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Simultaneous determination of ochratoxin A and zearalenone in maize by reversed-phase high-performance liquid chromatography with fluorescence detection and β-cyclodextrin as mobile phase additive

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method was developed for the simultaneous determination of the usually co-eluting peaks of ochratoxin A and zearalenone by fluorescence detection due to specific inclusion phenomena using β-cyclodextrin as a mobile phase additive. Additionally four different clean-up techniques were studied in order to optimize the recoveries of ochratoxin A and zearalenone in the range OS-1000 ppb (w/w) in maize, including liquid-solid extraction using Florisil, C_{18} and plain silica solid-phase extraction mini-columns and a base-acid liquid-liquid partitioning clean-up procedure.

INTRODUCTION

The infection of cereal grains with several species of fungal genera has been recognized as a potential threat to animal and human health. The contamination of animal feed with fungal secondary metabolites, the so-called mycotoxins, can cause serious reproductive problems and feeding difficulties, especially in pig-fattening stations and poultry farms. The effects of reduced litters, stillbirths, miscarriages [1], increased prenatal mortality, enteritis, diarrhoea, anorexia, kidney damages (known as mycotoxic porcine nephropathy) [2], cessation of milk production, hormonal disorders, sterility and numerous other anomalies in swine and cattle could directly be related to toxin content of the feed [1,3].

There is also a potential risk to human health by direct contamination of human food with toxigenic mould or indirect contamination by 'carry-over' of toxin residues in animal tissues [4], milk or meat [5,6] after feeding with contaminated feed, at the worst reported as Balkan endemic nephropathy [4,7].

Fungal infections of corn occur preharvest by field-growing *Fusarium species* and under storage conditions by *Aspergillus* and *Penicillium* species. The most frequently isolated field-growing fungi on Austrian corn are *Fusarium sacchari* var. *subglutinans* and *Fusarium graminearum* (Table I) [8], producing mycotoxins of the trichothecene family [especially deoxynivalenol (DON)], zearalenone (ZON) and the two diastereomers α- and β-zearalenol (ZOL) (Fig. 1), which have been suggested as precursors in biosynthetic pathway leading to ZON. α-Zearalenol possesses even 3-4 times more estrogenic activity than ZON, although it has been found only at low levels on Austrian maize. Ochratoxin A and B (Fig. 2) contamination, especially by *Penicillium verrucosum*, can appear when corn of high moisture content (23–40%) is stored at low temperatures [9], although such

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TABLE I

FUSARIUM SPECIES AND MYCOTOXINS MOST FREQUENTLY FOUND ON AUSTRIAN CORN

Taken in part from ref. 8. *Fusarium* strains were isolated from 48 samples of oats, wheat and maize harvested in 1985-87 in different Austrian production areas and were tested for the production of mycotoxins on autoclaved moistened maize. The original grain samples were also analysed for *Fusarium* mycotoxins [8].

Fusarium species	Deoxynivalenol positive/ total range (ppb)	Zearalenone positive/total range (ppb)	Zearalenol positive/total range (ppb)
F. graminearum	36/38 (25–310)	38/38 *(5-1200)*	9/38 *(1-125)*
F. culmorum	0/1	1/1 *(25)*	1/1 (2)
F. sambucinum	l/l (25)	1/1 (5)	O/l
F. avenaceum	0/27	0/27	*0127*
F. sacchari var. subglutinans	0/25	0125	0/25
F. tricinctum	0/10	0/10	0110
F. poae	0/22	0/22	0/22
F. verticillioides	*014*	*014*	0/4
F. oxysporum	0/3	0/3	0/3
F. equiseti	0/1	0/1	0/1

storage conditions are rare in Austrian maize cultivation regions, contrary to the situation in northern parts of Germany and the Scandinavian countries [10,11].

Many analytical methods for the determination of zearalenone and ochratoxin A have been developed, including thin-layer chromatography (TLC) [12–15], high-performance liquid chromatography (HPLC) [16–19], gas chromatography (GC) [20–24], enzyme immunoassay (EIA) [25] and enzyme-linked immunosorbent assay (ELISA) [26], but for highly sensitive and reliable detection there is still a need for further improvements to the existing methods.

