Purity Determination of Fusidic Acid Using Physico-chemical Methods

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When a new drug product is prefomulated and later is accepted as a future commercial product is important to test stability and also higher purity of medical substances which should be processed during drug production. In present paper was checked the purity of fusidic acid, a relatively new antibiotic acting on gram positive bacteria, and is used as treatment for acne vulgaris. Purity and also thermal stability of this new class of antibiotics was tested using physicochemical test methods, such as: Fourier transform Infrared Spectroscopy (FTIR), XRay Diffraction (XRD), Scanning Electron Microscopy (SEM), and also Thermal Analysis.

Keywords: FTIR spectroscopy, RX diffraction, thermal analysis, scanning electron microscopy

Fusidic acid was firstly discover and synthesized more than 40 years ago, but was not a popular and spread used antibiotic. His role was changed when was observed the increase of bacterial resistance at classical antibiotics; during last years was observed an increase of interest for usage of fusidic acid in antibacterial drugs formulation [1-4].

Fusidic acid represents an antibiotic used against gram positive bacteria, when he is acting onto the protein synthesis, inhibiting the synthesis of new protein structures. In actual stage of development, fusidic acid is available on market as topical and systemic formulations, but usual is used as topical treatment. In order to prevent that the staphylococcus get resistance at fusidic acid action is recommended usage of this antibiotic in combination with other antibiotics, excepting the quinolonic antibiotics [5]. While, near the antibiotic action, fusidic acid have also a chemotherapeutical action due to his sterol structure able to block the protein synthesis by linking the elongation factor of polypeptides chain, or to the guanosine phosphate nucleotide, and also by binding ribosomes. In figure 1 is presented the structure of fusidic acid.

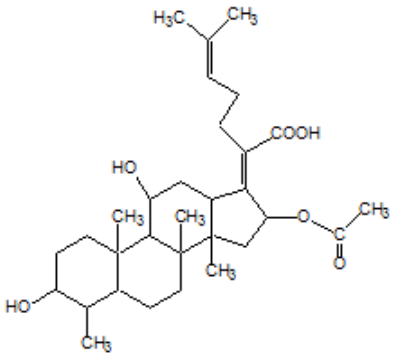

Fig. 1. Chemical structure of fusidic acid

Right now, almost all bacteria develop a high resistance at usual antibiotics, transforming in this way the fusidic acid in a valuable antibiotic for next decades. In this context, dermatologists start to explore new classes of antibiotics, such as fusidic acid. During clinical test was observed that the usage of fusidic acid lead at rapid and also significant amelioration of disease symptoms.

During commercial drug formulation is really important to test purity and also stability of used drug. Purity and thermal behavior of used drugs are determined by physicochemical testing methods such as: Fourier Transformed Infra Red Spectroscopy (FTIR), X-Ray Diffraction (XRD), Electron Scan Microscopy (SEM), and also Thermal Analysis.

Common and usual technique used for organic compound identification is represented by Fourier Transformed Infra Red Spectroscopy (FTIR), which can be used also for identification of any drug. Main advantage of such technique is represented by his non-destructive character, when the molecule integrity is preserved. Also, each functional group, any bond form each organic molecule and also any intermolecular bond present a characteristic adsorption in IR spectra. Due to that can consider the IR spectra as a specific mark of studied molecule, which make the identification much easier.

A really spread and used analytic technique is represented by scanning electron microscopy [6-9], usually used for investigation of organic tissues, entire cells, human organs fragments, observation and characterization of inorganic and also organic substances. Also, structure and purity of molecules with crystalline or polycrystalline (powders) substances can be identified by X-Ray diffraction technique [10-13]. Using such technique is possible to identify and characterize entire range of elements and also chemical compounds from different areas such as: chemistry, physics, mineralogy, metallurgy, biology, art in order to see the authenticity, and in paintings restoration. Thermal analysis is used to check the thermal stability of compounds and in same time to determine the thermodynamic and also kinetic parameters of substances used in chemistry, biology, polymers and medicine.

Experimental part

Materials and methods

Materials involved during present study were of analytical purity used directly without any further purification, fusidic acid (FA) was purchased from Sigma Aldrich GmbH, Germany.

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FTIR spectra were recorded using a JASCO 6100 FTIR spectrometer by scanning the spectral domain between 4000 – 400 cm^{-1} with a resolution of 4 cm^{-1}, using the classical technique of KBr pellets.

