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Performance of modern sample preparation techniques in the analysis of *Fusarium* mycotoxins in cereals

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Abstract

The efficiency of modern sample preparation techniques are discussed and compared to well-established techniques with respect to the determination of zearalenone in corn and B-trichothecenes in wheat in the μg/kg range. This includes the use of immuno-affinity columns and of multifunctional Mycosep columns as well as the employment of supercritical fluid extraction for the trace analysis of these major *Fusarium* mycotoxins. In addition, the performance of new analytical methods was investigated in an interlaboratory comparison study only recently organized by our laboratory. From both the validation data, and from the results of the intercomparison study, the suitability and competitiveness of the described methods could be clearly demonstrated.

Keywords: *Fusarium* spp.; Sample preparation; Food analysis; Mycotoxins; Toxins; Zearalenone; Trichothecenes; Deoxynivalenol

1. Introduction

The determination of mycotoxins in food and animal feed is of great importance because of their variety of toxic effects on humans and animals. Since the 1960s analytical methodology for mycotoxins has developed considerably due to the death of some 100 000 turkey poults in Great Britain as a result of aflatoxin-contaminated feed [1]. However, rapid methods which allow sensitive, accurate, and precise analyses are still required. Zearalenone (ZON) and deoxynivalenol (DON), the most important representatives of the type B-trichothecenes are mycotoxins produced by a diverse variety of fungi imperfecti, especially by the genus *Fusarium*, which is known to attack various cereals [2,3]. Since both mycotoxins are potential health risks for humans and animals, several countries have regulated ZON and/or DON in food and feed at maximum tolerable levels [4]. In order to be able to monitor suspected commodities in the μg/kg and mg/kg range many laboratories established analytical methodology for DON and ZON in cereals and perform determinations of these mycotoxins more or less regularly.

2. DON and ZON analysis: state-of-the-art

For the determination of DON and ZON, several chromatographic methods are applied, which are preceded by a sequence of operations including sampling, sample preparation, extraction and clean-up. The results of the most sophisticated chromatographic procedure will be determined by the efficiency of these steps. As a large number of interfering compounds originally present in samples contaminate the primary sample extracts, these components must be removed as completely as possible. The conventional time-consuming clean-up methods

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include column chromatography, liquid–liquid extraction and the use of commercially available solid-phase extraction (SPE) and chromatography cartridges.

GC is the most commonly used means of separating and identifying trichothecenes [5]. Scott et al. described 1986 a GC method for the determination of DON and nivalenol (NIV) in cereals [6]. This method has been established in many laboratories worldwide and can be considered as the most frequently used procedure for the trace analysis of B-trichothecenes (μg/kg range) in the last decade. Similar procedures are still being employed for the analysis of DON [7,8]. A number of methods has also been developed for the determination of ZON in different foods, feeds, animal tissues, blood and urine. Although there are TLC [9–11] and GC [11–13] methods available, LC is considered as the usual choice for the determination of ZON. Most of the LC methods have been developed for corn and other cereals. Recent HPLC methods for ZON have employed reversed-phase chromatography with direct fluorescence detection [14–16].

The described methods are well established and have been widely used for the assessment of various commodities. However, these methods are highly time-consuming and mostly require a very specialised experience, which is especially problematic for newcomers. Therefore, new approaches for the optimization of the clean-up step have been made. Combinations of polar and non-polar materials for solid-phase extraction (SPE) become increasingly popular. Most frequently the combinations of charcoal with alumina or charcoal with alumina and Celite are used. A quick method for the determination of B-trichothecenes using various adsorbents in combination was presented by Romer and coworkers [17,18]. While conventional methods for ZON generally use liquid–liquid partitioning during sample clean-up, a recently developed technique employs immuno-affinity columns for sample preparation [19,20]. Supercritical fluid extraction (SFE) may also be a useful alternative to these conventional techniques for extraction and sample purification in which a great amount of potentially hazardous solvents are employed.

