International Symposium for Thin-Layer Chromatography

BASEL, 06th-08th July 2011

Occurrence of some mycotoxins in wheat seeds from northwest Romania using HPTLC

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Purpose: comparative study of extraction methods and purification of the mycotoxins applied for the determination of mycotoxins occurrence in wheat seeds from north-west Romania using HPTLC

Abstract

The contamination level of Aflatoxins (B1, B2, G1, G2) in wheat seeds produced in northwest Romania during the last two years (2009 and 2010) was investigated using different HPTLC revealing techniques. The first step in mycotoxins isolation and identification was to establish the most efficient method. In this purpose we have chosen the protocol using two method of extraction described by Braicu *et al.* [1] and Morar *et al.* [2] and modified by our research group. We compared the obtained results and we selected the HPTLC Method 2 for quantification. 120 samples were investigated two consecutive years and 27 % from the total were positive but under the setting MRL value for the grain.

Introduction

It has been estimated that, annually, about 25% of the world`s food crops are affected by mycotoxin contamination. In this situation it is not surprising that the mycotoxins know two possible approaches:

- **from the farmer's perspective**: problems of prevention of mycotoxin formation; Their attention will turn to the application of HACCP to mycotoxin contamination at all stages in the commodity supply chain, with discussions on both the pre- and post-harvest situations.
- **from the analyst perspective**: much research is centered around surveillance, occurrence and the development of new analytical methods. From this point of view, the sampling plays a very important role, because the distribution of mycotoxins in a batch is quite heterogeneous. The sampling was made according with (CE) 401/2006 Reglementation.

Materials and Methods

- The samples were collected from grain producers from three districts from North-West of Romania.
- The samples were taken from different grain varieties and different batches, two consecutive years, following during the study to determine the influence of mycotoxins contamination on the wheat quality parameters.
- The distribution of analyzed samples depending on the geographical area is presented in the used table.
- For analytical reasons, the first step was to establish the best method from the two methods: TLC Method 1 and HPTLC Method 2, and then the presence of mycotoxins by confirmatory screening
- Densitometry was used for quantification with CAMAG TLC Scanner 3
- The selected working conditions are presented in the HPTLC Method 2.

TLC Method 1

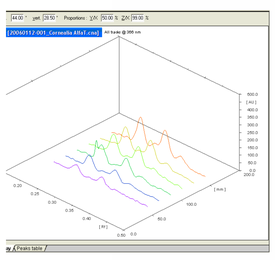
Extraction method 1

2 -3 g sea sand → Homogenization (15 - 20 min.) ← 10 g sample; 100 mL chloroforme
Leave to stand for 10 min.
Filtration
Residue ← 10 mL chloroforme
Filtrate
Drying col. Na_2SO_4 anh. Ø = 1,5 cm ← + 2 x 10 ml cloroform
Extract
Evaporate to dryness ← 55⁰ – 60 ⁰C
Dissolve residue with 10 mL chloroforme
Purification ← 5g Na_2SO_4 anh. + 10g SiO_2 + 15g Na_2SO_4 anh.
Elution with 150 ml methanol:chloroform (3:97, v/v)
Eluat 2 | Residue
Evaporate to dryness ← 55⁰ – 60⁰C
Dizolve with 1 ml chloroform
TLC application
Development ← + chloroform:acetone (92:8, v/v)
Plate drying ← 10 – 15 min. In dark, 20⁰-22⁰C
View plate under UV (366 nm)
Quantitative and qualitative analysis CAMAG Fotodensitometry

