ukraine, 13 T. Shevchenko Boul., Kyiv, 01601, Ukraine

Abstract

Forensic chemistry examines medicinal products from the point of view of their toxicity and degree of poisoning. Medicinal products in therapeutic doses are drugs, and in toxic or lethal doses - these are poisons. The most common causes of poisoning in Europe and America are: among children - domestic poisoning and poisoning with pharmaceuticals; among adolescents - poisoning due to the abuse of lethal substances; among adults - poisoning with analgesics, psychotropic drugs, barbiturates and hypnotic non-barbiturate type. Pharmaceutical counterfeits are often found in pharmacies, which are often counterfeit pharmaceuticals from well-known pharmaceutical companies. Forensic chemistry plays an important role in the diagnosis of poisoning, in the fight against crime.

Keywords: *forensic chemistry, poisons, alkaloids, medicines, analysis, toxicity, chemical-toxicological analysis*

1. INTRODUCTION

According to WHO, counterfeit medicines were found in 28 countries. Of the 951 cases, 25% of counterfeit production was attributed to industrialized countries, 65% - to developing countries, and 10% - to unknown sources. 59% of these drugs did not contain any active substance, 17% - the content of the active ingredient did not meet the requirements of analytical normative documentation, 16% of these medicines contained as active, completely different substances than those indicated on the packaging and in the documentation. In 7% of the active ingredient content was normal, but packaging, marking of these medicines did not meet the requirements and differed from the packaging of these original medicines. The use of such medicines can cause serious negative consequences for human health, as counterfeit medicines do not pass the control for legal medicines in its production and implementation (Meshkovskyi, 2002; Ushakova, 2004). Fundamentally new scientific concepts of the development of original medicines, modifications of known medicines, and the synthesis of new biologically active substances lead to the improvement of methods of chemical-toxicological analysis, as drugs are increasingly becoming the cause of poisoning and even death of patients. Welchinska (2017, pp. 3-10), Welchinska (2003, pp. 20-25), Efimov and Bora (1995), Aleksandrov and Emelianov (1990) claimed it remains relevant to create new and improve existing methods of express-analysis, necessary for the identification of toxic substances not only in laboratory conditions, but also at the scene, in the field. In 2011, Ukraine signed the Convention of Council of Europe (MEDICRIME - The Medicrime Convention Combating counterfeiting of medical products and similar crimes) about cases of counterfeiting medical products and similar atrocities (Regional conference on the MEDICRIME Convention 2013, Parliamentary conference on the MEDICRIME Convention 2015). This step sets new tasks for the country: strengthening the work of regulators to combat counterfeit pharmaceutical products, unifying requirements for the quality and conditions of drug supply, since counterfeit drugs or drugs stored in improper conditions can cause poisoning and death of patients. All these listed tasks help to solve the forensic chemistry, and especially, its part - chemical-toxicological analysis. Chemical-toxicological analysis is a set of scientifically grounded methods that are used in practice to isolate, detect and quantify toxic substances, including "medicinal" poisons. According to forensic chemistry classification "medicinal" poisons – are substances that are isolated by polar solvents. There are main four groups of "medicinal" poisons: «medicinal» poisons of acidic, neutral and weak alkalinous nature, «medicinal» poisons — alkaloids, natural toxins, synthetic «medicinal» poisons of alkalinous nature. The most important representatives of the alkaloid group are: derivatives of pyridine and piperidine, derivatives of tropane, derivatives of quinoline, derivatives of

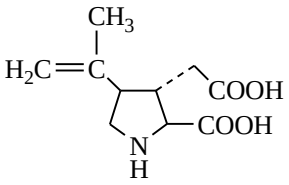
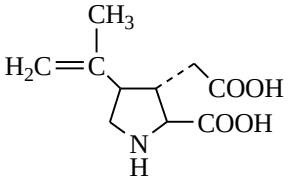
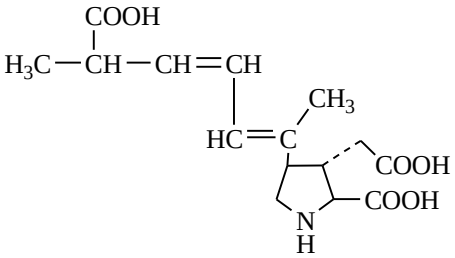
quinolizidine, derivatives of isoquinoline, derivatives of γ-pyrone, derivatives of pyrrolidine (rare sea origin alkaloids), derivatives of indole, derivatives of purine (xanthine), acyclic (aromatic) alkaloids, alicyclic compounds (cycloalkanes) – cardiac glycosides, derivatives of cyclic monoterpenes (substituted lymonenes), derivatives of imidazole. Many plants are a source of raw materials for the production of alkaloids, and alkaloids, in turn, are used not only as reagents in chemical analysis and synthesis, but also as components of medicinal preparations. In addition, many of them are toxic substances and poisons. Therefore, it is so important to know by what methods it is possible to confirm the presence of alkaloids in the material of a person in order to provide urgent medication, to establish the cause of poisoning or death.