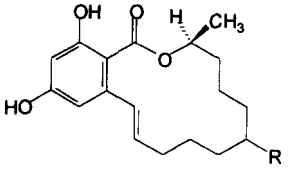

Fig. 1. Structures of zearalenone (ZON) (R = 0), zearalanone (ZAON) (R = 0), no double bond in position 1 and the two diastereomers a - and β-zearalenol (ZOL) (R = OH), which can be classified as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactones. IUPAC name: zearalenone = 3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-[*S*-(*E*)]-1*H*-2-benzoxacyclotetradecin-1,7(8*H*)-dione.

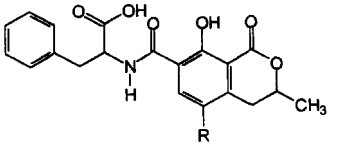

Fig. 2. Structures of ochratoxin A (R = Cl) and ochratoxin B (R = H), both closely related derivatives of isocoumarin linked to L-phenylalanine: (*R*)-N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-L-phenylalanine.

As has been shown, HPLC can cover the requirements of a multi-toxin simultaneous screening method for the major toxins (DON, ochratoxin A, ZON) [27,28] better than all the others, but the trichothecenes possess only poor UV absorption (DON maximum at 220 nm) allowing determination in real samples only in the high ppb range) [29]. A higher sensitivity for DON can be gained by fluorescence detection after pre- or postcolumn derivatization procedures [30,31], but this complicates the overall analytical procedure, which might adversely influence its ruggedness, and therefore high-sensitivity trichothecenes analysis is performed these days by GC methods using electron-capture detection (ECD) [32–34] or MS detection [35,36].

The simultaneous determination of aflatoxin, ochratoxin A and ZON in feeds by HPLC with fluorescence detection was demonstrated by Howell and Taylor [37], incorporating selective

sample pretreatment procedures. For improvement of ochratoxin A recovery, Langseth et al. [38] described a modified HPLC method after purification of sample extracts using solid-phase extraction (SPE) columns. Ochratoxin A and ZON were eluted from the column in two fractions and finally chromatographed using an RP-HPLC system with separate injections.

In this paper, a method for simultaneous determination of zearalenone (ZON), zearalanone (ZAON), a- and β-zearalenol (ZOL), ochratoxin A (OA) and ochratoxin B (OB) in a single HPLC run with fluorescence detection is described. Two basic approaches are covered: chromatographic separation of mycotoxins in a single run and improvement of sample pretreatment procedures, including extraction and clean-up.

The separation of all toxins in a single run was achieved by forming inclusion complexes with β-cyclodextrin added to the mobile phase (Fig. 3), which renders possible a more accurate determination of the major toxins OA and ZON without interferences from related compounds (ZAON, ZOL, OB). In addition, four different clean-up procedures were developed and compared for optimization of the recovery of OA and ZON from maize in the range of OS-1000 ppb. Evaluation of the final assay for recovery and accuracy of the other related mycotoxins, which, however, are of minor importance in Austrian feeds [8], will be the subject of further investigations.

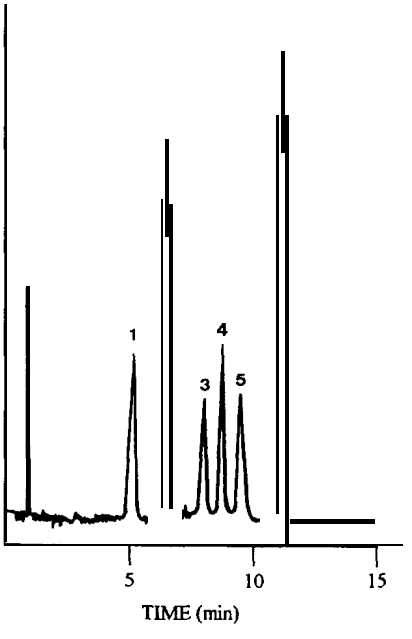

Fig. 3. HPLC of mycotoxin standard solution demonstrating the separation of 500 ng of β-zearalenol (1), 8 ng of ochratoxin B (2), 80 ng of a-zearalenol (3), 90 ng of zearalanone (4), 8 ng of zearalenone (5) and 4 ng of ochratoxin A (6) on a Merck LiChrospher 60, RP Select-B, 5 μm, 125 × 4 mm I.D. column. Mobile phase: methanol–water (45:55), pH 2.5 (12 m*M* phosphoric acid), containing 0.2 m*M* β-cyclodextrin. Fluorescence detection: λ_{ex} = 315 nm and λ_{em} = 465 nm.

