Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

SPECIAL PHARMACEUTICAL CHEMISTRY

Benzodiazepine derivatives

Lesson 8

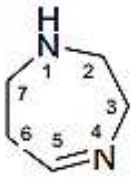
VII term

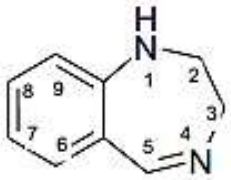
Volgograd, 2023

INTRODUCTION

Benzodiazepines are a class of psychoactive substances with soporific, sedative, anxiolytic (anxiety reduction), and anticonvulsant effects. Most are tranquilizers; some are used as sleeping pills. To a greater or lesser extent, benzodiazepines have anticonvulsant effects, and some are used exclusively for epilepsy. Benzodiazepines are part of a broad group of central nervous system depressants. They are used to treat and relieve symptoms of mental anxiety, insomnia, agitation, epileptic seizures, muscle spasms, as well as physical withdrawal syndrome (alcohol, drugs). Benzodiazepines are known to be effective for the treatment of panic attacks induced by hallucinogen drugs.

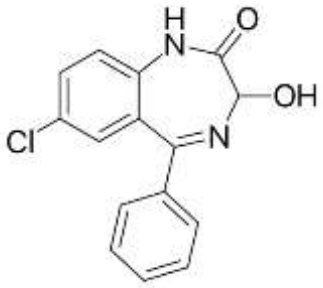
Benzodiazepine is a heterocyclic system including a benzene nucleus and a seven-membered hesterocycle, 1,4-diazepine, containing two nitrogen atoms (at positions 1 and 4):





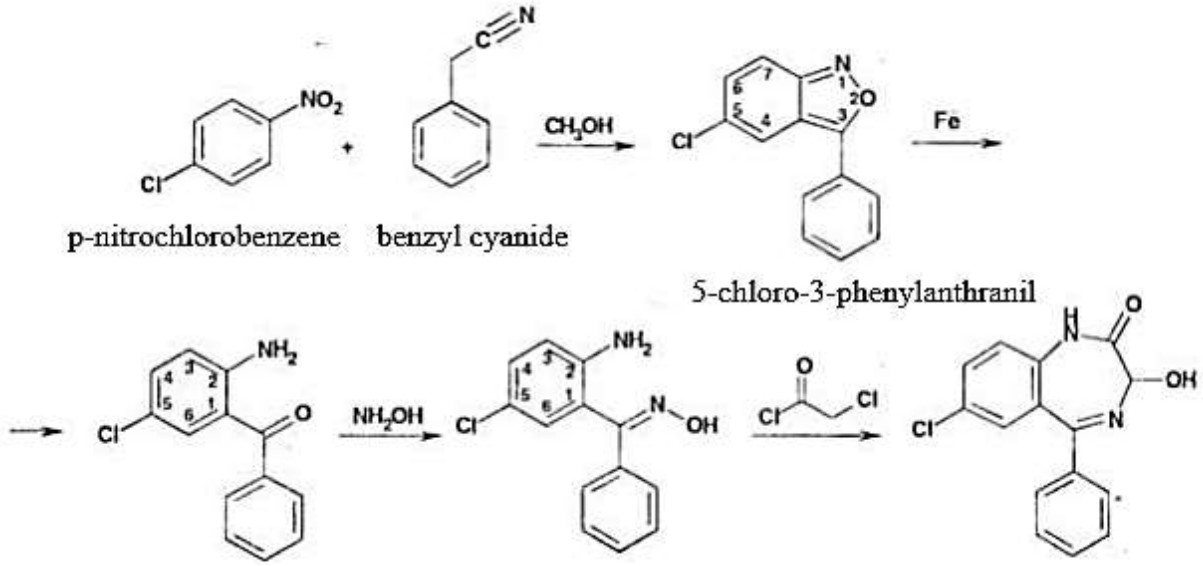
diazepine *benzodiazepine*

OXAZEPAM



OBTAINING

The starting material for oxazepam synthesis is p-nitrochlorobenzene, which forms 5-chloro-3-phenylanthranil when interacting with benzyl cyanide in methanol medium. After its reduction, the obtained 2-amino-5-chloro-benzophenone is condensed with hydroxylamine to form oxime, which interacts with monochloroacetic acid chlorohydride to form oxazepam:



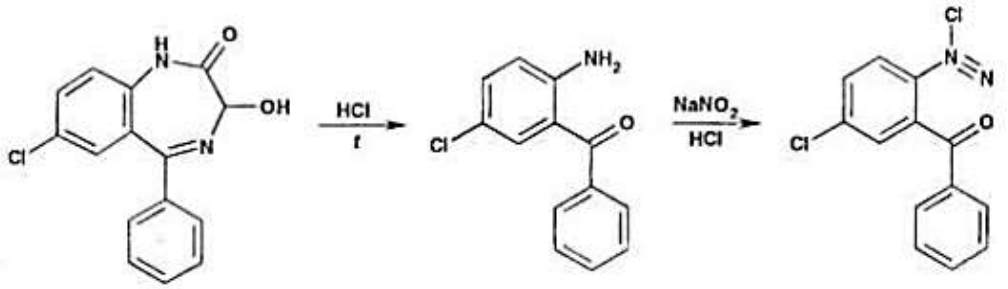
PHYSICAL PROPERTIES

White or slightly yellowish fine crystalline odorless powder, practically insoluble in water, slightly soluble in ethanol and in chloroform.

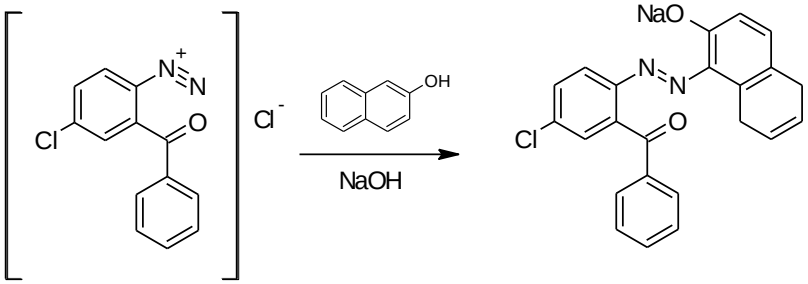
IDENTIFICATION

1. ***UV spectrometry***
2. ***Infrared spectroscopy***
3. ***Reaction of azo dye formation*** *(general reaction)*

   Acid hydrolysis produces 2-amino-5-chlorobenzophenone, which is then diazotized:



   β-Naphthol is used to produce azo dye. A red coloration appears:



4. ***Pyrolysis***

When heating about 0.01 g of drug substance in a dry test tube over a torch flame, a green colored fusion is formed which retains its color after addition of ethanol irrespective of the pH of the medium.

5. Alloying with alkalis

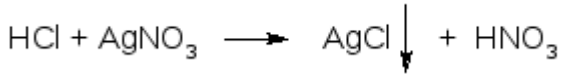
Exposure to alkali under harsh conditions (fusion with sodium hydroxide) leads to destruction of oxazepam molecule and release of ammonia from the amide group, which is detected with litmus paper. Oxazepam under these conditions forms an emerald green colored plaque on the walls of the test tube.

6. Beilstein's test

Organically bound chlorine atom is detected by the Beilstein test. The essence of this test is that a grain of substance introduced on a copper wire into a colorless burner flame, coloring it green. The coloration is due to the formation of volatile copper halides.

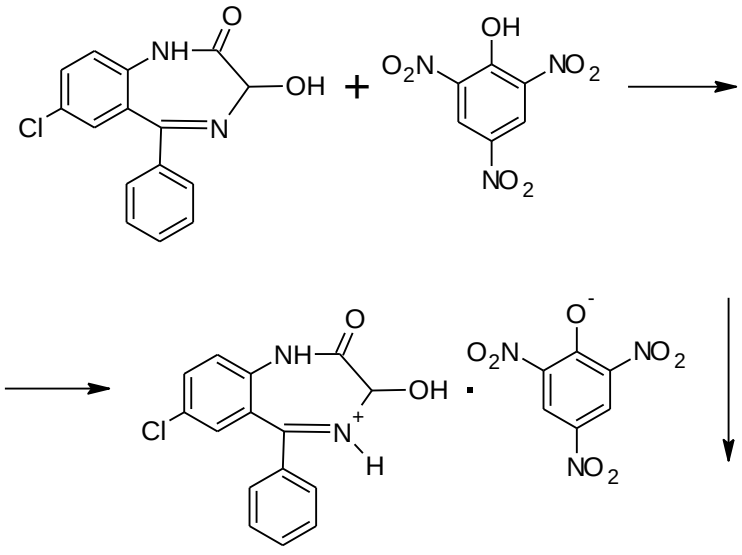
7. Mineralization of halogens

Chlorine atoms can also be detected by combustion in a flask with oxygen, using sodium hydroxide solution as an absorbing liquid. The resulting solution is then acidified with sulfuric acid and the chloride reaction is performed. A white curd-like precipitate is formed.