2. MATERIALS AND METHODS

2.1. Materials

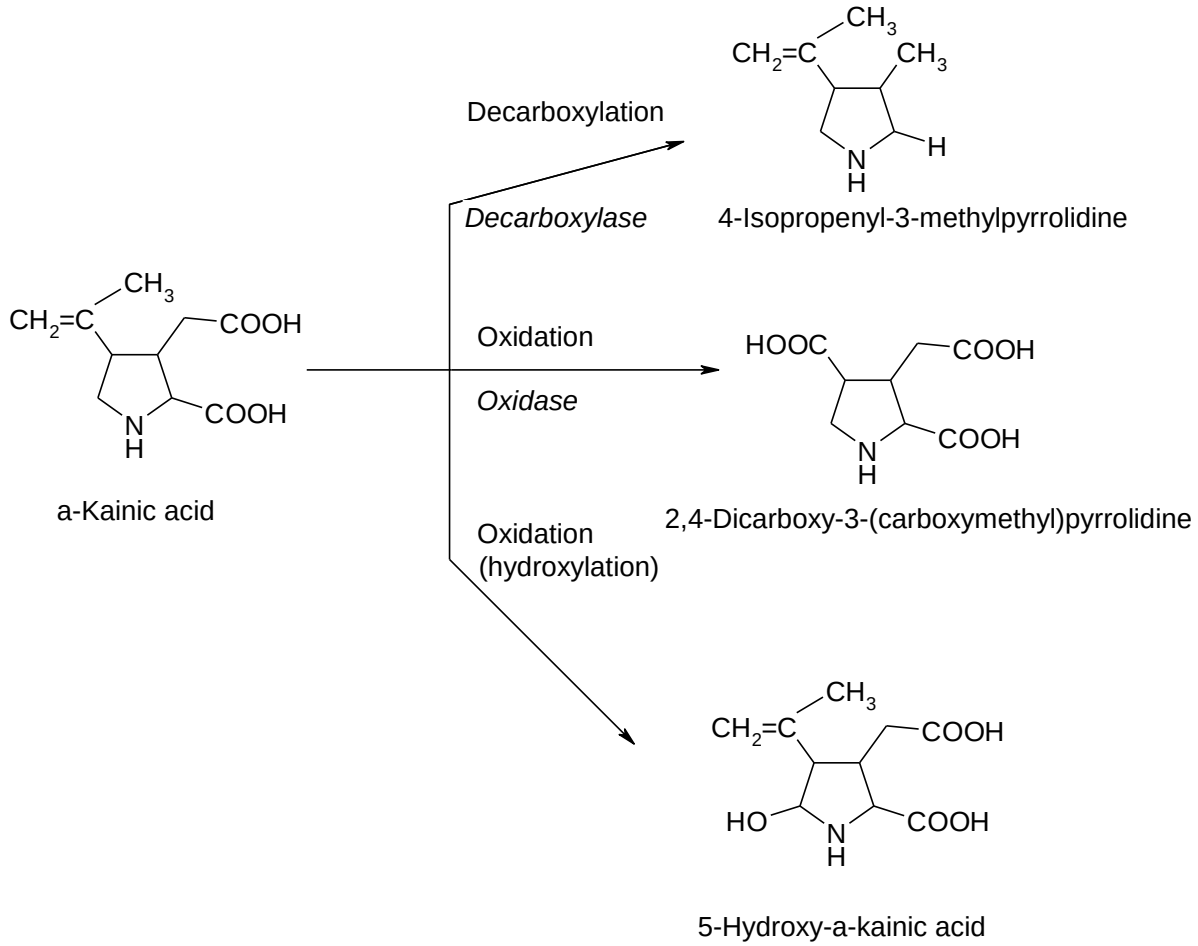
One of the least studied groups of alkaloids is the group of alkaloids of marine origin - pyrrolidine derivatives. Rare alkaloids of marine origin in recent times have been isolated from the red alga *Degenea simplex*. It includes derivatives of pyrrolidine: α-kainic, α-alo-kainic and domoic acids. α-Kainic acid differs from the α-alo-kainic acid space arrangement of isopropenyl group. Domoic acid, unlike the first two, is a tricarboxylic acid. Chemical formulas of derivatives of pyrrolidine are shown in Table 1.

