DISCRIMINATION AND QUANTIFICATION OF GLYCOSAMINOGLYCANS IN PHARMACEUTICAL FORMULATIONS USING micro-RAMAN SPECTROSCOPY

Eleni Kamilari[1], Christos Kontoyannis[1, 2], Fotini Lamari [1] and Malvina Orkoula [1,*]

[1]Department of Pharmacy, University of Patras
[2]ICE/HT-FORTH, Patras

Keywords: Glycosaminoglycans, Raman spectroscopy, pharmaceutical formulations

Glycosaminoglycans (GAGs) are heteropolysaccharides of great biological importance. They regulate many cell functions in physiological and pathological conditions. GAGs are found in biological systems, pharmaceutical formulations, as well as in food supplements. Methods developed for the structural characterization and quantitative analysis of GAGs rely on enzymatic digestion followed by disaccharide analysis and employ high performance liquid chromatography (HPLC), anion exchange chromatography, capillary electrophoresis and gel electrophoresis with fluorescence detection. Extensive consumption of time and chemicals and difficult application are their major disadvantages.

The purpose of the present study was to develop an easy to use, accurate and rapid method for the analysis of mixtures of GAGs. Raman spectroscopy was chosen because it provides information on the chemical composition of the examined specimen and it complies with all previous requirements. Raman analysis is done simultaneously for all constituents and no previous separation is needed. The collected Raman signal is the sum of individual signals of the components of the specimen. In the case of GAGs, spectral differentiation the various types, as well as quantification of mixtures, was proved burdensome, due to the structural similarities of the analyzed molecules and the consequent similar Raman spectra. However, a methodology based on Raman spectroscopy was constructed and successfully applied for the qualitative and quantitative analysis of a Chondroitin Sulfate and Hyaluronan mixture found in a pharmaceutical formulation administrated by intrarticular injection in osteoarthritis.

Identification of GAGs in the pharmaceutical formulation was performed by comparing the Raman spectra with those of pure substances. Discrimination was achieved based on characteristic vibrational frequencies of the functional groups of these molecules. The main distinguishing feature is chondroitin's sulfate band at 1071 cm^{-1} arising from sulfate group as hyaluronan has no sulfate groups. In order to quantitate chondroitin sulfate or hyaluronan in the formulation, mixtures of chondroitin sulfate with increasing amounts of hyaluronan (1%, 2%, 3% and 4% w/v) were prepared and analyzed. The calibration curve was constructed using the ratio of the peak amplitudes at 1071 cm^{-1} and 1128 cm^{-1}, characteristic bands arising from chondroitin sulfate and hyaluronan respectively.

Conclusively, Raman spectroscopy was successfully employed for the qualitative and quantitative determination of chondroitin sulfate and hyaluronan in pharmaceutical formulations. The most important advantage of the technique is that it offers the possibility of simultaneous and rapid analysis of all components with no need for previous separation.