

**NONDESTRUCTIVE DETERMINATION OF THE AMPICILLIN AND AMOXICILLIN TABLETS BY RAMAN SPECTROSCOPY****N. B. Saidkarimova^{*1}, A. N. Yunuskhudjaev²**¹PhD Student, Tashkent Pharmaceutical Institute, Republic of Uzbekistan, 100015, Tashkent, Oybek 45.²Professor, Doctor of Pharmaceutical Science, Med Standard, Scientific and Testing Center of Medicines and Medical Products, Republic of Uzbekistan, 100034, Tashkent, Karasaroy 343.***Corresponding Author: N. B. Saidkarimova**

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ABSTRACT

Antibiotics are one of the most widely used drugs in medical practice. The main pharmacological properties of penicillin antibiotics are due to the selective bacteriostatic and bactericidal influence on gram-positive and gram-negative microorganisms. The effect of beta-lactam antibiotics on such a wide spectrum of organisms is directly dependent on their structure. Differentiation of such drugs from related substances and limiting counterfeiting requires development of highly sensitive methods of analysis. Therefore, improvement of pertinent analytical methods is one of the most urgent tasks that needs to be addressed. **Objectives:** To establish Raman spectroscopy as a method of analysis for the penicillin group antibiotics. **Materials and methods:** The research involved selecting ampicillin and amoxicillin for analysis as representatives of penicillin group antibiotics. Analyses were performed on Raman spectrometer produced by USA company "Enhanced Spectroscopy", model "R-532". **Results:** Characteristic wavelengths of penicillins are located at 1500-1800 cm⁻¹ and 2900-3100 cm⁻¹ in the Raman spectrum. **Conclusion:** This article highlights the possibility of using Raman spectroscopy for determining the authenticity, as well as the quantitative analysis of penicillin antibiotics.

KEYWORDS: Raman spectroscopy, β-lactam ring, ampicillin, amoxicillin.**INTRODUCTION**

It is widely accepted that pharmaceutical products should be safe and efficient in order to meet consumers' expectations. This can be achieved through various processes, including analysis of raw materials, intermediate and final products and formulations, detection of impurities, as well as pharmacokinetics of drugs and their metabolites in biological samples. One should take into consideration not only the physical and chemical properties of the drug, but also the effect of the auxiliary substances. Incompatibility of active pharmaceutical ingredients and excipients may reduce or even fully negate the biological effectiveness of the drug. Antibiotics also belong to such group of chemotherapeutic agents of complex structure. Therefore, Raman spectroscopy is considered to be an effective method of molecular structural analysis of such compounds.^[1]

The analysis of drugs and their metabolites in biological fluids is important in monitoring such factors as concentration, absorption and toxicity. Various analytical methods (volumetric, gravimetric, spectroscopic, electrochemical) can be used to identify these factors. Among these methods, Raman spectroscopy is one of the

popular techniques that has gained a lot of interest and led to the advancement of pharmaceutical analysis in recent years. As a highly sensitive method, it is widely used in the pharmaceutical industry to control the quality of final and semi-final products, as well as to detect adulterated products.^[2]

Purpose of the research is to establish Raman spectroscopy as a method of analysis for the penicillin group antibiotics.

It is known from the pharmacopoeia analysis methods of the penicillin group antibiotics that the authenticity of such drugs can be determined with the help of color reaction, based on hydrolyses the β-lactam ring of the antibiotic. A good example would be the formation of a green-colored copper hydroxamate salt with Cu(NO₃)₂ in alkaline conditions, and dark red ferric hydroxamate salt with FeCl₃ in acidic medium.

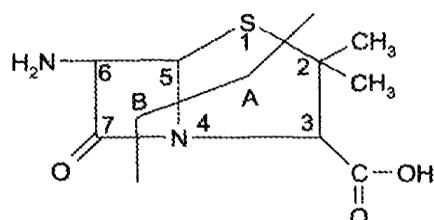
The reaction of formation of hydroxamate salts is not specific to β-lactamides, but can also be used to identify complex ethers, lactones and carboxylic acids.

