Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical, Toxicological Chemistry
Pharmacognosy and Botany

SPECIAL PHARMACEUTICAL CHEMISTRY

Cephalosporins

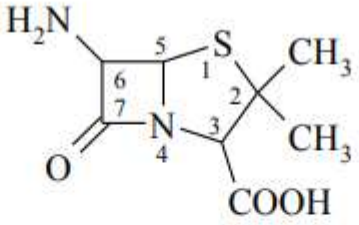
Lesson 11

IX term

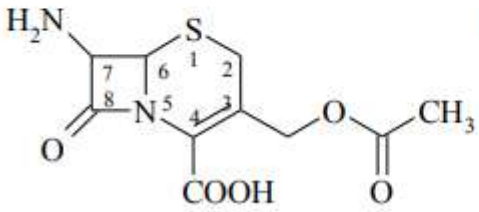
Volgograd, 2024

General characteristics

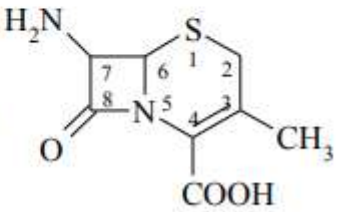
Cephalosporins are similar in chemical structure to penicillins. Whereas penicillins are acylated derivatives of 6-aminopenicillanic acid (6-APA), cephalosporins are - derivatives of 7-aminocephalosporanic acid (7-ACA) and 7- amino-deacetoxycephalosporanic acid (7-ADCA):



6-APA

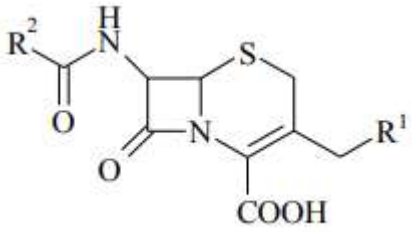


7-ACA



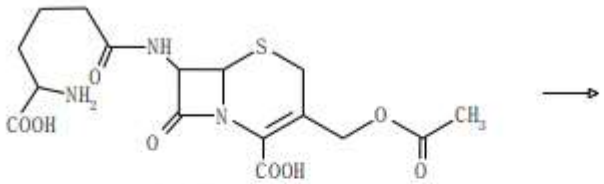
7-ADCA

The biosynthesis of cephalosporins and penicillins is similar to each other. The structural basis of cephalosporins is a condensed system including dihydrothiazine and β-lactam cycles:

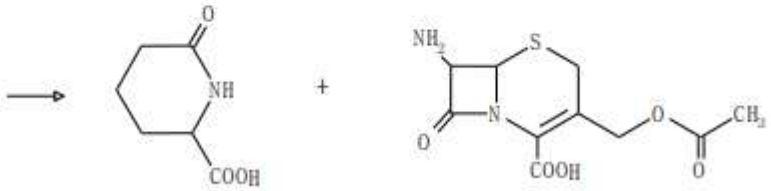


A large number of semisynthetic cephalosporins have been synthesised by modification of residues at positions 3 and 7. The antibiotic activity of cephalosporins is thought to be due to the presence of the β lactam cycle, the inductive effect of the acyl substituent and the steric effect of the molecule.

The source for obtaining semisynthetic cephalosporins is natural cephalosporin C. Cephalosporin C was isolated in 1945 from the products of the mould fungus *Cephalosporium salmosynnematum*. Due to its relatively low activity, cephalosporin C has not found independent application, but it is of interest as a source for obtaining 7-ACA on the basis of intramolecular aminolysis:

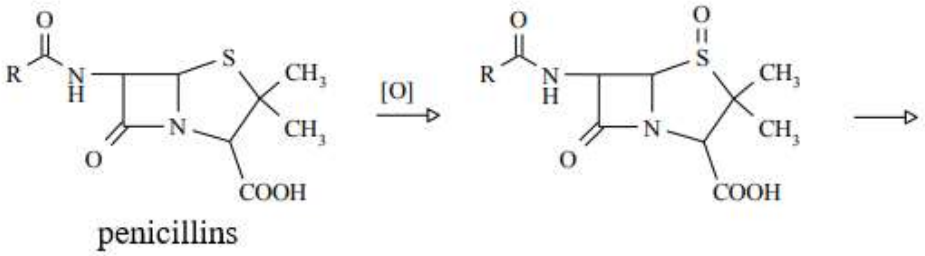


Cephalosporin C

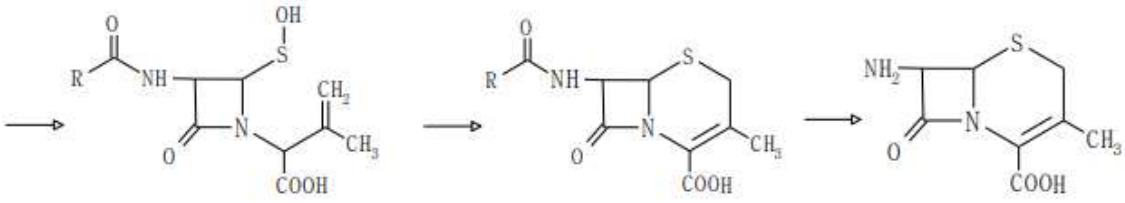


7-ACA

7-ADCA can be obtained by chemical transformation of penicillins:

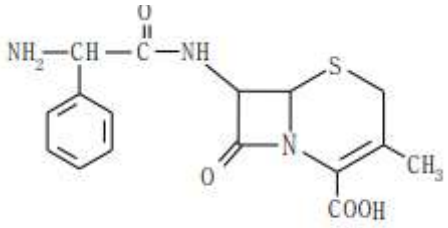


penicillins



7-ADCA

CEPHALEXIN



PHYSICAL PROPERTIES

White or slightly yellowish powder with a characteristic odour. Practically insoluble in chloroform and ether, hardly soluble in water and practically insoluble in ethanol.

IDENTIFICATION

Spectral methods

1. ***UV spectroscopy*.** The presence of conjugated double bonds in the molecule of cephalosporins causes a characteristic absorption band in UV-spectra with absorption maximum in the region of 260 nm. In addition, cephalexin has another chromophore - phenyl radical.
2. ***Infrared spectroscopy***. Objective conclusion about the identity of cephalosporin antibiotics can be made by IR spectra in the region of 4000 - 400 cm^{-1}. With their help it is possible to establish the absence of acetoxy group in the C3 side chain of dihydrothiazine cycle and confirm the attribution of the tested drug to the number of 7-ADCA derivatives (cephalexin). The bands in the vibrational region of carbonyl groups (1800-1500 cm^{-1}) and carboxyl group (1620-1600 cm^{-1}) are common for all cephalosporins. In the region of higher frequencies (3500-2500 cm^{-1}), caused by valence vibrations of amino and amido groups, the IR spectra have significant differences. The most specific spectral curves of cephalosporin antibiotics are located in the "fingerprint" region (1500-650 cm^{-1}).
3. ***NMR spectroscopy***. The method of proton NMR spectroscopy using dimethyl sulfoxide as a solvent proved to be promising for the identification of cephalosporins. The signals of protons of β-lactam and dihydrothiazine cycles were isolated, according to which the analyzed compounds were attributed to cephalosporin antibiotics. In addition, the method allows us to reliably identify each of the cephalosporin antibiotics by the characteristic signals of the protons of acyl residues in the side chain of the β-lactam fragment and radicals in position 3 of the dihydrothiazine cycle.

Chemical Methods.

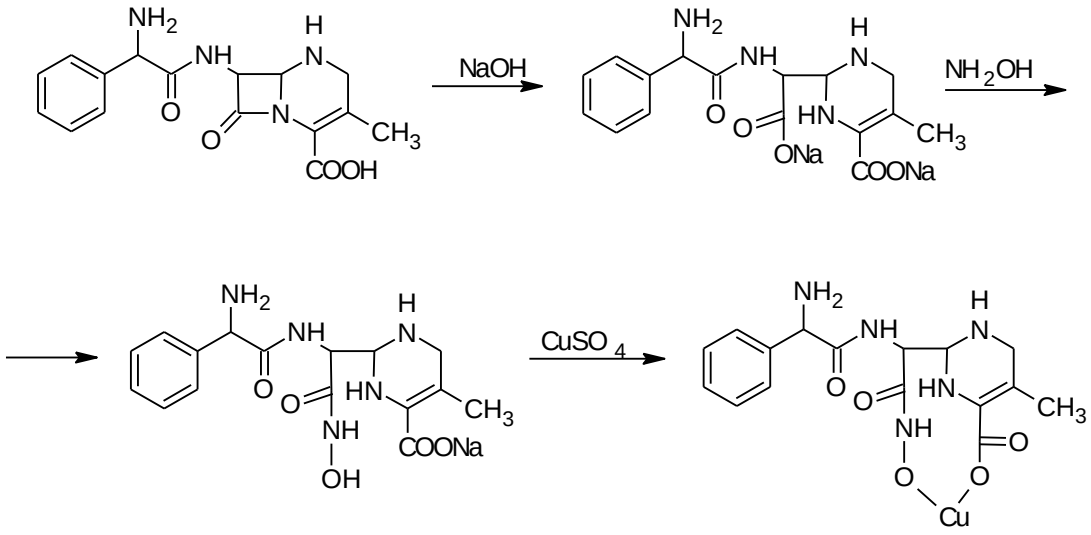
General chemical properties of cephalexin and cephalotin are due to the presence of sulfur atoms in the dihydrothiazine ring (oxidation ability) and β-lactam ring (hydroxamic reaction) in the composition of the molecules.