In this paper, the efficiency of the so-called Mycosep columns for the determination of trichothecenes in wheat, and a method employing immuno-affinity columns with respect to the determination of ZON in corn, are discussed. The performance of these methods was evaluated through validation figures and through the results obtained from an interlaboratory comparison study. Moreover, an analytical method for the determination of DON in wheat, based on SFE and HPLC–diode array detection (DAD) end-detection, is discussed as a new alternative for the analysis of DON. The calibration data of this work were subjected to statistical analysis utilizing the Microsoft Excel macro Validata [21]. This program calculates the limit of detection (LOD) from the *y*-intercept and its confidence interval after linear regression [22].

3. Analysis of trichothecenes by use of Mycosep columns

3.1. Introduction

The Mycosep (Romer Labs. Inc., Union, MT, USA) multifunctional clean-up columns consist of packing material, which contain various adsorbents, such as charcoal, Celite, ion-exchange resins and others [18]. The packing material is housed in a plastic tube between filter discs with a rubber flange on the lower end containing a porous frit and a one-way valve (see Fig. 1). When the column is inserted into the culture tube the flange seals tight, thus forcing the extract through the packing material

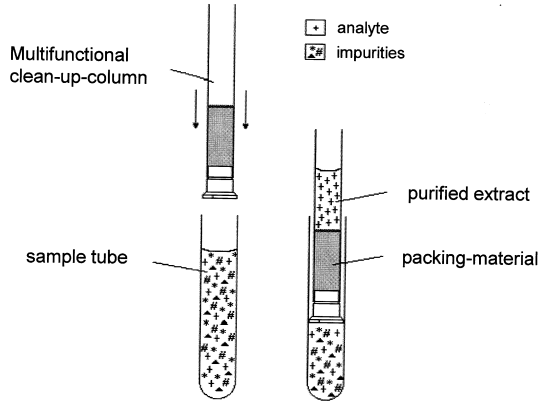

Fig. 1. Principle of Mycosep multifunctional clean-up columns.

of the column. On the top of the plastic tube the pure extract appears. The Mycosep column allows quick sample purification within 10 to 30 s. A major advantage of this column is that there are no time-consuming rinsing steps required as in SPE. In addition, nearly all analytical interfering substances are retained on the column while the trichothecenes are not adsorbed on the packing material. In this work the performance of this modern method is described. In one of our recent studies, a detailed description of the Mycosep clean-up, in particular the comparison with a conventional clean-up procedure, can be found [23].

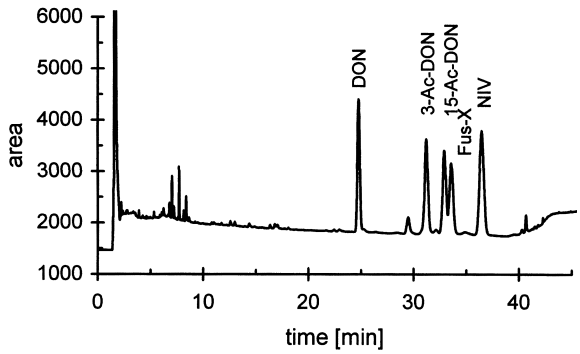

Fig. 2. GC–ECD chromatogram obtained from wheat spiked with 500 μg/kg of DON, NIV, 3-Ac-DON, 15-Ac-DON and FusX after clean-up with a Mycosep column.

3.2. Experimental

Twenty-five g of ground wheat are weighed into a 250-ml Erlenmeyer flask and extracted with 100 ml acetonitrile–water (84:16) for 80 min on a shaker. After filtrating a volume of about 8 ml the extract is transferred to glass culture tubes and is purified by pushing the flange end of the Mycosep column into the culture tube until about 3–4 ml have passed the column. Then 2 ml of this clear extract are transferred to vials and evaporated to dryness under a stream of nitrogen at 50°C. After derivatization with Sylon BTZ separation and detection in the μg/kg range are performed on GC with electron-capture detection (ECD) and a capillary column (e.g., type HP 5, 30 m×0.32 mm, 0.25 μm) as described by Weingärtner et al. [23] and Scott et al. [6].

