**Medicinal plants and raw material containing glycosides.**

**Medicinal plants and material containing cardiac glycosides**

Glycosides are the natural compounds consisting of sugar component and non-sugar group. The sugar component is called the glycone, and the non-sugar group is known as the aglycone or genin. The sugar component is attached to aglycone via oxygen, nitrogen, sulfur, carbon and respectively O-; N-; S-; C-glycosides.

O

H

O

-

R

O

H

N

H

-

R

O

H

S

-

R

O

H

 O-glycosides N-glycosides S-glycosides C-glycosides

If the sugar part is linked with an oxygen atom of aglycone, these glycosides are called O-glycosides, if the sugar moiety is linked directly to carbon atom of aglycon, they are called C-glycosides. C-glycosides are very resistant to hydrolysis. Glycosides are divided into monoglycosides (1 sugar residue), diglycosides (2 sugar residues), triglycosides (3 sugar residues) depending on the number monosaccharide residues. Glycosides with two monosaccharide residues joined together by chain are called biosides, but in diglycosides the sugar moieties are linked to different positions.

There are α- and β- glycosides based on the configuration of glycosidic bond (connection of aglycone to monosaccharide)

C

H

2

O

H

H

O

H

H

H

O

H

H

O

H

O

C

H

3

H

O

C

H

2

O

H

H

O

H

H

H

O

H

H

O

H

H

O

-

C

H

3

O

Methyl-α-D-glucopyranoside Methyl-β-D-glucopyranoside

Glycosides are divided into furanosides and pyranosides according to the size of carbon residue cycle.

O

C

H

O

H

O

-

R

H

H

O

H

H

O

H

C

H

2

H

O

C

H

2

H

O

H

H

H

O

H

H

O

H

H

O

-

R

O

α-glucofuranoside β-glucopyranoside

According to the monosaccharides included in the glucoside molecule, the monosaccharides may be named as glucosides (glucose), galacturonosides (galacturonic acid), galactosides (galactose) and oth. Glycosides may contain deoxysugars. The hydroxyl groups are replaced by hidrogen atoms (for example: D-rhamnose, digitoxose, cymarose).

Glycosides are classified according to the chemical nature of the aglycone. According to this classification they are divided into the following groups:

Aliphatic glycosides (glycosides of fatty acids, fatty alcohols and glycerol);

Alicyclic glycosides (cardenolides, bufadienolides, triterpenoid and steroidal saponins, mono-, di- and sesquiterpene glycosides, iridoides, glycoalkaloids);

Aromatic glycosides (phenolic glycosides, coumarins, antraglycosides, flavonoids and oth.)

Heterocyclic glycosides (nucleotides, nucleosides and oth.).

Pure glycosides usually are crystalline compounds. They are hydrolised by enzymes and acids.

The rate of acid hydrolysis depends on the structure of aglycone, configuration of sugar residue, position of the sugar attached to the aglycone and type of bond. Furanosides are hydrolyzed approximately 100 times faster than pyranosides. β-glycosides are more resistant to hydrolysis than α-glycosides. Some phenolic glycosides are characterized by the alkaline hydrolysis. Enzyme hydrolysis is specific and used for the study of glycoside structure.

During the procurement of plant material for prevention of enzyme hydrolysis the raw material is required to dry at the temperature 60°С. At this temperature the coagulation of protein occurs, but the hydrolysis does not occur.

Pharmacological activity of glycosides is directly associated with the aglycone. That’s why the plant material are classified by the type of aglycone. The presence of sugar component leads to the increased hydrophily and bioavailability.

###  Medicinal plants and material containing cardiac glycosides

Cardiac glycosides (cardiotonic glycosides) are the organic compounds and steroidal in nature containing cyclopentanoperhydrophenanthrene nucleus in aglycone.They are different from other steroids due to the presence of С17 saturated lactone ring. They are classified into cardenolides and bufadienolides according to the value of lacton cycle and thee degree of saturation..

The chemical structure of cardiac glycosides are established by the works of american scientists V.A. Jacobs, P. Tschesche and oth. in 1930s of XX century.

Most plants that produced steroidal lactones contain cardenolides. Only the plants of liliaceae (bowiea, scilla, drimia genus), iridaceae (homeria genius), ranunculaceae (helleborus genius) and melianthaceae contain bufadienolides.

The aglycone structure of cardiac glycosides is based on cyclopentanoperhydrophenanthrene..

1

3

1

4

1

5

1

6

1

7

1

8

1

9

2

0

D

C

H

1

2

3

4

5

6

7

8

9

1

0

1

1

1

2

A

B

Cyclopentanoperhydrophenanthrene

Cardiac glycosides are compounds contained the lactone ring at C17.