Table 1. Chemical formulas of pyrrolidine derivatives.

Pyrrolidine derivatives	Chemical formula
α-Kainic acid, 4-isopropenyl-2-carboxy-3-(carboxymethyl)pyrrolidine	
α-alo-Kainic acid, 4-isopropenyl-2-carboxy-3-(carboxymethyl)pyrrolidine, space isomer of α-kainic acid	
Domoic acid, 2-carboxy-3-carboxymethyl-4-(1-methyl-5-carboxy-1,3-hexadienyl)pyrrolidine	

These alkaloids have found their use as effective antihelminthic agents. Antihelminthics or antihelminthics are a group of antiparasitic drugs that expel parasitic worms (helminthes) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. Domoic acid is responsible for amnestic shellfish poisoning. It is a structural analogue of glutamic acid and kainic acid and, therefore, will act as an excitatory neurotransmitter. If poisoning alkaloids in this group there are nausea, vomiting, severe sweating, agitation, muscle aches and stomach, tremors, tachycardia, convulsions. The clinical manifestations of domoic acid poisoning

are nausea, vomiting, abdominal cramps, diarrhea, headache, ophthalmoplegia, grimacing, bronchorrhea, seizures, coma, and death (Holstege et al. 2009). Data on the metabolism of this group of compounds in the literature are absence. According to the specific chemical structure these compounds will metabolized by next ways: 1) oxidation to form the corresponding hydroxy derivatives; 2) decarboxylation. The general ways of metabolism of these alkaloids (as example – a-kainic acid) are illustrated in Scheme I.

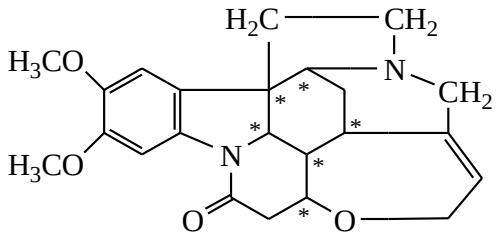
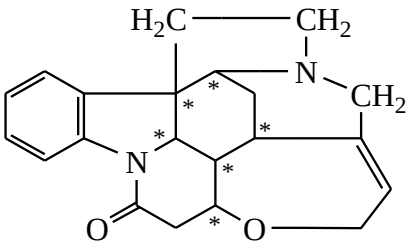


