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Simultaneous Determination of Ochratoxin A and Cyclopiazonic, Mycophenolic, and Tenuazonic Acids in Cornflakes by Solid-Phase Microextraction Coupled to High-Performance Liquid Chromatography

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A solid-phase microextraction (SPME) method, coupled to liquid chromatography with diode array UV detection (LC-UV/DAD), for the simultaneous determination of cyclopiazonic acid, mycophenolic acid, tenuazonic acid, and ochratoxin A is described. Chromatographic separation was achieved on a propylamino-bonded silica gel stationary phase using acetonitrile/methanol/ammonium acetate buffer mixture (78:2:20, v/v/v) as mobile phase. SPME adsorption and desorption conditions were optimized using a silica fiber coated with a 60 μm thick polydimethylsiloxane/divinylbenzene film. Estimated limits of detection and limits of quantitation ranged from 3 to 12 ng/mL and from 7 to 29 ng/mL, respectively. The method has been applied to cornflake samples. Samples were subjected to a preliminary short sonication in MeOH/2% $KHCO_3$ (70:30, v/v); the mixture was evaporated to near dryness and reconstituted in 1.5 mL of 5 mM phosphate buffer (pH 3) for SPME followed by LC-UV/DAD. The overall procedure had recoveries (evaluated on samples spiked at 200 ng/g level) ranging from 74 $\pm$ 4 to 103 $\pm$ 9%. Samples naturally contaminated with cyclopiazonic and tenuazonic acids were found; estimated concentrations were 72 $\pm$ 9 and 25 $\pm$ 6 ng/g, respectively.

KEYWORDS: Mycotoxins; SPME; penicillic acid; cyclopiazonic acid; mycophenolic acid; tenuazonic acid; ochratoxin A; cornflakes

INTRODUCTION

Mycotoxins are secondary toxic metabolites produced by different genera of fungi, ubiquitous and widespread at all levels of the food chain, that can contaminate a wide range of foods and feeds. Their presence is considered to be unavoidable and, despite many years of research and the introduction of good manufacturing practices in the food production, storage, and distribution chain, mycotoxins continue to be a problem. Some mycotoxins can also be metabolized by animals fed contaminated grains and excreted into, for example, milk, or accumulated in eggs, meat, and/or other tissues, thus re-entering the food chain.

The mycotoxigenic fungi involved in the human food chain belong mainly to *Fusarium*, *Aspergillus*, *Penicillium*, and *Alternaria* genera. Whereas *Fusarium* and *Alternaria* species are destructive plant pathogens producing mycotoxins before harvest or immediately postharvest, *Penicillium* and *Aspergillus* species are more commonly found as contaminants of commodities and foods during drying and subsequent storage.

The simultaneous presence in the same commodity of mycotoxins produced by fungi belonging to different genera is not uncommon (*1*−*7*). Thus, there is an increasing need for analytical methods for the simultaneous determination of mycotoxins arising from different genera that could provide also a great saving in analysis time and other laboratory resources. Existing papers (*8*−*14*) on this topic are mainly based on chromatographic separations and liquid−liquid (LLE) or solid-phase extraction (SPE) procedures that are, however, intrinsically laborious and time-consuming and employ large amounts of toxic organic solvents. These drawbacks can be avoided by adopting solid-phase microextraction (SPME) techniques that have been mainly applied (*15*−*17*) in combination with GC. However, a growing interest in SPME coupled to HPLC has been observed, especially in the past few years, as demonstrated by a number of recently published papers (*18*). Applications of SPME in the field of mycotoxins are substantially lacking, if one considers that, to the best of our knowledge, there are only two examples from our laboratory (*19*, *20*).

In the present work, an SPME-HPLC-UV/DAD method for the simultaneous determination of acidic mycotoxins, produced by *Aspergillus*, *Penicillium*, and *Alternaria*, namely, cyclopiazonic, mycophenolic, and tenuazonic acids and ochratoxin A (**Table 1** and **Figure 1**), was developed for the first time. The chromatographic separation of the target analytes was obtained using, as a starting point, a recent LC-UV method for the