They are divided into cardenolides (α, β- unsaturated five membered lactone ring) and bufadienolides ( a doubly unsaturated six-membered lactone ring) according to the structure of lactone ring.

1

3

1

4

1

5

1

6

1

7

1

8

1

9

2

0

2

1

2

2

2

3

O

O

H

H

O

H

O

1

3

1

4

1

5

1

6

1

7

1

8

1

9

2

0

2

1

2

2

2

3

O

H

O

H

H

O

O

2

4

1

2

3

4

5

6

10

9

8

7

11

12

1

2

3

4

5

6

10

9

8

7

11

12

Cardenolide Bufadienolid

Glycosyl part of cardiac glycosides contain 1-5 monoosaccharides and they are linked to aglycone at C3. D-glucose, D-galactose, D-xylose, L-arabinose, 6-dezoxysugar (L-rhamnose), 2,6-dezoxysugar and its 3-O-methyl ether (D-digitoxose, cymarose and oth.)are often found:

C

H

3

H

O

C

H

3

H

H

O

H

O

H

H

O

H

O

C

H

3

H

H

H

O

H

H

H

O

H

O

H

O

H

H

C

H

3

H

H

H

O

C

H

3

H

H

O

H

O

H

O

H

β-D-digitalose β-D-digitoxose D- cymarose

**Biosynthesis of the cardiac glycosides**

The study of steroid skeleton , substituents at different positions and orientations allow an understanding of the biochemical process of cardiac glycosides formation in plant kingdom. Phytosterols are made from squalene in plants as the result of the processes occured in molecule. β-sterol is widely distributed in the plant kingdom. It is supposed, that two types of cardiac glycosides as cardenolides and bufadienolides are formed by the side chain structure changes in molecule at С17.

These glycosides have tonic property exerted on the heart, that’s why they are called the cardiotonic glycosides. The cardiac tonic properties are attributed to lactone ring located at С17. The rupture or isomerization of lactone ring lead to total loss of biological activity. Cardiosteroids, in contrast of other steroids, are characterized by the specific spatial orientation of molecule. The ring C occupies a trans- configuration along to the ring B. The ring C/D has always cis junction. The rings A/B can be as cis as trans spatial orientation. Glycosides with the A/B cis junction are active.

The sugar component in the cardiotonic glycosides is attached to the hydroxyl group at С3 steroid part of the molecule. Cardioglycosides are characterised by the linear structure of hydrocarbon chain. The specific dezoxysugars as digitoxose, acetyldigitoxose, acetyldigitoxose, cymarose and oth are most often connected with the aglycones.

β-oriented methyl (digitalis group), aldehyde (strophanthus group), carbinol or carboxyl groups can be at С10.

The chemical structure of the cardiac glycosides has an effect on the cardiotonic activity. The compounds with cis position of the A/B and C/D rings, the b-position of lactone ring and othe functional groups (hydroxyl groups at C3) are the most active.

Introduction of hydroxyl group into C11, C12 positions increases the activity, to C16 position decreases the acitivity, acetylation of this group increases the toxicity, the presence of CHO- group at C10 enhances the effect of glycosides, accelerates their action and increase the toxicity of glycosides; the presence of methyl group in this position decreases the action of the cardiac glycosides and the cumulation occurs in the organism. The character of hydrocarbon component has an impact on the rate and strength of cardiotonic effect. Monosides have the strongest, but short-term action; the aciton becomes more milder and longer by the elongation f hydrocarbon chain.

The pure aglycones are poorly held by heart muscle, that’s why they have a momentary effect, in addition, they are toxic ( except for bufadienolides).

The widely known medicinal plants contain the following compounds: various species of digitalis, strophanthus, drimia, convallaria, adonis, olenader and oth. They were used to treat heart and other diseases by people of different countries during many centuries. Ancient egyptians and romans used drimia, greeks and romans used wallflower, english and scots used digitalis for therapeutic properties. Africans and Asian used some plants contained the cardiac glycosides to produce arrow poisons and spears.

The amount of glycosides in plants and effect of the appropriate medications depend on the environmental factors, plant growth, time of collection, methods of drying and storage.

The cardiac glycosides are found in the plants of convallariaceae, scrophulariaceae, ranunculaceae, fabaceae, apocynaceae, euphorbiaceae, liliaceae, iridaceae, moraceae, brassicaceae, sterculioideae, celastraceae, asclepiadoideae and cruciferae families.

At the present more than 400 cardiac lycosides were extracted, most of them are cardenolides (380).

The amount of well known bufadienolids in plants is small.  They are mainly found in the plants of Scilla, Drimia, Bowiea families and specoes of Helleborus genius*.* Cardiac glycosides are contained in cell sap of various plant organs in dissolved form. They occur in strophanthus seeds, convallaria herba, underground plant organs of apocynum cannabinum and oth. The amount of cardiac glycosides in plants, grew at an altitude (in mountains, upland areas) is significantly higher.