EXPERIMENTAL

Instrumentation, chromatography

HPLC was performed using a Jasco 880-PU intelligent HPLC pump and a Jasco 820-FP intelligent spectrofluorimeter (λ_{ex} = 315 nm and λ_{em} = 465 nm). Separations were achieved on a Merck LiChroCART 125 × 4 mm I.D. column filled either with LiChrospher 60, RP-18 Select-B, 5 μm, or alternatively with LiChrospher 100, RP-18, 5 μm, or on a Shiseido Capcell PAK C_{18}, SG 120, 5 μm, 250 x 4.6 mm I.D. column. All columns were thermostated at 65°C in a water-bath. Samples were injected on to the column by a Rheodyne Model 7125 six-port valve equipped with 20-μl loop. Peak areas were calculated with a Merck-Hitachi D-2500 chromate-integrator. The mobile phase was methanol-phosphoric acid (5.95%, pH 1) (45:55) with addition of 0.1 m*M* β-cyclodextrin. The eluent was filtered and degassed before use and the flow-rate was set at 1.0 ml/min.

Standards and reagents

All solvents were of HPLC grade from Merck. C_{18} SPE columns (200 mg) and silica SPE columns (500 mg) were Bond Elut from Varian (Darmstadt, Germany). Florisil (0.15-0.25 mm, 60-100 mesh) was obtained from Serva (Heidelberg, Germany). Ochratoxin A, zearalenone, zearalanone, α-zearalenol, β-zearalenol and β-cyclodextrin (cycloheptaamylose, water content = 7.0 mol/mol) were purchased from Sigma (Deisenhofen, Germany). Ochratoxin B was obtained from R.A. Learmonth (Food Science

and Technology, Pretoria, South Africa). Methanolic standard solutions were stored at 5°C in a freezer.

Sample preparation

Maize samples were stored in a dry room at ambient temperature, conditions that did not allow any growth of toxigenic mould. An amount of at least 500 g of maize was finely ground in a grain mill prior to each sample clean-up and analysis.

Extraction and clean-up

A 5-g amount of maize was weighed into a 50-ml centrifuge tube and after adding 3.35 g (19 mmol) of ascorbic acid (pH 2.5–3.0, 0.6 M), was extracted with 30 ml of methanol-water (90:10) for 30 min with automatic shaking. After centrifugation (5 min, 3000 rpm) the process was repeated with another 20 ml for 15 min. The combined extracts were transferred into a 50-ml volumetric flask and diluted to volume with methanol-water (90:10).

Florisil. *An* aliquot of 5 ml of the methanolic extract was concentrated by rotary evaporation, dissolved in 2 ml of chloroform and transferred into a glass column (I.D. 9 mm; 5 ml) tilled with 500 mg of Florisil [prerinsed with 2 ml of chloroform-methanol (70:30) and 4 ml of chloroform]. The mycotoxins were eluted with 20 ml of chloroform-acetic acid (90:10), and after evaporation to dryness by a gentle stream of nitrogen the residue was dissolved in 500 μl of mobile phase.

Silica. An aliquot of 5 ml of the methanolic extract was concentrated to dryness and the residue was dissolved in 2 ml of chloroform and transferred into a glass column (I.D. 9 mm; 5 ml) filled with 250 mg of silica gel 60 (0.040-0.063 mm), prerinsed with 2 ml of chloroform–methanol (70:30) followed by 4 ml of chloroform. The mycotoxins were eluted with 16 ml of chloroform-acetic acid (90:10) and, after evaporation to dryness, the residue was dissolved in 500 μl of mobile phase.

Alternatively, a commercially available SPE column was used. The column, after addition of 2 g of anhydrous Na_2SO_4 to the top, was connected to a vacuum manifold and preconditioned with 5 ml of chloroform–methanol–acetic acid (70:25:5) and 5 ml of dichloromethane. The residue was dissolved in 2 ml of dichloromethane and quantitatively transferred on to the column; according to protocol I the column was then washed with 2 ml of chloroform and the mycotoxins were eluted with 5 ml of chloroform-methanol-acetic acid (70:25:5).