1. ***Oxidation***

   Cephalexin turns yellow when exposed to a mixture of 80% sulphuric acid and 1% nitric acid.

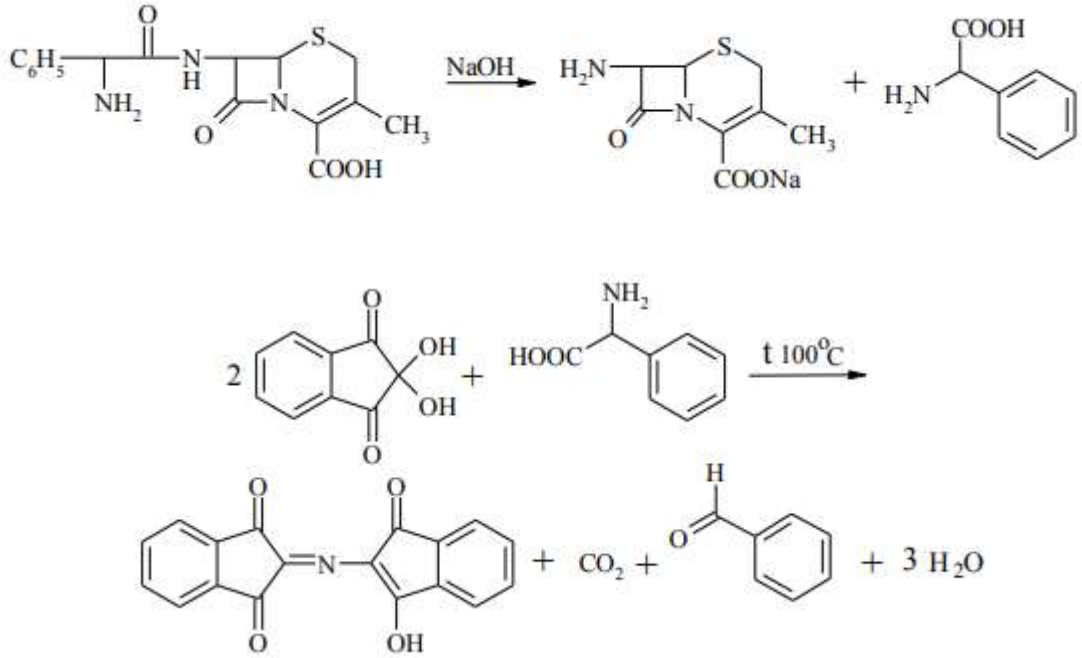
2. ***Hydroxamic reaction***

   The presence of β-lactam cycle in cephalosporin preparations, and in cephalotin also a complex ester group, determines the hydroxamic reaction. Colored copper (II) and iron (III) hydroxamates are formed.



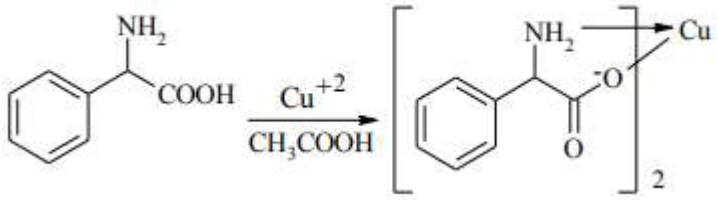
3. *Reaction with ninhydrin*

Cephalexin is characterised by the presence of an aliphatic amino acid residue in the molecule, which can be detected by ninhydrin.