8. Interaction with common alkaloid reagents

Common precipitation reagents for alkaloids are Dragendorf's K[BiI4], Bouchard's I_2 in KI, and picric acid.

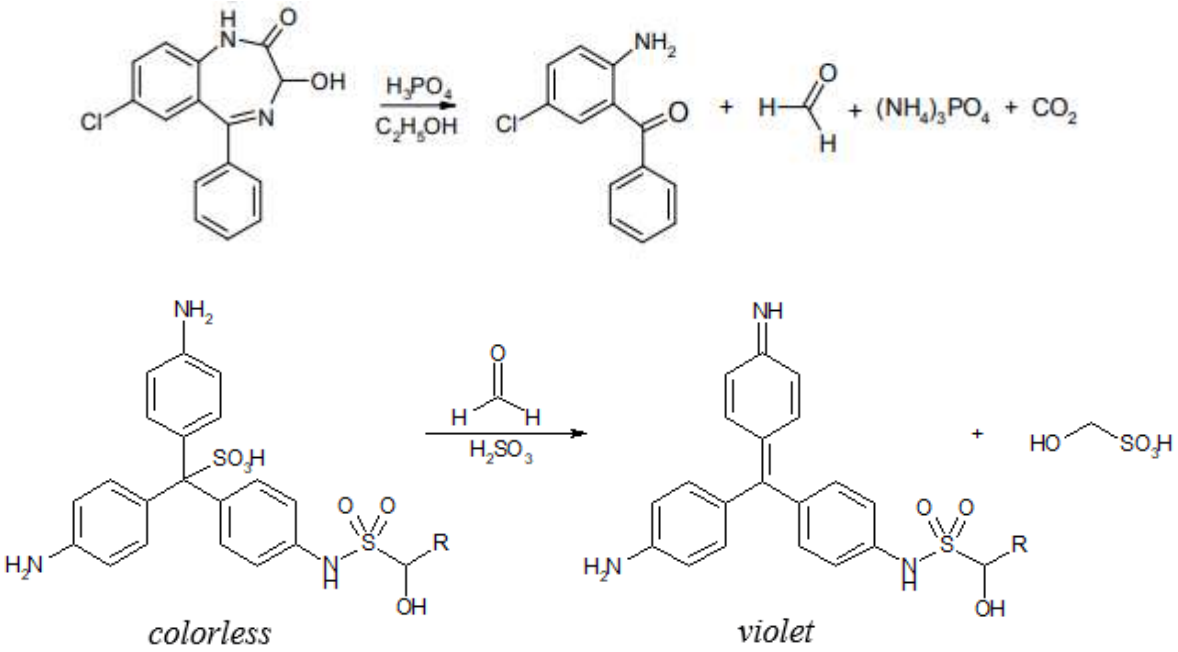


yellow precipitate

9. *Detection of acid degradation products of oxozepam*

After heating an alcoholic solution of oxazepam with concentrated phosphoric acid and adding fuchsinsulfuric acid, a violet coloration appears.

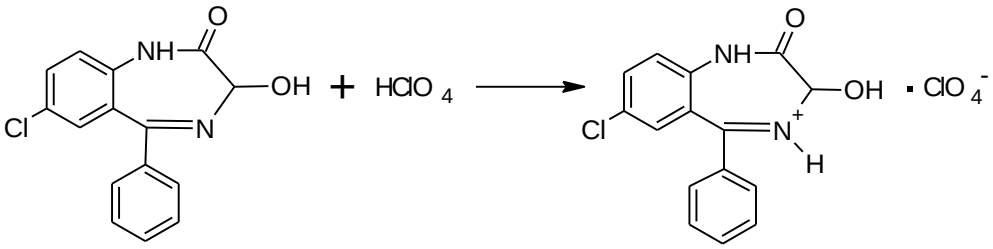
The reaction is based on the hydrolysis of oxazepam to form formaldehyde, which binds with fuchsinsulfuric acid while reducing the quinoid structure of the dye (in the presence of sulfuric acid):



QUANTIFICIFICATION

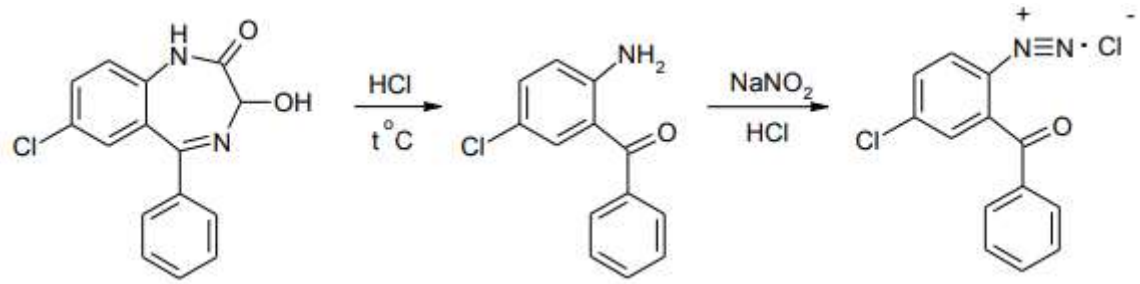
1. *Non-aqueous titration*

Formic acid combined with acetic anhydride is used as a solvent. The titrant is 0.1 M chloric acid solution. The equivalent point is established using crystal violet indicator or potentiometric method.



2. *Nitrite titration*

The diazotization reaction is the basis for the nitritometric determination of benzodiazepine derivatives:



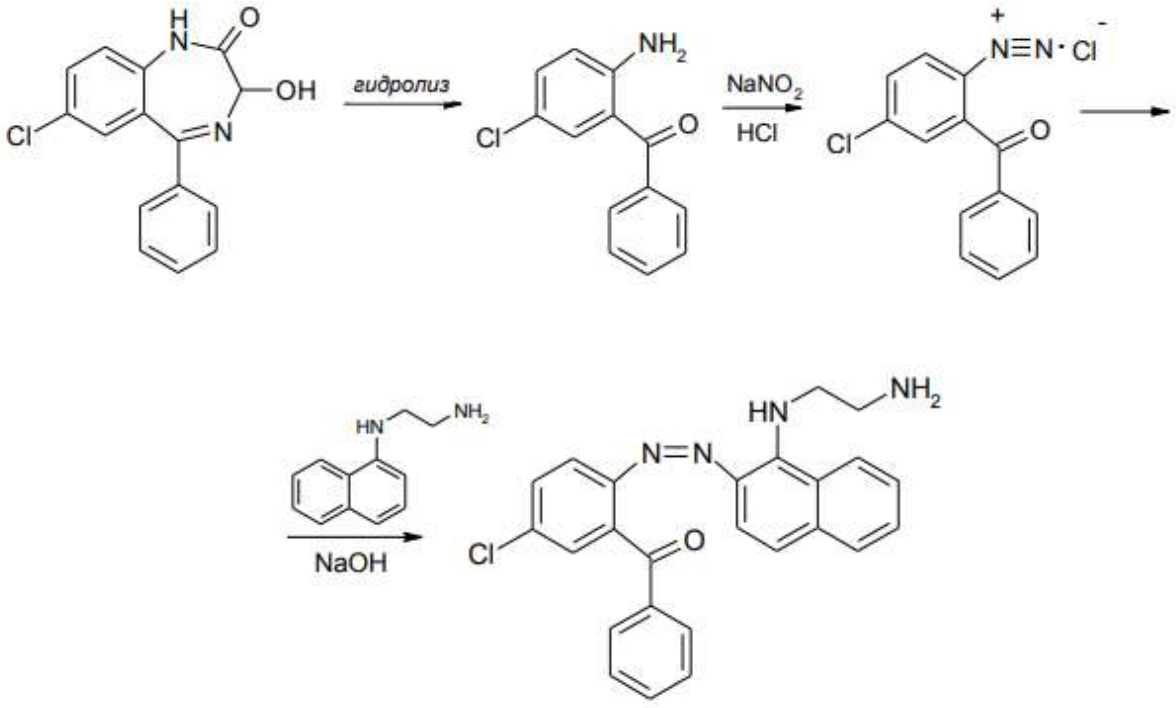
The working solution is sodium nitrite. In direct titration the equivalence point is determined potentiometrically; in reverse titration - iodometrically (indicator - starch):

$$NaNO_2 + KI + H_2SO_4 \longrightarrow I_2 + NO + K_2SO_4 + Na_2SO_4 + H_2O$$

$$I_2 + Na_2S_2O_3 \longrightarrow NaI + Na_2S_4O_6$$

3. Photocolorimetric method

The quantitative content can be determined spectrophotometrically by the intrinsic absorption of solutions at the indicated absorption maxima, as well as by photocolorimetric method using the azo-coupling reaction (after preliminary hydrolysis and diazotization) or other color reactions.



