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THE USE OF FORENSIC CHEMISTRY METHODS IN THE DETERMINATION OF COUNTERFEIT DRUGS

Elena Welchinska¹, Volodymyr Velchynskyi²

Abstract: The problem of illegal circulation of drugs, their support and falsification are relevant not only in Ukraine. Experts believe that about 10% of the global medicinal drug market is fake and fraud. Counterfeit drugs may contain the active ingredient with its exact content as well as non-relevant ingredients, may not contain the active ingredient at all or may contain unrefined ingredients. In any case, this leads to a lack of therapeutic effect in the treatment, poisoning and, even, death of patients. As a result of our research, the presence of highly dangerous and toxic components in the composition of drugs (antifreeze, tar, arsenic, mercury and others) was established. In case of poisoning or death of a patient after the use of a counterfeit medicinal drug, an examination is carried out, and the presence of the active and additional ingredients of the drug is confirmed by chemical toxicological analysis. Chemical-toxicological analysis is a part of the science "Forensic chemistry" and allows us to confirm the presence of the drug in the body, the purity of the drug, the presence of the active ingredient of the drug, and therefore allows us to confirm the fact of poisoning or death of a person under the action of a chemical substance (for example, a falsified drug).

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Keywords: forensic chemistry, salicylic acid, toxicity, chemical-toxicological analysis, poison.

Introduction

Counterfeiting and falsification of medicines in Ukraine has recently reached a large scale (Spivak, 2013). According to sources, at least a third of the drugs in Ukraine are fake and counterfeit (Ovcharenko, 2017). According to the WHO definition, counterfeit medicinal drugs are drugs that are deliberately unsatisfied with respect to their identity and the name of the manufacturer. Both original medications and generics can be falsified. Often, such preparations may contain an inappropriate composition, may not contain the active ingredient or contain an active ingredient in insufficient quantities. Packaging for medicinal drugs is also often faked. The manufactures of counterfeits often use cheap, overdue, poorly purified substances. In the manufacture of counterfeit drugs, the production technology is broken and the requirements of regulatory documents for the quality of the drug are not met. There are four main ways of falsifying pharmaceutical products: non-active medicines bimbos (empty medicines, not containing the active substance), medicines-imitators, modified medicines, medicines-copies. In Ukraine, medicines are most frequently falsified, using indifferent substances instead of active ingredients in the process of their manufacture (Lisenko, 2013). Thus, the main biologically active substances, which provide a therapeutic effect, are completely absent in the composition of drugs. Chalk, talc, and starch are used as indifferent substances — they are affordable and cheap. The price of the drug, which is paid by the patient, includes the cost of all of these active and non-active components of this drug. Thus, manufacturers of counterfeit drugs not only damage people's health, but also deceive them, forcing them to pay for drugs that do not meet quality and standards. One of the terrible methods of falsification of drugs is the imitation of the active substance, namely, replacing it with a cheaper and non-standard substance. Most often, expensive imported and most advertised drugs ("Omez", "Festal") are falsified, among them antibiotics (42%), hormonal drugs (20%), cardiac drugs, and others. All these listed facts confirm that counterfeit medicines are hazardous to health and pose a threat to the lives of patients.

The role of forensic chemistry in the analysis of counterfeit drugs

In case of poisoning or death of a patient as a result of the use of a qualitative (overdose) or falsified medicinal product, it is necessary to conduct an examination that confirms or denies the availability of the drug and the degree of its quality. The presence of the active and additional ingredients of the drug is confirmed by chemical toxicological analysis. Chemical-toxicological analysis is a part of the science "Forensic chemistry" and allows us to confirm the presence of the drug in the body, the purity of the drug, the presence of the active ingredient of the drug, and therefore allows us to confirm the fact of poisoning or death of a person under the action of a chemical substance (for example, a falsified drug).