In addition, a number of foreign pharmacopoeia describe that penicillins result in colored compounds (i.e.,

ampicillin and amoxicillin yellow orange) when mixed with the Marki reagent (formalin solution in concentrated sulphate acid).^[3]

The chemical structure of penicillins is based on 6-APC, which is a heterocyclic system that consists of 2 condensed rings:

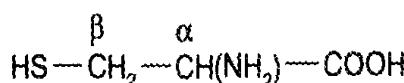
- Four membered - β -lactam (B)
- Five membered - thiazolidine (A).



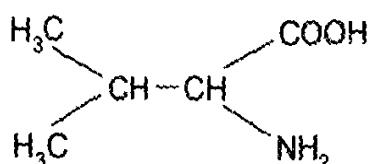
6-APC

6-aminopenicillin is considered to be a dipeptide, which is formed by combination of two amino acids.

L-cysteine (β -mercaptopropanoic acid):



L-valine (L- α -aminoisovaleric acid):



When aliphatic amino acid residue is combined with ampicillin and amoxicillin ninhydrin solution and heated, violet staining is observed.

Nevertheless it is very difficult to distinguish between penicillin group antibiotics with high degree of accuracy using the above-mentioned methods.^[4]

MATERIALS AND METHODS

Ampicillin and amoxicillin were selected for analysis as representatives of penicillin group antibiotics. Analyses were performed on Raman spectrometer produced by USA company "Enhanced Spectroscopy", model "R-532" with the following technical characteristics: spectral range from 100 to 6000 cm^{-1} , spectral resolution of 5-8 cm^{-1} , laser wavelength 532 nm, laser power 30 mW, detector type linear CCD array, pixel number 3648, focal length 75 mm, entrance aperture 20-30 microns, holographic diffraction grating 1800 lines/mm. The measurements were conducted at room temperature with samples placed in special cuvettes and illuminated by a laser beam with power of 30 mW.

RESULTS AND DISCUSSION

Characteristic wavelengths of penicillins are located at 1500-1800 cm^{-1} and 2900-3100 cm^{-1} in the Raman spectrum. It can be seen that C = O bond of the β -lactam ring, which is attached to thiazolidine heterocycle, is found to be at 1685 cm^{-1} , the scissor vibrations of primary aminogroup at 1600-1615 cm^{-1} , and the -COOH functional group at about 1770 cm^{-1} . Ampicillin and amoxicillin molecules have two -CH₃ groups, which are directly linked to the thiazolidine ring. Their symmetric valence vibrations are located at 2890 cm^{-1} , asymmetric valence vibrations at 2965 cm^{-1} , and the C-H bond of the aromatic ring at 3080 cm^{-1} . Presence of C-N bond of aromatic nitrogen can be demonstrated by vibrations at medium 1380 cm^{-1} and 1260 cm^{-1} relatively high intensities.^[5]

The C-H deformation vibrations of the monosubstituted benzene ring of the ampicillin molecule that are observed at high intensity 1000 cm^{-1} and the wavelength of 580 cm^{-1} indicate the presence of the C-S bond (Fig.1).

In contrast to the ampicillin, in amoxicillin spectrum vibrations of the para-substituted benzoic ring produce peaks of high intensity at 850 cm^{-1} (Fig.2). In addition, C-N bond and the deformation vibrations of C-C bond can be seen as stronger than in the ampicillin spectrum.^[6]