Another washing and elution procedure (protocol II), reported by Langseth *et al.* [38], was also applied, whereby the SPE column was preconditioned with 5 ml of hexane and 5 ml of dichloromethane. After sample loading the column was washed with 10 ml of dichloromethane, 10 ml of hexane and 10 ml of toluene. Mycotoxins were eluted with 6 ml of toluene-acetic acid (9:1).

C_{18}. An aliquot of 5 ml of the extract was adjusted to *ca.* pH 7 (checked by pH paper strips) with 30% sodium hydroxide solution and concentrated by rotary evaporation. This should prevent any matrix interactions with the mycotoxins, especially observed under acidic conditions (for discussion, see later). The residue was dissolved in 2 ml of 5% acetic acid and transferred onto a C_{18} SPE column (prewashed with 4 ml of 5% acetic acid). The column was first washed with 200 μl of methanol-water-acetic acid solution (50:46:4) (pH 2.9) and the mycotoxins were eluted with 4.8 ml of methanol–water-acetic acid (80:15:5). The final extract was concentrated to dryness by a gentle stream of nitrogen and the residue was dissolved in 500 μl of mobile phase prior to HPLC injection.

pH-controlled liquid-liquid partition. A 30-g amount of finely ground sample was weighed into a 500-ml erlenmeyer flask and, after adding 20 ml of water and 5 g (28 mmol) of ascorbic acid (pH 3.2, 0.1 M), extracted by tumbling for 15 min with 250 ml of chloroform. A 100-ml volume of the organic extract was filtered into a centrifuge tube and centrifuged for 5 min at 3000 rpm. A 50-ml volume of the clear chloroform extract was transferred into a 250-ml separating funnel and, after adding 10 ml of aqueous saturated sodium chloride solution, treated with 50 ml of aqueous sodium hydroxide (2%). After complete phase separation, the lower (organic) phase was discarded. For analysis of mixed feed

an additional washing step with 50 ml of chloroform is recommended. The upper alkaline phase was mixed with 8.6 g of ascorbic acid and 6 ml of phosphoric acid (pH 1) to adjust the pH to cu. 2-2.5 and extracted twice with 50 ml of dichloromethane. The combined organic phases were dried over 2 g of sodium sulphate and the clear extract was concentrated to dryness. The residue was dissolved in 500 μl of mobile phase.

RESULTS AND DISCUSSION

Extraction

Chloroform-water (250:20) with 0.1 *M* ascorbic acid or methanol-water (90:10) with 0.6 *M* ascorbic acid turned out to be an effective extraction medium for zearalenone and ochratoxin [27,28,37,38], ensuring complete conversion of the possibly ionized ochratoxin A to the non-ionized form (estimated pK 3.5, similar to the pK values of phenylalanine-containing dipeptides). The use of ascorbic acid should protect the phenolic OH groups of the molecules from oxidation and interactions with the sample matrix, which might occur when using strong inorganic acids such as phosphoric acid alone. This was especially true for zearalenone; the recoveries fell below 50% after concentration of a phosphoric acid-acidified extract (no added ascorbate) prior to SPE clean-up. Acidification with acetic acid (no added ascorbate) was also successful in extracting all toxins without deterioration of zearalenone, but an additional neutralization step before solvent evaporation is recommended.

Clean-up

The recoveries of the toxins and the effectiveness of purification using a liquid-liquid and C_{18}, silica) clean-up techniques were studied. The use of silica SPE columns together with chloroform–acetic acid, (90:10) elution required frequent exchange of the guard columns of the HPLC system, although showing no interferences from the maize matrix. The recovery was 85% at high ppb levels, but it dropped to 61% for ochratoxin A and 64% for zearalenone at low ppb levels. Also, the elution volume of 16 ml is rather high (Fig. 4A).

The use of a commercially available silica SPE column and elution with only 5 ml of chloroform-methanol-acetic acid (70:25:5) (protocol I) resulted in an increased ochratoxin A recovery of 75%.

"Clean" chromatograms and an increased lifetime of the guard columns were obtained by washing the silica SPE clean-up column with dichloromethane, hexane and toluene (protocol II) as reported by Langseth *et al.* [38].