Most cardiac glycoside-containing plants grow up in tropical places (strophanthus) or in warm climate regions (digitalis, wallflowers, adonis and oth.).

The presence of manganese and molybdenum in soils increases the amount of the cardiac glycosides in a particular area.

The chemical properties of the cardiac glycosides are attributed to the presence of glycosidic bond (hydrolysis with enzymes and acids), lactone ring (isomerization under alkalines, the formation of coloured products with aromatic nitoderivatives in alkaline medium), steroid nature (the formation of coloured products with acid reagents: acetic anhydride, concentrated sulfuric acid, trichloroacetic acid and oth.).

The time of collection of cardiac glycoside- containing plant material is individual. It is necessary to collect the plant material in dry weather and deliver it to drying place, in order to prevent the self-heating of material. Most cardiac glycosides-containing material should be dried instantly at the 50-70С temperature, in order to inactivate the enzymes that hydrolyze the cardiac glycosides. Air drying is acceptable for some kinds of material (convallaria species, adonis vernalis, drimia maritima and oth.). Sometimes for the same plant material various drying schedules are established according to the neccessary glycoside.

The biological activity of cardiac glycosides- containing material is tested annually.

*Physical and chemical properties of cardiac glycosides*

Cardiac glycosides are colorless or white crystals, less randomly amorphous solid. These substances are odorless, bitter taste, optically active and they have certain melting point (100-270 0C). Most cardiac glycosides are slightly soluble in ethyl ehter, chloroform, water, but highly soluble in aqueous solutions of mehtanol and ethanol. Glycosides consisted of long hydrocarbon chain are more soluble in water and aqueous alcohol solutions and aglycones – in organic solvents.

The polarity and solubility of cardiac glycosides are enhanced by the increasing of hydroxyl groups.

The cardiac glycosides are hydrolyzed by acids and enzymes. It is soft stepped technique of splitting during the enzymatic hydrolysis. The secondary glycosides are formed from the primary glycosides by the enzymatic hydrolysis, they are different by the length of hydrocarbon chain. For example, during the enzymatic hydrolysis of purpureaglycoside A the digitoxin and one molecule of glucose are formed, then the digitoxigenin and three molecules of digitoxose are formed. Completed splitting into algycone and sugar components occurs in acidic hydrolysis.

The destruction of aglycone of cardiac glycosides is occurred in alkaline medium due to the rupture of the lactone ring leads to the loss of cardiotonic effect. Most cardiac glycosides fluoresce under ultraviolet light. For example, the lanatosides of digitalis purpurea fluoresce under ultraviolet light: A lanatoside – yellowish-green; B lanatoside – blue-green, C-lanatoside- blue colour.

 Cardenolides

The major element of cardenolides is a five-membered α, β- unsaturated lactone ring at C17, that gives the coloured reactions with picric acid (Balyetta reaction) in an alkaline medium, m-dinitrobenzene (reaction Raymond), m-dinitrobenzoic acid (reaction Kedde), sodium nitroprusside (reaction Legal) and oth. Add several drops of freshly prepared 5% sodium nitroprusside solution and 1 ml of sodium hydroxide solution to 1-2 ml alcohol solution of 3-5 mg glycosides for Legal reaction and the red colour is formed that disappears fast.

The reaction of Kedde and Balyetta with the derivatives of динитробензолсульфио is used for the quantitative determination of cardenolides, and the Raymond reaction is used for detection of cardenolides on the chromatograms.

The Liebermann-Burchard color reaction is used for determination of steroid nucleus of cardenolides. 1 g of substance is dissolved in 2 drops of glacial acetic acid, then add 3 ml of the mixture of glacial acetic anhydride (5parts) and concentrated sulfuric acid (1 part). Formation of pink color through red to green or blue-green shows the stereoid nature of the substance.

The cardiac glycosides and aglycones are characterised by the specific reaction with concentrated sulfuric acid and its 84% solution. They are used for the identification of individual components, sometimes for the quantitative determination of certain glycosides in the mixture.

The oxygroups are found at different positions as the substituents of steroid nucleus in the cardenolides: oxy-groups, acyl residues, epoxide bridges, ketogroups, aldehyde and carboxyl groups. Hydroxyl groups in IR-spectrum, non-associated with hydrogen bonds, are found at 3625—3600 см-1, associated with intermolecular hydrogen bonds at 3600—3200 см-1, associated with intramolecular hydrogen bonds at 3200—2500 см-1. Aldehyde and ketone groups are easily detected by UV-spectroscopy and dispersion of optical rotation. The absorption maximum of the ketones is located at 280—295 nm and the maximum absorption of aldehyde group – at 303—307 nm.

