**Lesson 8**

**Analysis of preparations containing azepine and benzodiazepine derivatives.**

**CONDENSED DERIVATIVES OF AZEPIN AND DIAZEPIN**

Azepine is a seven-membered heterocycle with one nitrogen atom (cycle numbering starts from the nitrogen atom). Depending on the location of the nitrogen atom due to the double bonds, Azepin can be described in several isomeric forms, for example:

H

b

f

1

0

f

5

6

7

1

1

1

2

3

4

1

N

H

4

3

9

2

g

6

7

8

N

H

5

6

7

1

2

3

4

N

H

a

b

c

d

e

1H-azepine 3H-azepine 5H-dibenz [b,f] azepine

The state of double bonds is indicated by the help of "outside" hydrogen atoms (1H, 2H, 3H, etc.), which correspond to saturated carbon or nitrogen atoms. According to the accepted rule, states formed by nuclei as a result of condensation are indicated by Latin letters.

Diazepine and thiazepine are seven-membered heterocycles, but the diazepine molecule has two nitrogen atoms and the thiazepine molecule has one nitrogen and one sulfur atom. In diazepine and thiazepine molecules, nitrogen and sulfur atoms are usually located in the 1, 4 or 1, 5 positions relative to each other:



Azepine, diazepine, and thiazepine derivatives are chemically fused systems with one or two benzene rings. According to their chemical structure and pharmacological effects, these derivatives are divided into several groups.

The first antiepileptic drugs - dibenzazepine derivatives, 10,11-dihydrobenzcycloheptene derivatives, which are similar to dibenzazepine derivative antidepressants according to their chemical structure and pharmacological effect, and benzdiazepine derivatives, which are highly effective tranquilizers, are distinguished:



Dibenzdiazepine derivatives, which have a strong neuroleptic and sedative effect, and triazolebenzdiazepine derivatives, which are highly effective tranquilizers, are also widely used in medical practice:



Unlike diazepine, the presence of a sulfur atom in the thiazepine molecule also changes its pharmacological activity. Thus, benztiazepine derivatives are used as calcium ion antagonists, hypotensive and antiarrhythmic agents:



**10, 11 – dihydrodibenzcycloheptene derivatives**

**Imipramine - Imipramine**

Drugs with 10,11-dihydrobenzcycloheptene derivatives are chemically similar to dihydrobenzazepine derivatives, differing only in the absence of nitrogen in the seven-membered ring:



In 1957, a substance called our (N-(3-dimethylaminopropyl)-iminidibenzyl-hydrochloride) was found to have an antidepressant effect. Later, similar active substances similar to this substance in chemical structure were synthesized: dibenzazepine, dihydrodibenzazepine and dibenzcycloheptene derivatives. Since there are 3 cycles in the molecule of these substances, these substances were called tricyclic antidepressants. Such substances include amitriptyline and imipramine (our).

**Amitriptyline-hydrochloride – Amitriptyline Hydrochloride**

**(Tryptizole)**



***5-(3-dimethylaminopropylidene)-10,11-dihydrobenz-[α,d]-***

***1,4-cycloheptadiene-hydrochloride***

Acquisition

Amitriptyline is obtained by the synthesis method based on phthalic anhydride and phenylacetic acid according to the following scheme:



Fenilsirkə turşusu

 ftal anhidridi 3-benzalftalid 2-(2-feniletil)benzoy turşusu



10, 11-dihydrodibenz-

 [α,d]cyclohepten-5-one

Amitriptyline hydrochloride is a white crystalline powder. Easily soluble in water, ethanol and chloroform, practically insoluble in ether. The melting point is 195-199 0C.

Determination of identity

IR spectroscopy: IR spectrum of amitriptyline hydrochloride standard

should be the same as the IR spectrum of the sample.

UV spectrophotometry: amitriptyline hydrochloride in methanol

solution should give maximum absorption at 239 nm d.u.

Gives reactions related to chlorides.

Determination of cleanliness

Dibenzsuberone and tsicobenzprin compounds (not more than 0.25%) are compared with standard samples by NTX method. Silica gel sorbent and cyclohexane-ethylacetate-diethylamine (85:12:3) solvent system are used. Chromatograms are observed in UV light after treatment with formaldehyde - sulfuric acid (4:96) solution.

Quantification

Alkalimetry is performed using ethanol as a solvent.

