

ANALYSIS OF DRUGS CONTAINING ALKALOIDS PART II

Proof of presence of alkaloids in drugs

From the analytical point of view, alkaloids are similar to each other only by general nature of the organic bases. This fact forms the basis of numerous group reactions, which can be divided into two types:

Precipitation reactions:

1. Hydroxide, carbonate and alkali solutions and ammonia solutions are used as basic precipitants of alkaloids. It should be noted that stronger bases, especially alkali hydroxides, can form soluble phenolates with alkaloids containing free phenolic groups.
2. a) The pharmacopoeial Mayer reagent, containing the neutral tetraiodomercurate $(\text{HgI}_4)^{2-}$ or the analogic Dragendorff reagent, containing tetraiodobismutite $(\text{BiI}_4)^-$ solution, belong among the group reagents containing complexes with heavy metals in the anions.

Precipitates of alkaloids occur with Mayer reagent in solutions slightly acidified with diluted sulfuric acid, and are of dirty greenish color. Some alkaloids, like slightly basic purines and colchicine, tend to form precipitates only in larger concentrations. Composition of the resulting precipitate varies depending on the conditions of precipitation, however, it can be attributed to the approximate composition $\text{Base}_{1-2}(\text{HgI}_4)$. The precipitates of alkaloids with Dragendorff reagent are mostly orange-red, and correspond to the structure $\text{Base}(\text{BiI}_4)$. The Lugol's solution, containing polyiodide $(\text{I}_3)^-$ solution, may be assigned to these two reagents.

2. b) From the reagents, which have the character of heteropoly acids, can be mentioned eg. phosphowolframic acid $[\text{P}(\text{W}_3\text{O}_{10})_4]^{3-}$, phosphomolybdic acid, silicowolframic acid and others. These reagents form precipitates in the base anion ratio 1:1 to 1:5 in acidic or neutral environment. The phosphomolybdic acid forms yellow precipitate, which turns blue over time – reduction to molybdenum blue. The phosphowolframic acid forms an off-white, eventually crystallizing precipitate.

3. Some organic acids, for example tannin solution (tannic acid) form insoluble beige precipitates with alkaloids. Picric acid forms crystalline salts of a defined melting points with alkaloids.

If these group reactions serve as evidence, it is necessary to link them with the microscopic assessment of the crystalline systems of precipitates, or with defining the melting point of defined derivatives.

Color reactions:

Reagents used most often for characteristic color reactions of alkaloids are:

1. Strong inorganic acids, eg. concentrated sulfuric acid, nitric acid, eventually their mixtures.
2. Strong inorganic acids with an admixture of some condensing, oxidizing or reducing reagents.

Ad 1. For example the Erdmann reagent, which is sulfuric acid with a trace of nitric acid. The importance of nitric acid as a basic reagent to alkaloids comes from its ability to dissolve organic compounds, especially those of alkaline character. However, these compounds often tend to change while being dissolved, and the resulting products are sometimes characteristically colored. The same goes for the nitric acid, which eg. with brucine provides an orange color, whereas with colchicine violet. Exposing alkaloid to sulfuric acid can

sometimes cause only sulfonation, other times the effects are stronger, for example the whole molecule can be rearranged. Color products resulting from the reactions with sulfuric acid are very unstable when water is present – the resulting salts of weak bases can be easily hydrolyzed.

Ad 2. Reagents containing strong mineral acids (mostly sulfuric acid) as basis, and in addition various oxidizing, reducing or condensing agents. The Marquis reagent is a solution of formaldehyde in sulfuric acid. It is very often used as evidence of alkaloids. The Mandelino reagent is 0.5% ammonia metavanadate solution in sulfuric acid, the Froede reagent is molybdenum sulphuric acid and finally the Wasicki reagent is a solution of *p*-dimethylaminobenzaldehyde in sulfuric acid.

Individual reagents usually provide color reactions with higher number of alkaloids. Even though these reactions sometimes tend to be quite diversified in regards to the color, they still tend to be the same or at least very similar with the majority of alkaloids. Still, some of them, contrary to the precipitation reactions, tend to have the character of selective reactions. In most cases the alkaloid can't be identified using only one reagent and the orientation about its character is thus quite difficult. For the purpose of the orientation diversification of alkaloids, several reagents are usually used and the results of color reactions are compared.

For example the Marquis reagent usually reacts with alkaloids containing phenolic, or phenol ether groups. Positive reaction thus usually occurs with alkaloids from isoquinoline group, especially the group of opium alkaloids. Further also colchicine, lobeline, scopolamine, cotarnine, narcotine and papaverine react positively.