The value of the frequencies of carbonyl groups in IR-spectrum depends on the carbonyl nature and its position in the steroid nucleus. It is  1750—1700 см-1 for carbonyl groups of saturated ketones, and 1719 см-1 is for aldehyde groups.

The cardenolides of plants are mainly represented as the glycosides, the sugar component of them is attached to hydroxyl at C3 of the steroid skeleton. The color reactions are used for the determination of sugar components. During the glycoside hydrolysis the free sugar is formed, that determined by Fehling’s reagent or silver mirror reaction.

2-dezoxysugars contained in glycoside molecules, give a positive reaction with Keller-Killiani’s test. 5 mg of glycosides are dissolved in 1 ml of glacial acetic acid, contiained 0,01 ml of 5% iron chloride and carefully pour 1 ml of concentrated sulfuric acid along the test tube wall. Upper layer gives a bright-blue or blue-violet colour. Dark brown colour is formed at the border between two layers.

However, di- and triglycosides, contained 2-desoxysugars, do not give this reaction. In such a case the glycosides are hydrolyzed by trichloroacetic acid, and free 2-desoxysugars form blue color with nitrophnylhydrazine and alkaline. It is recommended to use the mixture of p-dimethylaminobenzaldehyde solution in alcohol and phosphoric acid or vanillin solution in alcohol with phosphoric acid for detection of 2-desoxysugars on paper. The glycoside spots with 2-desoxysugars are revealed as a blue colour. The monosaccharides are identified by paper chromatography with aniline phthalate.

The pentose spots are revealed as a pink colour, but hexose – as brown colour.

Bufadienolides

In bufadienolids, it is a six-membered lactone ring with two conjugated double bonds at C17 of steroid skeleton. A six-membered lactone ring gives a positive reaction with saturated antimony trichloride solution in chloroform, and a pink-violet color is formed under heat. Liebermann-Burchard, Rosenheim and oth. reactions are used to identify the steroid cycle in a molecule. UV-spectroscopy is used for determination of bufadienolides, absorption maximum is at 300 nm (lg E=3,7). Absorption in this region is used to manifest in the chromatogram under UV-light at 290— 360 nm.

The six-membered ring is characterised by three absorption bands in IR-spectrum: at 1730 cm"1 (С0-group); at 1640 и 1540 см^1 (conjugated -С-bonds).

The sugar components contained in bufadienolids are represented by three sugars: D-glucose, l-rhamnose and L- tevetose.

*The characteristics of aglycone*

The base of aglycone structure of the cardiac glycosides is fully or partly hydrogenated cyclopentanoperhydrophenanthrene ring system. A/B rings are cis or trans fused. C ring is in a trans configuration. But C/D rings in contrast to other natural steroids are always cis fused.

There are hydroxyl groups at C3 and C14 in all of the aglycones, but the methyl group – at C13.

In aglycones of cardiac glycosides the substituents can be at the following carbon atoms: 3, 5, 10, 12, 13, 14, 16, but an unsaturated lactone ring - at С17

The hydroxyl groups can be present at 1, 2, 5, 11, 12, 15 and 16 in the cardiac glycosides aglycones, they can be acylated with formic, acetic and isovaleric acids. The cardiac glycosides aglycons contained double C=C bonds, ketogroups, epoxide rings, are also isolated from plants.

The substituents at С3 are in a-position, at C5 and C14 are in cis-configuration; a lactone ring can be in a- and b-positions.

Biological activity of cardiac glycosides depends on stereochemic structure of glycosides.

Only the glycosides with a cis- fused A/B rings are active. The presence of unsaturated lactone ring is characterised by their specific effect on cardiac muscle. Any changes in the structure of the lactone ring lead to loss of characteristic cardiac action. These changes can be : 1) splitting of the lactone ring under alkaline conditions; 2) formation of hydrolactone during hydrogenation

The structure of sugar moiety

The sugar components are attached to aglycone by alcohol hydroxyl group at C3. The cardiac glycosides contain 1-5 monosaccharides.

About 30 different monosaccharides are found in various cardiac glycosides, the majority of them (except glucose, fructose and rhamnose) are highly specific, they are not found in other natural substances of plant origin. The characteristic property of the specific sugars contained in cardiac glycosides: they are linked with oxygen atom and referred to deoxysugars. These are 6-deoxy- and 2,6-dideoxyhexoses that contain methoxyl or acetyl groups in different positions. For example, digitoxose, cymarose, гапдроза, diginose and oth.

Carbohydrate components of natural cardiac glycosides have a linear position, the deoxysugars are first attached to aglycones, and the glucose is a terminal monosaccharide.

**Extraction of cardiac glycosides from plant material**

The main difficulty during the extraction of the cardiac glycosides form the plant material is their high lability, that’s why any violation of temperature requirements leads to destruction.