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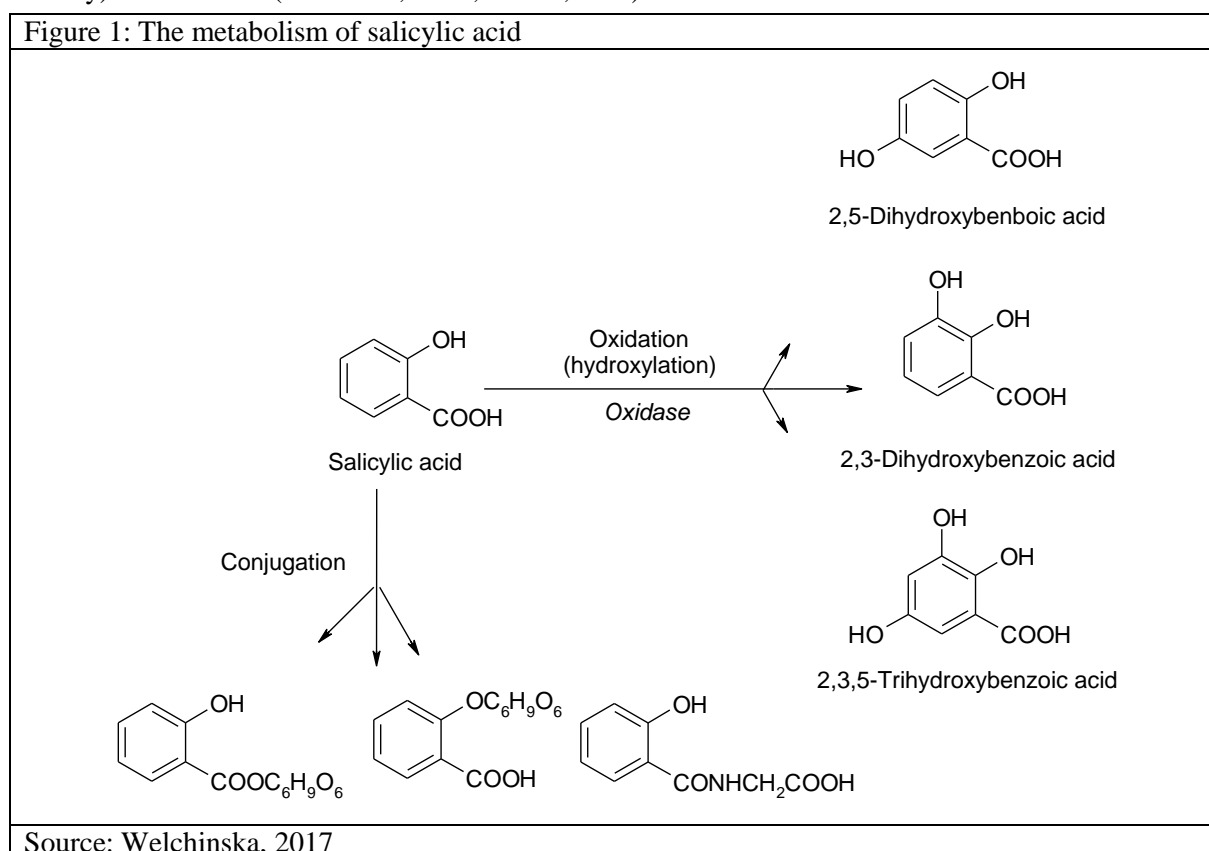
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As Bayerman (1987) and Welchinska (2017) write, to date, "Forensic chemistry" show cases some of the most specific chemical methods for the detection of medicinal products in the body. "Forensic chemistry" has methods for the qualitative and quantitative determination of drugs in various biological objects: urine, blood plasma, liver, kidneys, brain tissue and other organs of the body. These methods are required to be known not only by criminologists and chemists-toxicologists, but also by pharmacists.

Only pharmacists are working directly with drugs, both in pharmacies and in laboratories for quality control of medicinal drugs. Therefore, the discipline "Forensic chemistry" is studied at the pharmaceutical faculties of the medical universities and at the pharmaceutical universities of Ukraine (Moskalenko, 2011).