4. *Complexation*

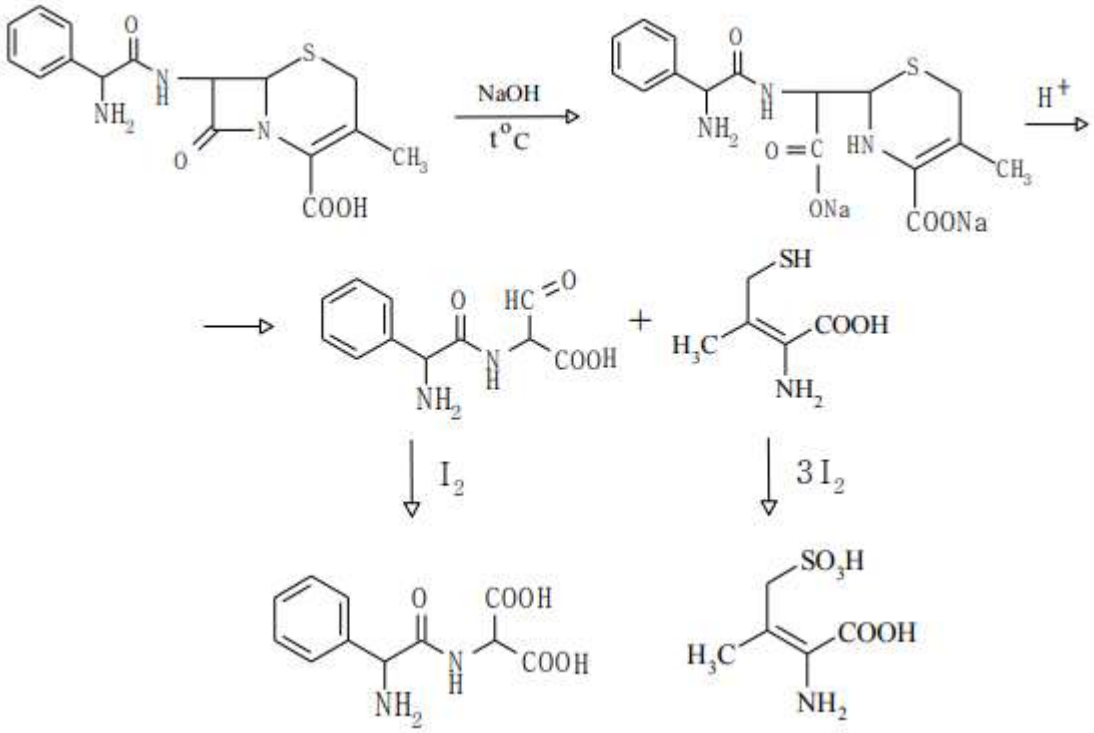
The amino acid residue makes it possible to identify cephalexin by complexation reaction with copper (II) ion in acetic acid medium. After addition of sodium hydroxide solution an olive green coloration appears.



ASSAY

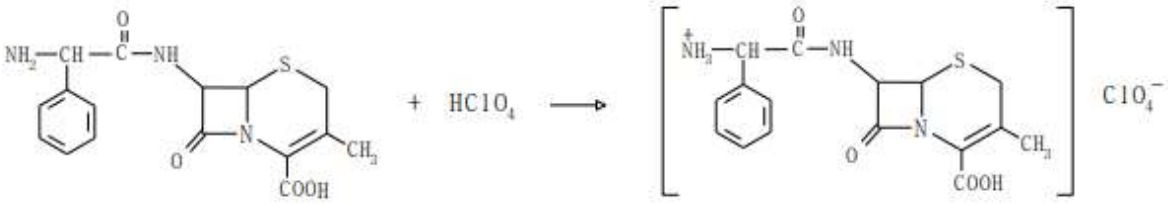
1. *Iodometric determination*

The quantitative determination of cephalexin is based on preliminary alkaline hydrolysis to 7-ADCA. The hydrolysis products are then oxidised with titrated iodine solution. In parallel, a standard sample of the drug is analysed under the same conditions.



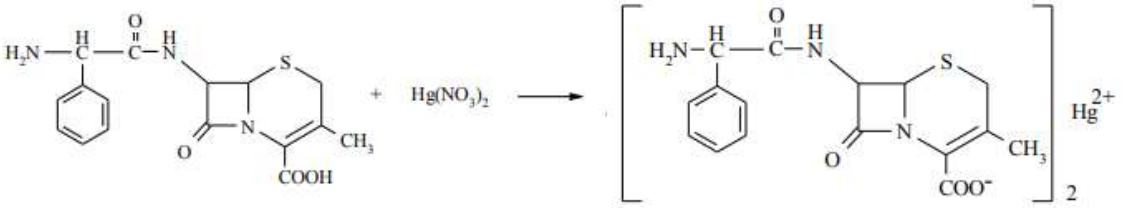
2. *Non-aqueous titration*

Cephalexin in solution exhibits amphoteric properties due to the presence of carboxyl and amino groups in the molecule. Therefore, quantitative determination can be performed by non-aqueous titration using formic acid and glacial acetic acid mixed with acetone as solvents. This enhances the basic properties of cephalexin and it is titrated with a solution of perchloric acid in dioxane:

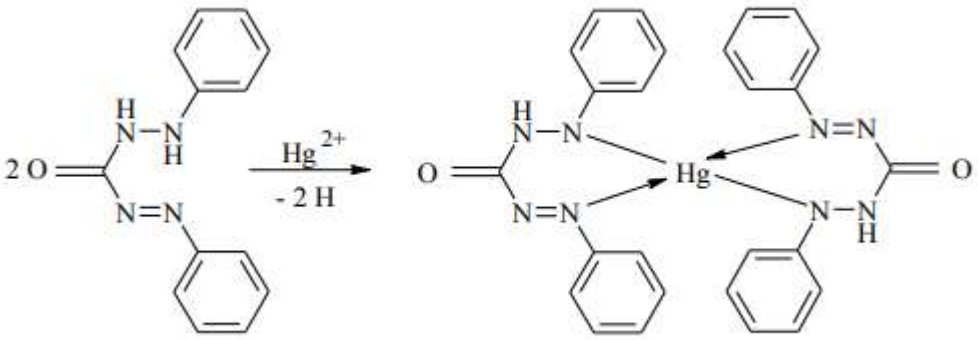


3. *Mercurimetric titration*

The mercurimetric method is also used for the quantitative determination of cephalosporin antibiotics. This method is based on the formation of low-dissociated compounds of mercury (II). The working solution is mercury (II) nitrate.



At the equivalence point, the excess of free Hg2+ titrant ions is detected potentiometrically or with the indicator diphenylcarbazone, which forms a blue complex with mercury ions (at the equivalence point):



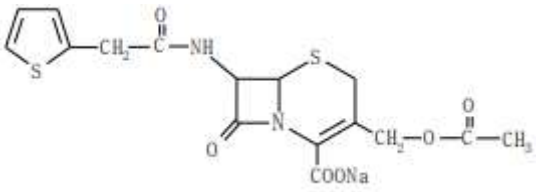
4. *Spectrophotometric determination*

The method of spectrophotometric determination of cephalexin based on the formation of colored complexes with copper (II) ion and change of light absorption at pH 6.55 in the region of 640-675 nm is described. The reagent is copper (II) acetate

STORAGE

Cephalexin is stored in a dry place protected from light at room temperature. Cephalexin is available in 0.25 g capsules

CEFALOTHIN SODIUM



Physical properties

White or almost white crystalline powder. Sensitive to light. Practically insoluble in chloroform and ether, readily soluble in water, 0.9% sodium chloride solution, 5% glucose solution and sparingly soluble in ethanol.

IDENTIFICATION

Spectral methods

1. ***UV spectroscopy***

   The presence of conjugated double bonds in the molecule of cephalosporins causes a characteristic absorption band in UV spectra with an absorption maximum at 260 nm. To test the identity of cefalotin sodium, the presence of absorption maximum at 237 nm in the UV-spectrum of aqueous solution is established, as well as the "shoulder" at 265 nm, the presence of which is associated with the cyclic system of 7- aminocephalosporanic acid (7-ACA). According to the requirements of the pharmacopoeia 0.002 % solution of cefalothin sodium at a wavelength of 237 nm in a cuvette with a working length of 10 mm should have an optical density of not less than 0.65 and not more than 0.72 (reference solution - water).

2. ***Infrared spectroscopy***

   Objective conclusion about the identity of cephalosporin antibiotics can be made by IR spectra in the region of 4000 - 400 cm^{-1}. They can be used to determine the presence of an acetoxy group in the C3 side chain of the dihydrothiazine cycle and confirm the attribution of the tested drug to the 7-ACA derivatives (cephalothin sodium salt). The bands in the vibrational region of carbonyl groups (1800-1500 cm^{-1}) and carboxyl group (1620-1600 cm^{-1}) are common for all cephalosporins. In the region of higher frequencies (3500-2500 cm^{-1}), caused by valence vibrations of amino and amido groups, the IR spectra have significant differences. The most specific spectral curves of cephalosporin antibiotics are located in the "fingerprint" region (1500-650 cm^{-1}).