The cardiac glycosides are mainly extracted from plant material with 70-80% methanol or ethanol.

The extraction of the cardiac glycosides from the plant material is the multiple process. It includes: the preparation of plant material, purification from fats by petroleum ether or petrol (for seeds), the extraction with the mixture of alcohol-water, removal of organic solvents, precipitation of lipophilic substances and filtration (chlorophyll and other pygments, resins, wax, sterols and oth.), carrying through the layer of oxide aluminium to separate from phenolic compounds and carry out the fractional extraction with vaious organic solvents with different degrees of polarity: diethyl ether, chloroform and mixture of chloroform-alcohol (3:1-2:1). The separation of a sum of the cardiac glycosides into the individual components is carried out in column chromatography filled with the sorbents (oxide aluminium, silica gel). Necessary ranges are eluated with the certain solvents. Then necessary zones are eluated with the certain solvents. The obtained eluate is evaporated to dryness in vacuum at the temperature 50 °С, then recrystallise to obtain the individual components. Strict control of ph and temperature must be in all steps of the separation of the cardiac glycosides. Because they are very sensitive to these factors. Isocompounds without pharmacological activity are formed in alkalescent medium. Glycosides can be hydrolyzed in an acid medium, sometimes the separation of tertiary hydroxyl groups occurs by the formation of anhydroforms of steroid nucleus. Acetyl and formyl functional groups, presented in the aglycones of some cardiosteroids, are splitted of in an acidic and an alkaline medium. Aldehyde group is subject to oxidation, even by oxygen the air.The cardiac glycosides are sensitive to heat. Before the extraction of natural glycosides it is necessary to deactivate the enzymes contained in raw material. High temperature, alcohol vapours, ammonium sulphate and oth. are used for this. The plant enzymes are used for the extraction of the secondary glycosides. Defatted plant material is wetted and stored for several days at 25-37 C temperature. Then the glycosides are extracted with alcohol-aqueous solution.

Qualitative reactions of the cardiac glycosides

In spite of absence of the specific reactions, the application of the following reactions enables to determine the quality of the cardiac glycosides. Qualitative reactions are carried out with individual substances or purified extraction from plant material. Several drops of extraction are evaporated to dryness on clock glass or bowl.

The colored reactions are often used for detection of the cardiac glycosides in plant extracts.  They are divided into three groups: steroid nucleus; lactone ring; carbohydrate part of the molecule.

*Reactions of steroid nucleus*

- Liebermann-Burchard reaction. The blue-green color is formed by the addition of mixture of acetic anhydride and the concentrated sulfuric acid (50 parts of acetic anhydride and 1part od the concentrated sulfuric acid).

- Reaction with Chugayev’s reagent (zinc chloride and acetyl chloride in acetic acid). The pink color is formed with absorption maximum at λ = 562 nm during the reaction.

- Rosenheim reaction. The cardenolides containing a dien group or are able to form it, give this reaction under trichloroacetic acid. The pink color is formed (λ = 562 nm), changing to violet or blue.

Reactions of five-membered unsaturated lactone ring. This reaction is carried out with aromatic nitrogen derivatives in alkaline medium:

- Kedde reaction. The violet-red color is formed by the reaction with 3,5-dinitrobenzoic acid, it is specific for γ-lactone ring of the cardenolides.

- Legal reaction. The red coloor is formed with sodium nitropruisside.

- Raymond reaciton. The violet color is formed with m-dinitrobenzene in benzene solution.

-Bailey reacton. The reacton is carried out with picric acid.

The specific reactions are not found for double unsaturated six-membered lactone ring.

The UV-spectrum is taken for the identification of bufadienolides, it has the characteristic absorption band at 300 nm. The five-membered lactone ring in this conditions has the intense absorption at 215-220 nm.

*Reactions for deoxysugars.*

- Keller- Killiani reaction. This reaction is with the mixture of two reagents: glacial acetic acid contained the traces of iron (III) sulfate and the concentrated sulfuric acid with the traces of iron(III) chloride. The dark blue color is formed.

K-strophanthin and strophanthaside (di- and triglycosides) do not give this reaction. In such cases a more sensitive method is used. The glycoside is hydrolyzed with trichloroacetic acid, and free 2-deoxysugar is found by blue color after the reaction with n-nitrophenylhydrazine in analkaline medium. The vairous physical and chemical methods are used for the determinaiton of the cardiac glycoside’s structure : UV-; IR-; NMR- spectroscopy.