The formation of diphenylmethane derivatives, whose molecule can grow into high molecular units, which is manifested for example as a formation of amorphous precipitates, especially in the presence of bigger amount of formaldehyde, probably occurs during the reaction.

If the substances react slowly or just when exposed to heat, they usually provide a sequence of color, which usually consists of magenta or red. Substances which react too quickly provide only a dark brown solutions, they are burned up.

The Mandelino reagent acts mainly oxidatively while forming often characteristically colored products. It reacts positively with ephedrine, cocaine, cinchonine, strychnine and pilocarpine.

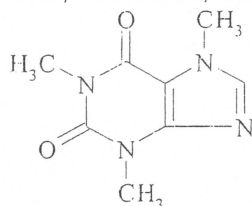
Hydrogen peroxide solution acts oxidatively as well. Molecule of hydrogen peroxide as dihydrol also has the capability to act like a substitution, similarly to an elementary halogen molecule. That is how simple phenols are converted to di- to poly- hydroxy-compounds, to which the progress of color changes often corresponds.

Description of the color and sequence of its changes is difficult to capture and the assessment may differ. It is therefore recommended to follow precise instructions in carrying out qualitative reactions, and to compare the results with a known drug, used as a „standard“.

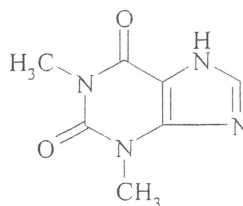
Theae folium

Thea sinensis, Theaceae

Content compounds: purine alkaloids (mainly caffeine, traces of theophylline and theobromine), tannins, flavonoids, essential oils



Caffeine



Theophylline

Coffeae semen

species of the *Coffea* genus, especially *Coffea arabica* and *Coffea liberica*, *Rubiaceae*

Content compounds: purine alkaloids (caffeine), tannins, chlorogenic acid, fats, sugars and pentosans

Yerba maté

Ilex paraguariensis, *Aquifoliaceae*

Content compounds: purine alkaloids (caffeine, theobromine...), phenolic acids (chlorogenic acid, para-coumaroylquinic acid...), tannins, vitamins A, B, C, carbohydrates, essential oils

Identification:

1- Murexide reaction

0.5 g of the powdered drug is heated to boiling with 5.0 ml of 1M sulfuric acid solution. While hot, the mixture is filtered. 5 ml of 6M ammonia solution and 5 ml of chloroform are added to the filtrate. After thorough shaking, the chloroform layer is filtered through a cotton wool into a porcelain dish and evaporated to dryness. The residue is dissolved in several drops of 6M hydrochloric acid, small amount of potassium chlorate is added and the mixture is evaporated to dryness on a water bath. Pink-red residue occurs in the presence of purine alkaloids. This color passes into purple after adding a few drops of 6M ammonia solution.

Strong oxidizing agents (in addition to hydrogen peroxide, chlorates and chlorine water can be used) break down the imidazole ring in acidic environment, with the occurrence of alloxan methyl derivative and uramil methyl derivative. These form oxybarbituryluramide methyl derivative by condensation, which changes into purple acid methyl derivative through the evaporation of water. After the addition of ammonia, the red colored purple acid changes into murexid, which is colored purple.

2- Perform microsublimation.

The drug offers colorless sublimate, consisting of long needles (caffeine). We dissolve the sublimate in a drop of chloroform and apply it on TLC.

3- Thin layer chromatography

Tested solution: sublimate from task 2 dissolved in a drop of chloroform

Developing mixture: ethyl acetate : methanol : water (77 : 15 : 8)

Reference solution: caffeine solution

Apply both the tested and the standard solution on silicagel layer separately and allow them to develop. After developing, dry it well and examine under UV at 254 nm.