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   The method of proton NMR spectroscopy using dimethyl sulfoxide as a solvent proved to be promising for the identification of cephalosporins. The proton signals of β-lactam and dihydrothiazine cycles were isolated, which were used to assign the analyzed compounds to cephalosporin antibiotics. In

addition, the method allows us to reliably identify each of the cephalosporin antibiotics by the characteristic signals of the protons of acyl residues in the side chain of the β-lactam fragment and radicals in position 3 of the dihydrothiazine cycle.

Chemical Methods

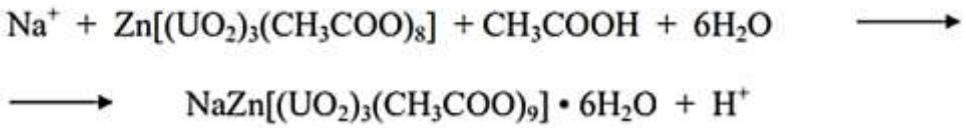
General chemical properties of cephalexin and cephalotin are due to the presence of sulfur atoms in the dihydrothiazine ring (oxidation ability) and β-lactam ring (hydroxamic reaction) in the composition of the molecules.

1. ***Oxidation***

   When exposed to a mixture of 80% sulfuric acid solution and 1% nitric acid solution of cephalothin sodium salt turns olive green, changing to red-brown.

2. ***The discovery of sodium***

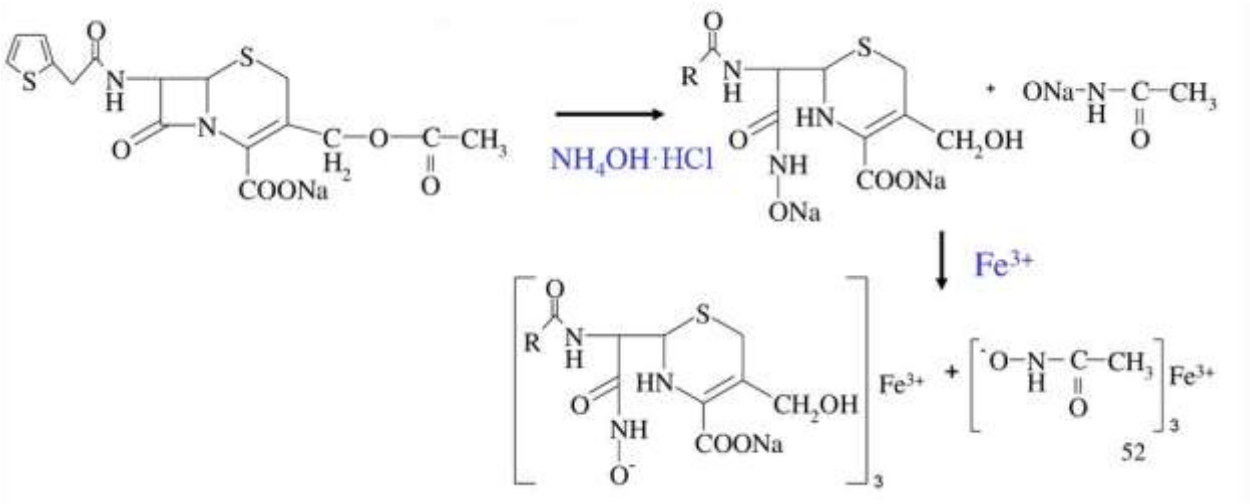
   a) Sodium salts form a yellow crystalline precipitate ***with zinc uranyl acetate***. The precipitate is insoluble in acetic acid.



   b) sodium salt introduced into a colorless flame turns it yellow.

3. ***Hydroxamic reaction***

   The presence of β-lactam cycle in cephalosporin preparations, and in cephalotin also a complex ester group, determines the hydroxamic reaction. Colored hydroxamates of copper (II) and iron (III) are formed.