Scheme I: Metabolism of alkaloids – pyrrolidine derivatives

Source: Author

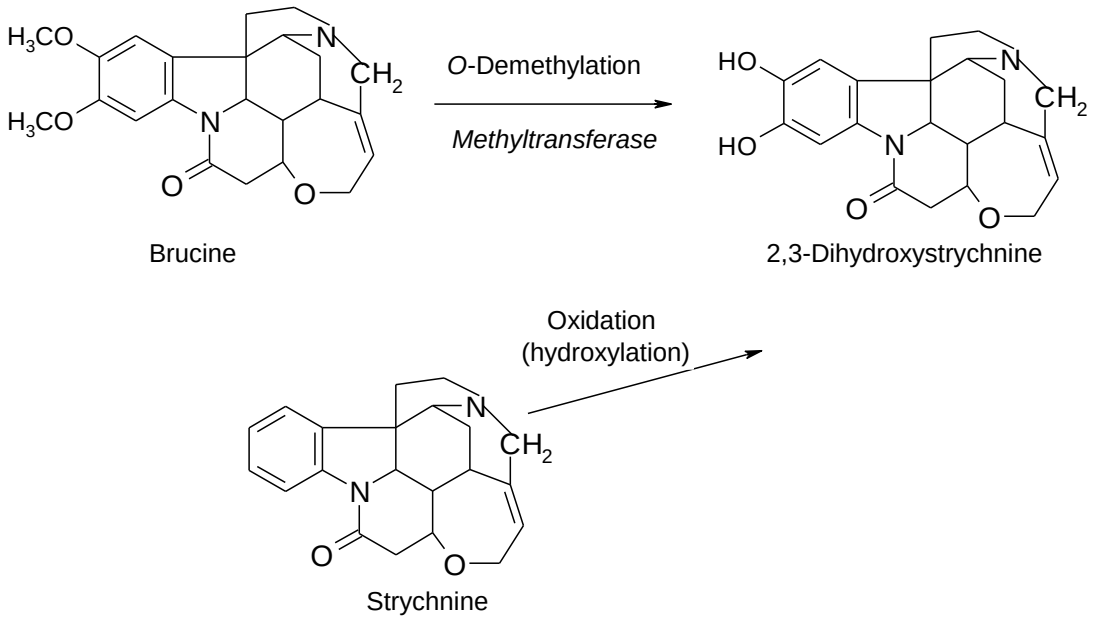
Of great interest is the group of alkaloids, which are derivatives of indole and contain a (benzo[*b*]pyrrole) cycle in the molecule. Brucine and strychnine are indole derivatives (indole alkaloids with heptacyclic structure). Strychnine in high doses is highly toxic. Upon receipt of the toxic dose of strychnine in the body will soon show signs of poisoning with this alkaloid. Strychnine blocks glycine and as result — spastic paralysis with convulsions and paralysis of the respiratory muscles, and death. There is a characteristic curve of the body, arch back, and his face appears a famous *risus sardonicus* — «sardonic bare one's teeth». Brucine not used in medicine. It is used in analytical chemistry for the determination of nitrates, in toxicological analysis for the determination of toxic substances. Determination of brucine in extracts from the organs of the corpse indicates that the poisoning was due to the ingestion of drugs (tinctures, infusions) from plant material, which contains strychnine and brucine simultaneously. Chemical formulas of indole derivatives are shown in Table 2.

Table 2. Chemical formulas of indole derivatives.

Indole derivatives	Chemical formula
Brucine	
Strychnine	

* — asymmetric Carbon atoms

When poisoning with strychnine there are frequent seizures (convulsions), which in many cases end in death (asphyxia). The cramps easy to occur under the influence of various irritations (sound, touching the body of the victim, etc.). Strychnine is very dangerous for people with heart disease, liver disease, kidney disease, as well as for children. The lethal dose of strychnine: 15-30 mg (for children), 50-100 mg (for adults). Brucine is about 20 times less toxic than strychnine. Strychnine is rapidly absorbed into the bloodstream from the digestive tract, can easily penetrate into the bloodstream through the mucous membranes and intact skin. About 80% of the dose of strychnine is metabolized in the liver; the remainder is excreted in the urine in unchanged form. The accumulation action of unchanged strychnine is a result of slow release from the body of this alkaloid. Strychnine metabolites have not been identified, but believe that it molecule is oxidized at positions 2 and 3 of the aromatic ring to form 2, 3-dihydroxystrychnine. Metabolite of the same structure described for brucine. Strychnine is persistent in human cadaver. It can be defined in the corpses exhumed several years after the death. Brucine is metabolized to produce metabolites — hydroxy compounds of strychnine. Brucine excreted in the urine in unchanged form (in smaller quantity). The general ways of metabolism of the indole derivatives are illustrated in Scheme II.



Scheme II: Metabolism of alkaloids – indole derivatives

Source: Author

2.2. Methods

Peculiarity of the alkaloids isolation is extraction from the water acidic and alkaline solutions.