HPTLC Method 2

Extraction method 2

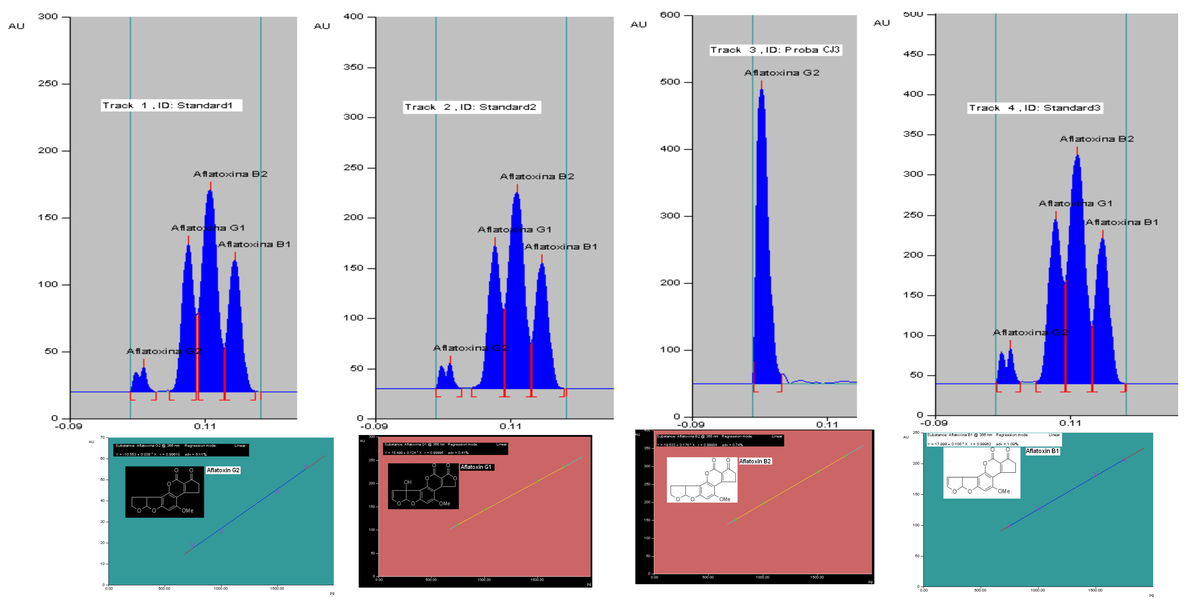
2 g sample
Homogenization (3 min.) ← + 100 mL methanol
Mixing (4 min.) ← + 40 mL water
Leave to stand for 10 min.
Filtration
Residue | Filtrate (20 mL)
Mixing (2 min.) ← 20 mL NaCl sol. 10% + 20 mL petroleum ether
Leave to stand for 10 min.
Organic phase | Water phase
Shake ← + 50 ml dichlormethane
Leave to separate
Water phase | Organic phase
Drying on 5 g Na_2SO_4
Evaporate to dryness ← 55⁰ – 60 ⁰C
Dissolve residue with 0.5 ml toluene:acetonitrile (98:2, v/v)
HPTLC application
chloroform:acetone (92:8, v/v) → Development
10 – 15 min. in dark, 20⁰-22⁰C → Plate drying
View plate under UV (366 nm)
Quantitative and qualitative analysis CAMAG Fotodensitometry

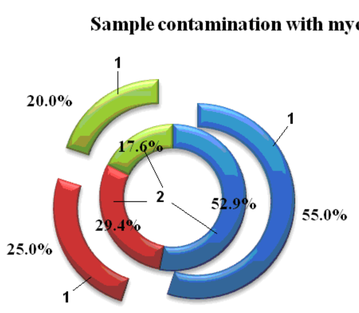


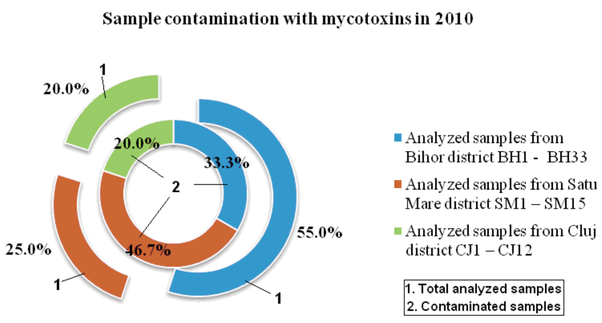
G1 and AFLA G2 Absorbances
5 nm in different concentrations

Results

District from producers	Cod probe	Year 2009		Year 2010	
		Total	Positive Contaminated With AFLA	Total	Positive Contaminated With AFLA
Analyzed samples from Bihor district	BH1 - BH33	33	9	33	5
Analyzed samples from Satu Mare district	SM1 – SM15	15	5	15	7
Analyzed samples from Cluj district	CJ1 – CJ12	12	3	12	3
Total of analyzed samples		60	17	60	15
		120			







Literature

[1] Braicu Cornelia, Puia C., Bele C., Bodoki E., Socaciu C., *Optimization of Screening Systems to Evaluate Relevant Mycotoxins From Cereals and Bread*, Buletinul USAMV Cluj-Napoca, vol. 61, 2005, 144-149.
[2] Morar Oana Anita, Cornelia Braicu, Constanta Modoran, V. Florian, *Evaluation of the mycotic and mycotoxic content and bakery features of wheat from Transylvania in the conditions of the year 2005*, Buletinul USAMV Cluj-Napoca, vol. 63, 2007, 596-601.

Acknowlegement:
This study has been financed by Romanian Ministry of Education and Research, PN II Project 52132/2008