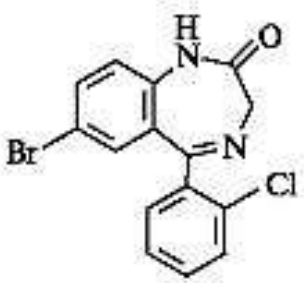
STORAGE

Store in a dry place protected from light.

MEDICAL USE

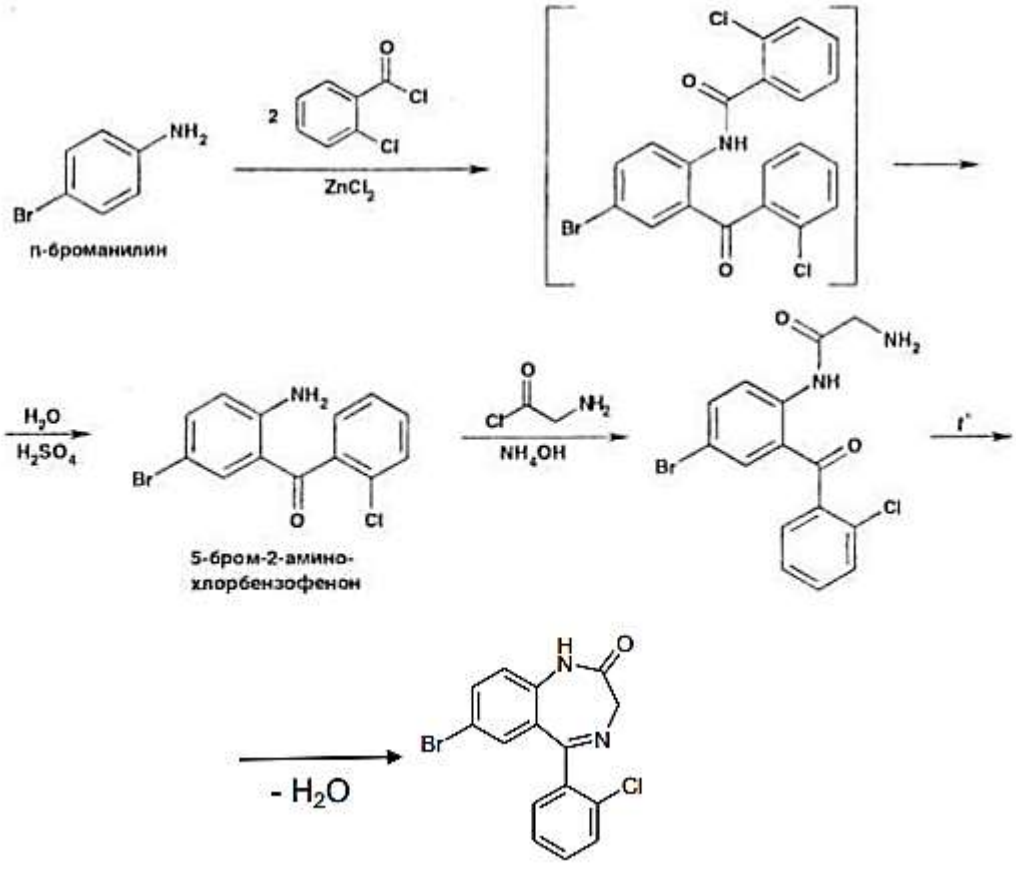
Used as anxiolytic. Used in neuroses; states of anxiety, tension, fear, excitability (in chronic alcoholism); sleep disorders.

PHENOZEPAM



OBTAINING

The synthesis of phenazepam is based on the reaction of C-acylation of p-bromaniline with benzoyl chloride and N-acylation with aminoacetic acid chlorohydride. The final step of the synthesis is intramolecular thermal cyclocondensation of diaryl ketone:



PHYSICAL PROPERTIES

White crystalline powder without odor. Melting point225-230 °C. Slightly soluble in ethanol, practically insoluble in water and ether, slightly soluble in chloroform.

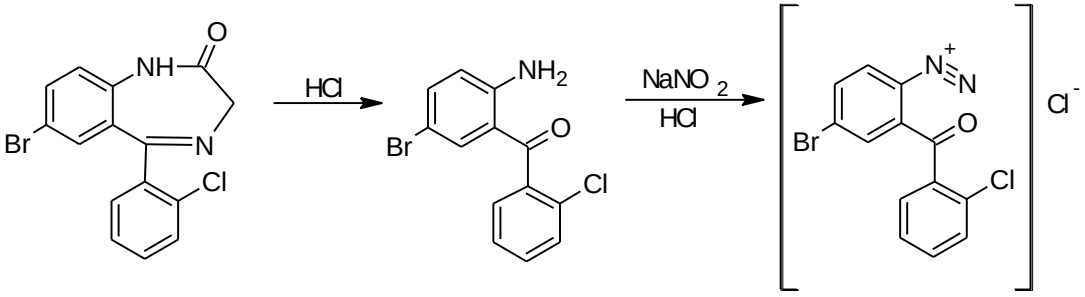
IDENTIFICATION

1. ***UV spectrometry***

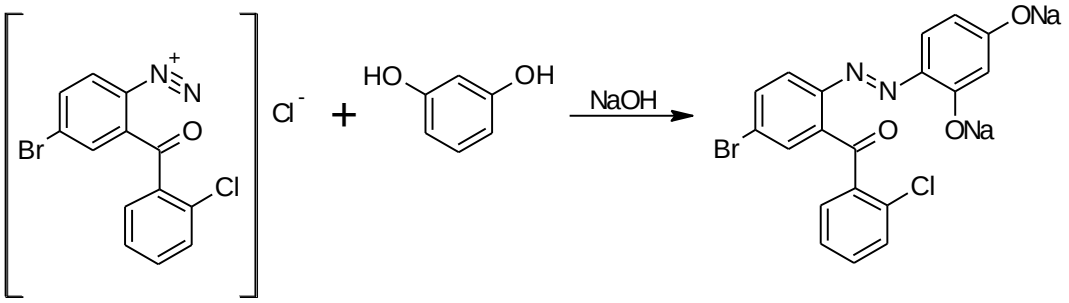
2. ***Infrared spectroscopy***

3. ***Formation of azo dye***

Acid hydrolysis of fenozepam produces a 2-aminobenzophenone derivative, which is then diazotized:



In obtaining the azo dye resorcinol. A red coloration appears:



4. ***Pyrolysis***

Heating phenazepam in a dry test tube over a torch flame produces a violet or red-violet colored fusion, which changes depending on the pH of the medium. After addition of ethanol and sodium hydroxide solution, the fusion acquires blue-violet color, and when adding dilute sulfuric acid - blue-green, changing to yellow. This allows phenazepam to be distinguished from other benzodiazepine derivatives.

5. ***Alloying with alkalis***

Exposure to alkalis under harsh conditions (fusion with sodium hydroxide) leads to destruction of the fenozepam molecule and release of ammonia from the amide group, detectable with litmus paper.

6. ***Beilstein's test***

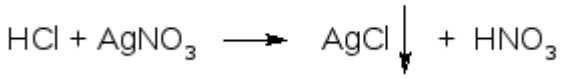
Organically bound chlorine and bromine atoms are detected by the Beilstein test. The essence of this test is that a grain of substance introduced on a copper wire into a colorless flame of a burner, coloring it green. The coloration is due to the formation of volatile copper halides.