One of the most popular drugs among patients is Aspirin. This drug is produced by many foreign manufacturers, it is widely advertised. This drug is in the "risk" group — a group of possible falsified drugs. In addition, the active ingredient of this drug — acetylsalicylic acid may undergo hydrolysis in violation of the rules of storage of the drug. As a result, the patient will buy a drug containing the product of the hydrolysis of acetylsalicylic acid — salicylic acid. Salicylic acid is used to treat skin diseases for disinfecting and hyperhidrosis purposes. Salicylic acid in small amounts can be found in berries (cherry, raspberry, strawberry), in tomatoes, in grape must; in the household and in industries can be used as a preservative for wine production, for vegetable canned food, for juice and jam. There are possible side effects during use of therapeutic doses of salicylic acid derivatives — tinnitus, hearing loss, swelling, heartburn, vomiting. In case of *chronic poisoning*, there may occur nonspecific symptoms — hallucinations, metabolic acidosis, dehydration, pulmonary or brain edema that lead to death. *Toxic doses* cause exacerbation of asthma. In case of *acute intoxication*, there appear vomiting, digestive tract bleeding, hyperpnoea, tinnitus, then — alkalosis and metabolic acidosis, convulsions and pulmonary edema. Death occurs as a result of cardiac and respiratory failure, damage of the central nervous system. Poisoning symptoms may occur in the first 1–2 hours after the acute poisoning. Commonly, symptoms severity reaches its maximum after 12–24 hours following the drug intake. Currently there are no antidotes to treat salicylic acid derivatives poisoning. Lethal dose of salicylic acid derivatives: 2–4 g — for children and about 15–20 g (300–500 mg/kg – severe toxicity) — for adults (Ellenhorn, 2003; Moffat, 2011).