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Table 1. Main *Alternaria*, *Aspergillus*, and *Penicillium* Species Potential Producers of the Test Mycotoxins; the Most Commonly Contaminated Commodities Are Also Reported

genera	fungal species	mycotoxin	commodity
Aspergillus, Penicillium	*A. flavus, P. comune, P. camemberti, P. verrucosum*	cyclopiazonic acid	cereals, feedstuffs (*12*), meat, eggs (*7*), milk cheese (*19*), tomatoes (*13*)
Penicillium	*P. viridicatum, P. roqueforti, P. brevicompactum*	mycophenolic acid	cheese (*20*)
Alternaria	*Alternaria alternata*	tenuazonic acid	cereals, feedstuffs (*7*, *12*). oilseeds (*7*), tomatoes (*13*), pepper
Penicillium	*P. comune, P. roqueforti, P. camemberti, P. expansum*	penicillic acid	maize, cereals, feedstuffs (*12*)
Aspergillus, Penicillium	*A. ochraceus, P. viridicatum, P. verrucosum, P. cyclopium*	ochratoxin A	maize (*12*), cereals (*7*, *12*), eggs, meat (*7*), milk

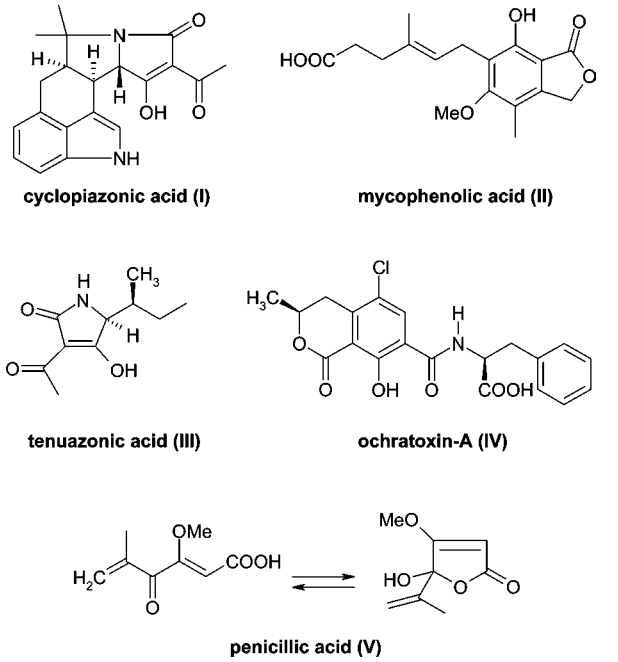

Figure 1. Chemical structures of investigated mycotoxins.

determination of cyclopiazonic acid based on the use of propylamino-bonded silica gel stationary phase (*21*). Then, SPME conditions (extraction and desorption) were optimized. Finally, the potential of the method was demonstrated by the analysis of naturally contaminated cornflake samples.

MATERIALS AND METHODS

Chemicals. Ochratoxin A and penicillic, cyclopiazonic, mycophenolic, and tenuazonic acids were purchased from Sigma (St. Louis, MO). Stock solutions were prepared in methanol and stored at 4 °C in the dark. Dilute solutions were prepared just before use. Organic solvents (Carlo Erba, Milan, Italy) were of HPLC grade. The mobile phase was filtered through a 0.45 μm membrane (Whatman Ltd., Maidstone, U.K.) before use.

Apparatus. The SPME interface (Supelco) consisted of a standard six-port Rheodyne valve equipped with a fiber desorption chamber (total volume = 60 μL), installed in place of the sample loop. The HPLC system consisted of a Spectra System pump, model P2000 (Thermo Finnigan, San Jose, CA), a 250 × 4.6 mm i.d., 5 μm packing Supelcosil LC-NH_2 column (Supelco), protected by a 20 × 4.6 mm i.d., 5 μm packing, Supelguard LC-NH_2 precolumn (Supelco), and a Spectra System model UV6000LP photodiode array detector (Thermo Finnigan) controlled by ChromQuest software running on a personal computer.

Chromatographic and Detection Conditions. The mobile phase composition was, unless otherwise specified, an acetonitrile/methanol/ammonium acetate buffer (50 mM, pH 7) mixture (78:2:20, v/v/v). The flow rate was 1 mL/min, and the temperature was ambient. The mobile phase was degassed on-line by an SCM1000 vacuum membrane degasser (Thermo Finnigan). Spectra were acquired in the 220−380 nm range using a 2 Hz frequency and a 5 nm bandwidth. Chromatograms were monitored on three wavelength channels, 232, 250, and 282 nm, using a 10 Hz frequency and a 5 nm bandwidth.

