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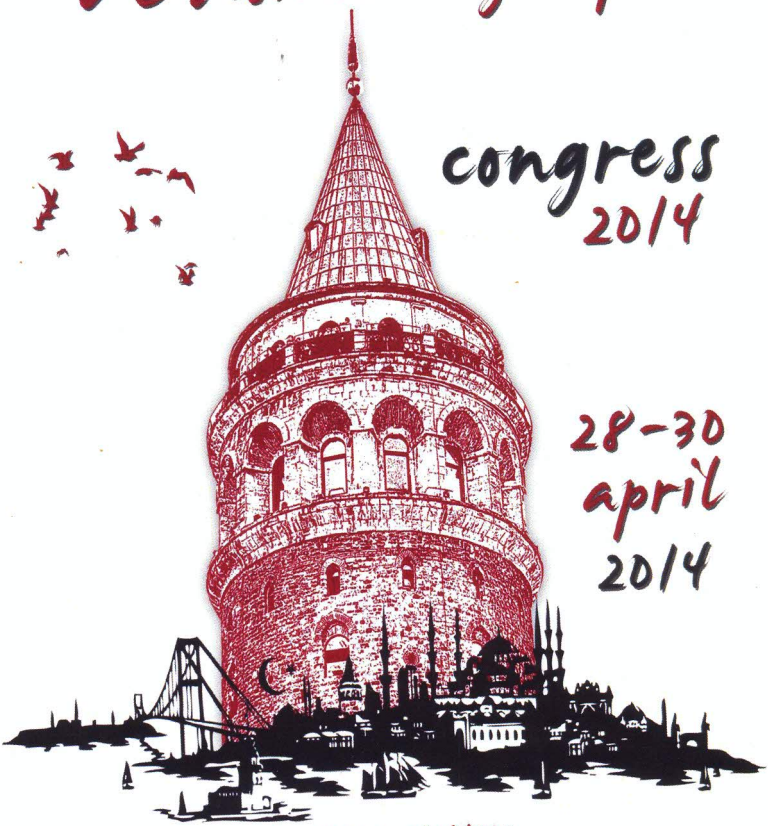

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VALIDATION PROCEDURE FOR DETERMINATION OF ZEARALENONE IN CEREALS IMPLEMENTING COMMISSION REGULATION 2006/401 AND COMMISSION DECISION 2002/657/EC

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The *Fusarium* fungi are probably the most prevalent toxin-producing fungi in the world. They produce a number of different mycotoxins. One of them, zearalenone (ZEA) is nonsteroidal, estrogenic mycotoxin witch is found worldwide in a number of cereals: corn, maize, barley, oats, wheat, even bread and pastries. It is implicated in reproductive disorders and hyperoestrogenic syndromes. ZEA is stable and not degraded during storage, grinding and high-temperature processing. IARC made classification of ZEA together with other Fusarium toxins in Group 3 (not carcinogen to humans). Content of ZEA in foodstuffs is regulated by legislation worldwide. MRL varies in the range of 50-200 µg/kg. The HPLC-FD method with immunoaffinity column clean-up is the method of choice for determination of zearalenone in order to achieve accurate and reliable results.

For the experiment purposes, ZEA-free corn samples (previously determined with HPLC-FD) were spiked with known amount of ZEA at three concentration levels (37.5; 50.0 and 100 µg/kg). All reagents used were HPLC grade. For clean-up procedure EasiExtract Zearalenone immunoaffinity columns (R-Biopharm) were applied. The extraction and purification of samples was done according to modified method of Visconti and Pascale (1998). HPLC analysis was performed with Perkin Elmer chromatographic system with fluorescence detector.

The validation procedure was performed according to Regulation 401/2006/EC and Decision 2002/657/EC. Six working standard solutions in range of 10-250 ng/ml were used for the linearity testing and a good coefficient of correlation (R^2) was found (0.9999) with following equation y=2009.7x-12037. Limit of detection (LOD) and limit of quantification (LOQ) were obtained through the standard deviation of the signal values for 20 blank samples and slope value estimated from the calibration curve. The established values for LOD and LOQ were 1.34 µg/kg and 4.06 µg/kg, respectively, and they were acceptable. Recovery was assessed according to the method of standard addition at three spiking levels (37.5; 50.0 and 100 µg/kg) and the results are in the range of 97.19-111.39%. Repeatability was estimated on SD and RSD values using the data from the recovery and RSD_r was in the range of 2.46-11.35%. RSD_R results (within-laboratory reproducibility) show good correlation between two days (1.99% and 1.17%).

The performed validation procedure (according to Regulation 401/2006/EC and Decision 2002/657/EC) provides satisfactory values for the performance criteria. They show that the method is simple, precise and accurate and can be implemented for determination for routine analysis of zearalenone in cereals.