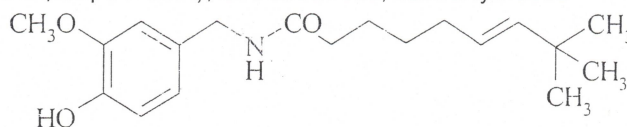
Determination of purines using the gravimetric method

5 g of the powdered drug is shaken with 40 ml of chloroform for 10 minutes, 1 ml of concentrated ammonia is added and the mixture is left for 1 hour with occasional shaking. 18 ml of the filtrate is poured into a chromatographic column filled with 4 g of Al_2O_3 with the addition of 0.1 g of activated charcoal. After dripping the filtrate, the adsorption substances are washed twice with 15 ml of chloroform, the combined chloroform extracts are evaporated on a water bath to the volume of 3 ml and 25 ml of hot water is added. The extract is then heated on a water bath until the rest of the chloroform evaporates. The hot extract is immediately filtered into weighed evaporating dish, the container is rinsed twice with 10 ml of water each time. The solution is then filtered into a previously weighed evaporating dish through the same filter. The combined aqueous extracts are evaporated, the residue is dried at 100°C and after cooling, it is weighed. The weight of the residue multiplied by 33.3 gives the caffeine content in %.

Capsici fructus

Capsicum annum, Solanaceae

Content compounds: alkaloids capsaicinoids (mainly capsaicin, further homocapsaicin, dihydrocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin.), ascorbic acid, thiamine, carotenoids (capsanthin, capsorubin), essential oils, carbohydrates



capsaicin

Identification:

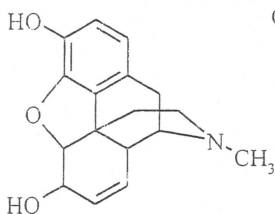
1- 0.2 g of the powdered drug is shaken with 3 ml of water acidified with 3 drops of sodium chloride solution for 3 minutes and then the liquid is filtered. Adding 5 drops of Mayer reagent to the filtrate causes the occurrence of the alkaloid precipitate.

2- 1 g of powdered drug is shaken with 5 ml of acetone, filtered, acidified with 4 drops of concentrated hydrochloric acid, then 0.1 g of ammonium metavanadate is added and everything is mixed up. The solution turns green (capsaicin).

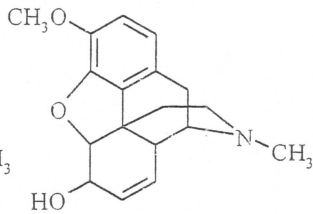
Papaveris fructus maturus

Papaverum somniferum, Papaveraceae

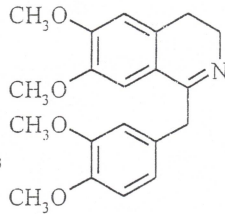
Content compounds: morphine alkaloids (morphine, codeine, thebaine, noscapine in the form of meconic acid salts etc.), other types of alkaloids (papaverine, narcotine, berberine...), sugar, organic acids (lactic, fumaric, meconic...)



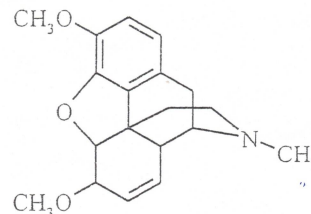
Morphine



Codeine



Papaverine



Thebaine

Identification:

1- 1.0 g of powdered drug is shaken with 5 ml of water for 5 minutes and is then filtered. The filtrate is mixed with 0.25 ml of ferric chloride solution while red color occurs, which doesn't change after adding 0.5 ml of diluted HCl.

2- Thin layer chromatography on silicagel

Tested solution: 5 g of the powdered drug is boiled with 30 ml of Ca(OH)_2 (5% w/V) under reflux for 30 minutes. After cooling to room temperature, the pH is measured and Al_2O_3 is added up to pH of 6 – 7 (approximately 10 g). The suspension is centrifuged for 3 minutes at 3000 rev/min. The supernatant is poured into a flask, the rest in the centrifugal tubes is washed with chloroform, centrifuged again and added to the aqueous phase in the flask. After shaking, the organic phase is separated and the aqueous phase is extracted two more times with 15 ml of chloroform. Combined chloroform extracts are dried with anhydrous sodium sulfate and after filtration evaporated on a rotary vacuum evaporator to a volume of about 1 ml. The concentrated chloroform extract is used for TLC and the rest is left for the determination of the content.

Reference solution: chloroform solution of narcotine, papaverine and codeine (5 mg/ml)

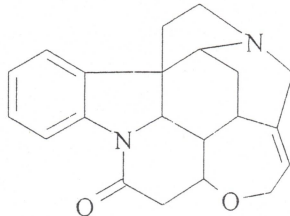
Mobile phase: toluene : ethyl acetate : diethylamine (10: 10: 2) – always freshly prepared!

