



A RAPID ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF ZEARELENONE IN CEREALS

Francesca Diana, Lidija Persic and Maurizio Paleologo

Tecna S.r.l, Area Science Park, Padriciano 99, 34012 Trieste, Italy
E-mail: tecna@tecnalab.com; francesca.diana@area.trieste.it

Introduction

Zearalenone, a mycotoxin produced by moulds of the genus *Fusarium*, is a common worldwide contaminant of all major cereals. Because of its estrogenic properties, contaminated feed cause urogenital disorders in animals, whereas toxic effects in humans are under investigation. European Community fixed different maximum levels of zearalenone in cereals and their derivatives for feed and food. According to Commission Recommendation 576/2006, 2000 ppb is indicated as guidance value of zearalenone for cereals in products intended for animal feeding. On the other hand, the Commission Regulation 856/2005 provides the following maximum levels of zearalenone for food: 200 ppb for unprocessed maize and 100 ppb for other unprocessed cereals. Celer ZON is a direct competitive enzyme immunoassay for the quantitative determination of zearalenone in cereals. The range of measurement is 10-1000 ppb or 50-5000 ppb, to meet the need to test food as well as feed, respectively.

Materials and Methods

- Test principle:** direct competitive enzyme immunoassay.
- Assay time:** 20 minutes.
- Samples:** cereals.
- Dosage range:** 10-1000 ppb, optional: 50-5000 ppb (by dilution of the sample extract).
- Samples preparation:** to 5 g of finely ground samples 1 g of NaCl (apart for wheat samples) and 25 ml of a solution of 70% methanol in distilled water were added. Samples were extracted by shaking thoroughly for 3 minutes and then centrifuged at 3500 g for 5 minutes. Supernatants were directly tested in the assay, for a dosage range of 10-1000 ppb.
- Samples:** 20 maize samples whose zearalenone content was less than 10 ppb (HPLC analysis) were used for Celer ZON specificity determination. 3 contaminated maize samples (reference materials supplied by FAPAS), one negative maize and one negative wheat sample (both <100 ppb, HPLC analysis) spiked with zearalenone at different levels were tested for accuracy and precision study.

Results

Immunoassay performance

An example of calibration curve is shown in Figure 1. IC₅₀ value (ppb corresponding to 50% of maximum absorbance signal inhibition) is in the range of 90-140 ppb (mean value : 112±16 ppb, n = 28). Cross-reactivities % towards zearalenone metabolites are reported in Table 1.

	CROSS-REACTIVITY %
Zearalenone	100
α-zearalenol	58
β-zearalenol	16
Zeranol	7
Taleranol	6
Zearalalone	4

Table 1: Celer ZON cross-reactivities

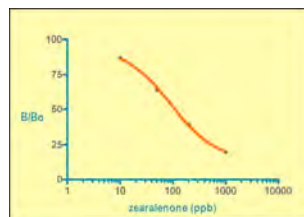


Figure 1. Celer ZON calibration curve

Validation results

- Specificity:** 20 HPLC negative maize samples were analysed and a Decision Limit of 44 ppb was determined (mean of B/B₀ - 2SD). At the established cut-off, the specificity of the assay was 100%.
- Sensitivity:** The EIA concentration value of both maize and wheat samples fortified with 100 ppb of zearalenone was always higher than the established Decision Limit (n = 10 determinations), indicating that the EIA limit of detection (LOD) is ≤100 ppb. The sensitivity at 100 ppb was 100%.
- Accuracy:** The analysis of contaminated maize samples (concentration range: 100-500 ppb) showed a high correlation between the assigned value (HPLC analysis) and the EIA concentration (Figure 2), with a R value of 0.983. The mean recovery of all the samples was 95±12%. Moreover, negative maize and wheat samples were fortified with 100, 250 and 500 ppb of zearalenone. The correlation between spiking level and EIA concentration is shown in Figure 3: the R value was higher than 0.991 for both maize and wheat samples. Mean recoveries were 108±7% for maize and 118±8% for wheat.

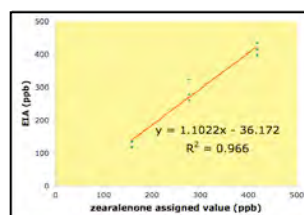


Figure 2. Assigned value-EIA correlation for contaminated maize samples (n = 3 determinations for each sample)

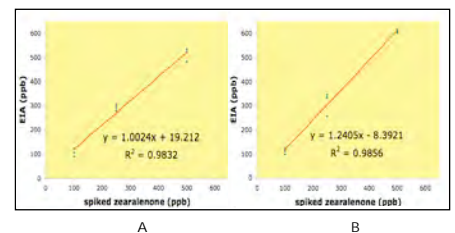


Figure 3. Spiking level-EIA correlation for maize (A) and wheat (B) (n = 3 determinations for each sample)

- Precision:** Precision was calculated as Coefficient of Variation % (CV %) of EIA values of spiked maize and wheat samples. Intra-assay CV% (n = 3 determinations for each sample) was in between 1 and 16%; inter-assay CV% (n = 3 different experiments) was in between 8 and 20% (Table 2).

	Spiking level	Celer ZON determination	
		Intra-assay CV%	Inter-assay CV%
M A I Z E	100 ppb	15.3	13.9
	250 ppb	3.7	8.3
W H E A T	500 ppb	5.2	10.3
	100 ppb	10.0	11.9
	250 ppb	15.7	10.4
	500 ppb	1.2	20.0

Table 2: Celer ZON intra- and inter-assay precision for spiked maize and wheat samples (n = 3)

Conclusions

Celer ZON is a rapid and simple EIA: ten minutes for sample preparation and twenty for assay performance. Results obtained for specificity, accuracy and precision indicate that Celer ZON can be used as a rapid and cost effective screening method for the quantitative detection of zearalenone in cereals. Thanks to its ranges of measurement, the assay can be used to test both human food and animal feed, meeting the sensitivity needs of EU Regulations.

Acknowledgements

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