7. ***Mineralization of halogens***

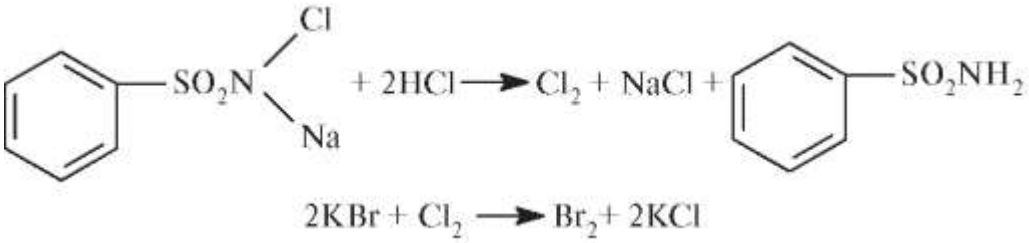
Halogen atoms can also be detected by combustion in a flask with oxygen, using sodium hydroxide solution as an absorbing liquid. The resulting

solution is then acidified with sulfuric acid and the reaction for chlorides or bromides is performed.

Chloride identification. A white precipitate is formed:



Identification of bromides. When chloroform is added, its layer is colored red-orange.



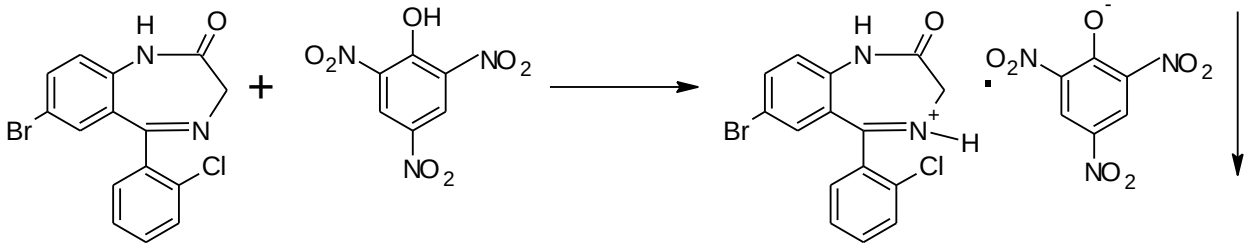
8. Formation of fluorescent products

Phenazepam can be detected by the greenish-yellow coloration and fluorescence in UV light of its solution in a mixture of chloroform, ethanol, and 2 drops of perchloric acid solution.

9. Interaction with common alkaloid reagents

Due to the presence of tertiary nitrogen atoms in the molecules, benzodiazepine derivatives give positive reactions with precipitating (obshealkaloid) reagents (Dragendorf, Bouchard, picric acid), as well as with Reineke's salt - $NH_4[Cr(NCS)_4(NH_3)_2]$.

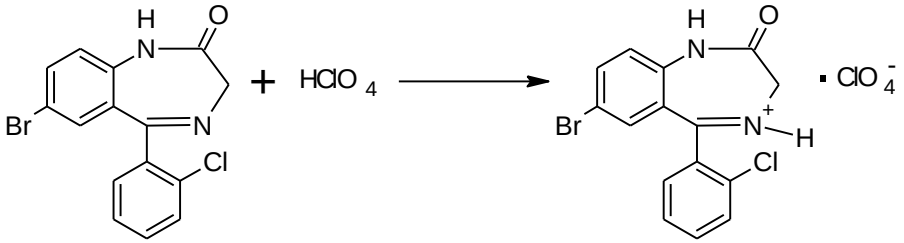
A yellow precipitate forms with picric acid:



QUANTIFICIFICATION

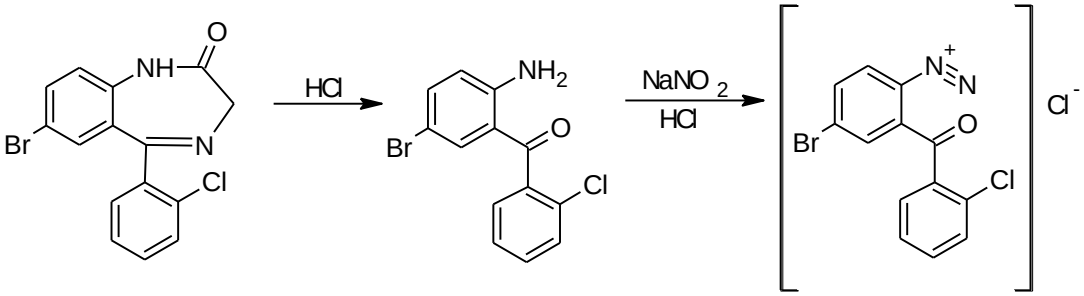
1. Non-aqueous titration

Formic acid combined with acetic anhydride is used as a solvent. The titrant is 0.1 M chloric acid solution. The equivalent point is established using crystal violet indicator or potentiometric method.



2. *Nitrite titration*

The diazotization reaction is the basis for the nitritometric determination of benzodiazepine derivatives:



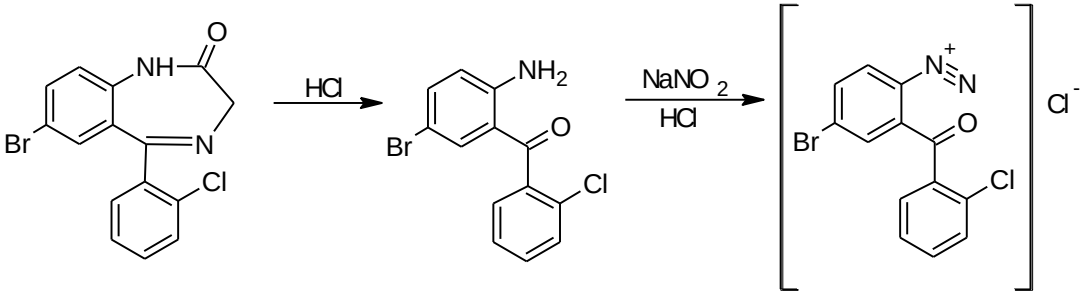
The working solution is sodium nitrite. In direct titration the equivalence point is determined potentiometrically; in reverse titration - iodometrically (indicator - starch):

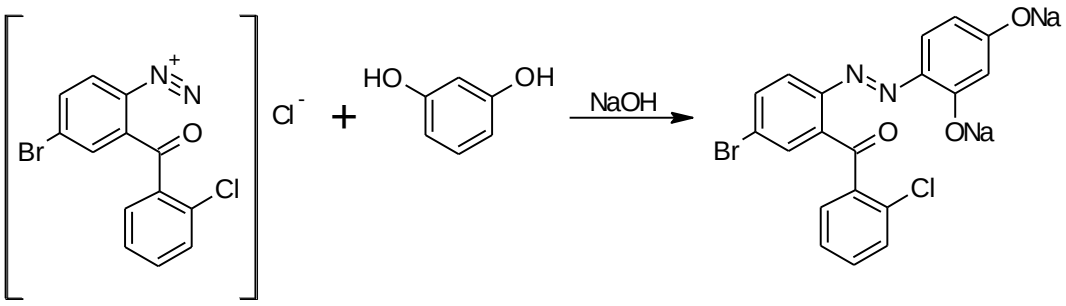
$$NaNO_2 + KI + H_2SO_4 \longrightarrow I_2 + NO + K_2SO_4 + Na_2SO_4 + H_2O$$

$$I_2 + Na_2S_2O_3 \longrightarrow NaI + Na_2S_4O_6$$

3. *Photocolorimetric method*

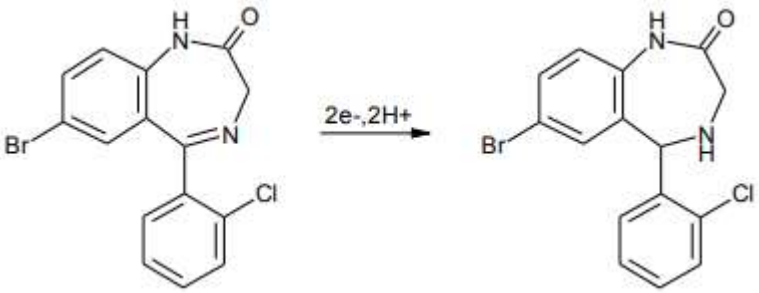
The quantitative content of benzodiazepine derivatives in dosage forms can be determined spectrophotometrically by the intrinsic absorbance of solutions at the indicated absorption maxima, as well as photocolorimetrically using the azo-coupling reaction (after prior hydrolysis and diazotization or other color reactions).





4. Polarographic determination

The ability of phenazepam to reduce can be used for analytical purposes:



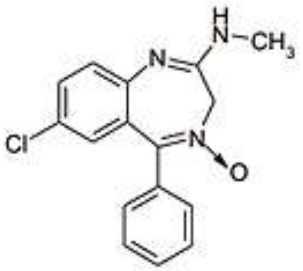
STORAGE

Store in a dry place protected from light.