3.3. Results and discussion

Fig. 2 shows a GC–ECD chromatogram obtained from a sample spiked with 500 μg/kg of DON, nivalenol (NIV), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON) and fusarenon X (FusX) each, after a clean-up with a Mycosep 225 column. The mean recoveries which are usually obtained with these columns (working range 100–2000 μg/kg, spiked samples) range from 95 to 103% for DON, 3-Ac-DON, 15-Ac-DON and FusX (see Table 1). Only for the more polar NIV are the average recoveries lower (66%). This is obviously due to the strong adsorption on polar sites of the packing material since similar poor recoveries are observed when the Mycosep clean-up is applied to pure standard solutions. The relative standard deviation of the recoveries range from 6.4% for DON to 10.4% for 3-Ac-DON for the working range from 100 to 2000 μg/kg. In our studies the mean recovery for 47 samples spiked with DON only (spiking levels, 100, 250, 480, 500, 1000 and 2000 μg/kg) was 96.6% with a standard deviation of 9.5%. The analysis of a certified reference material (CRM 379) showed a mean value of 643 μg/kg with a standard deviation of 25.1 for six determinations. The certified value is 673 μg/kg determined as mean value of six independent determinations by six laboratories. The according confidence interval at 95% probability ranges from 617 to 670 μg/kg. The LODs for the trichothecenes analyzed in this work are in the range of 40 μg/kg with a reasonable uncertainty of 10%.

3.4. Conclusion

The clean-up procedure with Mycosep 225 columns is a quick, reliable and rugged method for the determination of B-trichothecene mycotoxins. The major advantages are the saving of time, the good accuracy, precision and recovery of the method because of the minimum number of clean-up steps which avoid possible sources of error. Using acetonitrile–water (84:16) allows recycling of the solvent due to the azeotropic nature of the solvent system. For all the investigated trichothecenes except NIV higher mean recoveries (ca. 10% higher), and thus better precision of the final results (corrected for recovery), are obtained with the Mycosep columns

Table 1
Mean recoveries (%) of trichothecenes from wheat obtained with the Mycosep method for four different spiking levels (100, 500, 1000, 2000 μg/kg, $n=3$)

Trichothecene	DON	3-Acetyl-DON	15-Acetyl-DON	Fusarenon X	Nivalenol
Mean recovery	102.8	96.1	95.3	97.1	66.4
S.D.	6.36	10.41	8.87	7.66	8.45
R.S.D.	6.18	10.84	9.31	7.89	12.73

compared to a well-established method using liquid–liquid extraction and Extrelut technique (e.g., R.S.D.=6.4% instead of 11.3% for DON) [8,23]. This amendment is obviously due to the easy one-step clean-up.

4. Analysis of trichothecenes by use of SFE

4.1. Introduction

In contrast to the use of the mostly hazardous solvents employed for the extraction of mycotoxins from cereals, supercritical CO_2 is nontoxic, non-flammable and chemically inert. Furthermore, by means of modern SFE approaches, both extraction and clean-up can be performed in one step [24,25].

However, in particular for polar substances, e.g., mycotoxins, the recoveries obtained with CO_2-SFE are generally very poor (<20%) [26–29]. Only two publications can be found in the literature which deal with SFE of *Fusarium* mycotoxins [30,31]. However, no values for the obtained DON recoveries are given in the work which deals with SFE of DON [30]. The goal of a recent investigation in our lab was to develop an analytical method for the determination of DON in wheat based on SFE and HPLC–DAD end-detection [32]. In the following the suitability of this new SFE-method for the analysis of B-trichothecenes is discussed.

4.2. Experimental

Supercritical fluid extractions are performed by using modified carbon dioxide (5.2-SFC quality) on an SFE module (type HP 7680 T, Hewlett-Packard) (see Fig. 3). The extraction chamber having a volume of 5 ml is filled with 1 g wheat sample between two layers of glass wool. The modifier (methanol) is added to the CO_2 with a T-connected HPLC pump prior to the cryogenic high-pressure pump of the SFE apparatus. Analyte trapping is carried out with an SPE silica trap. After centrifugation, aliquots of the extracts containing DON can be analyzed either by HPLC [32] or GC [33].

4.3. Results and discussion

Since the efficiency of the SFE is strongly dependent on the analyte–matrix interaction, optimization of the SFE parameters has to be carried out with naturally contaminated cereals. It is useful to refer to the values obtained with a conventional method as 100% recovery for these naturally contaminated samples.

