Study Of Clean Up Procedures Using Charcoal-Alumina-Celite Column, Immu noaffinity Column And Strata x Column To Determine Deoxynivalenol By High Performance Liquid Chromatography In Wheat

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Fusarium graminearum is the most common toxic fungal species affecting grains in Uruguay. Since 1977, due to favorable climate conditions, there have been harvests with prominent Fusarium Head Blight in wheat. This were in 1984, 1990, 1993, 1996 and 2001. Natural Toxin Department of Technological Laboratory of Uruguay as National Reference Laboratory, is continuously improving the analytical methods in order to have a good response to the industry requirements and to the monitoring programs for import and exports commodities. The objective of this work was to compare different clean up methods in order to select the best one for routine determination of deoxynivalenol (DON) in wheat (grain and flour). Charcoal-alumina-celite (7+5+3), immunoaffinity columns DONPREP R-Biopharm Rhone and Strata X 33µm polymeric sorbent Phenomenex columns were used to perform the study. Considering as reference analytical method the internal protocol PEC.TOX.063 accredited by United Kingdom Accreditation Service (UKAS) following the ISO 17025 requirements, and based on AOAC method 986.17(chapter 49, 2002) for extraction and clean-up and on J.AOAC 70(3), 1987:479-483 for the high performance liquid chromatography (HPLC) detection, two more clean up methods were evaluated. In all of them PEC.TOX.063 detection procedure was carried out.
PEC.TOX.063 used for the clean up an in house column chromatography prepared with charcoal-alumina-celite (7+5+3) . Extraction was performed using acetonitrile- water (84+16) and an aliquot of the extract was passed through the column. Extract was dryed under vacuum and DON detected by HPLC using photodiode array detector. For the method that used immunoaffinity columns, water was the extraction solvent, and manufacture protocol was followed up. An aliquot of the extract was passed through the column. Column was washed using water and DON eluated using methanol 100%.
For the method that used Strata X column for the clean up, the extraction solvent was acetonitrile- water (84+16). Column was conditioned with methanol 100% and water. Prior to pass an aliquot of the extract through the column it was necessary to reduce the acetonitrile to 10% using vacuum. Column was washed using methanol 15 %, and DON eluated using methanol 100%. An internal reference flour material (2010 µg/kg) was analysed following the three procedures. For PEC.TOX.063 the historical recovery percentage of 91% was maintained, for immunoaffinity column protocol 84 % average was obtained, and for Strata X column 101% was obtained. Chromatograms and spectrums showed appropriated results. As the recoveries were acceptable it was decided to start with wheat samples analysis. Taking approval of the reference material and the IAC, the reuse of the columns was also estimated. Columns were regenerated with phosphate buffer solution (PBS) and storage in the fridge for 24 hours. At least three uses were undertaken with accepted recovery results (91, 69 and 104%) respectively.
A total of 21 samples including wheat and reference internal material were analysed following PEC.TOX.063 (charcoal-alumina-celite) and immunoaffinity column method (first, second and third use) . Nine samples from the 21 were also analysed using Strata X columns. PEC.TOX 063 and the immunoaffinity method showed appropriate chromatograms. This didn't occur with Strata X columns method. Chromatograms presented interferences at DON retention time. Due to chromatograms and results obtained no more samples were passed through Strata X columns.

As conclusion charcoal-alumina-celite (7+5+3), as well as immunoaffinity column would be a good option to analysed wheat samples by HPLC. For the Strata X Phenomenex columns more studies need to be done in order to optimize the elution solvent
The advantage of the first one is the low effective cost, the possibility to make them in house and the fact that no lifetime is involved. As future study, validation of immunoaffinity column method should be carry out in order to compare the data obtained at this moment and to calculate the correlation coefficient between PEC.TOX.063 and immunoaffinity column method .
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