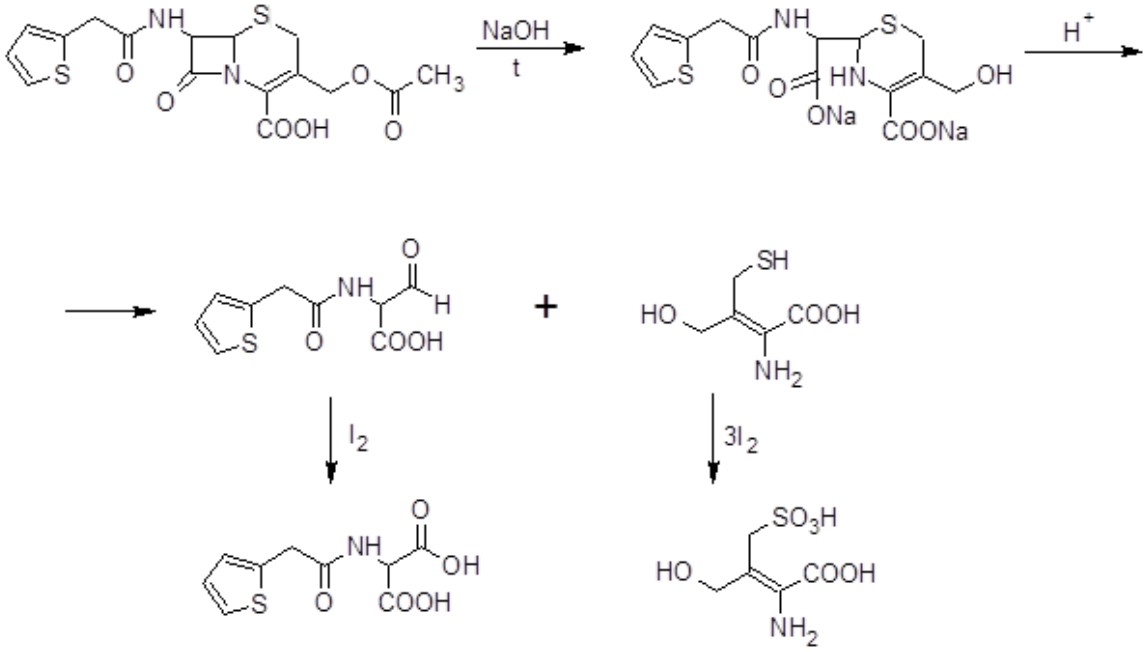
PURITY TEST

The presence of specific impurities in cephalothin sodium salt (not more than 5 %) is determined by GC method by the sum of areas of all peaks on the chromatogram, except for the peak of the main substance. The same method determines the residual content of solvents: ether and 1-propanol.

ASSAY

1. *Iodometric determination*

Quantitative determination of cephalothin sodium salt is based on preliminary alkaline hydrolysis to form 7-ACA. The hydrolysis products are then oxidized with titrated iodine solution. In parallel, a standard sample of the drug is analyzed under the same conditions.



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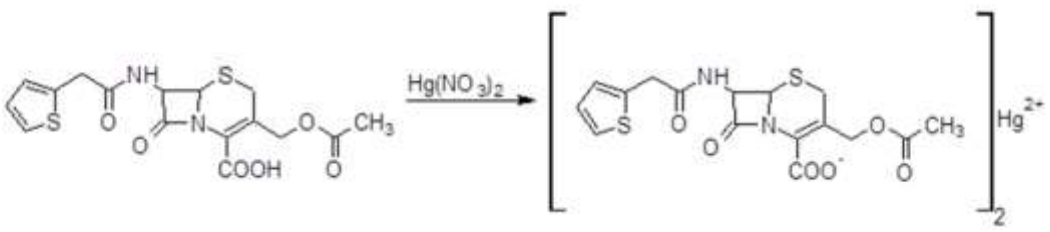

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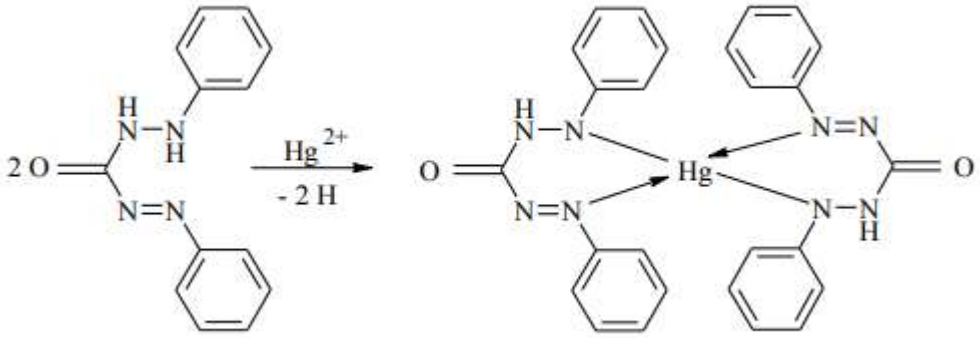

3. Spectrophotometric determination

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STORAGE

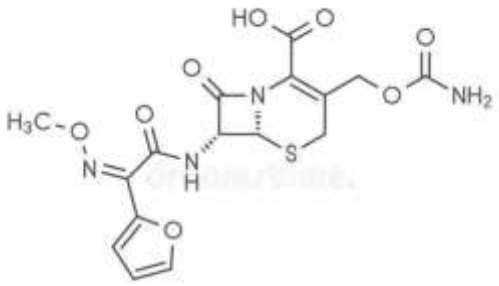
Cefalothin natrium is stored in a dry place protected from light at room temperature.