Titration with 0.1 M sodium hydroxide solution is carried out, the end point is determined by potentiometry.

Determined by titration in anhydrous medium according to the US Pharmacopoeia.

The determination is carried out in the medium of glacial acetic acid (in the presence of mercury-2-acetate). Titrant: 0.1 M perchloric acid solution; indicator: crystal violet is used.

Amitriptyline is one of the main representatives of antidepressants, it has cholinolytic, antiepileptic and sedative effects. It is prescribed for depression, enuresis and emotional disorders. In capsules and tablets of 0.025, 0.05 and 0.075 g, 1% solution is released for injection in the amount of 2 ml.

The preparation is stored in tightly closed containers at a temperature not higher than 25 0C.

**Imipramine-hydrochloride**

**Imipramine-Hydrochloride**

**(Imizin, Melipramine, Pryleugan)**

7

8

2

C

H

2

2

H

C

6

.

N

C

H

3

C

H

3

H

C

l

b

1

0

f

1

1

1

N

4

3

9

C

H

2

5-(3-dimethylaminopropyl)-10,11-dihydro-5H-dibenz

[b,f] azepine hydrochloride or

3-(10,11-dihydro-5H-dibenz[b,f]azepin-5-yl)

propyldimethylamine-hydrochloride

It is a white crystalline powder. Easily soluble in water and alcohol.

Determination of identity

1) Azepin core inspection. Solid HNO3 is added to the crystals of the preparation; an intense blue color is formed; the color changes to dirty green and then turns brown.

2) Taking imipramine picrate: dissolve 0.2 g of the drug in 10 ml of water, add 2.5 ml of saturated picric acid solution and wait for 1 hour. The obtained sediment is filtered through glass filter No. 3, washed with water, dried at a temperature of 100-1050C, and they determine. The melting temperature of imipramine-picrate should be 140-1420C.

3) A 0.0025% solution of the drug in 0.01 M hydrochloric acid gives maximum absorption at 251 nm d.u.

4) The solution of the preparation in water is alkalized with NaOH solution and filtered. The filtrate reacts with chlorides.

Determination of cleanliness

The presence of imindibenzyl and aliphatic amines is checked.

Quantification

It is done in several ways.

1) Aqueous titration method. The preparation etc. dissolved in a mixture of acetone and mercury 2-acetate (30:5) and titrated with 0.1 M perchloric acid until pink (T=0.03169 g/ml) with the presence of a solution of methyl orange in acetone.

2) It is carried out by the method of neutralization (alkalimetry). The preparation etc. dissolve in alcohol neutralized by phenolphthalein and titrate with 0.05 M NaOH solution until pink color (indicator-phenolphthalein; T=0.01584 g/ml).

It refers to cyclic antidepressants. 0.01; 0.025; 0.05; It is available in 0.075 g tablets and 2 ml of 1.25% solution for injection.

**Carbamazepine - Carbamazepine**

**(Finlepsin, Tegretol)**



5-carbamoyl-5H-dibenz [b,f]azepine

M.k. 236.9

Purchase:

Carbamazepine is synthesized on the basis of o-nitrobenzyl chloride. First, dibenzazepine is obtained from 0-nitrobenzyl chloride, which is then treated with carbamic acid chloroanhydride to obtain carbamazepine:



dibenzazepine carbamazepine

It is a white crystalline powder. Insoluble in water. Hardly soluble in ethanol, easily soluble in propylene glycol and chloroform.

Determination of identity

1) The IR spectrum of the drug should correspond to the IR spectrum of standard carbamazepine.

2) UV-spectrophotometry: a solution of carbamazepine in ethanol should give a maximum absorbance at 238 and 285 nm d.u., and a minimum at 257 nm d.u. Solutions of the preparation give blue fluorescence under the influence of UV rays.

3) A solution of carbamazepine in strong sulfuric acid gives an orange-red color when heated in a water bath.

4) Azepin core inspection. The preparation is heated in a water bath with nitric acid for 3 minutes; an orange-red color is formed.

5) The melting temperature of the preparation is 183-1930C.

Determination of cleanliness

Imindibenzyl compounds are checked by NTX method.

Quantification

It is carried out by the spectrophotometry method. The optical density of 0.01% solutions of the drug and the standard sample of carbamazepine in ethanol is measured at a wavelength of 285 nm (E\_1cm^(1%)490).

It is an anti-seizure (epilepsy) and anti-depressant. It is released in 0.2 and 0.4 g tablets.