A five-membered lactone ring has an intense absorption in UV-spectrum at 215-220 nm, and IR-spectrum is characterised by the split band at 1750 см-1 (‑С=О group) and at 1625 см-1 (–С=С-bond).
The absence of specific reagents for six-membered lactone ring requires to take UV-spectra for these substances, where they are characterised by the absorption band at 300 nm. The absorption in this region is also used for chromatogram with wavelength range 290-360 nm by UV-light. In infrared region of spectrum the six-membered lactone ring is characterised with absorption band at 1730 см-1 (С=О-group) and two bands at 1640 and 1540 см-1 (conjugated С=С-bonds). The determination of the number of hydroxyl groups and acetylating (primary and secondary). Total hydroxyl groups are determined by Cerevitinov method (determination of active hydrogen) or IR-spectroscopy. But the first method requires a large number of substance. 1-2 mg of substance are enough for the second method.

Unfortunately, the application of these methods to the cardiac glycosides encounters obstacles. Cardenolides and bufadienolides as high molecular polyhydric alcohols give complicated spectrums for identification.

**Chromotographic detection***.* In the references there are many systems for the cardiac glycosides separation by TLC and paper chromatography, that can be divided into the following groups:

For low-polarity glycosides and aglycones. 2. For polarity glycosides and aglycones. Universal TLC systems of the cardiac glycosides are ethylacetate-methanol-water in different proportions.

The cardiac glycosides show a fluorescensce in UV-light. That’s why the colour reactionos are used for detection. Chromatograms of the cardenolides are sprayed by the reagents of Kedde, Legal, Raimond and Baljet. The universal reagents for the cardenolides and bufadienolides are: antimony chloride solution under heat; the mixture of chloramine and trichloroacetic acid; the concentrated sulfuric acid under heat. Liebermann- Burchard reaction can be used for the detection of any steroids and the cardiac glycosides.

Fyurjak and Lurje. 2-deoxysugars are first hydrolyzed for detection, then the paper is sprayed with n-dimethylaminobenzaldehyde alcohol solution in phosphoric acid or vanilin alcohol solution in phosphoric acid. The spots of the cardiac glycosides with 2-deoxysugars give a blue color.

*Quantitative determination of the cardiac glycosides.*

Neither of quantitative determination of the cardiac glycosides is not perfect, each of them has its disadvantages. The quantitative determination of quite labile cardiac glycosides is needed in case-by-case approach.

All methods for quantitative determination of the cardiac glycosides can be divided into twow groups: biological and physical – chemical.

Biological methods of the cardiac glycosides are based on the determination of biological activity on animal (cats, frogs, pigeons, oth.) and expressed in units (cat, pigeon, frog units). 1 unit represents the minimal quantity of the preparation to be assayed, expressed in terms of mg of the substance or ml of plant extract, caused cessation of the heartbeat of the animal within 1 hour. Number of units of action in 1 g is called valor.

Spectrophotometric and colorimetric methods are based on the determination of optical density of reaction products of cardiac glycosides with different chromogen reagents.

Polarographic method is based on the ability of cardenolides and bufadienolides to reduce at the dropping mercury electrode.

Titrimetric assay is based on the reaction between hydroxylammonium chloride and carbonyl group of cardioglycoside, as a result hydrochloric acid is produced, that interacted with diethylamine, and excess is titrated with perchloric acid in methanol.

A combination of chromatography and spectrophotometry, photometry, potentiometry, polarography, fluorimetry and oth. methods for the quantitative determination of the cardiac glycosides are considered to be prospective.

The best results are obtained by paper chromatography impregnated with formamide. In this case the benzene – chloroform (9:1); (7:5); (3:7) is used or toluene- k-butanol ((9:1); (4:1); (2:1); (1:1), ethyl acetate – benzene – water (84:16:50), chloroform- benzene – n-butanol (78:12:5) or chloroform as mobile phase. This system of solvents enables to get a clear separation of the cardiac glycosides. In recent years thin-layer chromatography is widely used. Silica gel, kieselgur, talc and oth. are used as a sorbent. The system of solvents described above are used.

The reagents which give fluorescence or colour are used for detection of the glycosides on chromatograms. The mixture of 25% trichloroacetic acid and 3% chloramine solution in ethanol (15:1) are most frequently used. Digitoxigenin derivatives give a clear golden- yellow fluorescence; gitoxigenin — blue; digoxigenin – silver.

The simplest and most accessible methods for the quantitative determination of the cardiac glycosides are considered to be photometric method: spectrophotometry and photocolorimetry and its genins in visible spectrum. These methods are based on the color reactions between the cardiac glycosides and nitro compounds (picrate sodium, 3,5-dinitrobenzene and oth.) or xanthydrol. Crystalline glycosides can be used to make a standard solution, also it is possible to use the solutions of potassium dichromate and sodium sulfite.

Fluorimetric methods are based on the ability of the cardiac glycosides to give a fluorescence with strong acids (concentrated sulfuric, phosphoric acids) and oxidants (iron perchlorate, ron chloride) after short-term irradiation with ultraviolet light.