β-cyclodextrin

(9:1)ne-acetic acid

Na_2SO_4,

Na_2SO_4.

with Florisil and C_{18} SPE mini-columns were very successful in removing matrix interferences from maize samples, as also demonstrated for the GC-ECD determination of trichothecenes in maize [34]. Using chloroform-acetic acid (9:1) together with a Florisil SPE column the elution volume was high (20 ml; see Fig. 4B) and the toxin recoveries were still unsatisfactorily low in the low ppb range (ochratoxin A 49%; zearalenone 96%). Elution with 12 ml of chloroform-acetic acid-methanol (9:1:1) (Fig. 4C) gave recoveries of 66% for ochratoxin A and 87% for zearalenone.

A clean-up with a C_{18} SPE column allowed the removal of co-eluting interferents in the final HPLC system (Fig. 5) by successively washing the cartridge with 4 ml of 5% acetic acid and 200 μl of methanol-water-acetic acid (50:46:4). The final elution was performed with methanol–water-acetic acid (80:15:5) (Fig. 4D), yielding an 83% recovery for both toxins. Reproducibility tests of this SPE clean-up procedure indicated a relative standard deviation (R.S.D.) of 3.9% for ochratoxin A and 6.5% for zearalenone.

A pH-controlled base-acid liquid-liquid partitioning technique reported for zearalenone and

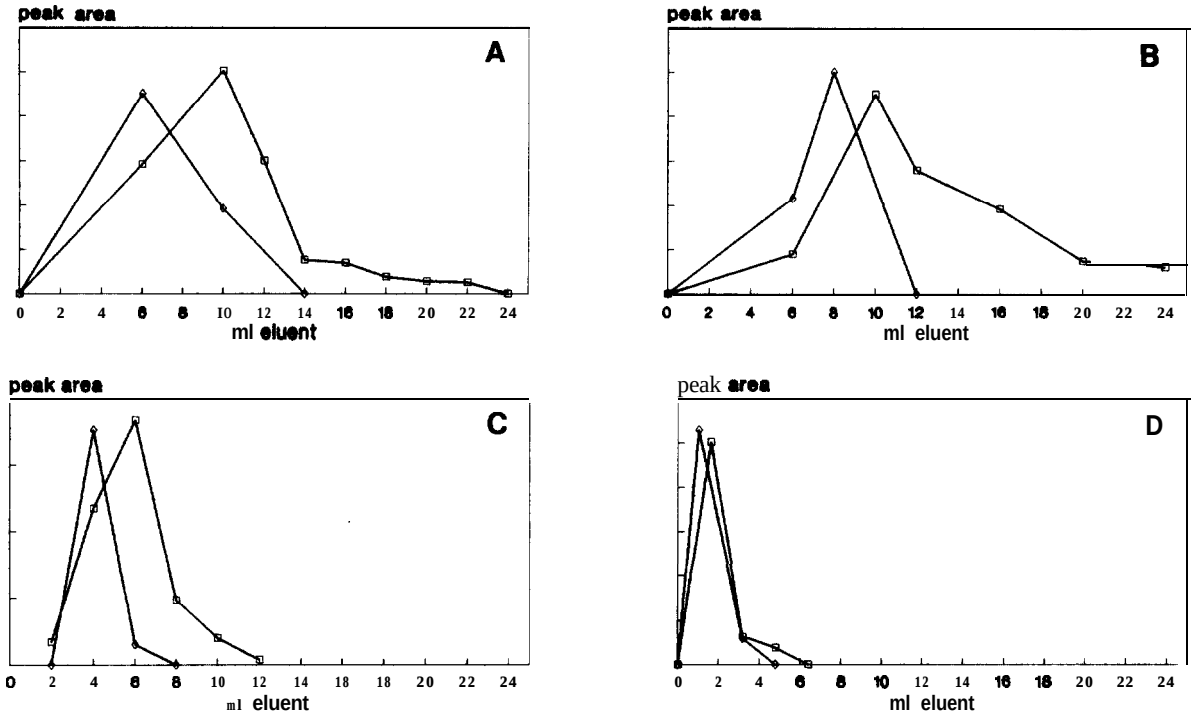

Fig. *4.* Elution profiles of mycotoxins (0) ochratoxin A and (0) zearalenone eluted with (A) chloroform-acetic acid (9:1) from a silica gel 60 SPE column; (B) ochratoxin A and zearalenone eluted with chloroform-acetic acid (9:1) from a Florisil SPE column; (C) chloroform-methanol-acetic acid (9:1:1) from a Florisil SPE column; (D) methanol-water-acetic acid (80:15:5) from a C_{18} SPE column.