Figure 1: The metabolism of salicylic acid



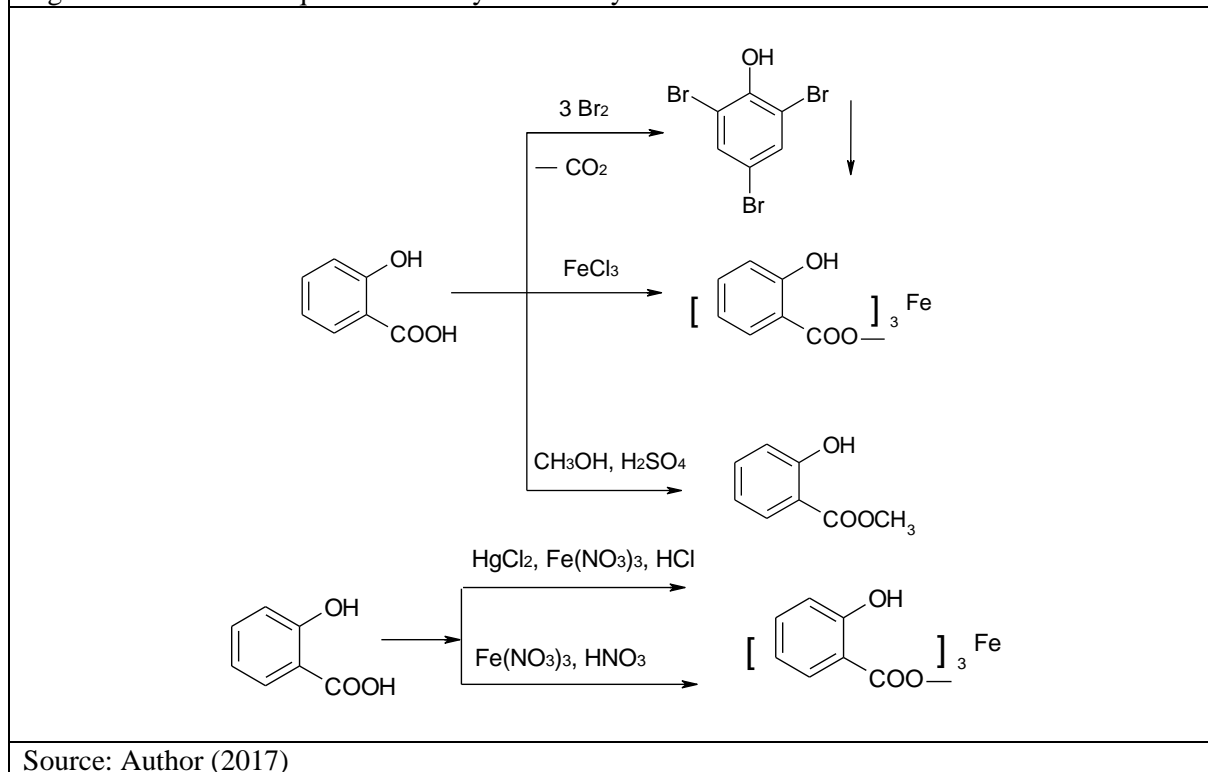
The main method for isolation of “medicinal” poisons is extraction. Stages of poisons isolation by extraction with polar solvents: preparation of sample for study: the crushing of corpse’s bodies with the help of scissors, grinder, homogenizer; weigh out and taking the sample (1—100 g depending on the content of toxic substance and sensitivity of the methods of study); 2—3 times extraction of poison by acidulated solvent or solvent without acidification; unification, filtration and centrifugation of the extract; extraction of poisons from acidic aqueous phase with organic solvents — getting “acidic” chloroform extraction; extraction of poisons from alkaline aqueous phase by organic solvents — getting “alkaline” chloroform extraction. Peculiarities of the isolation of salicylic acid derivatives from the objects is A.A. Vasileva’s method — water extraction with acidification containing oxalic acid. Methods of extract purification: straining; centrifugation. Qualitative analysis of salicylic acid is a group of chemical reactions: sedimentary reactions, colour reactions, reactions of formation of the products with specific smell, reactions in the blood and urine without salicylic acid isolation. Quantitative analysis of salicylic acid is carried out by instrumental methods: spectral methods, chromatography.

As Miners (1989) writes salicylic acid is rapidly absorbed and distributed. It is partially excreted with urine in the unchanged form. It is metabolized by conjugation with glucuronic acid and glycine. Salicylic acid and its derivatives are metabolized in the liver. Esters of salicylic acid are partially metabolized in the small intestine. Metabolism is carried out in three main phases: hydrolysis (of esters or amides of salicylic acid under action of esterase and amidase); oxidation (hydroxylation); formation of conjugates with glucuronic acid and glycine. Oxidation (hydroxylation) of salicylic acid under action of oxidase gives a 2, 3- (or 2, 5-) or 2, 3, 5- hydroxy derivatives of salicylic acid (Welchinska, 2017) (Figure 1).

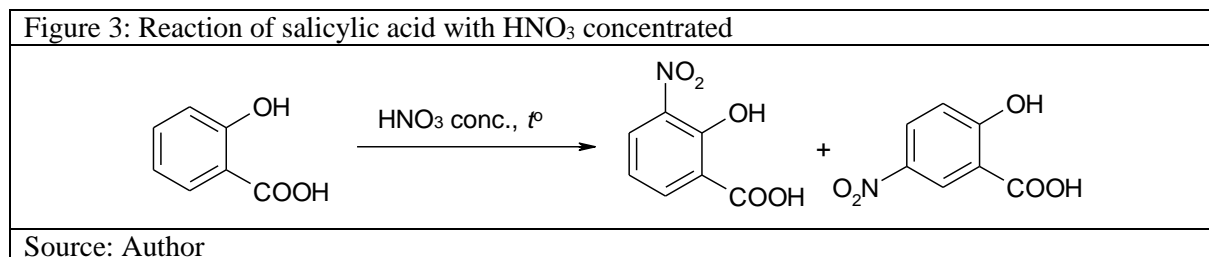
Reactions of qualitative analysis of salicylic acid and its derivatives are:

- *sedimentary reaction*: the reaction with bromine water — a white precipitate;
- *colour reaction*: the reaction with ferric (III) chloride — colour complexes (depends on pH):
 - 1.8-2.5 – a blue-violet colour, 4-8 – a red-brown colour, 8-11 – a yellow colour;
- *reaction of methyl salicylate formation*: the reaction with methanol in the concentrated sulfuric acid medium — a specific smell of methyl salicylate;
- *reactions of detection in blood and urine*:
 - with ferric (III) nitrate in urine — a purple colour;
 - with Trindler’s reagent in urine or blood plasma — a purple colour (Figure 2):

Figure 2: Reactions of qualitative analysis of salicylic acid and its derivatives

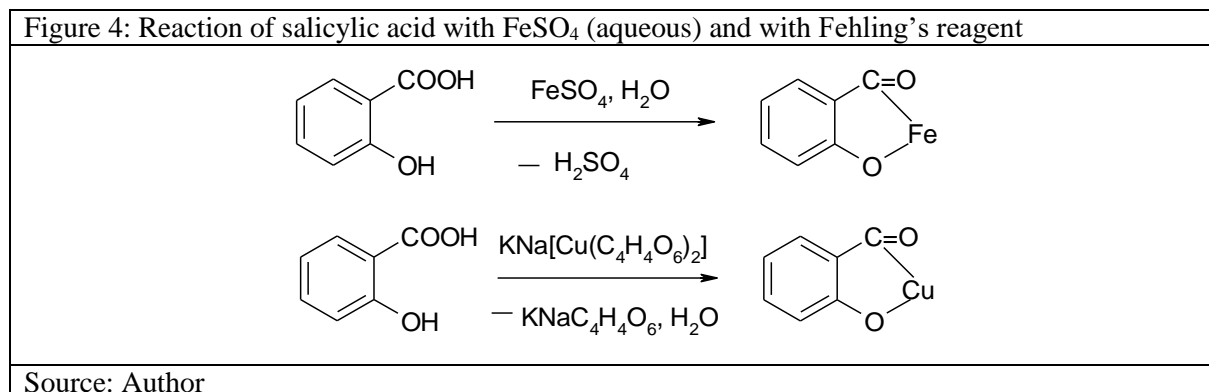


In the process of developing new methods for identifying salicylic acid (substance), we carried out the reaction of salicylic acid (0.5% alcohol solution in 70% ethanol and 0.25% aqueous solution) with HNO_3 conc., not previously described in the literature and Governmental pharmacopoeia of Ukraine (GFU) (GFU, 2004, 2008; Kramarenko, 1995). This reaction belongs to the group of colour reactions with mineral acids. When concentrated nitric acid is added to the salicylic acid solutions (0.5% alcohol solution in 70% ethanol and 0.25% aqueous solution), a yellow colour appears in both cases when heated. Since the final product was not isolated, it can be assumed that as a result of the electrophilic substitution reaction, a mixture of coloured *ortho*— and *para*— substitution products of salicylic acid are formed (Figure 3).



In order to expand the scope of the possibilities of identification of salicylic acid in the practice of pharmaceutical and chemical-toxicological analysis, we proposed new methods for the qualitative detection of the substance's salicylic acid (0.5% alcohol solution in 70% ethanol and 0.25% aqueous solution) with FeSO_4 (aqueous) and with Fehling's reagent, which had not previously been described in the literature and Governmental pharmacopoeia of Ukraine. These reactions belong to the category of salt formation reactions. The formation of a light violet colour was after adding of FeSO_4 (aqueous) to the salicylic acid solutions, which is deepened with the addition of excess of reagent without heating.