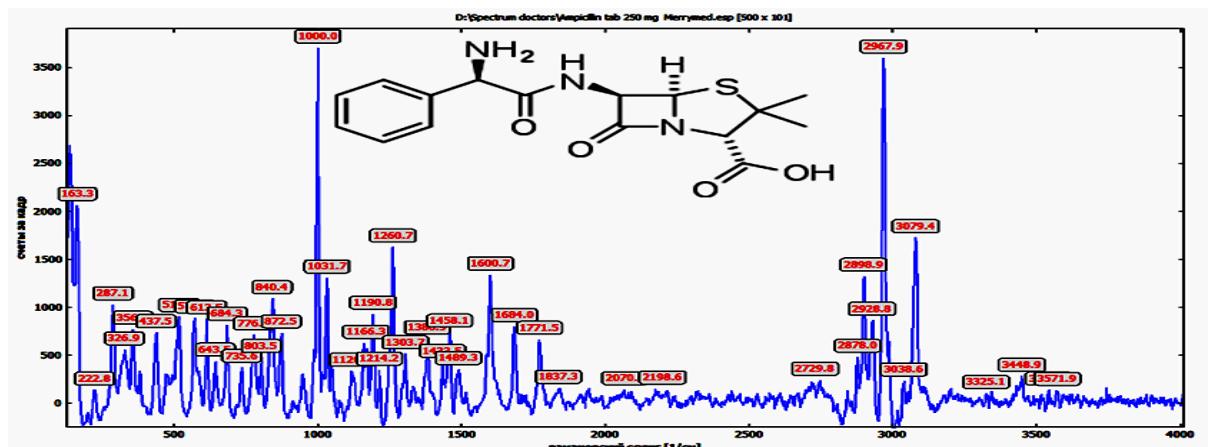


Figure 1: Raman spectrum of Ampicillin tablet.

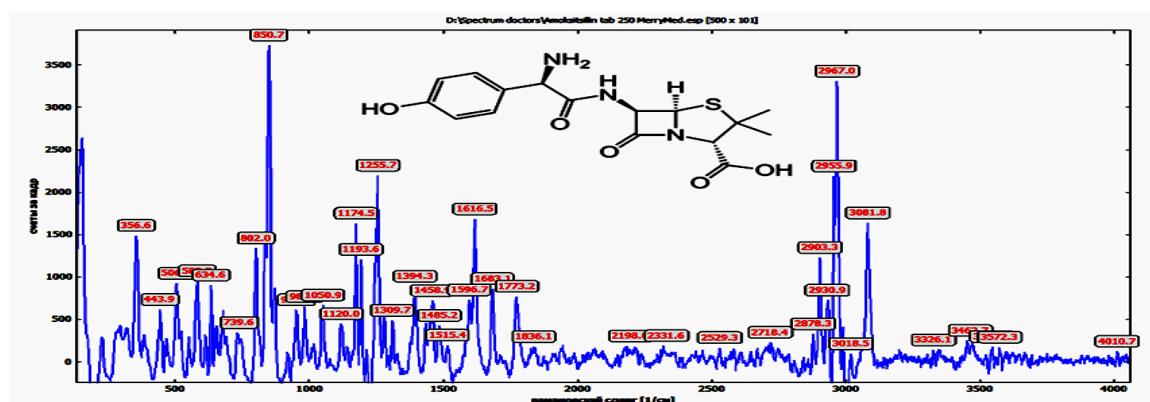


Figure 2: Raman spectrum of Amoxicillin tablet.

It is known that the amount of penicillin is determined in two phases. At the first step, the total amount of penicillin in the drug is determined by the iodometric method. In that case, other derivatives of penicillin, which can be formed during the biosynthesis process, may also be included.

In the second phase of quantitative analysis, each of the penicillin salts is identified separately by gravimetric method.

Moreover, their quantity can also be determined by spectrophotometry. This method is based on the measurement of optical density of penicillin in the acidic conditions by hydrolysis with solution of copper (II) sulfate (which is added to increase reaction sensitivity).

Existing pharmacopoeia methods are complex and require much time and effort.^[7] Establishing the most sensitive express method for quantitative analysis of penicillins remains important.

The figure below pictures the characteristic Raman spectrum of amoxicillin tablets in doses of 250 mg and 500 mg including specific high intensity peak measurements (Fig.3-4). It should be noted that while the x-axis value of the specified wavelength remained unchanged, the y-axis value was nearly doubled. These data indicate a possibility of quantitative analysis of amoxicillin with high degree of sensitivity and specificity.