MEDICAL USE

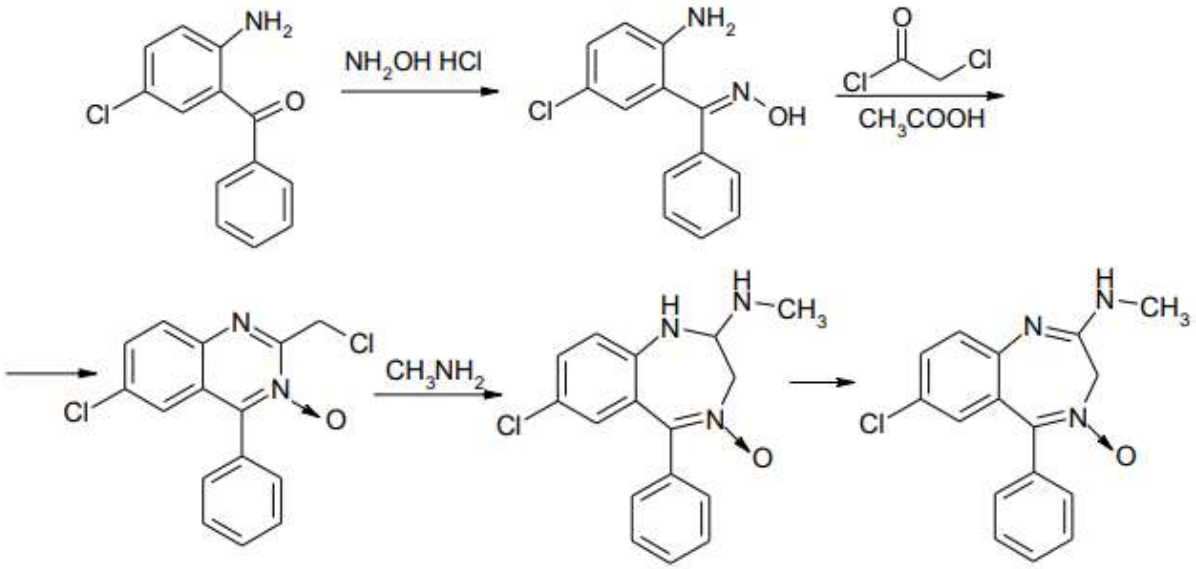
Fenozepam has anxiolytic, soporific, sedative, anticonvulsant, and myorelaxant actions.

CHLORDIAZEPOXIDE



OBTAINING

The synthesis of chlordiazepoxide is somewhat different from other benzodiazepine derivatives. Using the same starting products, it is carried out by first obtaining quinazoline derivative and then chlordiazepoxide from it:



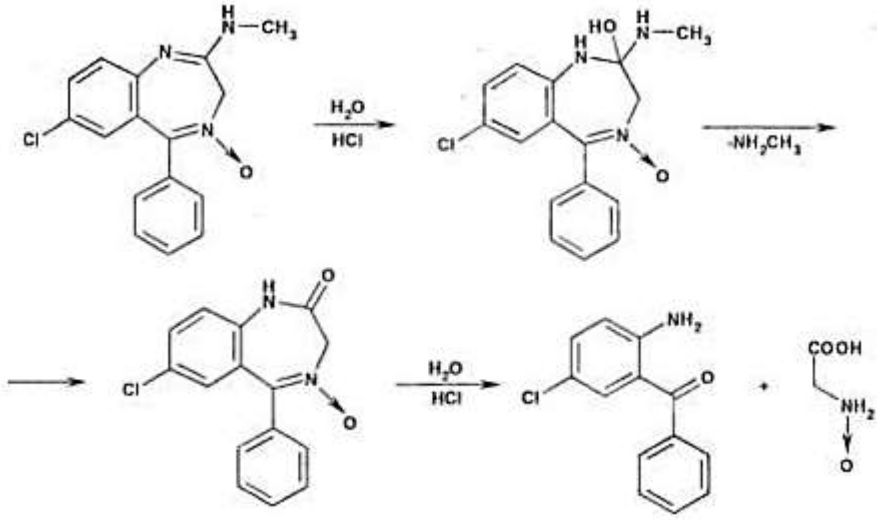
PHYSICAL PROPERTIES

White or light yellow odorless crystalline powder, practically insoluble in water, slightly soluble in ether, ethanol, and chloroform.

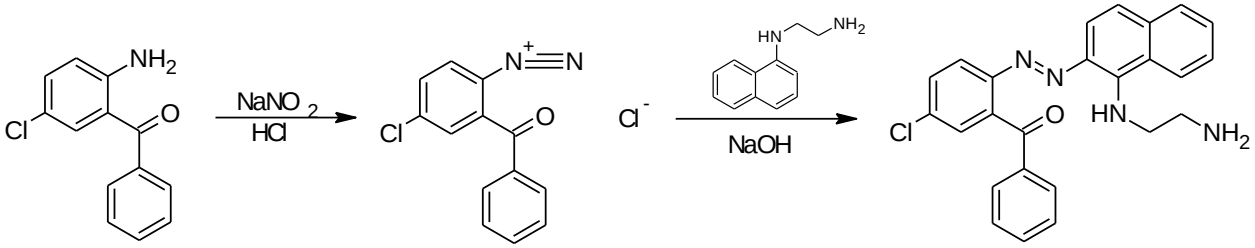
IDENTIFICATION

1. ***UV spectrometry***
2. ***Infrared spectroscopy***
3. ***Formation of azo dye***

   Hydrolysis of chlordiazspoxide on heating to boiling in hydrochloric acid proceeds somewhat differently. At first, a water molecule is attached to the double bond 1-2, then methylamine is detached and an amide bond is formed. The latter is hydrolyzed to give 2-amino-5-chlorobenzophenone:



After cooling the solution, the diazotization and azo-coupling reactions are carried out. When α-naphthyl-ethylenediamine dihydrochloride is used as a reagent, an intense red-violet coloration occurs.



4. *Pyrolysis*

When heating about 0.01 g of drug substance in a dry test tube over a torch flame, a green colored fusion is formed which retains its color after addition of ethanol irrespective of the pH of the medium.

5. *Alloying with alkalis*

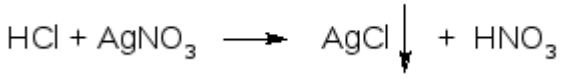
Exposure to alkalis under harsh conditions (fusion with sodium hydroxide) leads to degradation of chlordiazeproxide and release of ammonia from the amide group, detectable with litmus paper.

6. *Beilstein's test*

Organically bound chlorine atom is detected by the Beilstein test. The essence of this test is that a grain of substance introduced on a copper wire into a colorless burner flame, coloring it green. The coloration is due to the formation of volatile copper halides.

7. *Mineralization of halogens*

Chlorine atoms can also be detected by combustion in a flask with oxygen, using sodium hydroxide solution as an absorbing liquid. The resulting solution is then acidified with sulfuric acid and the chloride reaction is performed. A white curd-like precipitate is formed.



8. *Interaction with common alkaloid reagents*

Due to the presence of tertiary nitrogen atoms in the molecules, benzodiazepine derivatives give positive reactions with precipitating (obshealkaloid) reagents (Dragendorf, Bouchard, picric acid), as well as with Reineke's salt - $NH_4[Cr(NCS)_4(NH_3)_2]$.