Detection reagent: potassium tetraiodobismutite solution (Dragendorff reagent)

Strychni semen

Strychnos nux vomica, Loganiaceae

Content compounds: indole alkaloids (mainly brucine, strychnine, further α -colubrine- β -colubrine, icajine, 3-methoxyicajine, protostrychnine, vomicine, novacine, N-oxystrychnine, ψ -strychnine, isostrychnine), chlorogenic acid, glycosides (loganin, secologanin), lipids



Strychnine

Identification:

1- Add 5.0 ml of water and 5 drops of concentrated hydrochloric acid to about 0.2 g of the powdered drug (IV), boil the mixture and filter it after cooling. Add 5 drops of Mayer's reagent to the filtrate, yellowish precipitate (alkaloids) occurs.

2- Add 10 ml of ether and 3 drops of diluted ammonia solution to about 0.5 g of the powdered drug and shake vigorously for 1 minute. The liquid is filtered in a porcelain dish and evaporated to dryness. The residue is also used for test 3. A drop of ammonium metavanadate is placed on a part of the residue, after a while it turns reddish (strychnine).

3- A drop of concentrated nitric acid is placed on the rest of the residue from test 2, orange-red color occurs (brucine).

4- The filtered liquid from the quantitative determination is evaporated to about 5 ml, potassium dichromate solution is added dropwise to the cold solution as long as a pale orange precipitate is excreted. The precipitate is filtered, washed with small amount of water, and part of the precipitate is moistened with concentrated sulfuric acid in a small porcelain dish. Blue-violet, then dark red and finally orange color appears (strychnine).

5- Thin layer chromatography

Tested solution: 1g of powdered drug is mixed with 10 ml of 60% alcohol and heated for 15 minutes in a water bath under a reflux, and is filtered after cooling.

Reference solution: 10 mg of strychninum nitrate and 10 mg of brucine is dissolved in 10 ml of methanol

Developing solution: diethylamine : acetone : chloroform (10 : 40 : 50)

The layer is left to develop and is observed under UV at 254 nm.

Detection reagent: potassium tetraiodobismutite solution (Dragendorff reagent)

The layer is sprayed with the detection reagent and observed in daylight.

Apply 10 μ l of both the tested and the reference solution on a TLC plate and let it develop over a distance of 12 cm. After drying, it is observed under UV at 254 nm. There are clearly visible dark brown spots of narcotine and codeine, and a yellow-blue fluorescing papaverine stain between these two on the chromatogram. Above the start of the chromatogram, there is an apparent dark-blue fluorescing morphine spot, as well as less pronounced spots of codeine, papaverine and narcotine, some other less visible spots can be also present on the chromatogram. We spray the chromatogram with the detection reagent, pink alkaloid stains are visible on the light-orange background, other substances don't produce any color. Evaluation of the chromatogram after Dragendorff reagent detection must be carried out quickly, since the whole chromatogram turns black relatively soon.

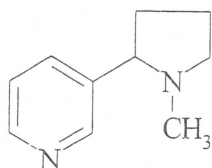
Determination of content

We dilute the chloroform extract from the test 2 with 10 ml of chloroform, quantitatively transfer it into a volumetric flask of 25 ml and complete with chloroform up to the mark. We measure the absorbance at 420 nm compared to pure chloroform and we calculate the morphine content from the calibration curve.

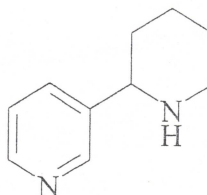
Nicotianae folium

Nicotiana tabacum, Solanaceae

Content compounds: alkaloids (nicotine, anabasine)



Nicotine



Anabasine

Identification:

1- We moisten the drug with ammonia solution of chloroform, shuffle and apply the released dark brown extract to a small square of filter paper with a capillary tube. We dry the excess of chloroform and detect the paper with Dragendorff reagent. Red spot appears in the presence of nicotine.

2- Thin layer chromatography

Tested solution: 5 cigarettes are moistened with 3 ml of 20% NaOH and extracted in the Soxhlet apparatus with ether or petroleum ether for 1 hour. Alkaloid solution in organic solvent is shaken three times with 25 ml of 11% HCl. United acidic extracts are alkalized with 20% NaOH to pH of 11 and the filtrate is shaken quantitatively (three times) with 25 ml of ether each time. United ether extracts are evaporated on a water bath and the oily residue is evaluated on TLC.

Developing system: benzene – methanol (9: 1).

Detection reagent: potassium tetraiodobismutite solution (Dragendorff reagent)

Tested solution is applied to the layer and it's allowed to develop over the distance of 12 cm.

The layer is dried, sprayed with detection reagent and observed in daylight.