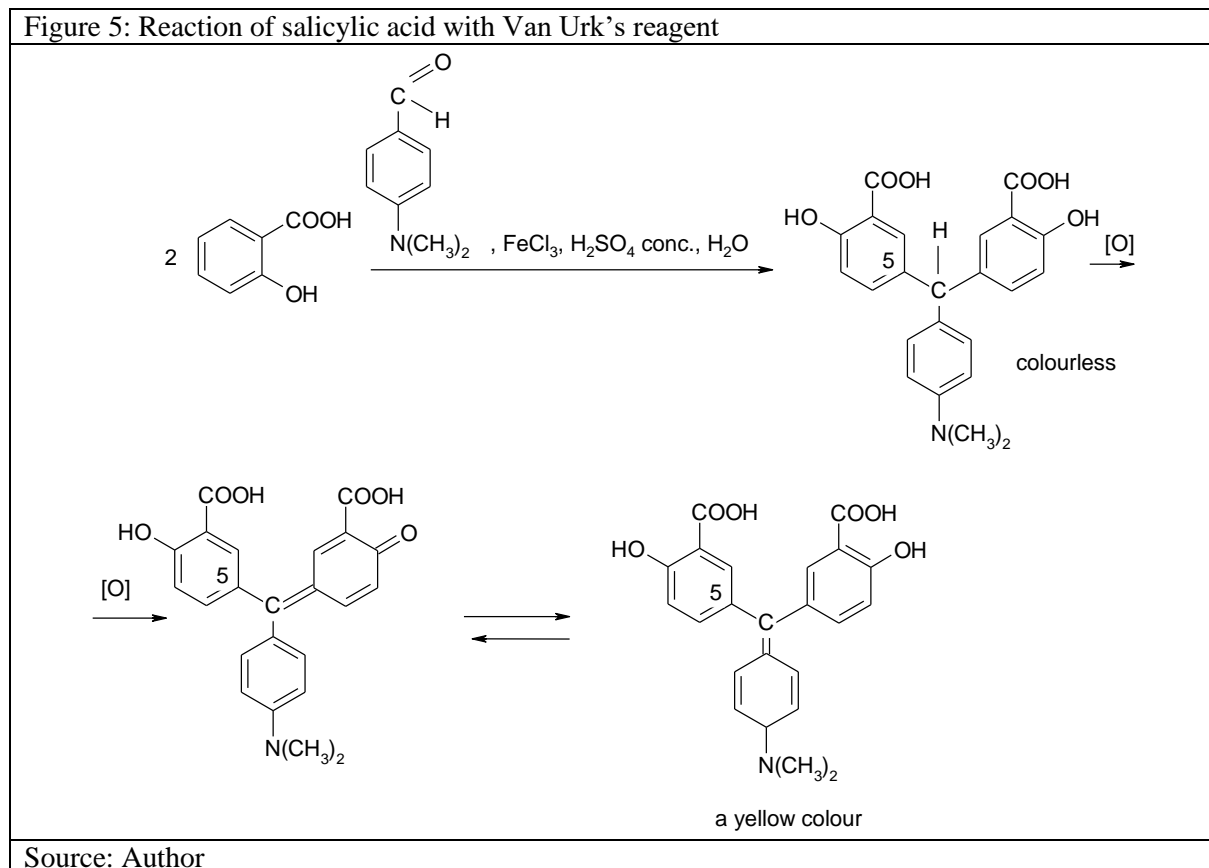
It is known that salicylate-ions react with heavy metal salts. A precipitate with green colour is formed in reaction of sodium salicylate with copper sulfate (Belikov, 1996). We carried out the salt formation reaction of salicylic acid (0.5% alcohol solution in 70% ethanol and 0.25% aqueous solution) with a Fehling's reagent. The appearance of a light green colour allows us to assume the formation of copper salicylate, and Fehling's reagent is considered as a carrier of Cuprum (Figure 4).