The drug is stored in tightly closed containers, in a dry place protected from light.

Benzodiazepine derivatives

Diazepines are seven-membered heterocycles with two nitrogen atoms in the ring. Depending on the position of the nitrogen atoms in the cycle, 1,2-diazepines, 1,3-diazepines and 1,4-diazepines can exist:



1H-1,2-diazepine 1H-1,3-diazepine 1H-1,4-diazepine

Benzodiazepines are a heterocyclic system consisting of a 1,4-diazepine with a benzene nucleus and two nitrogen atoms (in positions 1 and 4). Currently, the medical importance of benzodiazepines and some dibenzdiazepine derivatives is increasing.

In the benzazepine system, the position of the benzene ring according to the heteroatoms is indicated in Latin letters according to the serial number:



1H-benz[f]-1,4diazepin 5H-dibenz [*b,e*] [1,4] diazepin

 (1,4-benzodiazepin)



 1,5-benzodiazepin 5H-2,3-benzodiazepin

It was established in the 60s of the 20th century that substances from this group have high tranquilizing activity. So, the first drug from this group - chlordiazepoxide - was purchased in 1962.

In subsequent periods, numerous benzodiazepine derivatives with the following general chemical structure (I and II) were synthesized:



R1$-$Cl; Br; NO2; R2$-$Cl; R3$-$H; OH və s.

Chlordiazepoxide and medazepam are widely used in medical practice.

Substances belonging to the general formula II are called 1H-benzodiazepine derivatives because they have a keto group in the 2-position. Chlordiazepoxide is a 3H-1,4-benzodiazepine, and the other substances are 1H- or 2H- derivatives. Of the numerous benzodiazepine derivatives, oxazepam, phenazepam, nitrazepam and diazepam are used in medical practice.

**Chlordiazepoxide – Chlordiazepoxide\*\***

**(Chlorzepide, Chlozepil, Elenium)**

C

H

2

l

C

C

N

C

C

6

H

5

N

N

H

C

H

3

O

7

6

4

2

3

1

5

**7-chloro-2-methylamine-5-phenyl-3H-1,4-benzodiazepine-**

**-4-oxide**

**M.k. 299.8**

**The melting point is 239-2430 C.**

**Diazepam – Diazepam\*\***

**(Sibazonum, Relanium, Seduxen, Valium)**

C

H

2

l

C

C

N

C

C

6

H

5

N

C

H

3

O

7-chloro-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepine-4-one

M.k. 284.7

**Phenazepam - Phenazepamum**

C

H

2

r

B

C

N

C

O

N

H

C

l

M.k. 340.62

7-bromo-5-(ortho-chlorophenyl)-2,3-dihydro-1H-

-1,4-benzodiazepine-2-one

The melting temperature is 225-2300 C.

**Nitrazepam**

**(Radedorm, Eunactin)**



**7-nitro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepine-2-one**

**Oxazepam – Oxazepam**

**(Nozepam, Tazepam)**



**7-chloro-2,3-dihydro-3-oxy-5-phenyl-2H-1,4-benzodiazepine-2-one**

**Medazepam – Medazepam**

**(Mezapam)**



These substances are white or light yellow (or light greenish-yellow) pomegranate crystalline powder. Practically insoluble in water, slightly soluble in 95% alcohol and ether, moderately soluble in chloroform.

Benzodiazepine derivatives are amphoteric compounds. Thus, they show a weak basic property due to the nitrogen heteroatom in the 4th position in the molecule, and a weak acidic property due to the lactam bond in the 1st, 2nd position.

Purchase:

Benzodiazepine derivatives are obtained by synthesis method.

During the synthesis of chlordiazepoxide, quinazoline is first obtained from 5-chloro-2-aminobenzophenone, and then the drug:



 5-xlor-2-aminbenzofenon 5-xlor-2-amin- 2-xlormetil-6-xlor-3-oksid-4-

 benzofenonun oksimi fenilxinozolin

The synthesis of phenazepam is based on the reactions of C-acylation of p-bromaniline with benzoyl chloride and N-acylation of aminoacetic acid with chloroanhydride. The course of the reaction can be shown on the basis of the following scheme:



 *p*-bromanilin



Industrial synthesis of oxazepam is carried out on the basis of p-nitrochlorobenzene according to the following scheme:





Tranquilizers are effective substances (calming, hypnotic, enhancing the effect of analgesics, anticonvulsants, used in anesthesiology, in the post-surgery period).