Solid-Phase Microextraction. Silica fibers (Supelco) with three different coatings, 85 μm thick polyacrylate (PA) film, 50 μm thick Carbowax/Template resin (CW/TPR-100) film, and 60 μm thick polydimethylsiloxane/divinylbenzene (PDMS/DVB) film, respectively, were employed for comparative studies. The fibers were conditioned as suggested by the producer. A manual SPME device (Supelco) was used to hold the fiber. The SPME device has been extensively described elsewhere (*15*−*17*). Working solutions were prepared by spiking 1.5 mL of phosphate buffer (5 mM, pH 3) with different amounts of the target compounds into 2 mL clear vials (Supelco). Then, the vials were sealed with hole caps and Teflon-faced silicone septa (Supelco). The extraction was carried out at 50 °C for 60 min under magnetic stirring in order to improve mass transfer from the aqueous sample into the fiber coating. Desorption was performed in "static mode" (*18*) (mobile phase as extraction solvent) for 5 min followed by 4 s of injection time. To avoid possible memory effects, the fiber was fully desorbed in "dynamic mode" before the next extraction.

Cornflake Samples. Cornflake samples were collected from a local market. Sample aliquots (0.5 g) were previously crumbled in a vial, 5 mL of MeOH/2% $KHCO_3$ (70:30, v/v) was added, and the resulting mixture was sonicated for 5 min and centrifuged at 5000 rpm for 5 min. The supernatant was then evaporated to dryness and the residue reconstituted in 1.5 mL of 5 mM phosphate buffer (pH 3) for the SPME step.

Recoveries were calculated on samples spiked at the 200 ng/g level; spiked samples were equilibrated at room temperature for at least 12 h and then analyzed as described above. Quantitation was performed according to the standard addition method.

RESULTS AND DISCUSSION

The simultaneous separation of cyclopiazonic, penicillic, tenuazonic, and mycophenolic acids and ochratoxin A has never been attempted; these mycotoxins are generally analyzed on different stationary phases under different separation mechanisms, for example, normal phase, ion exchange, reverse phase, and ion pairing. This is quite surprising because all of the mentioned compounds (see **Figure 1**) have a common characteristics; that is, they are weak acids due to the presence of a carboxylic group. This implies that they could be simultaneously separated on the same phase system like the one recently developed for cyclopiazonic acid (*21*). Retention of cyclopiazonic acid on propylamino-bonded silica gel stationary phase under acidic conditions (protonated amino groups and deprotonated analyte) is governed by a mixed mechanism (*21*). Ion exchange operates simultaneously with a reverse phase mechanism, especially in strongly polar mobile phases, due to possible interactions between the solute and the short hydrocarbon spacer that links the amino groups to the silica gel. Polar interactions

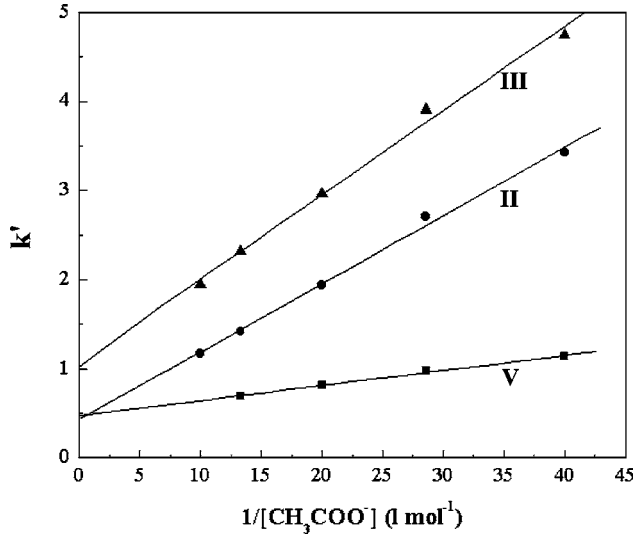

Figure 2. Plots of the capacity factors *k′* of penicillic (**V**), mycophenolic (**II**), and tenuazonic (**III**) acids versus the inverse of CH_3COO^- concentration. The mobile phase was CH_3CN/CH_3COONH_4 (pH 7, 80:20, v/v).

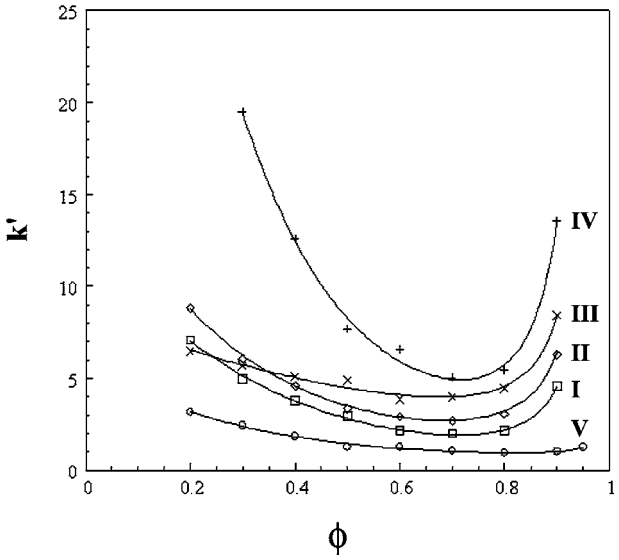

Figure 3. Dependence of the capacity factors *k′* of the target analytes on the volume fraction φ of CH_3CN at constant pH and ionic strength. The mobile phase was CH_3CN/50 mM CH_3COONH_4 (pH 7). Symbol legend is as in **Figure 1**.