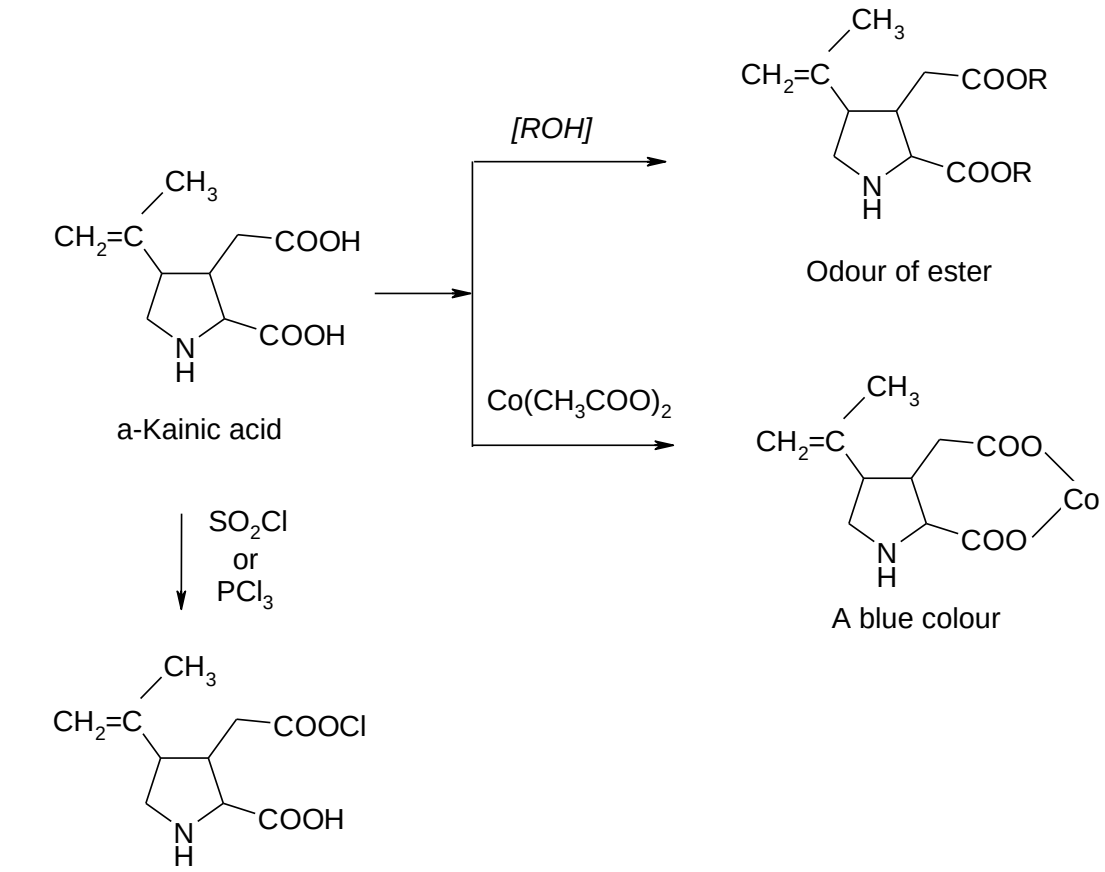
α-Kainic, α-alo-kainic and domoic acids are isolated from the studied objects by the method of organic solvents extraction from the acidified aqueous solution, in small quantities they are isolated from weakly alkaline aqueous solutions. Strychnine and brucine are isolated from the studied objects by the method of organic solvents extraction from water acidic and alkaline solutions (more effective).

2.2.1. Qualitative and quantitative determination of pyrrolidine derivatives

For the qualitative determination of these alkaloids use the following types of reactions:

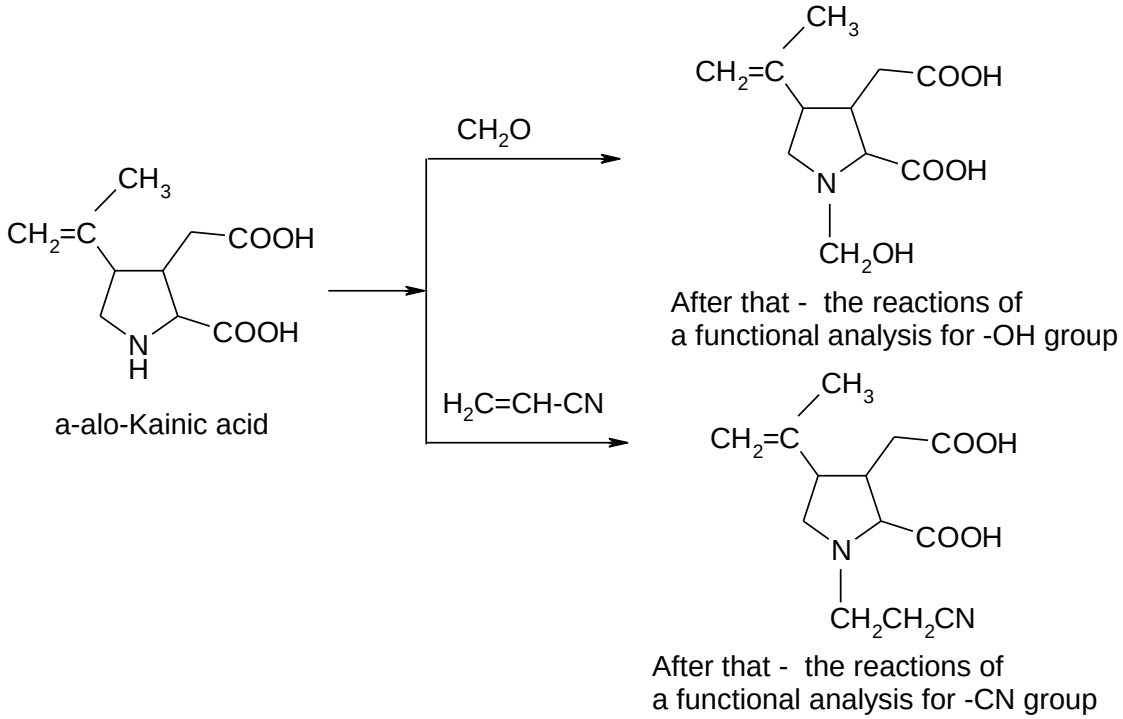
- *the precipitation reactions* with general reagents of group precipitation: with Dragendorff's reagent; with Nessler's reagent (of tertiary Nitrogen atom);
- *the reactions of carboxylic group:* esterification reactions, salts formation, halogen anhydrides formation;
- *the reactions of heterocyclic part of the molecular:* with formaldehyde, with acrylonitrile.

The reactions of qualitative analysis of the pyrrolidine derivatives (as example – a-kainic acid, α-alo-kainic acid) are illustrated in Schemes III, IV.



Scheme III: The reactions of qualitative analysis of the pyrrolidine derivatives – the reactions of carboxyl groups

Source: Author



Scheme IV: The reactions of qualitative analysis of the pyrrolidine derivatives – the reactions of heterocyclic part of the molecular

Source: Author

Quantitative determination of alkaloids may be carried out using instrumental methods. Quilliam *et al.* (1998) analyzed domoic acid using TLC. Tissues were extracted and concentrated using a strong ion-exchange resin. Silica-gel TLC plates were used and developed with a butanol-acetic acid-water mixture (3:1:1, Rf = 0.45 for domoic acid). Lawrence *et al. (*1994) analyzed domoic acid using reversed-phase HPLC method with an existing radioimmunoassay. James *et al.* (2000) described fluorimetric HPLC method for determination of domoic acid in seafood and phytoplankton. Zhao *et al.* (1997) described capillary electrophoresis with UV absorbance detection of the analysis of domoic acid. Besides that spectral methods used for analysis of pyrrolidine derivatives: UV-spectrum: 209, 230—270 nm.

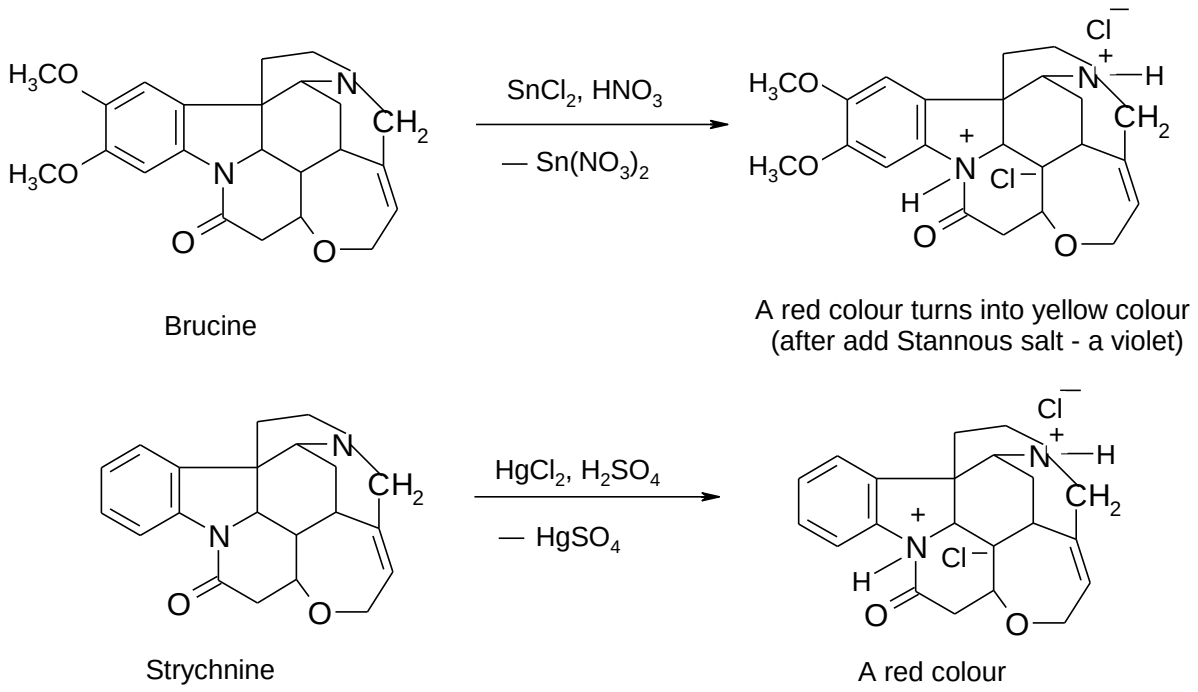
2.2.2. Qualitative and quantitative determination of indole derivatives