X-ray diffraction patterns were obtained using a X'Pert PRO MPD Diffractometer equipped with Cu anode X Ray tube, PixCEL detector and a vertical theta – theta goniometer. All XRD spectra were recorded at room temperature in 2θ rage of 0 to 80 degrees.

Scanning electron microscopy data were recorded by using FEI Inspect S High resolution scanning electron microscope, equipped with a high stability Schottky field emission gun, and a specimen room with 379 x 280 mm door size.

TG/DTG/DTA data were recorded on Netzsch – STA 449 TG/DTA instrument into the temperature range of 20 to 500°C using a platinum crucible with containing approximately 5 mg sample. All tests were carried out under dynamic nitrogen atmosphere, 20 mL min^{-1}, and heating rate of 10 K min^{-1}.

Results and discussions

Recorded FTIR spectrum is presented in figure 2. Analyzing data plotted into the spectrum can observe the presence of bands associated with presence of different functional group into the molecular structure of studied compound.

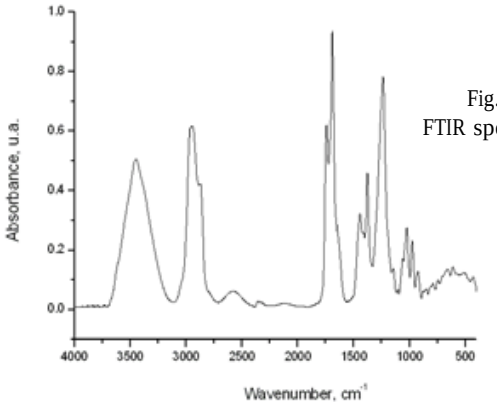


Fig. 2. Recorded FTIR spectra of fusidic acid

So, at lower wave numbers can observe presence of some vibrations associated with presence of planar deformations of double bond C = C at 607 cm^{-1}, C = C – C at 746 cm^{-1}, C – H at 850 and 913 cm^{-1}, C = C – H at 970 cm^{-1}, and also deformation of C – H bonds outside of the plane at 781 cm^{-1}.

An intense and relatively large vibration observed at 3446 cm^{-1} can be correlated with the stretching of O – H bond, and in same time other intense vibrations observed at 1686 and 1742 cm^{-1} can be assigned to the symmetrical and also asymmetrical stretching of the functional group C = O.

In same time was observed the presence of intense bands at 2869 and 2953 cm^{-1}, associated with symmetric and also asymmetric stretching of methyl group and also symmetrical and asymmetrical deformation of methyl group at 1380 and 1445 cm^{-1}.

X-Ray diffraction technique is used to determine phase, structure of crystal, and also purity of analyzed substances. In figure 3 is presented the recorded XRD pattern recorded for pure fusidic acid. Analyzing presented spectra and comparing it with the ICDB data base spectra, can observe presence o fusidic acid characteristic peaks. Also, because identified peaks are perfectly matching the standard data for pure compound can say that analyzed product is pure and in same time present a high crystallynity.

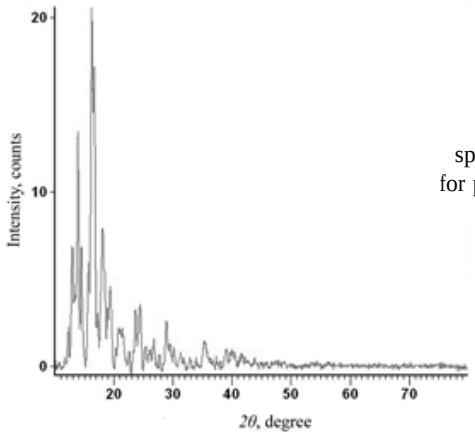


Fig. 3. XRD spectra recorded for pure fusidic acid

Other important property of any commercial drug is thermal stability, so in case of studied compound was also tested thermal behavior, presented in figure 4.

From data presented can observe that between 84 and 110°C studied compound present a small mass loss, around 1.38 %, associated with evaporation of adsorbed water. When temperature increase, can observe the good stability of studied compound until 170°C, at higher temperature can observe onto TG and DTG curves initiation of thermal decomposition of fusidic acid. So, in first stage of thermal decomposition, between 170 and 215°C was counted a mass loss of 10.80 %, followed by a mass loss of 86.25 % between 270 – 440°C.