The optimum SFE conditions have been found at a pressure of 318 bar and an extraction chamber temperature of 40°C, employing a dynamic mode of merely 15 min, a fluid flow-rate of 2.0 ml/min, 3% methanol as modifier, and a preceding static mode of 30 min. In order to improve the swelling of the matrix and to thus accelerate the solute diffusion, 500 μl methanol are applied to the sample directly in the cell before running the SFE. Fig. 4 shows the HPLC chromatogram obtained after SFE of wheat naturally contaminated with DON monitored at 220 nm. It can be seen that there are no interferences from the remaining matrix components at the retention time of DON although no further clean-up has been applied. This clearly demonstrates the strength of SFE compared to classical extraction techniques based on solid–liquid extraction, which require at least one further clean-up step to obtain similar chromatograms.

The recoveries which can be obtained with this new method for DON contaminated wheat flour are 53.0±3.2% ($n=5$). For the determination of the mean recovery of the method, SFE experiments with

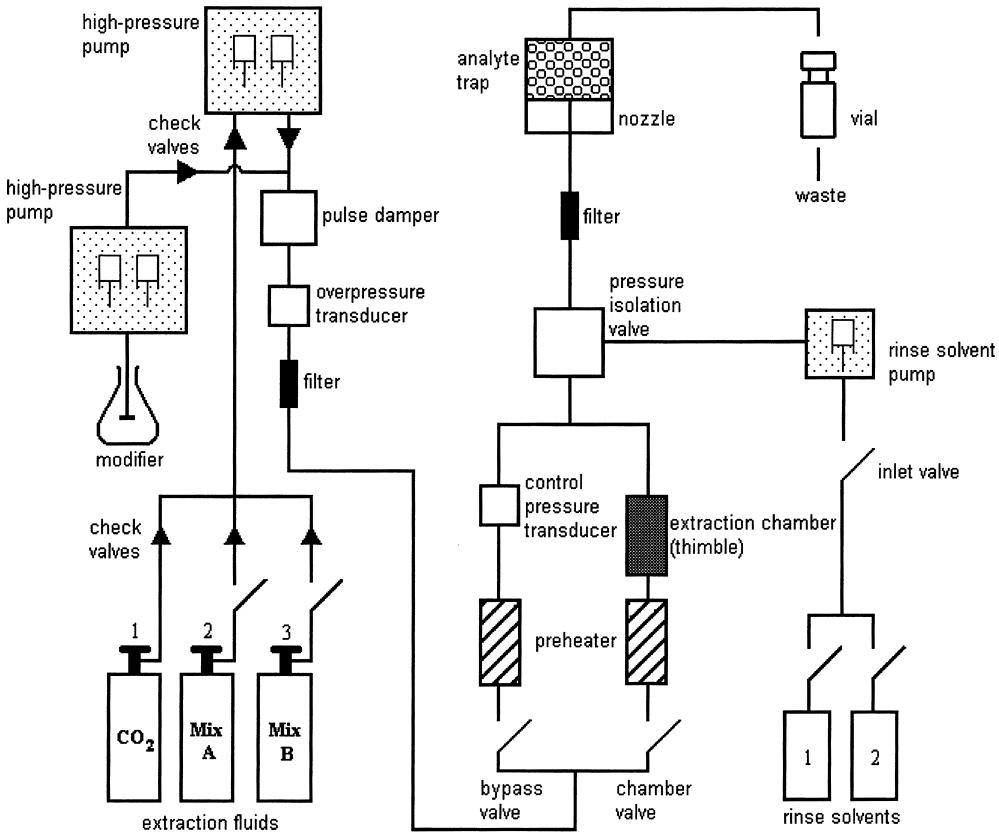

Fig. 3. SFE system HP 7680T.

wheat flour naturally contaminated with DON at four concentration levels (2.35, 5.73, 11.42 and 16.75 mg/kg) were employed. The sensitivity of the developed SFE method is only limited by the use of the UV detection. When GC–ECD after derivatization with TRI-SIL-TBT [5] is employed subsequent to the SFE procedure, an LOD of 250 μg/kg can be achieved [33], which meets the requirements of several guidelines in Europe and the USA that regulate the maximum DON concentration in wheat [4].