For the qualitative determination of these alkaloids use the following types of reactions:

- *colour tests* : with Mandelin's reagent (strychnine, brucine), with Erdman's reagent (brucine), with Frede's reagent (brucine), with $SnCl_2$ and HNO_3 (brucine), with $K_2Cr_2O_7$ and H_2SO_4 (strychnine), with $HgCl_2$ and H_2SO_4 (strychnine), Vitali—Moren's reaction (strychnine) (results of the reactions are shown in Table 3 and illustrated in Scheme V).

Table 3. Results of colour tests for indole alkaloids.

Reaction	Strychnine	Brucine
With Mandelin's reagent	A blue-violet → red A violet (for strychnine sulphate)	A red → yellow
With Erdman's reagent	—	A red → yellow
With Frede's reagent	—	A red → yellow
With $SnCl_2$, HNO_3	—	A red → yellow, after adding of $SnCl_2$ — a violet
With $K_2Cr_2O_7$, H_2SO_4	A violet → wine-red (for strychnine nitrate)	—
With $HgCl_2$, H_2SO_4	A red	—
Vitali—Moren's reaction	A red	—



Scheme V: The reactions of qualitative analysis of the indole derivatives – colour tests

Source: Author

Beyer *et al* (2009), Moffat *et al.* (2011) demonstrated instrumental methods of the quantitative determination of these alkaloids. Spectral methods used for analysis of indole derivatives: spectral data are shown in Table 4.

Table 4. Spectral data of indole derivatives.

Indole derivatives	UV-spectrum	IR-spectrum
Brucine	267, 301 nm (in C_2H_5OH) 265, 300 nm (in 0.1 *N* H_2SO_4 solution)	1190, 1285, 1450, 1500, 1649 cm^{-1}
Strychnine Strychnine sulphate	255 nm (in C_2H_5OH or 0.1 *N* H_2SO_4 solution) 254 nm (aqueous acid) 255, 278 nm (aqueous alkali)	1664, 764, 1392, 1480 cm^{-1} 1664, 764, 1050, 1110, 1282, 775 cm^{-1}

Strychnine sulphate may be detected by:

- *mass spectrum* (principal ions at *m/z* 334, 335, 162, 120, 107, 144, 143, 130), *TLC* (system TA–R_f 0.26; system TB–R_f 0.08; system TC–R_f 0.19; system TE–R_f 0.32; system TL–R_f 0.02; system TAE–R_f 0.08; system TAF–R_f 0.11; system TAJ–R_f 0.04; system TAK–R_f 0.02; system TAL–R_f 0.44 (acidified iodoplatinate solution – positive; Dragendorff spray – positive);
- *GC* (system GA-RI 3116);
- *HPLC* (system HA–*k* 13.0 (tailing peak); system HS–*k* 2.43; system HX–RI 302; system HY–RI 257; system HZ–RT 2.2 min; system HAA–RT 9.2 min; system HAX–RT 7.5 min; system HAY–RT 3.9 min;
- *GC – MS;*
- *LC – MS.*

3. RESULTS

The range of qualitative determination reactions and instrumental methods used in pharmaceutical analysis and chemical-toxicological analysis is extensive. Using the above methods of determination, we can confirm the high quality of medications, non-compliance with the requirements of the manufactured medicines. And finally, using well-known methods of analysis in the complex, you can confirm the poor quality of the medicine, if it caused the patient to become poisoned or died.