Analyzing the DTG curve can observe that an endothermic transformation is taking place between 165 and 375°C, which have two prominent peaks at 183.7 and also at 359.1°C. These two peaks are associated with the endothermic decomposition of fusidic acid.

Next step in fusidic acid characterization was represented by the Scanning Electron Microscopy test. SEM picture recorded at different magnifications are presented in figure 5.

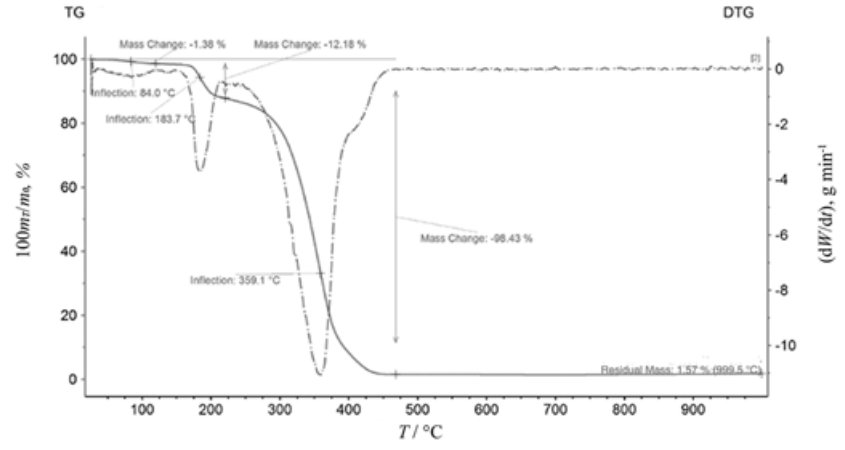
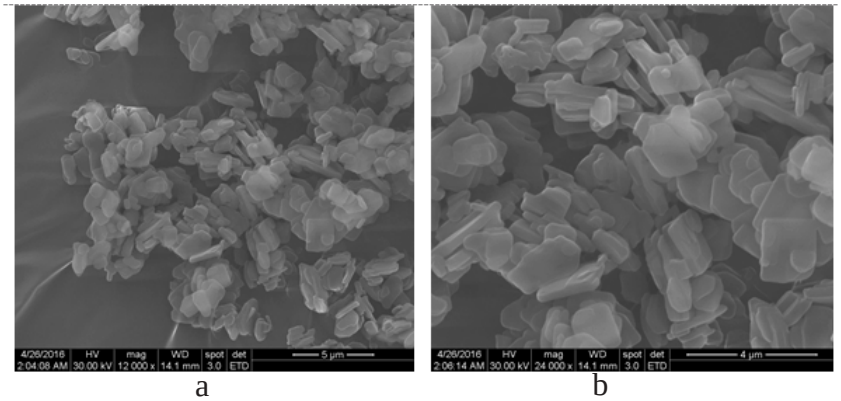


Fig. 4. Fusidic acid thermal behaviour



a b

Fig. 5. Fusidic acid SEM: a) 12000X, b) 24000X

From micrographs can observe that fusidic acid is a compound with a high crystallinity degree and in same time can observe the compact and complex form of his structure. Such complex structure can be associated with heterogeneous crystallization of fusidic acid molecules. Such heterogeneous process, lead at formation of different planar structures with irregular forms of fusidic acid particles.

Conclusions

Carried out studies of stability and purity are mandatory study during preformulation and also during formulation of commercially drugs.

In present paper was determined the purity of fusidic acid, which represent an antibiotic that acts on gram positive bacteria, used especially in treatment of acne vulgaris, when is used in combination with other antibiotics, excepting the quinolonic one.

Purity evaluation and also thermal behavior of fusidic acid were evaluated using phisico-chemical techniques. FTIR spectroscopy was used to determine the purity of fusidic acid, confirmed by the presence of specific vibrations characteristic for every functional group present into the compound molecule. High purity of studied compound was also confirmed by using XRD technique. Based on SEM micrograph recorded at different magnifications was observed that the studied compound present a complex and high crystalline structure. Thermal behavior of fusidic acid reveal that during manipulation time was adsorbed a small quantity of water form neighboring (1.38%). These reveal the necessity to eliminate included water during drug preformulation or formulation process.

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