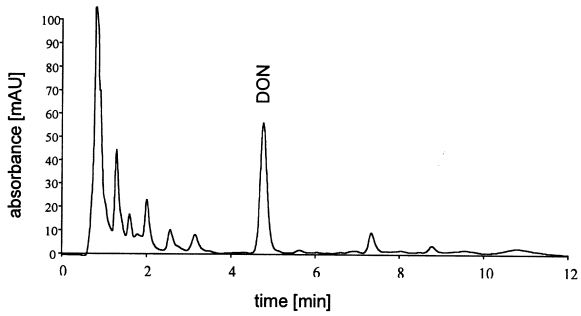

Fig. 4. HPLC–DAD chromatogram obtained from wheat spiked with 1.6 mg/kg of DON after SFE.

4.4. Conclusion

The results achieved with this SFE method are much more promising than previously published results on the SFE of the even less polar aflatoxins. Due to the simplicity of the method, SFE seems to be ideally suited for the screening of trichothecenes, such as DON. The tremendous improvement of the extraction efficiency for DON and the good repeatability of the method indicates that the potential of SFE for the extraction of more polar substances, e.g., mycotoxins, is probably greater than expected.

However, further investigations are required to verify this conclusion.

5. Analysis of ZON by use of immuno-affinity columns

5.1. Introduction

Since 1976, when the first immunoassays were described for aflatoxin B1, there has been rapid development of these assays for mycotoxin detection. The initial stages involve the production of antibody, but as mycotoxins are low-weight molecules they are not immunogenic. However, following conjugation to a protein carrier the toxins may be used for immunization to induce antibody production. Using this approach a number of antibodies have been developed to mycotoxins such as the aflatoxins, ochratoxin A, ZON, T-2 toxin, and many more [5]. With the availability of these antibodies, simple and rapid immunoassay methods have been developed for the determination of these mycotoxins in food samples, animal feed samples, and also within biological fluids such as milk and urine.

Perhaps one of the most applicable and adaptable procedures for mycotoxin detection is the immuno-affinity column. This method is simple, robust and can be used as a semi-quantitative method for the screening of mycotoxins or it can be coupled with physicochemical equipment such as HPLC for the complete and accurate estimation of mycotoxin quantities [5]. After preconditioning of the column with a buffer (see Fig. 5, step 1) the sample is applied to the column (see Fig. 5, step 2). As the solution passes through the column the mycotoxin molecules bind specifically to the antibodies attached to the solid-phase of the column. Other components in the solution are unaffected by the antibodies and therefore pass directly through the column. After washing with a buffer solution or distilled water at neutral pH to remove unwanted substances, the mycotoxin is eluted (desorbed) (see Fig. 5, step 3) by the use of an appropriate solution which causes antibody denaturation (e.g., methanol, acetonitrile) (see Fig. 5, step 4).

The conventional and the immuno-affinity method for ZON differ mainly during clean-up procedure. In contrast to the one-step clean-up with immuno-affinity columns employing an acetonitrile–phosphate buffer mixture, sample purification according to conventional methods demands successive extractions with chloroform–aqueous NaOH and chloroform again. In both cases chromatographic separation and quantification of ZON can be carried out by RP-HPLC with fluorescence detection (FLD) (275/450 nm).

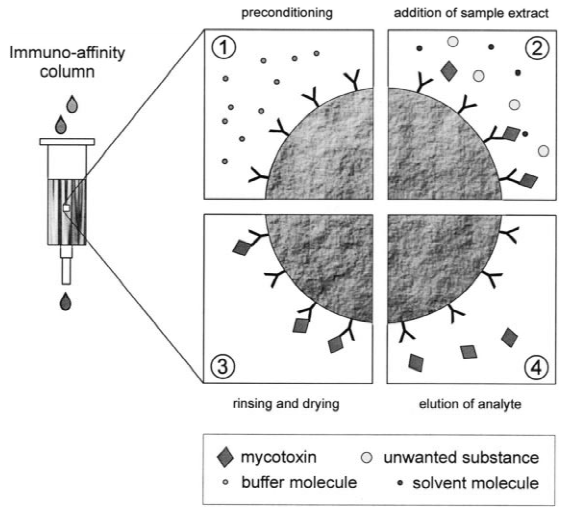

Fig. 5. Principle of immuno-affinity columns.