(Write a reaction with one of them)

QUANTIFICIFICATION

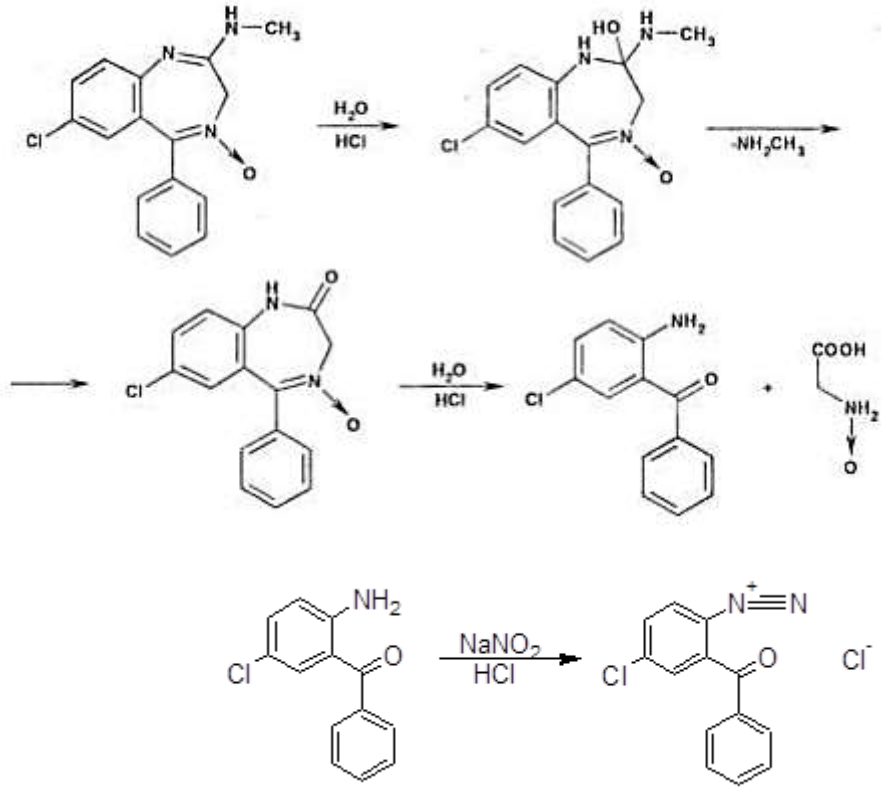
1. *Non-aqueous titration*

Glacial acetic acid is used as solvent. The titrant is 0.1 M chloric acid solution. The equivalent point is established by means of crystal violet indicator or potentiometric method.

(Write a reaction)

2. *Nitrite titration*

The diazotization reaction is the basis for the nitritometric determination of benzodiazepine derivatives:



The working solution is sodium nitrite. In direct titration the equivalence point is determined potentiometrically; in reverse titration - iodometrically (indicator - starch):

$$NaNO_2 + KI + H_2SO_4 \longrightarrow I_2 + NO + K_2SO_4 + Na_2SO_4 + H_2O$$

$$I_2 + Na_2S_2O_3 \longrightarrow NaI + Na_2S_4O_6$$

3. *Photocolorimetric method*

The quantitative content of benzodiazepine derivatives in dosage forms can be determined spectrophotometrically by the intrinsic absorbance of solutions at the indicated absorption maxima, as well as

photocolorimetrically using the azo-coupling reaction (after prior hydrolysis and diazotization or other color reactions).

(Write the reaction for the formation of azo dye)

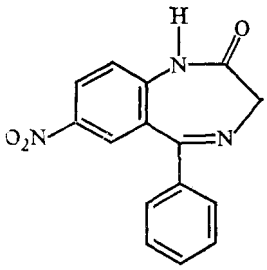
STORAGE

Store in a dry place protected from light.

MEDICAL USE

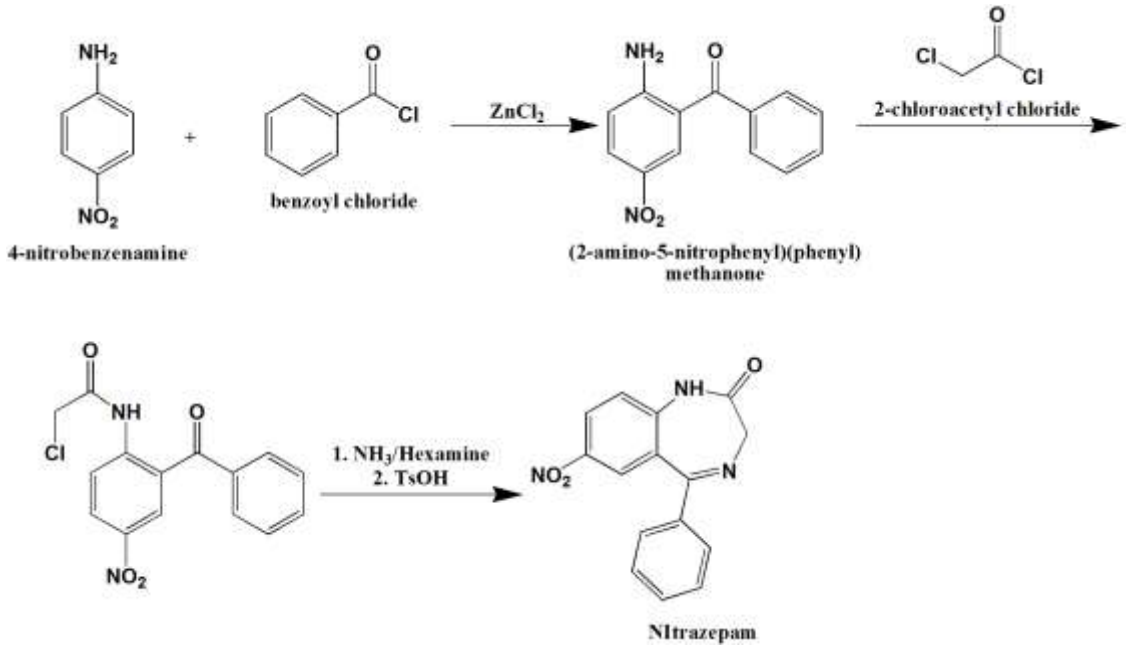
It has anxiolytic, sedative, sleeping, anticonvulsant, anticonvulsant, central myorelaxant effect. Indications for use: Neuroses, sleep disorders, nervous tension, anxiety, irritability, reactive depression, withdrawal syndrome, convulsive state or its threat, NCD, menopausal syndrome, premenstrual tension syndrome, for preparation for surgery.

NITRAZEPAM



OBTAINING

Nitrazepam is synthesized according to the scheme:



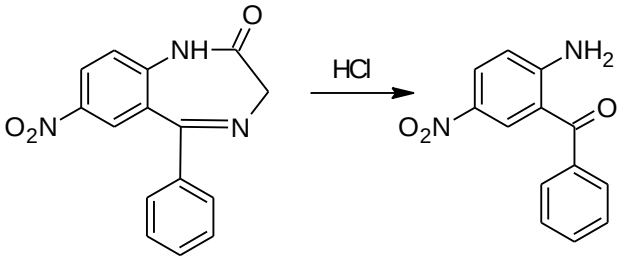
PHYSICAL PROPERTIES

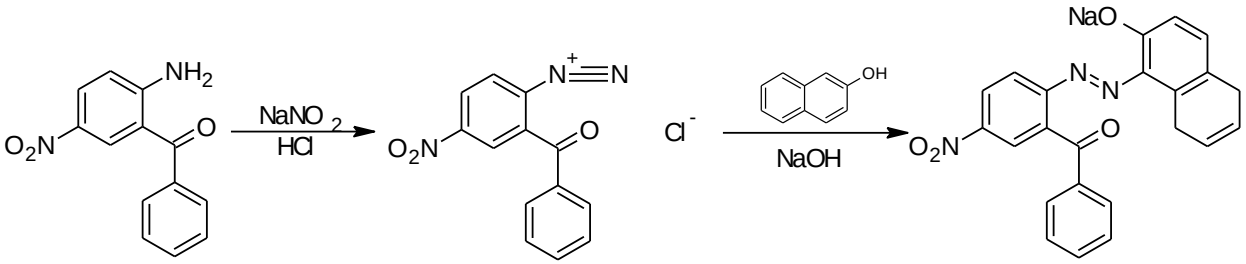
Light yellow or greenish tinged crystalline powder without odor. Melting point is 226- 230 °C. Practically insoluble in water, slightly soluble in ether, ethanol, and chloroform.

IDENTIFICATION

1. ***UV spectrometry***
2. ***Infrared spectroscopy***
3. ***Formation of azo dye***

   Acid hydrolysis of nitrosepam produces 2-amino-5-nitrobenzophenone, which is then diazotized:





   A red coloration appears

4. ***Pyrolysis***

   When heating about 0.01 g of drug substance in a dry test tube over a torch flame, a green colored fusion is formed which retains its color after addition of ethanol irrespective of the pH of the medium.

5. ***Alloying with alkalis***

   Exposure to alkalis under harsh conditions (fusion with sodium hydroxide) leads to degradation of chlordiazeproxide and release of ammonia from the amide group, detectable with litmus paper.

