CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

Fusarium Toxins : Extraction procedure and test strip detection

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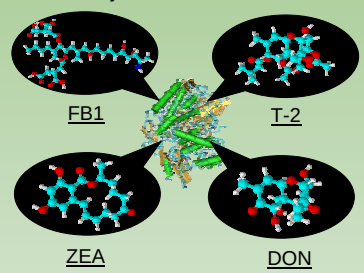
I) Introduction

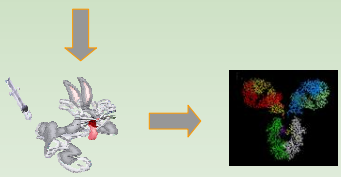
Fusarium species are plant pathogens commonly associated with cereals that, under favourable environmental conditions, can produce several secondary toxic metabolites. *Fusarium* toxins are widely distributed in the food chain in the EU and the major sources for their dietary intake are cereal products, mainly based on wheat and maize. The major *Fusarium* toxins found in cereals and cereal-based products that can be harmful to both human and animal health are deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2), zearalenone (ZEA) and fumonisins (FB1, FB2). In order to protect human health from exposure to these mycotoxins, the European Commission has recently established regulatory limits for DON, ZEA and fumonisins (sum of FB1 and FB2) in cereals and cereal-based foods and feeds, while permissible levels of T-2 and HT-2 are under discussion (EC Regulations No 1881/2006 and 1126/2007). The aim of mycotoxin research within CONffIDENCE project is to develop dipstick tests for the determination of the *Fusarium* toxins DON, ZEA and T-2/HT-2 toxins in wheat and oats; DON, ZEA, T-2/HT-2 and FBs in maize and maize by-products for feeds; and DON, ZEA, T-2/HT-2 and FBs in cereal based food.

II) Antibodies production

Antibodies raised against T-2/HT-2, DON, ZEA and Fumonisins were produced on rabbits. The sera were collected after injections of a mixture of modified-Hemocyanin immunogens and Freund's adjuvant.



Immunogen synthesis



pAb characterization

ELISA	FB1	T-2	DON	ZEA
IC_{50} (ng/ml)	0.5	2.5	3	0.1

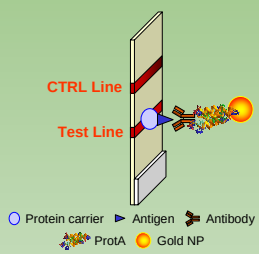
III) Extraction of Mycotoxins

A simplified sample preparation protocol has been developed for raw wheat, oat and maize cereals for recovering the four mycotoxins of interest. In each kind of matrices, no more than 6 minutes of extraction in alcoholic medium are necessary to recover more than 70% of each mycotoxin (Table 1).

	Recovery %* (RSD %, n=3)			
	FB1	T-2/HT-2	DON	ZEA
Spiking level	-	500 µg/kg	1400 µg/kg	80 µg/kg
Wheat	-	73(6)	97(8)	103(6)
Oat	-	73(6)	93(4)	107(9)
Spiking level	3200 µg/kg	500 µg/kg	1400 µg/kg	280 µg/kg
Maize	94(3)	85(3)	103(5)	87(3)

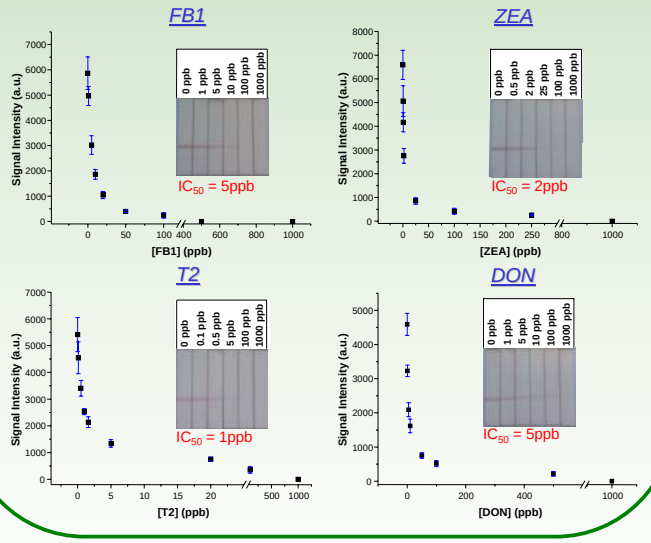
Table 1 : Mycotoxin recoveries using the described extraction procedure
*Analysis made by LC-MS/MS

IV) Dipsticks design



The indirect dipstick format involves the use of a coated antigen on the test line. When the sample is free of contaminant, the antibody will link the test line and a dark red colour will appear. However, when contaminants are present in the sample, no colour appears.

- Test sequence in spiked buffer (n=6)
- Incubation with 200µl of diluted extract during 5 min at room temperature
- Migration of 5 min at room temperature



V) Conclusions

In this work, we have described the first steps towards the design of four rapid, specific and highly sensitive lateral flow devices for FB1, T-2/HT-2, DON and ZEA. The single extraction protocol is designed to extract all targeted mycotoxins in one step with satisfactory recovery percentages. The total time of analysis doesn't exceed 16 minutes, extraction procedure included. The final step of this work will be to add to each test strip a control line that will allow first to validate each strip and second to compare the test line to a reference included in the same strip.

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