5.2. Experimental

Twenty-five g ground corn are homogenized and extracted in 100 ml acetonitrile–monodistilled water (75:25) with an Ultra Turrax T25 mixer for 3 min at 24 000 rpm. The extract is filtered through a Whatman GF/A filter and 20 ml of the filtrate are pipetted to 80 ml of phosphate-buffered saline (PBS) (pH 7.4). The immuno-affinity column from Rhône-diagnostics technologies (also available from VICAM) are preconditioned with 15 ml of PBS buffer and the diluted sample extract is sucked through the immuno-affinity column with a flow-rate of approximately 1.5 ml/min. The column is rinsed with 2×10 ml water and dried by employing a gentle vacuum for about 5 min. ZON is eluted with 5 ml of acetonitrile for HPLC. After gentle evaporation of the solvent (nitrogen stream, 50°C) the sample is dissolved in 250 μl acetonitrile–water (4:6), filtered

through a 0.22-μm membrane filter (Millex GV13) and 20 μl are injected into the HPLC. Separation and detection of ZON is carried out with acetonitrile–water (4:6) (0.5 ml/min) using an RP-HPLC column (e.g., 100×2.1 mm, 5 μm, ODS-Hypersil) and an FLD at 275/450 nm. Additionally, the identity of ZON can be confirmed by measuring UV spectra with diode array detection (DAD).

5.3. Results and discussion

Fig. 6 shows a typical chromatogram for a naturally contaminated sample, which was obtained when the immuno-affinity method was employed for the analysis of ZON in corn. The chromatogram clearly demonstrates that this modern clean-up procedure is well suited for subsequent HPLC–FLD analysis of corn samples. The LOD of the immuno-affinity method is 6 μg/kg. In the examined concentration range (10–200 μg/kg ZON) the method gives a high mean recovery rate of 94% (n=10).

One of the major advantages of the conventional methods lies in the relative low material costs of about US$8–9 compared to approximately US$25 for the immuno-affinity method [19]. On the other hand, these methods require more practical experience to achieve precise and accurate analytical results. In particular, the exposure time of ZON with aqueous NaOH must be minimized because the ZON lactone ring can be hydrolyzed under alkaline conditions. Cleavage of the ester bond would result in loss of ZON during clean-up procedure.

Using immuno-affinity columns, eight samples/day can be analyzed with an average recovery of >90%. But it should be noted that, according to the information provided by Rhône-diagnostics technologies, clean-up can be speeded up by applying higher flow-rates (up to 5 ml/min) to the immuno-affinity column. In our laboratory this would mean a sample throughput of approximately eight to 10 analyses per day. However, own experiments revealed that recoveries could deteriorate to approximately 50% by employing a flow-rate of 5 ml/min.

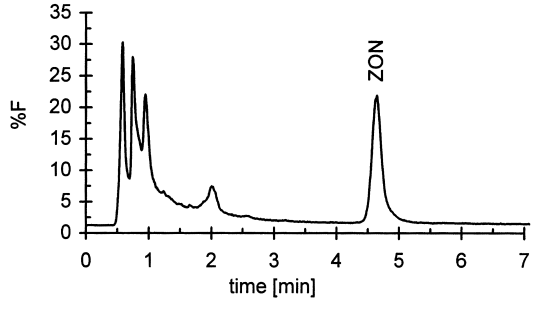

Fig. 6. HPLC–FLD chromatogram obtained from corn naturally contaminated with ZON (c=111±6.7 μg/kg) after IAC-clean-up.

5.4. Conclusions

The analytical results demonstrate that the immuno-affinity columns are well suited for the determination of ZON in corn in the μg/kg range. Although the material costs per analysis are higher than for conventional methods, sample preparation under the described conditions usually gives better precision for the immuno-affinity method (e.g., R.S.D.=6% instead of 10.5%) [11,19]. From the environmental point of view a major drawback of the conventional method is the use of a chlorinated solvent (chloroform), which is not needed for the immuno-affinity method. Furthermore, ZON analysis by means of immuno-affinity columns is easy to learn and to employ.

