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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF AZLOCILLIN IN PHARMACEUTICAL FLUID

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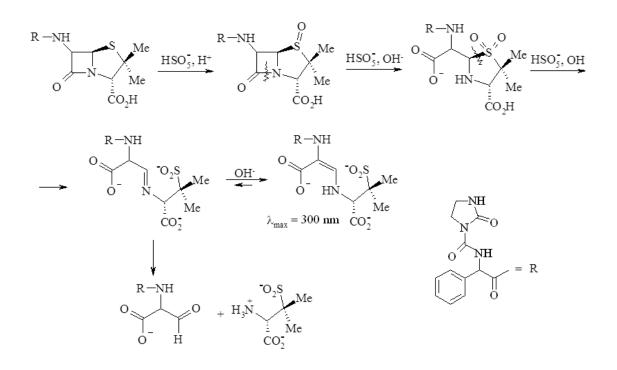
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Lyme disease is one of most common vector-borne diseases, reporting more than 300,000 cases annually in the United States. Treating Lyme disease during its initial stages with traditional tetracycline antibiotics is effective. However, 10-20% of patients treated with antibiotic therapy still shows prolonged symptoms of fatigue, musculoskeletal pain, and perceived cognitive impairment. When these symptoms persists for more than 6 months to years after completing conventional antibiotics treatment are called post-treatment Lyme disease syndrome (PTLDS). Though the exact reason for the prolongation of post treatment symptoms are not known, the growing evidence from recent studies suggests it might be due to the existence of drugtolerant persisters. In order to identify effective drug molecules that kill drugtolerant borrelia we have tested two antibiotics, azlocillin (Azl) and cefotaxime that were identified by us earlier. The in vitro efficacy studies of Azl and cefotaxime on drug-tolerant persisters were done by semisolid plating method. The results obtained were compared with one of the currently prescribed antibiotic doxycycline. We found that Azl completely kills late log phase and 7-10 days old stationary phase B. burgdorferi. Our results also demonstrate that azlocillin and cefotaxime can effectively kill in vitro doxycycline-tolerant B. burgdorferi. Moreover, the combination drug treatment of Azl and cefotaxime effectively killed doxycycline-tolerant B. burgdorferi. Furthermore, when tested in vivo, Azl has shown good efficacy against B. burgdorferi in mice model. These seminal findings strongly suggests that Azl can be effective in treating B. burgdorferi sensu stricto JLB31 infection and furthermore in depth research is necessary to evaluate its potential use for Lyme disease therapy [1].

(2S,5R,6R)-3,3-dimethyl-7-oxo-6-{[(2R)-2-{[(2-oxoimidazolidin-1yl)carbonyl]amino}-2-phenylacetyl]amino}-4-thia-1-azabicyclo[3.2.0]heptane-2carboxylic acid (Azl) belongs to the ureidopenicillin class and it is used for the treatment of serious infections caused by susceptible strains of microorganisms [2]. Literature review revealed enormous analytical method were reported for the estimation of azlocillin individually or in combination with other drugs [3]. International Pharmacopoeia recommends to determine penicillin summary in semisynthetic penicillin by neutralization method after preparation hydrolysis by excess of sodium hydroxide titrated solution at heating [4].



The scheme of peroxo acid oxidation and perhydrolysis conjugated reactions of Azl on the time are shown on Fig.

The following quantitative procedures of penicillin determination are described: using potentiometry titration and ionometry, spectrophotometry, extraction photometry, voltammetry and polarography, micelle electrokinetic capillary and paper chromatography, chemiluminescense and kinetic analysis methods [5-8].

A new procedure for the quantitative determination of Azl sodium in the Azlocillinum[®] preparation by the method of back spectrophotometric method using potassium peroxomonosulfate (KHSO₅) as an analytical reagent was developed [9].

Thus, the improvement of the known and development of new methods of quantitative determination of penicillin is rather important. The existing pharmacopoeial methods of penicillin preparations determination are quite complex, long-lasting and require the application complex and expensive devices. The disadvantage of the known simple enough in performance methods of spectrophotometric determination of penicillin, which are based on the determination of the final products of their hydrolytic cleavage, is the requirement of prolonged heating.

The developed method of Azl kinetic determination has several advantages: makes it possible to identify the preparation in much smaller quantities than the parmacopoeial iodometric method, it is applicable to the same range of concentrations, as in photometric determination of hydrolysis products, but it doesn't require prolonged heating of the reaction mixture, it is simpler and faster than the method of chromatographic analysis.

The reaction kinetics of the peroxyacidic oxidation and perhydrolysis of Azl with potassium caroate in the alkaline medium is studied. As an oxidizing agent, the potassium triple salt of peroxymonosulfuric acid, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, syn. "Oxone", was applied. The procedure was developed and the possibility of the quantitative determination of Azl in the Azlocillinum[®] preparation based on the results of the kinetic-spectrophotometric method with potassium caroate as reagent was shown. RSD = 2.0 %, $\delta = +1.1 \%$.

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