In recent years gas-liquid chromatography is used for the quantative determination of the cardiac glycosides. Firstly the volatile derivatives (heptafluorobutyrate) are obtained from the cardiac glycosides by acetylation, then they are analysed. Regulatory and technical documentation on the cardiac glycosides –containing material requires essential standardization of plant material along with the quantitative detemination by biological methods. The biological standardization of the caridac glycosides is carried out on frogs, cats and pigeons according to the State pharmacopoeia, X edition. Biological potency of cardiac glycosides is compared with potency of standard preparation  and expressed in units. One frog unit represents the minimal quantity of the preparation to be assayed and caused cessation of the heartbeat of the animal within 1hour (frog-male’s weight 28-33 g). If the material and preparation of digitalis are assayed – 1 hour, for material and preparation of strophathus, erysimium and oleander – 2 hours.

1 cat or pigeon unit represents the dosage of standard preparation per1 kg of animal’s weight.

Valor is necessarilly indicated in the regulatory and technical documentation for the cardiac glycosides. Valor of material — is a number of units in 1 g. The standardization on frogs are most frequently used. The main shircomings of this method are the following: 1) the study is carried out on cold-blooded animals; 2) the lethal dose is determined, although it is important to determine therapeutic dosages for clinic; 3) the species of frogs are not distributed in all regions of our country and all countries; 4) low accuracy of this method (± 20-25 %) due to sharply changed sensitivity of frogs according to season and other conditions. The biological standardization on cats accepted by the State Pharmacopoeia, X edition is more accurate (± 5—10 %), but it is time-consuming.

1

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4

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9

2

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D

C

H

1

2

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4

5

6

7

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9

1

0

1

1

1

2

A

B

cyclopentanoperhydrophenanthrene

1

3

1

4

1

5

1

6

1

7

1

8

1

9

2

0

2

1

2

2

2

3

O

O

H

H

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1

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3

4

5

6

10

9

8

7

11

12

 cardenolide

1

3

1

4

1

5

1

6

1

7

1

8

1

9

2

0

2

1

2

2

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12

 bufadienolide

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H

O

C

H

3

H

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O

H

O

H

H

O

H

O

H

β-D-digitalose

C

H

3

H

H

H

O

H

H

H

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H

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H

O

H

β-D-digitoxose

C

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H

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H

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H

D-cymarose

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O

B

A

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H

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H

C

D

 digitoxigenin

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 digoxigenin

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gitoxigenin

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digitoxigenin

 digitoxin

Purpurea glycoside A

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D

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D

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G

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H

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gitoxigenin

 gitoxin

Purpurea glycoside B

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H

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H

H

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Lanatoside A

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D

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β

G

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C

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H

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H

D

G

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c

α

Strophanthidin

К-strophanthin-β

Cymarine

 К-Strophantoside

K-strophanthoside-R=cymarose+β-glucose+β-glucose;

K-strophanthin-R=cymarose+β-glucose

Cymarine – R = cymarose

O

H

3

O

H

O

H

C

H

2

H

O

H

H

O

O

5

1

0

1

1

1

4

1

6

1

7

 Strophanthidol

O

O

O

H

O

Cymarose

C

H

2

O

H

O

H

Cymarole

O

O

O

H

O

R

a

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o

z

a

C

H

2

O

H

H

O

O

H

O

H

G-strophanthin (uabain)

R

3

O

O

O

H

O

H

R

2

R

1

Cardenolides

O

O

O

H

O

L

-

r

a

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n

ose

C

O

1

4

1

6

O

H

H

Adonitoxin

R1 = OH R2 = H R3 = CHO Strophanthidine

R1 = H R2 = OH R3 = CHO Strophadogenin

R1 = H R2 = OH R3 = CH2OH Adonitoxol

R1 = R2 = OH R3 = CHO Adonitoxigenin

HCO

O

O

O

H

O

H

H

OH

Strophanthidine

HCO

O

O

O

H

O

H

OH

H

Strophadogenin

H2CHO

O

O

O

H

O

H

OH

H

Adonitoxol

HCO

O

O

O

H

O

H

OH

OH

Adonitoxigenin

O

O

O

H

1

R

R

O

H

 Aglycon K-strophanthin

Convallotoxin R = CHO; R1 = L-rhamnose.

Cranvalloside R = CHO; R1 = L-rhamnose + D-glucose.

Convallotoxol R = CH2OH; R1 = L-rhamnose.

Glycoconvalloside R = CHO; R1 = L-rhamnose – D-glucose + D-glucose.