6. Intercomparison study for DON and ZON

6.1. Introduction

An interlaboratory comparison test, under the supervision of the Austrian Society for Analytical Chemistry (ASAC) [34], was carried out in 1996 with the objective to obtain information on the quality of analytical data and the comparability of ZON and DON results produced by different methods. Sixteen laboratories from six countries performed tests for ZON, thirteen for DON. Twelve of the 17 laboratories participated for both mycotoxins. For each mycotoxin, five different sample types were distributed. The participants received standard solutions, blank materials, spiked samples (75.1 and 378.3 μg/kg ZON in corn, 126.2 and 2519 μg/kg DON in wheat) and naturally contaminated corn (mean=78.7 μg/kg ZON) and wheat (mean=492.6 μg/kg DON).

Laboratories were asked to carry out two in-

dependent replicate determinations for each type of sample employing their usual analytical methods for DON and ZON. Fifteen of the 16 laboratories who participated for ZON sent in information about the methods employed. Except for one participant, who used pure ethyl acetate, mycotoxin extraction was carried out by employing a mixture of an organic solvent (acetonitrile, ethyl acetate, methanol, chloroform or acetone) with water or aqueous acidic solutions, respectively. Six participants performed a liquid–liquid extraction applying an organic solvent and an aqueous alkaline solution. Two laboratories used immuno-affinity columns for purification of raw extracts. For the final separation and determination of ZON most laboratories applied RP-HPLC with FLD; UV detection was chosen by one participant. Four participants quantified ZON by competitive enzyme-linked immunosorbent assay (ELISA) methods.

DON extractions were carried out in pure water (two laboratories) or in mixtures of water with either acetonitrile, methanol or acetone. For purification of raw extracts SPE techniques were employed, either with self-filled aluminium oxide–activated charcoal columns or with Mycosep columns (four laboratories). Additionally, the Extrelut technique in combination with SPE and, in one case, liquid–liquid extraction with dichloromethane, was applied for clean-up. Three out of four laboratories, who determined DON by ELISA did not use any clean-up at all. Beside ELISA methods, determination of DON was carried out by GC–ECD, RP-HPLC with UV detection and, in one case, by RP-HPLC followed by post-column derivatization–FLD.

6.2. Results and discussion

Divergent analytical performances for ZON in corn that might have been caused by applying different analytical methods could principally be found for all spiked and naturally contaminated samples, and shall be illustrated in an exemplary manner for the naturally contaminated maize sample. The two results derived by the immuno-affinity method lie within ± 1 s from the overall mean. Methods using liquid–liquid partitioning of ZON between an organic solvent and aqueous alkaline solution seem to be more difficult to control, since results obtained by these methods vary broadly (e.g., $c = 62.7–402.5$ μg/kg).

For DON a method dependence of the analytical results can also be demonstrated. In general, laboratories who employed the Mycosep columns for purification of raw extracts achieved good accuracy for the determination of DON in wheat. ELISA methods, however, tended to give too high DON concentrations. This can be illustrated for the naturally contaminated wheat samples. Three out of four results are more than 27% above the overall mean.

6.3. Conclusions

In the intercomparison study a method dependence of the analytical results was demonstrated for both mycotoxins. It became obvious that clean-up procedures for ZON by means of an alkaline aqueous extraction step seem to require much experience. While most data obtained by DON ELISA testkits did not show good accuracy, excellent results were obtained with new clean-up techniques employing, e.g., the Mycosep columns (for DON) and immuno-affinity columns (for ZON) in combination with chromatographic end detection.

7. Overall conclusions

The validation figures and the results of the interlaboratory comparison study clearly demonstrate the suitability of modern clean-up techniques for the analysis of the *Fusarium* mycotoxins DON and ZON. The clean-up procedures employing multifunctional Mycosep and immuno-affinity columns are quick, reliable and rugged methods for the determination of these mycotoxins. The major advantages are the saving of time and the achievement of better precision. Furthermore, less experience is needed compared to the usual analytical methods for these species. In addition, also rather innovative techniques, such as SFE, seem to have a greater potential for the analysis of mycotoxins than expected.