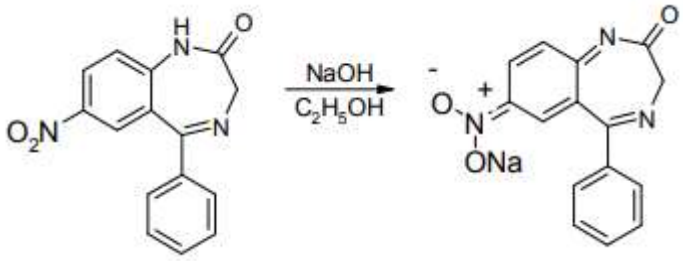
6. ***Interaction with common alkaloid reagents***

   Due to the presence of tertiary nitrogen atoms in the molecules, benzodiazepine derivatives give positive reactions with precipitating

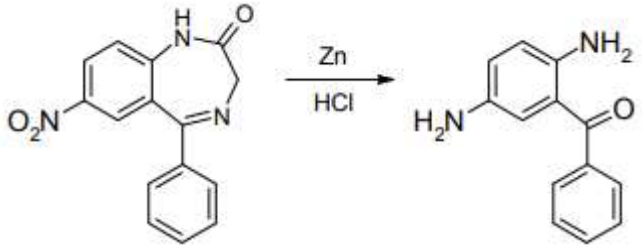
(obshealkaloid) reagents (Dragendorf, Bouchard, picric acid), as well as with Reineke's salt - $NH_4[Cr(NCS)_4(NH_3)_2]$. *(Write a reaction with one of them)*

7. ***Detection of nitrogroup***

- ✓ The nitro group in nitrazepam can be detected by the reaction of its alcoholic solution with sodium hydroxide solution. A yellow coloration appears, caused by the formation of acisole:



- ✓ Nitrazepam can also be hydrogenated with zinc dust in the presence of hydrochloric acid. Hydrolysis and hydrogenation of the aromatic nitro group to an amino group to form 2,5-diaminobenzophenone occurs:

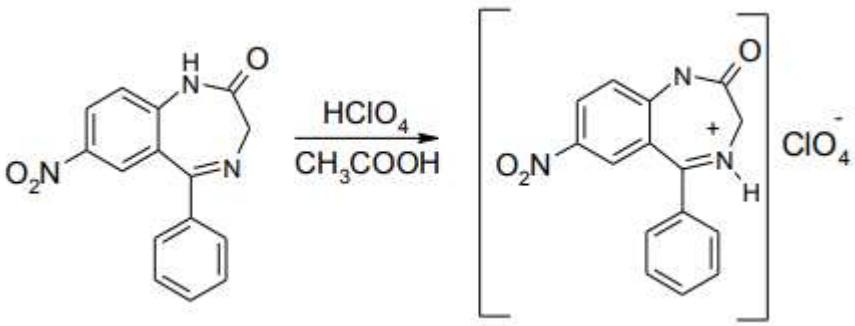


Both amino groups with a diazo reagent form a bis-azo compound (dark red coloring) *(write reaction).*

QUANTIFICIFICATION

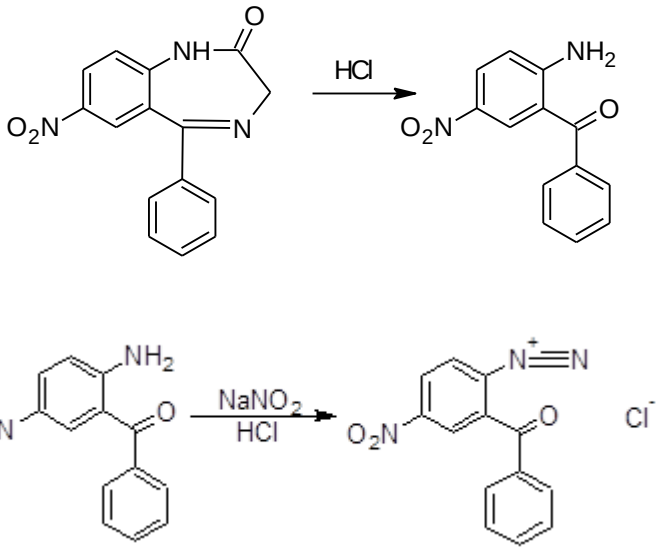
1. ***Non-aqueous titration***

Nitrazepam is dissolved in acetic anhydride. The titrant is 0.1 M chloric acid solution. The equivalent point is established using crystal violet indicator or potentiometric method.



2. *Nitrite titration*

The diazotization reaction is the basis for the nitritometric determination of benzodiazepine derivatives:



The working solution is sodium nitrite. In direct titration the equivalence point is determined potentiometrically; in reverse titration - iodometrically (indicator - starch):

$$NaNO_2 + KI + H_2SO_4 \longrightarrow I_2 + NO + K_2SO_4 + Na_2SO_4 + H_2O$$

$$I_2 + Na_2S_2O_3 \longrightarrow NaI + Na_2S_4O_6$$

3. *Photocolorimetric method*

The quantitative content of benzodiazepine derivatives in dosage forms can be determined spectrophotometrically by the intrinsic absorbance of solutions at the indicated absorption maxima, as well as photocolorimetrically using the azo-coupling reaction (after prior hydrolysis and diazotization or other color reactions).

(Write the reaction for the formation of azo dye)

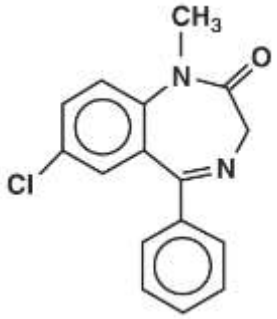
STORAGE

Store in a dry place protected from light.

MEDICAL USE

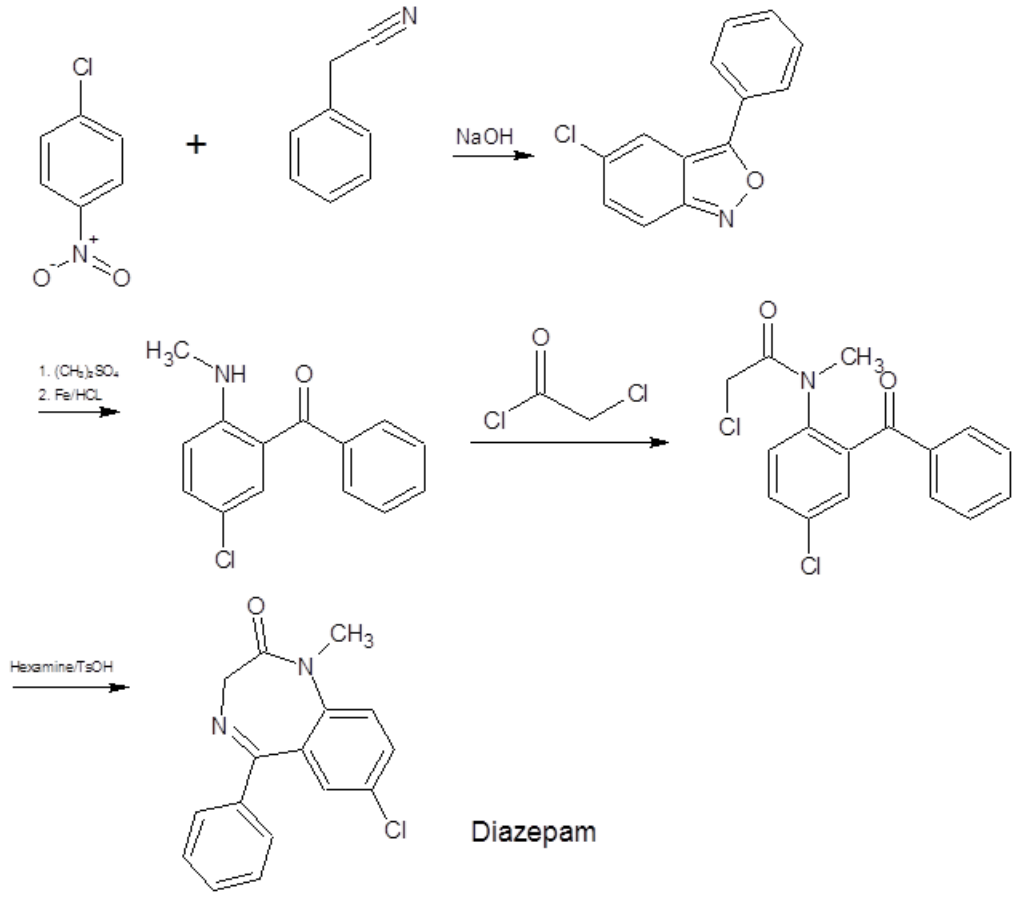
It has a pronounced soporific effect, as well as anxiolytic, sedative, anticonvulsant and central myorelaxant effects.