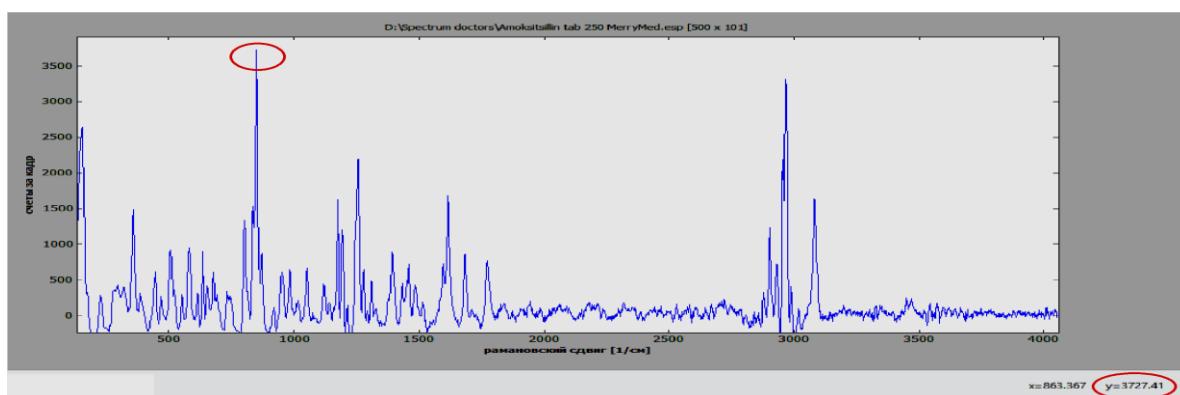


Figure 3: Raman spectrum of Amoxicillin tablet (250 mg).

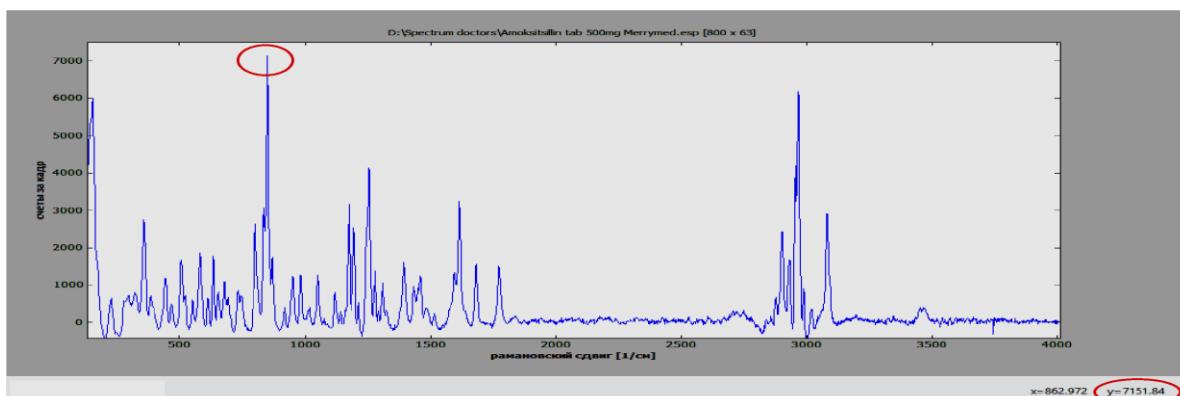


Figure 4: Raman spectrum of Amoxicillin tablet (500 mg).

CONCLUSION

1. Analysis of β -lactam group antibiotics by Raman spectroscopy demonstrated characteristic wavelengths at 1500–1800 cm^{-1} and 2900–3100 cm^{-1} .
2. The C-H bond of the benzene ring in the ampicillin molecule has the highest intensity at 1000 cm^{-1} . Because of para-substituted benzene ring in the amoxicillin molecule it was observed to have a hypsochromic shift.
3. Raman spectroscopy can be applied to both determining the authenticity, as well as the quantitative analysis of this group of antibiotics.

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