Development of spectrophotometric method for determination of lysozyme hydrochloride by specific absorbance

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Lysozyme is a natural component of many biological fluids (saliva, tears, serum, amniotic fluid, breast milk, sputum, gastric juice, as well as in the tissues of the heart, spleen, lymph nodes, etc.) and is an important integral part of non-specific and specific human immunity. There are lysozymes of different origins. More than 50 lysozymes from different sources have been isolated and studied. In pharmacy, lysozyme is used to treat chronic septic conditions and purulent processes, burns, frostbite, conjunctivitis, corneal erosions, stomatitis and other infectious diseases. A number of studies are devoted to the development and application of biotechnological approaches to the immobilization of lysozyme in polymers of synthetic and natural origin, in order to create new mucoadhesive systems with antibacterial action. Therefore, the development of methods for quality control of lysozyme in the dosage forms is quite relevant.

The aim of the study is to develop a spectrophotometric method for the identification of lysozyme hydrochloride by the method of specific absorbance in the dosage forms.

Two subtances of lysozyme hydrochloride Bouwhuis Enthoven B.V., Netherlands, C.N15K30 and C.N10316044, as well as Class A measuring vessels and reagents that meet the requirements of the State Pharmacopoeia of Ukraine were used in the study. Determination of physicochemical characteristics was performed using analytical scales AXIS ANG200, ionometer / pH meter Mettler Toledo S220, spectrophotometer EVOLUTION 60S Thermo Scientific.

The absorption spectrum of a solution of lysozyme hydrochloride in acetate buffer solution (pH 5.4) and phosphate buffers solutions pH 6.0; pH 6.2 and pH 6.8 (1 in 10000) was determined according to the instructions for ultraviolet spectrophotometry <2.24> (Fig. 1).The results of the study of optical absorption prove that the optical absorption of lysozyme hydrochloride is most stable in phosphate buffer solutions pH 6.0 and pH 6.2. A maximum at 281 nm was selected for analysis. The results of the study proved that the absorption spectra of substances of different series of the manufacturer under these conditions do not differ significantly ($\Delta A_{281} = \pm 0.001$) (Fig. 2).

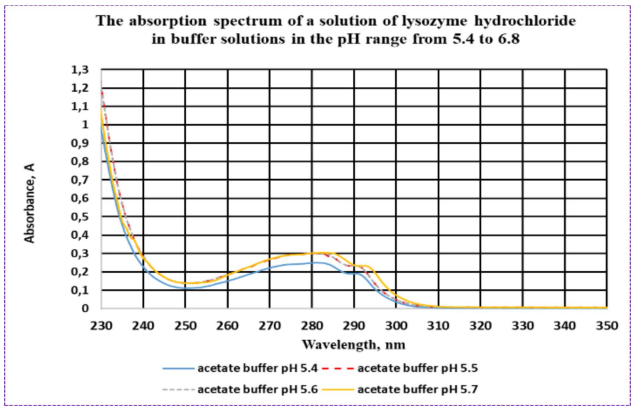

Fig. 1. Absorbance of lysozyme hydrochloride in buffer solutions with different pH

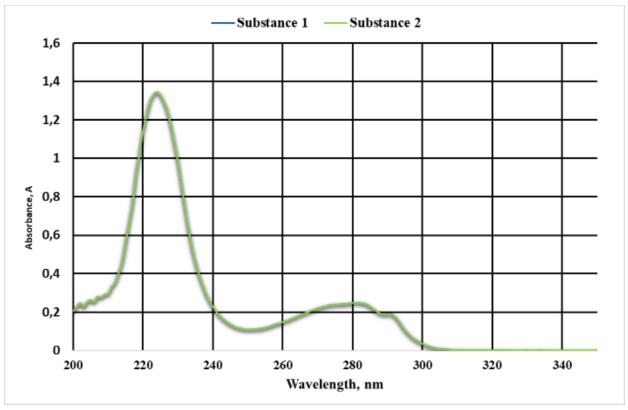

Fig.2. The absorption spectrum of LH of different series of the manufacturer

As a result of the study, the conditions for testing for the identification of lysozyme hydrochloride by spectrophotometry by the method of specific absorbance were determined. The average value of the specific absorbance $A_{1c}^{1\%}$ (25.29 ± 0.07, RSD = 0.12%, ΔAs = 0.28%) in the concentration range of the method from 80.0% to 120.0%. The maximum allowable value of the specific absorbance in the range from 24.80 to 25.80 is proposed.

The developed method according to certain metrological characteristics is characterized by acceptable accuracy and reliability and can be used in quality control of lysozyme hydrochloride and routine analysis of the final product in the manufacture of dosage forms.