4. DISCUSSION

“Forensic chemistry" allows getting knowledge about medicinal preparations from the standpoint of their toxicity and the possibility of determining these compounds in the human body. The qualitative and quantitative analysis of medications when performing chemical-toxicological research is somewhat different from the pharmaceutical analysis. With the help of chemical-toxicological analysis, the determination can be not only the presence of medical poison in the body, but also determine its metabolites. The objects that forensic chemistry investigates are qualitatively and quantitatively different from the objects of research in pharmaceutical analysis. If in pharmaceutical analysis medications in various medical forms and as substances are used as objects of research, the research objects of forensic chemistry are not only drugs, but also the biological material of a person who has been poisoned or died after ingestion of this drug. “Forensic chemistry” determines the presence of a medical product as a quality drug, and a falsified product, which very often becomes the cause of poisoning or death of a person.

5. CONCLUSIONS

"Forensic chemistry", especially, chemical-toxicological analysis is a promising direction of analysis not only of medical products, but also poisons of other origin. The nomenclature of chemicals increases annually and, therefore, the danger of the multi-vector contact of the human body with known and new substances increases. Therefore, it is imperative to develop methods for the qualitative and quantitative determination of toxic chemicals (including medical products, especially counterfeit ones) in order to ensure the obtaining of objective research results in extremely short periods of time and to provide medical assistance to the injured person.

REFERENCES

1. Meshkovskyi, AP 2002, "Realization of plan of actions in the fight against the counterfeit medications", *Pharmatheka,* no. 11, pp. 75–79. (Russian).
2. Ushakova, ED 2004, "Problems of counterfeit medications", *Pharmatheka*, no. 7, pp. 70–79. (Russian).
3. Welchinska, EV 2017, *Toxicological and forensic chemistry (criminal analysis). Poisonous substances and their biotransformation*. ADEF-Ukraine, Kiyv.
4. Welchinska, HeV, Piecuszak, B, Kovalenko, EA & Sharykina, NI (2003), "Biological activity of bacterial lectins and their molecular complexes with heterocyclic bis-adducts", *Microbiol. J.*, vol.65, no. 6, pp. 20-25.
5. Efimov, LK & Bora, VM 1995, *Medicinal poisonings of children*, Zdorovie, Kiyv. (Russian).
6. Aleksandrov, VN & Emelianov, VI 1990, *Poisonous substances*, Voenizdat, Moscow. (Russian).
7. Regional conference "Towards the practical implementation of the council of Europe convention on counterfeiting of medical products and similar crimes involving threats to public health (MEDICRIME Convention), 11-12 June 2013, Kyiv, Ukraine.
8. Parliamentary conference on the MEDICRIME Convention, 24 November 2015, OECD Conference Center, Paris, France.
9. Holstege, Ch, Borloz, M, Lawrence, D & Charlton, N 2009, *Toxicology recall*. Wolters Kluwer Health: Lippincott Williams & Wilkins.
10. Quilliam, MA *et al*. 1998, "Analysis of domoic acid in shellfish by thin-layer chromatography", *Nat. Toxins*, no.6, pp.147-152.
11. Lawrence, JF *et al*. 1994, "Comparison of high-performance liquid chromatography with radioimmunoassay for the determination of domoic acid in biological samples", *J. Chromatogr. A.*, no. 662, pp. 173-177.
12. James, KJ *et al.*, 2000, "New fluorimetric method of liquid chromatography for the determination of the neurotoxin domoic acid in seafood and marine phytoplankton", *J. Chromatogr. A.*, no. 871, pp. 1-6.
13. Zhao, JY *et al.*, 1997, "Analysis of domoic acid and isomers in seafood by capillary electrophoresis", *Electrophoresis*, no. 18, pp. 268-276.
14. Beyer, J *et al.*, 2009, "Analysis of toxic alkaloids in body samples", *Forensic Sci. Int.*, no.185, pp. 1-9.
15. Moffat, AC, Osselton, MD & Widdop, B (eds) 2011, *Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material*, 4th edn, Pharmaceutical Press, London, UK; Gurnee, USA.