MEDICAL USE

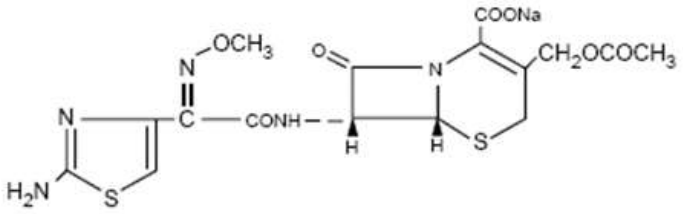
Cephalosporins have a broader spectrum of antibacterial action than penicillins against Gram-positive and Gram-negative cocci, spirochetes, sibiria bacilli. Sodium salts of cephalosporins are used intramuscularly, less often intravenously and intrapleural in acute and chronic diseases of respiratory organs, urinary tract, genital organs, postoperative and other infections. A number of cephalosporins (cephalexin) are effective when administered orally.

There are five generations of cephalosporins, each with a broader spectrum of antimicrobial activity than the previous one:

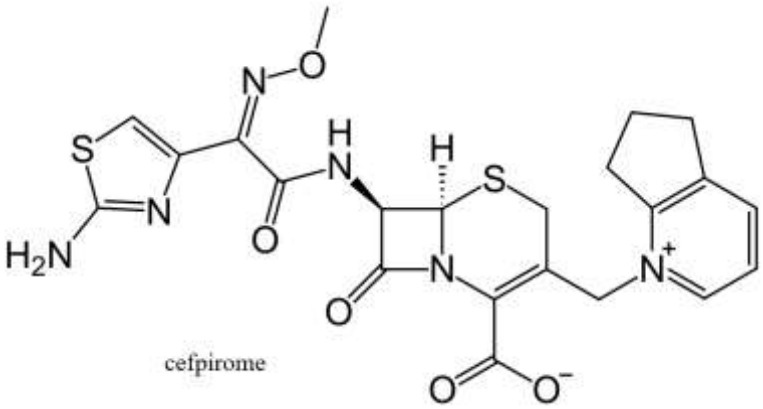
- ✓ I generation: cefazolin, cephalexin;
- ✓ II generation: cefuroxime, cefaclor;
- ✓ III generation: ceftriaxone, cefixime, cefatoxime, etc.;
- ✓ IV generation: cefepime, cefpirome;
- ✓ V generation: ceftaroline, ceftobiprole.



cefuroxime

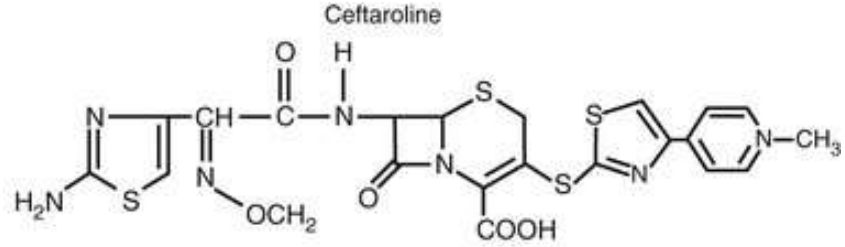


Claforan (Cefotaxime)



cefpirome

Ceftaroline



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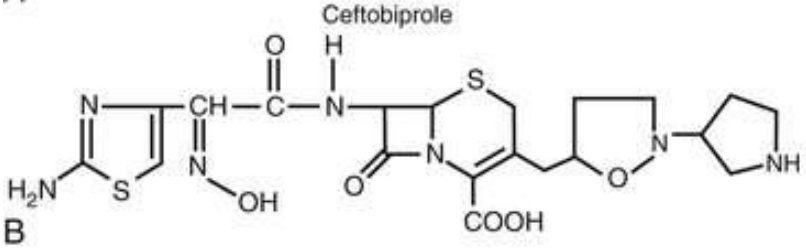
Ceftobiprole



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