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References

[1] J.D. Miller, H.L. Trenholm, Mycotoxins in Grain. Compounds other than Aflatoxin, Eagan Press, St. Paul, MN, 1994.
[2] L. Roth, H.K. Frank, K. Kormann, Giftpilze, Pilzgifte, Schimmelpilze, Mykotoxine, Ecomed, Landsberg, 1990.
[3] R.J. Cole, R.H. Cox, Handbook of Toxic Fungal Metabolites, Academic Press, New York, 1981.
[4] H. van Egmond, Worldwide Regulations for Mycotoxins 1995, FAO Food and Nutrition Paper, Rome, 1996.
[5] V. Betina, Chromatography of Mycotoxins. Journal of Chromatography Library, Vol. 54, Elsevier, Amsterdam, 1993.
[6] P.M. Scott, S.R. Kanhere, E.J. Tarter, J. Assoc. Off. Anal. Chem. 69(5) (1986) 889.
[7] V. Seidel, B. Lang, S. Fraissler, C. Lang, K. Schiller, K. Filek, W. Lindner, Chromatographia 37 (1993) 191.
[8] J. Chelkowski, H. Lew, H. Petterson, Mycotoxin Res. 10 (1994) 116.
[9] R.M. Eppley, J. Assoc. Off. Anal. Chem. 51 (1968) 74.
[10] M.T. Liu, P.B. Ram, L.P. Hart, J.J. Petska, Appl. Environ. Microbiol. 50 (1975) 1178.
[11] C.J. Mirocha, B. Schauerhamer, S.V. Pathre, J. Assoc. Off. Anal. Chem. 57 (1974) 1104.
[12] W.M. Hagler, C.J. Mirocha, S.V. Pathre, J.C. Behrens, Appl. Environ. Microbiol. 37 (1979) 849.
[13] P.M. Scott, T. Panalaks, S. Kanhere, W.F. Miles, J. Assoc. Off. Chem. 61 (1978) 593.
[14] D.B. Prelusky, R.M. Warner, H.L. Trenholm, J. Chromatogr. 494 (1989) 267.
[15] P.M. Scott, G.A. Lawrence, J. Assoc. Off. Chem. 71 (1988) 1176.
[16] K. Ranfft, R. Gerstl, G. Mayer, Z. Lebensm.-Unters. Forsch. 588 (1991) 47.
[17] T.R. Romer, D.E. Greaves, G.E. Gibson, 6th AOAC Annual Spring Workshop, 12–14 May 1981.
[18] T.R. Romer, J. Assoc. Off. Anal. Chem. 69 (1986) 699.
[19] R. Schuhmacher, R. Krska, M. Grasserbauer, W. Edinger, H. Lew, Fresenius J. Anal. Chem. (1997) in press.
[20] Biocode Technical Notes, SA-45 (V01).
[21] C. Rohrer, W. Wegscheider, Validata an Excel-Macro, Version 1.04, 1995.
[22] Guidelines of the German norm DIN 32645, May 1994.
[23] J. Weingaertner, R. Krska, W. Praznik, M. Grasserbauer, H. Lew, Fresenius J. Anal. Chem. 357 (1997) 1206.
[24] L.T. Taylor, Anal. Chem. 67 (1995) 364.
[25] B. Wenclawiak, Analysis with SF: Extraction and Chromatography, Springer, Berlin, 1992.
[26] A. Boenke, H.P. van Egmond, P.J. Wagstaffe, Fresenius J. Anal. Chem. 345 (1993) 224.
[27] S.L. Taylor, J.W. King, J.L. Richard, J.I. Greer, J. Agric. Food Chem. 41 (1993) 910.
[28] H. Engelhardt, P. Haas, J. Chromatogr. Sci. 31 (1993) 13.
[29] M.I. Selim, M.-H. Tsuei, Am. Ind. Hyg. Assoc. J. 54 (1993) 135.
[30] H.T. Kalinoski, H.R. Udseth, B.W. Wright, R.D. Smith, Anal. Chem. 58 (1986) 2421.
[31] R.D. Smith, H.R. Udseth, Govt. Reports Announcements Index 25 (1982) 1.
[32] R. Josephs, R. Krska, M. Grasserbauer, J.A.C. Broekaert, J. Chromatogr. A. 795 (1998) 297.
[33] R. Josephs, R. Krska, M. Grasserbauer, J.A.C. Broekaert, Cereal Res. Commun. 25(3/1) (1997) 363.
[34] R. Schuhmacher, R. Krska, J. Weingaertner, M. Grasserbauer, Fresenius J. Anal. Chem. 359 (1997) 510.