View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by University of Szeged

SEPARATION OF AFLATOXINS BY CENTRIFUGAL PARTITION CHROMATOGRAPHY

Gábor Endre[1,2], Zsófia Hegedüs[1,2], Mónika Varga[1], Csaba Vágvölgyi[1], András Szekeres[1]

[1]*University of Szeged, Faculty of Science and Informatics, Departement of Microbiology, Közép fasor 52. Szeged H-6726*
[2]*Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary*
egabcy@gmail.com, andras.j.szekeres@gmail.com

Abstract

Aflatoxins are mycotoxins, produced by several species of filamentous fungi. For this group of toxins, there are very low, ppb-level limits in the EU in food and feed products, thus as reference standards relatively high amounts of pure aflatoxins are required. One of the promising methods for their purifications, the centrifugal partition chromatography could be used successfully. Herein this study, the development of a liquid-liquid chromatographic method for the separation of both the aflatoxins and the impurities was involved.

Introduction

Mycotoxins are the secondary metabolites produced by certain filamentous fungi. Within these toxic compounds, aflatoxins are playing an outstanding role due to their high level toxicity, which cause remarkable problems in food industry and agriculture [1,2]. Plenty of methods are available for monitoring or measuring these compounds from various matrixes [3,4], which requires relatively high amounts of pure aflatoxins. Liquid-liquid chromatography, which is based on a distribution of components between two phases in a biphasic solvent system, could serve solutions in their purifications. One of the technical implementation of this technique is the Centrifugal Partiton Chromatography (CPC) [5], which was applied in our work for the separation of aflatoxins from the fermentation product.

Experimental

Aspergillus parasiticus (SZMC 2473) was cultivated on liquid media for 7 days in dark at room temperature containing malt extract. The produced aflatoxins were extracted three times using dichloromethane and the pooled crude extract was dispensed into 1.5 ml vials and were evaporated to dryness for the solvent system testing. Each examined solvent system was mixed in 10 ml test tube. Than equal amounts of both saturated phases were added into same vial, containing the dried extract. Aflatoxins and the impurities were distributed between the two phases, and their concentrations in the upper- and lower phases were measured by HPLC-UV technique. The partition coefficients (P) and separation factors (α) were calculated, and the best system was selected for the purification of aflatoxins.

Results and discussion

At the beginning of our work, the aflatoxins were extracted from the culture media of *A. parasiticus* (SZMC 2473). The extraction of the desired compounds was carried out and the examination of ternary systems was started. The constructed ternary systems are based on the *best solvent method*. For these purposes, a solvent, which is best to dissolve the aflatoxins, is selected as core component of the solvent system. Beside this bridge solvent, two other solvents are also selected including both a more polar- and less polar solvents, compared to the best one. Applying these solvents in different ratios to solve the crude extract, the valuable component and the impurities could be shifted from one phase into the other. For the best

solvents, chloroform, acetone and acetic acid were selected initially and altogether 108 systems were created. The most promising system possessed 0.25, 0.53, 0.79 and 1.8 partition coefficient values for aflatoxin B1, B2, G1 and G2, respectively. After the calculation of separation factors this system was applied for the instrumental optimization and purification. , the preparative separation of aflatoxins was carried out. At the end of our work the purity of our products were measured.

Conclusion

In our study, a liquid-liquid chromatographic separation of four aflatoxins was accomplished using a cost-effective Centrifugal Partition separation method. Based on the results of our examinations the developed procedures will be proper for the large scale production of these type of mycotoxins in high purity.

Acknowledgements

This work was supported by the Hungarian Scientific Research Fund by grants NKFI K-115690 and this work was connected to the project GINOP-2.3.2-15-2016-00012.

References

[1] J. W. Bennett, M. Klich, Mycotoxins. Clin. Microbiol. Rev. 16 (2003) 497.
[2] D. L. Eaton, E. P. Gallagher, Ann. Rev. Pharmacol. Toxicol. 34 (1994) 135.
[3] F. Hepsag, O. Golge, B. Kabak, Food Control 38 (2014) 75.
[4] O. Golge, F. Hepsag, B. Kabak, Food Control 59 (2016) 731.
[5] A. P. Foucalult, Centrifugal Partition Chromatography, New York: Marcel Dekker