Chlordiazepoxide 0.005 g in coated tablets, sibazone 0.001; 0.002; In tablets of 0.005 g and 0.5% solution for injection in the amount of 2 ml, and phenazepam 0.0005; It is released in 0.001 g tablets. Nitrazepam in 0.005 and 0.01 g tablets, and oxazepam in 0.01 g; It is released in 0.015 and 0.03 g tabs.

Determination of identity

In determining the identity of benzodiazepine derivatives, UV-spectrophotometry and IR-spectroscopy methods are widely used. In addition, hydrolysis, triple nitrogen determination, molecular cleavage, halogen atom determination, and oxidation reactions based on the chemical properties of benzodiazepine derivatives are used.

1) UV-spectrophotometry: 0.002% solutions of chlordiazepoxide and 0.005% solutions of phenazepam in chloroform give maximum absorption at 325 and 320 nm d.u., respectively. A 0.0005% solution of nitrazepam in 1 M hydrochloric acid and methyl alcohol (1:9) gives a maximum absorption at 280±2 nm d.u., and a minimum absorption at 240 nm d.u. A 0.005% solution of oxazepam in ethanol gives a maximum absorption at 229 nm, and a 0.005% solution of phenazepam in ethanol gives a maximum absorption at 231 nm d.u. A solution of diazepam in a mixture of ethanol and 0.1 M hydrochloric acid gives three absorption maxima at 240, 284 and 360 nm d.u., and minima at 262 and 330 nm d.u. Based on the indicators in the UV spectrum, these substances can be distinguished from each other.

2) The reaction of obtaining azo dye after acid hydrolysis. 0.02 g of the preparation (except diazepam; here, since it is substituted on the nitrogen atom in the 1st position, it gives yellow 2-methylamine-5-chlorobenzophenone, that is, it does not diazotize and does not enter the azo dye reaction) a mixture of solid hydrochloric acid and water (5:10 ) and heat until boiling. To the cooled solution were added 0.1% NaNO2 and 0.5% ammonium sulfamate (1:1) solutions, followed by 0.1% N-1-naphthylethylenediamine dihydrochloride (2% resorcinol in strong alkali to obtain azo dye - solutions are also used) add the solution; red (when using resorcinol), and when using N-phenyl-1-(or α)-naphthylene, a purple color is formed:

D

i

a

z

e

p

a

m

H

C

l

H

2

O

N

H

C

H

3

C

O

C

6

H

5

l

C

2-metilamin-5-xlorbenzofenon

(diazolaşmır və azoboya reaksiyasına daxil olmur)

F

e

n

a

z

e

p

a

m

+

2

H

O

H

(

H

C

l

)

-

N

H

2

-

C

H

2

-

C

O

O

H

r

B

N

H

2

C

O

C

6

H

4

C

l

+

(

N

a

N

O

2

+

2

H

C

l

)

-

(

N

a

C

l

+

2

H

2

O

)

r

B

N

C

O

C

6

H

4

C

l

N

+

Cl$¯\rightarrow $

 2amin-5-brom-α-xlorbenzofenon



 azoboya

β-naphthol in oxazepam, resorcinol in phenazepam, N-(1-naphthyl)-ethylenediamine-dihydrochloride, as well as N-phenyl-1-naphthylamine in oxazepam and phenazepam can be used to obtain azo dye. When β-naphthol is used, the reaction follows the following scheme:



Acidic hydrolysis of chlordiazepoxide occurs in a slightly different way. So, first, a water molecule is connected to the molecule due to the double bond in the 1,2 position, then methylamine is separated and an amide bond is formed. The latter is also hydrolyzed to form 2-amino-5-chlorobenzophenone. After that, the reaction of diazoization and obtaining azo dye is carried out.

3) When all 1,4-benzodiazepine derivatives are dissolved in the same amount of alkali, the released vapors (ammonia or methylamine) turn the water-soaked red litmus paper blue, and a colored residue is obtained on the wall of the test tube.

4) In order to determine the presence of halogen in benzodiazepine derivatives (chlordiazepoxide, sibazone, phenazepam, nozepam, etc.), they burn them on a copper wire; the flame turns green (Beilstein's test). The reason for the green color of the flame is the formation of copper halides, which are volatile compounds.

