SECOND INTERNATIONAL CONFERENCE «INTEGRATION NETWORK OF THE PHARMACEUTICAL ECOLOGY - 2024»

The study of the interaction of ochratoxin A with proteins using the method of fluorescence polarization analysis

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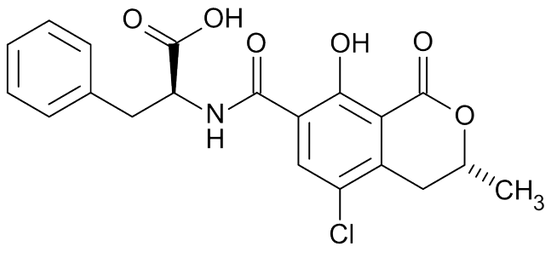
INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin that is produced by several Aspergillus and Penicillium species.

Mechanisms of its toxicity lie in lipid peroxidation, direct modification of DNA structure, inhibition of protein synthesis and mitochondrial energy production and disruption of calcium homeostasis. Ochratoxin A binds to blood serum proteins.

The content of OTA should be investigated in such pharmaceutical excipients as, for example, potato starch, as well as in certain pharmaceutical substances.



GOAL

Study the binding of ochratoxin A to serum proteins in order to develop a fast, cheap, one-step and simple method of quantitative determination using the method of fluorescence polarization analysis (FPA).

METHOD

Device: Sentry 200 from Ellie (USA)

Materials: Fluorescein-labelled ochratoxin A (OTA-AMF) was used as a tracer.

The method of fluorescence polarization analysis is based on the interaction of fluorophore-labelled antigen and antibody. All measurements were done in BB at pH=7.5

Method: to construct the saturation curve, human serum albumine (HSA) and bovine serum albumine (BSA) solutions were added to a constant amount of tracer, which were sequentially twice diluted.

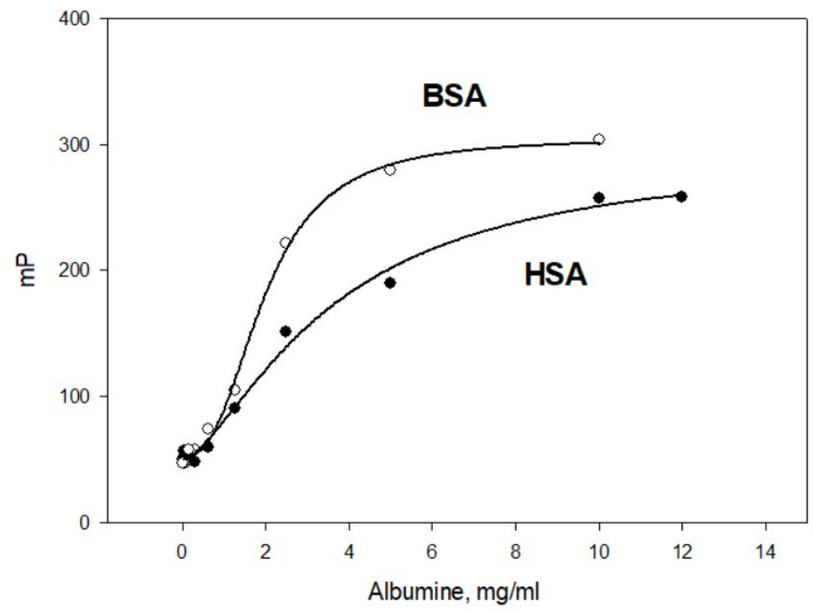


Figure 1: Calibration curve of polarization (mP) dependence on albumine concentration

RESULTS

To characterize the binding energy, we evaluated IC_{50}

IC_{50} (HSA) = 2.85 mg/ml

IC_{50} (BSA) = 1.96 mg/ml

CONCLUSION

1) The binding energy of ochratoxin A to HSA is higher than the binding energy to BSA.

2) BSA can be used to develop a technique for the quantitative determination of ochratoxin in pharmaceutical raw materials.