zearalenol by Bagneris et al. [9] could easily be adapted to the additional ochratoxin A determination, giving recoveries of 43% and 88% for ochratoxin A and zearalenone, respectively. Sodium chloride was added to break emulsions that appeared with mixed feed extracts. Acceleration of phase separation was performed by washing warm tap water around the separating funnel. The resulting chromatograms showed fewer interferences than obtained in all the other purification techniques, but the procedure is labour intensive and hardly suitable for automation.

Precision data were evaluated for the pH-controlled liquid-liquid partitioning procedure in comparison with a clean-up with C_{18} SPE columns, revealing better repeatability of the SPE technique. The R.S.D. of independent test results from a single maize sample was 3.9% for ochratoxin A and 6.5% for zearalenone with C_{18} SPE clean-up; using the pH-controlled liquid–liquid partitioning purification technique for ochratoxin A and zearalenone R.S.D. values of 8.7% and 7.5%, respectively, were obtained.

Quantification was performed by external standard calibration owing to the good linearity and correlation of the results obtained from spiked maize samples in the range OS-1000 ppb. The correlation coefficients for ochratoxin A were 0.995 for C_{18} SPE clean-up and 0.989 for the liquid-liquid partitioning procedure.

Chromatographic separation

The formation of inclusion complexes between mycotoxins and β-cyclodextrin (a cyclic oligosaccharide, consisting of seven α-1,4-linked glucose units arranged in a torus [39]) molecules is mainly responsible for altering mycotoxin retention in the RP-HPLC system. The formation of an inclusion complex depends on the shape, size and geometrical properties of the solute, the diameter of the CD cavity (6.4 Å for β-CD) and other factors [39,40]. There have been some

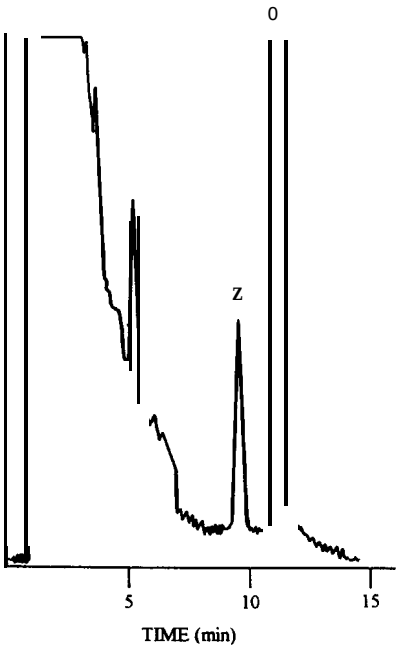

Fig. 5. HPLC of maize spiked with 20 ng/g each of xearalenone (Z) and ochratoxin A (0), after clean-up with C_{18} SPE columns, performed on a Merck LiChrospher 60, BP Select-B, 5 μm, 125 x 4 mm I.D. column. Mobile phase: methanol-water (45:55), pH 2.5 (12 m*M* phosphoric acid), containing 0.2 m*M* β-cyclodextrin. Fluorescence detection: A_{ex} = 315 nm and λ_{em} = 465 mn.

reports relating stability constants of inclusion complexes to retention characteristics [41–44]. For optimization of the chromatographic toxin separation, some parameters (β-cyclodextrin concentration, column temperature and mobile phase composition) that might have an influence on the ability to form inclusion complexes and on the secondary equilibria in the RP-HPLC system with β-cyclodextrin (β-CD) were studied. Changing only the β-CD concentration between 0.1 and 5 m*M* revealed no significant effect on toxin resolution (Fig. 6). Between 0.2 and 1 m*M* β-CD, surprisingly a slight increase in retention was observed compared with higher β-CD concentrations owing to specific phenomena of adjustment of secondary equilibria on the surface of the column packing material. However, lower β-CD concentrations with careful setting of equilibria are recommended in order to keep the system back-pressure reasonable. Raising the column temperature to 65°C resulted