5) Benzodiazepine derivatives react positively with general alkaloid precipitating reagents (Dragendorf, Bouchard, picric acid) due to the triple nitrogen atom in their molecule.

6) The melting temperature of sibazone should be 128-1340C.

7) A few drops of 42% or 57% perchloric acid are added to the solution of phenazepam in chloroform-ethanol mixture (2:5) and mixed; the solution is colored greenish-yellow. A clear-green fluorescence is observed under the influence of UV rays.

8) In nitrazepam, as a result of the reduction of the aromatic nitro group in the 7th position, (Zn+HCl) turns into an amine group, and then the determination is made based on the diazotization and azotization of both groups:

C

H

2

N

2

O

C

N

C

O

N

H

C

6

H

5

Z

n

+

H

C

l

N

H

2

C

O

C

6

H

5

N

2

H

nitrazepam 2,5-diaminebenzophenone

9) The melting temperature of nitrazepam should be between 225-2300C.

10) 0.01 g of oxazepam is heated with 5 ml of 95% ethyl alcohol and 4 drops of sulfuric acid in a water bath until it boils for 5 minutes and then cooled. 5 ml of water and 1 ml of fuchsin sulphitic acid are added to the obtained solution; a purple color is formed (reaction specific to formaldehyde obtained as a result of hydrolysis of the amidocarbinol group).



fuchsin-sulphitic acid (colorless) violet

11) Crushed tablets of Nozepam 0.07 g of pomegranate are mixed with 10 ml of chloroform and methyl alcohol (1:1) for 5 minutes and filtered. 0.01 ml (10 μg) of the solution prepared above and 0.01 ml (10 μg) of the solution of nosepam in chloroform and methyl alcohol (1:1) are placed on the "Silufol" plate at a distance of 2 cm from each other. After keeping the plate in the open air for 2 minutes, it is placed in a chamber containing a mixture of chloroform-methyl alcohol (1:1) and chromatography is carried out using the ascending method. After the solvent has risen to the bottom of the plate, it is removed from the chamber, air-dried, and viewed under ultraviolet light. The stain of the test substance should be at the same level as the stain of the standard sample. Determination of Medazepam in tablets is also performed by this method.

12) Determination of identity of diazepam is determined by reaction with ninhydrin. When a mixture of diazepam, ninhydrin, and ethanol is heated to boiling, a blue color is obtained, and when copper-sulfate solution is added to the mixture, the blue color turns red or orange-red. Chlordiazepoxide gives a brown color and nitrazepam a yellow-brown color under these conditions.

Determination of cleanliness

Extraneous impurities (2-aminobenzophenone derivatives) are checked by NTX method. Chromatography is performed with reference samples on "Silufol" plates.

Additions of residual solvents (isopropyl alcohol should not exceed 0.2%) are determined by the QMX method.

Quantification of benzodiazepine derivatives is carried out by several methods.

It is impossible to titrate benzodiazepine derivatives in aqueous or alcoholic solutions, because the basicity of the nitrogen in the 4th position in the molecule is greatly weakened due to its combination with the aromatic nucleus:



1) Aqueous titration method. Chlordiazepoxide, nitrazepam and phenazepam etc. dissolved in a mixture of glacial acetic acid and acetic anhydride (20:10) and titrated with 0.1 M HClO4 (indicator-purple crystal; in chlordiazepoxide T=0.02998 g/ml; in phenazepam T=0.03406 g/ml; and in nitrazepam T=0.02813 g/ml).

In parallel, a control experiment is carried out.

Diazepam and oxazepam are also prescribed by the indicated method. In the determination of oxazepam, formic and acetic acids are taken as solvents.

N

H

2

O

N

C

N

C

+

C

H

2

H

C

6

H

5

O

C

l

O

4

N

i

t

r

a

z

e

p

a

m

+

H

C

l

O

4

2) The spectrophotometry method is based on the 1st identity determination. The amount of benzodiazepine derivatives can be determined in different dosage forms.

3) Photocolorimetry method. The determination of nosepam by this method was shown in the 2nd and 8th identical determinations.

4) The US Pharmacopoeia recommends chlordiazepoxide quantification by the YEMX method. Methanol - water (60:40) is used as the mobile phase.

5) Polarography method.

The basis of determination of the amount of fenazepa by polarography method is its reduction:



Medicinal forms of benzodiazepine derivatives are stored in a dry place protected from light.