We propose a new qualitative reaction for the identification of salicylic acid (substance) with the Van Urk's reagent. This reaction is not described in the State Pharmacopoeia of Ukraine and in the literature on chemical-toxicological analysis, either. The Van Urk's reagent is prepared on the basis of *p*-dimethylaminobenzaldehyde, FeCl_3 , H_2SO_4 conc., H_2O . The reaction used salicylic acid (substance) 0.5% alcohol solution in 70% ethyl alcohol. When the reaction mixture is heated, a yellow color is observed (Figure 5).

The reaction with the Van Urk's reagent is used in pharmaceutical and chemical-toxicological analysis as a group reaction to derivatives of indole. Phenolic acids that have a free position in the aromatic ring at C_5 can enter into this reaction. Usually the reaction gives a blue colour. The main reagent in the reaction is *p*-dimethylaminobenzaldehyde. A mixture of FeCl_3 and H_2SO_4 conc. serves as an oxidizing agent.

Figure 5: Reaction of salicylic acid with Van Urk's reagent



Source: Author

Quantitative analysis of salicylic acid carried out by instrumental methods: spectral methods, chromatography. As Moffat (2011) writes, determination of salicylic acid by spectral methods: *ultraviolet (UV) spectrum*: aqueous acid — λ_{max} 236, 303 nm; aqueous alkali—salicylic acid — λ_{max} 298 nm; methanol—(choline salicylate) — λ_{max} 298 nm. 0.5 M solution of aqueous alkali (NaOH) — λ_{max} 300 nm; 0.1 M solution of aqueous acid (H_2SO_4) — λ_{max} 302 nm (Kramarenko, 1995; Welchinska, 2017). *Infrared (IR) spectrum* of salicylic acid: 758, 1657, 1288, 1210, 1250, 1150 cm^{-1} (KBr disk); 1587, 1724, 1176, 1515, 699, 1041 cm^{-1} (choline salicylate) (Moffat, 2011). Mass Spectrum of salicylic and salicylic acids: principal ions at m/z 120, 92, 138, 64, 39, 63, 121, 65 (salicylic acid); 121, 120, 69, 92, 195, 39, 93, 45 (salicylic acid).

As Moffat (2011) writes, determination of salicylic acid by chromatography: *Thin-Layer Chromatography (TLC)*: system TD—salicylic acid R_f 0.07, salicylic acid R_f 0.00; system TE—salicylic acid R_f 0.10, salicylic acid R_f 0.00; system TF—salicylic acid R_f 0.01, salicylic acid R_f 0.00; system TAD — R_f 0.24; system TAE — R_f 0.86; system TAJ — R_f 0.12; system TAK — R_f 0.71; system TAL — R_f 0.70 (acidified potassium permanganate — positive; ferric chloride — violet; location under UV light — violet fluorescence).

Gas Chromatography (GC): system GA—salicylic acid RI 1307, M (-Me) RI 1200, M (-Me₂) RI 1195, M (glycine conjugate) RI 1825, M (glycine conjugate—Me) RI 1810, M (glycine conjugate—Me₂) RI 1845; system GB—salicylic acid RI 1340, M (-Me) RI 1228; system GL—M (-Me₂) RI 1210, M (5-OH-Me₃) RI 1530.

High Performance Liquid Chromatography (HPLC): system HD—salicylic acid k 0.7, choline salicylate k 0.7; system HW— salicylic acid k 4.60, choline salicylate k 4.80; system HY—RI 355; system HAA—RT 12.1 min; system HAX—RT 5.2 min; system HAY—RT 4.4 min.

In addition, photoelectrocolorimetry and extractive photocolourimetry methods are also used.

Conclusion

It can be concluded, the results of qualitative and quantitative determination of salicylic acid — a substance or as a product of acetylsalicylic acid hydrolysis during a pharmaceutical or chemical-toxicological analysis confirm the degree of quality of the drug aspirin, the presence of the active

ingredient in its composition, the degree of purity of the test substance, and finally, the quality drug standards. We have expanded the possibilities for the qualitative determination of salicylic acid, which will be useful for studies of high-quality drugs and counterfeit drugs containing in their composition salicylic acid and its derivatives.

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