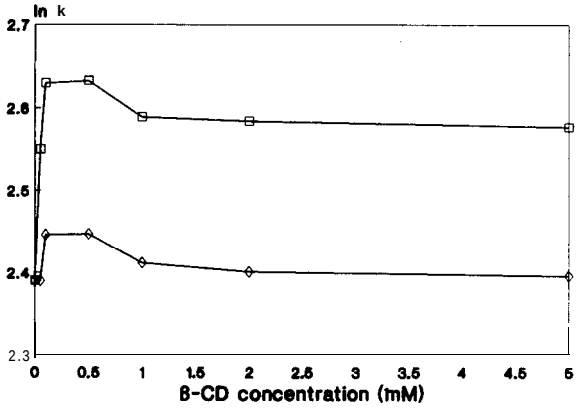

Fig. 6. Influence of β-cyclodextrin concentration on separation of (Cl) ochratoxin A and (0) xearalenone. Analytical column: BP-8 Select-B, 5 μm, thermostated at 60°C in a water-bath. Mobile phase: methanol-water (45:55), pH = 2-3 (12 m*M* phosphoric acid), with β-cyclodextrin as mobile phase additive.

in a decrease in retention times (Fig. 7) but with no loss of resolution combined with an improvement in peak shape.

The methanol content of the mobile phase turned out to be a crucial parameter for toxin separation using β-CD as a mobile phase additive (Fig. 8). Above 50% methanol no separation of zearalenone and ochratoxin A could be achieved, which can be explained by competition of mycotoxins and methanol for the formation of an inclusion complex with β-CD [45]. Lowering

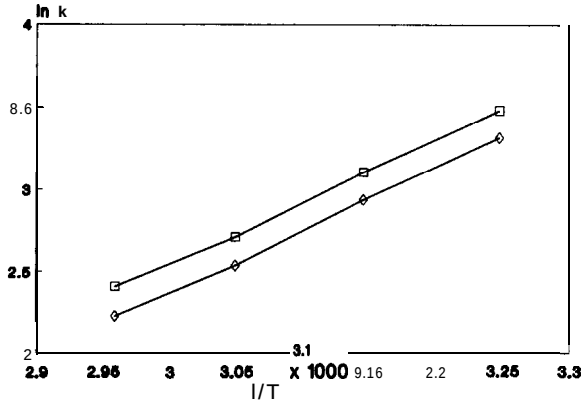

Fig. 7. Influence of column temperature on separation of (Cl) ochratoxin A and (0) xearalenone. using β-cyclodextrin as mobile phase additive. Analytical column: BP-8 Select-B, 5 μm. Mobile phase: methanol-water (45:55), pH = 2-3 (12 m*M* phosphoric acid).

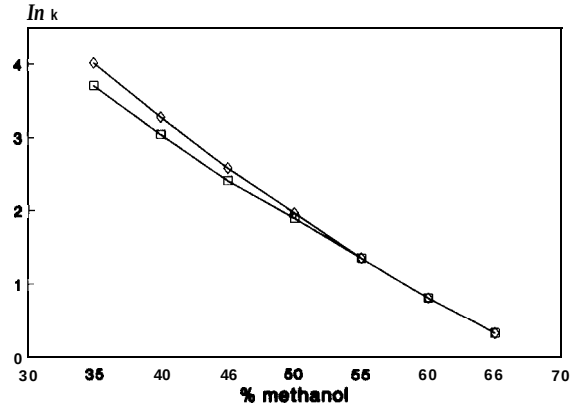

Fig. 8. Influence of methanol content of mobile phase on separation of (□) xearalenone and (0) ochratoxin A using a constant amount of β-cyclodextrin as mobile phase additive. Analytical column: RP-8 Select-B, 5 μm, thermostated at 60°C in a water-bath. Mobile phase: methanol-water, pH = 2-3 (12 m*M* phosphoric acid).