DIAZEPAM (SIBAZON)



OBTAINING

Diazepam is synthesized according to the scheme:



PHYSICAL PROPERTIES

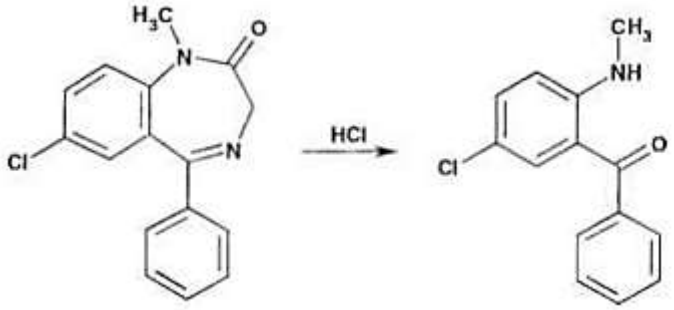
White or with light yellow tinge, odorless fine crystalline powder, practically insoluble in water, slightly soluble in ether, ethanol, easily soluble in chloroform.

IDENTIFICATION

1. ***UV spectrometry***
2. ***Infrared spectroscopy***

3. *Hydrolysis*

As a result of hydrolysis, diazepam forms 2-methylamino-5-chlorobenzo-phenone, which has a yellow color:



Further, in contrast to other benzodiazepine derivatives, diazepam does not form azo dyes.

4. *Pyrolysis*

When heating about 0.01 g of drug substance in a dry test tube over a torch flame, a green colored fusion is formed which retains its color after addition of ethanol irrespective of the pH of the medium.

5. *Alloying with alkalis*

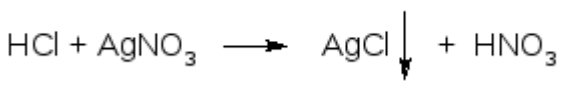
Exposure to alkalis under harsh conditions (fusion with sodium hydroxide) leads to the destruction of the diazepam molecule and the release of methylamine from the amide group, detectable with litmus paper.

6. *Beilstein's test*

Organically bound chlorine atom is detected by the Beilstein test. The essence of this test is that a grain of substance introduced on a copper wire into a colorless burner flame, coloring it green. The coloration is due to the formation of volatile copper halides.

7. *Mineralization of halogens*

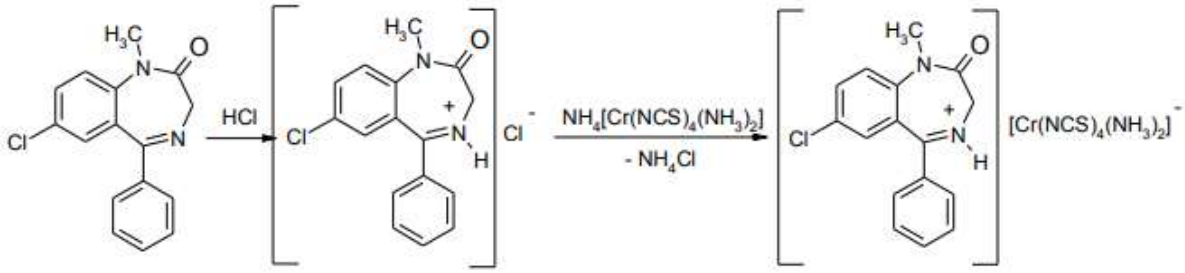
Chlorine atoms can also be detected by combustion in a flask with oxygen, using sodium hydroxide solution as an absorbing liquid. The resulting solution is then acidified with sulfuric acid and the chloride reaction is performed. A white curd-like precipitate is formed.



8. *Interaction with common alkaloid reagents*

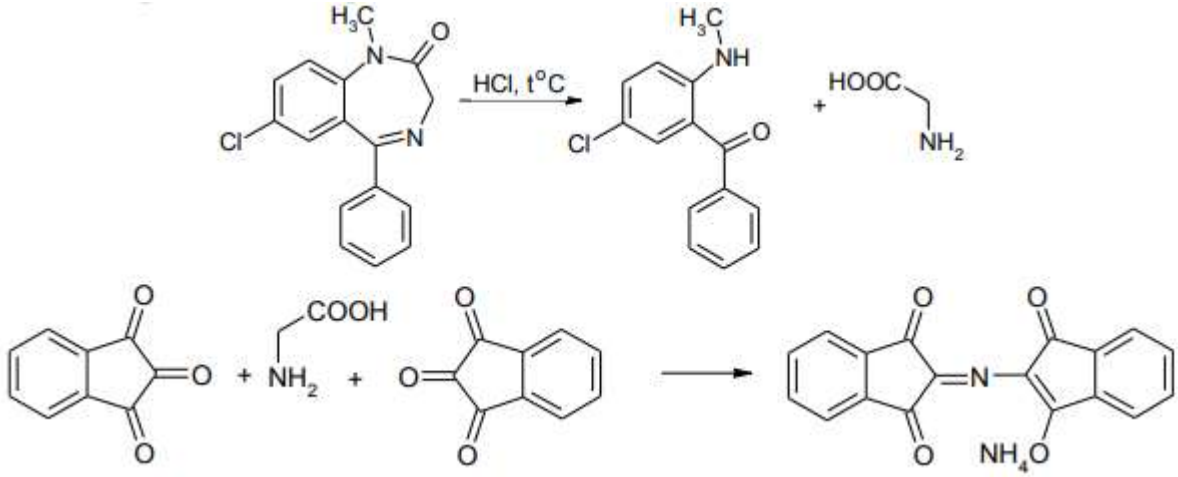
Due to the presence of tertiary nitrogen atoms in the molecules, benzodiazepine derivatives give positive reactions with precipitating (obshealkaloid) reagents (Dragendorf, Bouchard, picric acid), as well as with Reineke's salt - $NH_4[Cr(NCS)_4(NH_3)_2]$.

For example, from a solution of diazepam in dilute hydrochloric acid with the addition of ammonium reynecate precipitates pink precipitate, soluble in acetone.



9. Reaction with ninhydrin

The authenticity of diazepam can be established by color reaction with ninhydrin. When boiling a mixture of this reagent with diazepam and ethanol, a light blue coloration appears, changing to red or orange-red after the addition of copper sulfate solution.



QUANTIFICIFICATION

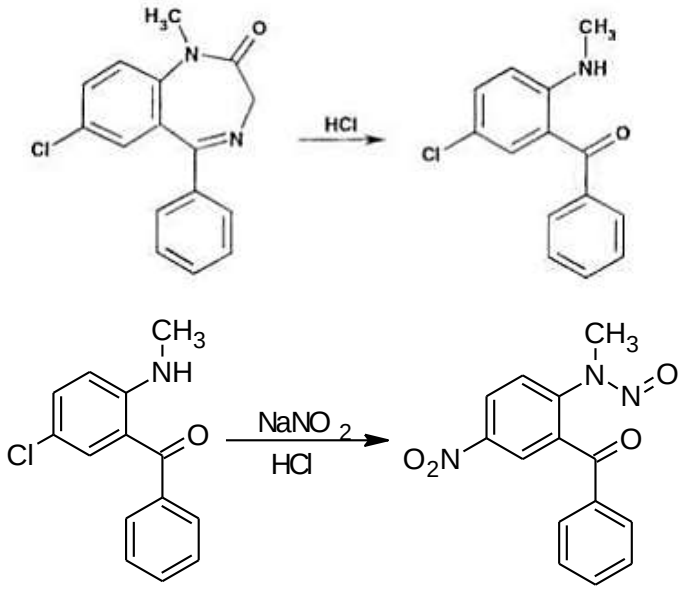
1. Non-aqueous titration

Diazepam is dissolved in acetic anhydride. The titrant is 0.1 m chloric acid solution. The equivalent point is established using crystal violet indicator or potentiometric method.

(write reaction).

2. Nitrite titration

The nitrosation reaction is the basis for the nitritometric determination of diazepam:



The working solution is sodium nitrite. In direct titration the equivalence point is determined potentiometrically; in reverse titration - iodometrically (indicator - starch):

$$NaNO_2 + KI + H_2SO_4 \longrightarrow I_2 + NO + K_2SO_4 + Na_2SO_4 + H_2O$$

$$I_2 + Na_2S_2O_3 \longrightarrow NaI + Na_2S_4O_6$$

3. Photocolorimetric method

The quantitative content of benzodiazepine derivatives in dosage forms can be determined spectrophotometrically by the intrinsic absorbance of solutions at the indicated absorption maxima, as well as photocolorimetrically using the color reactions.

STORAGE

Store in a dry place protected from light.

MEDICAL USE

It has a pronounced anxiolytic and anticonvulsant effect; somewhat weaker is the soporific and central myorelaxant effect.