Locundoside R = CH3; R1 = L-rhamnose

O

O

O

H

L-rhamnose

CHO

O

H

 Convallotoxin

O

O

O

H

CHO

O

H

L-rhamnose + D-glucose

Convalloside

O

O

O

H

L-rhamnose

H2CHO

O

H

Convallotoxol

O

O

O

H

CHO

O

H

L-rhamnose – D-glucose +D-glucose

Glycoconvalloside

O

O

O

H

L-rhamnose

CH3

O

H

Locundoside

O

O

O

H

O

R

C

O

H

C

H

3

O

H

Strophanthidin – R = H

Erysimine – R = digitoxose

Erysimoside – R = digitoxose + glucose

Desglycocheirotoxin – R = gulomethylose + glucose

Cheirotoxin – R = gulomethylose + glucose

Canestein – R = gulomethylose

Glycocanestein – R = glucomethylose + glucose

O

O

O

H

O

H

C

O

H

C

H

3

O

H

Strophanthidin

O

O

O

H

O

digitoxose –

C

O

H

C

H

3

O

H

Erysimine

O

O

O

H

O

C

O

H

C

H

3

O

H

digitoxose + glucose –

Erysimoside

O

O

O

H

O

gulomethylose + glucose –

C

O

H

C

H

3

O

H

Desglycocheirotoxin

O

O

O

H

O

C

O

H

C

H

3

O

H

gulomethylose + glucose –

 Cheirotoxin

O

O

O

H

O

gulomethylose –

C

O

H

C

H

3

O

H

 Canestein

O

O

O

H

O

C

O

H

C

H

3

O

H

glucomethylose + glucose –

 Glycocanestein

C

C

H

3

O

H

O

-

R

C

H

3

O

C

H

3

O

O

O

Oleandrigenin

 Oleandrin = R - oleandrose

C

H

3

O

O

O

H

O

-

R

C

H

3

Adinerigenin

Adinerin = R – diginose

C

C

H

3

O

H

O

-

Oleandrose

C

H

3

O

C

H

3

O

O

O

Oleandrigenin

C

H

3

O

O

O

H

O

-

diginose

C

H

3

Adinerigenin

O

H

O

O

O

-

R

O

H

 Periplogenin

 Periplocin R- cymarose + glucose

 Periplocymarin R- cymarose

O

H

O

O

O

-

cymarose + glucose

O

H

Periplocin

O

H

O

O

O

-

cymarose

O

H

 Periplocymarin

O

H

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C

Corelborin К

Hellebrigenin

Corelborin – P

R

3

O

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H

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H

R

2

R

1

Cardenolides

O

O

O

H

O

L

-

rhamnose

C

O

1

4

1

6

O

H

H

Adonitoxin

O

H

O

O

O

H

C

H

O

O

H

3

Hellebrigenin

*Biological activity of the cardiac glycosides*

 The cardiac glycosides are characterised by the specific aciton exerted on the cardiac muscle due to the unsaturated lactone ring. In small doses they accelerate the cardiac contractions, in high doses they supress the heart and cause cardiac arrest. The action of the cardiac glycosides are presented in the changes to the main functions of the heart

Under influence of cardioglycosides it is observed:

- Increase in contractility, shortening in the length of systole (positive inotropic aciton) ;

-lengthening in diastole, decreasing the heart rate, the blood flow to ventriculars are improved (negative chronotropic effect);

- increasing the myocard tonus (positive tonotropic action);

-decrease conduction velocity (negative dromotropic action);

-strengthening myocardial contractility: lengthening the interval between the contractions of the atria and ventricles. (positive batmotropic action).

Only first three effects appear in therapeutic guidelines, they determine the clinical value of the cardiac glycosides.

Last two effects are manifested in overdose (cumulative effect).

Biological action of the cardiac glycosides depends on the number of СН3  and especially OH groups at carbo atoms. The polarity enhances due to the increased number of hydroxyl groups and accordingly the solubility in water. Aglycones are little different from the parent cardiac glycosides by the cardiotonic action, but they are rapidly deactivated in liver. In addition, the cardiac glycosides have a cytostatic action, they calm the central nervous system.

Aglycone of the cardiac glycosides are not used in medical practice in free form. They are not hold by the cardiac muscle, that’s they have short-term effect, in addition they are toxic (except of bufadienolides).

**Methods of standardization of medicinal plant material and preparations, containing the cardiac glycosides.**

The regulatory and technical documentation on the material and preparations, containing the cardiac glycosides is carried out on frogs, cats, pigeons. Despite several lacks this method is indispensable in analysis of medicinal plant material and galenic formulation, because the different related substances can interact with the chemical reagents. Spectral methods are used for the purified preparations, but the complex preparations are analysed by chromatospectrophotometric method. The quantitativee determination of the cardiac glycosides is carried out by gas-liquid chromatography after complete hydrolysis and obtaining of the aglycone, or chromatography its trimethylsilyl ethers. In all cases the quantitative determination of preparation is carried out by physical-chemical method, or biological standardization indicated in the regulatory and technical documentation.