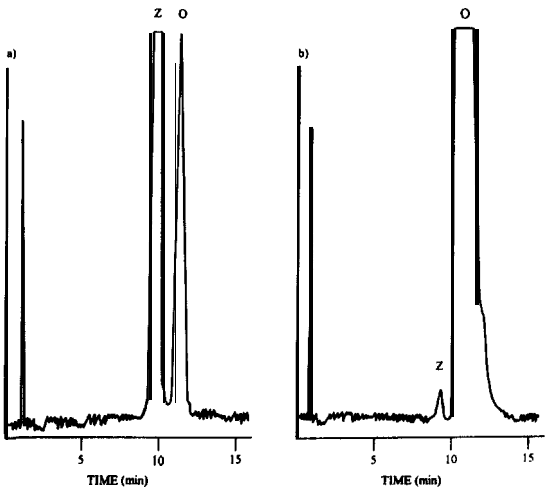

Fig. 9. HPLC of mycotoxin standard solution demonstrating the separation of (a) 100 ng absolute amount per 20 μl injection volume of xearalenone (Z) and 0.1 ng of ochratoxin A (0) and (b) 0.4 ng of zearalenone (Z) and 100 ng of ochratoxin A (0) on a Merck LiChrospher 60, RP Select-B, 5 μm, 125 x 4 mm I.D. column. Mobile phase: methanol-water (45:55), pH 2.5 (12 m*M* phosphoric acid), containing 0.2 m*M* β-cyclodextrin. Fluorescence detection: A,, = 315 nm and λ_{em} = 465 nm.

the methanol content was directly related to an increase in resolution. A Shiseido Capcell PAK C_{18} analytical column (polymer-coated RP material) gave similar results to conventional RP columns (LiChrospher 100, RP-18 or LiChrospher 60, RP-8 Select B).

A mobile phase composition of methanol-water (45:55) proved to be the best compromise of a good resolution of zearalenone and ochratoxin A and a short analysis time. The resulting separation factor (α) of 1.2 was sufficient for the simultaneous detection of zearalenone and ochratoxin A even when there was a large difference in concentration [Fig. 9: (a) 0.4 ng absolute amount per injection of zearalenone and 100 ng of ochratoxin; (b) 0.1 ng of ochratoxin and 100 ng of zearalenone]. This improvement gives rise to a marked increase in confidence in terms of determining especially zearalenone in addition to ochratoxin A, particularly because the fluorescence signal of zearalenone is 5-10 times lower than that of ochratoxin A.

In addition, a slight increase of 15% in the fluorescence sensitivity of ochratoxin A was observed with the use of β-cyclodextrin according to a higher UV absorption in the range 310-350 nm. Without β-CD in the mobile phase, all the RP-HPLC systems tested were not able to resolve these two mycotoxins and therefore all techniques rely totally on a perfect and selective preseparation of zearalenone and ochratoxin A in the course of sample clean-up and pretreatment prior to the final HPLC analysis. Even when the separation of both toxins seems to be possible, as reported for an Econosphere C_{18}, 5 μm, 150 x 4.6 mm I.D. analytical column (Alltech) [28], the use of β-CD as a mobile phase additive can improve the ruggedness of the RP-HPLC system, allowing also the use of all conventional RP-C,, analytical columns.

CONCLUSIONS

Using the above-described HPLC system and sample work-up procedure, we were able to reach detection limits of 400 pg absolute amount per 20-μl injection volume of zearalenone and 50 pg per 20 μl of ochratoxin A (A,, = 315 nm and λ_{em} = 465 nm), resulting in limits of determination of 5 ppb of zearalenone and 0.5 ppb of ochratoxin A in naturally contaminated maize. Mycotoxin detection could be improved by the use of a detector allowing time-programmed

setting of the excitation wavelength to the specific absorption maxima of 270 nm for zearalenone and 320 nm for ochratoxin A, giving a 20% increase in sensitivity for zearalenone and 10% for ochratoxin A (note: these values are strongly dependent on the physical properties of the light source in the fluorescence detector; therefore, diffferent values have been reported for different equipment [9,17,18,27,37,38]). An excitation wavelength of 315 nm was the best compromise for the highly sensitive and selective detection of zearalenone and ochratoxin A for the routine analysis of maize.

The method was successful in detecting zearalenone in several maize samples from southern parts of Austria in the range 28-660 ppb (mean = 274 ppb). Ochratoxin A and a-zearalenol were found at trace levels only. Ochratoxin B, zearalanone and β-zearalenol were not detected.

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