are also possible between the analyte and residual silanols, in nonaqueous mobile phases.

The first step of the present work was to investigate factors influencing retention and chromatographic efficiency in order to optimize the separation of the selected acidic mycotoxins. The mobile phase was buffered with ammonium acetate at a pH value of 7 to keep the analytes in the deprotonated form and the amino groups of the stationary phase in the protonated form. Acetonitrile was selected as organic modifier. **Figure 2** shows the influence of the buffer ionic strength on the retention of penicillic, mycophenolic, and tenuazonic acids, at constant pH and volume fraction of acetonitrile. As expected for a pure ion exchange mechanism, the capacity factor *k′* increases as the ionic strength decreases, but the nonzero intercept on the *y*-axis (infinite ionic strength) indicates, as already observed for cyclopiazonic acid (*21*), the contribution of an additional retention mechanism(s).

Figure 3 shows the dependence of the capacity factor *k′* on the volume fraction φ of acetonitrile (the less polar solvent), at constant pH and ion strength, that, as expected (*21*), shows a clear minimum for all of the investigated analytes. A further control of retention and especially of the chromatographic efficiency could be finally achieved by adding to the mobile phase a small amount of a second organic modifier, such as CH_3OH. **Figure 4** shows the HPLC-UV/DAD chromatograms obtained by direct injection of a standard solution of the target analytes (5 ng injected) using the optimized mobile phase composition, that is, an acetonitrile/methanol/50 mM ammonium acetate buffer (pH 7) mixture (78:2:20, v/v/v). Under these conditions theoretical plate numbers ranging from 16000 m^{-1} (tenuazonic acid) to 36000 m^{-1} (penicillic acid) and good peak symmetry factors (*22*) were achieved.

Once the separation was optimized, the second step was the development of the ideal SPME conditions. A strong dependence of the extraction yield on the pH value was observed for all of the acidic mycotoxins under study, indicating that the undissociated form of the analyte is preferentially extracted by the polymeric coating of the fiber. All of the extractions were then carried out at pH 3, where all of the target compounds (weak acids) were present in their undissociated form.

Extraction efficiencies of PA, CW/TPR-100, and PDMS/DVB coatings were evaluated and compared in order to select the best fiber. The relative extraction efficiencies of the target analytes on each fiber are reported in **Table 2**. As apparent, penicillic acid could not be extracted by any of the selected fibers, whereas the other mycotoxins were extracted to a different extent depending on the nature of the polymeric coating. The CW/TPR fiber was the most efficient toward ochratoxin A and cyclopiazonic and micophenolic acids but was poorly effective for tenuazonic acid. On the other hand, the polyacrylate coating was the less effective, being unable to extract penicillic and tenuazonic acids. The PDMS/DVB coating was then chosen for further experiments because it gave satisfactory extraction efficiencies for all of the extractable mycotoxins.

Figure 5 shows the extraction profiles obtained by plotting the peak area versus the extraction time. As can be seen, at a working temperature of 50 °C extraction times >90 min were necessary to reach equilibrium. Lower temperatures, for example, ambient, require even longer times. However, as long as the extraction was performed under reproducible conditions, an adsorption time of 60 min revealed a good compromise between sample throughput and chromatographic response. Under equilibrium conditions, the fiber−solution distribution coefficients, K_{f-s}, of each analyte could be calculated as the ratio between the concentration of the analyte in the fiber coating and in the solution; log K_{f-w} values ranging from 2.34 (tenuazonic acid) to 2.75 (ochratoxin A) were estimated for the PDMS/DVB fiber.

The addition of sodium chloride (up to 200 mg/mL) to the extraction solution caused an ∼20% signal enhancement (i.e., improved extraction efficiency) for mycophenolic acid and ochratoxin A, a slight signal decrease for cyclopiazonic acid, and only negligible effects for tenuazonic acid. In any case, the adjustment of the ionic strength was not further considered, because during the analysis of real samples it was found to improve the coextraction of sample components, giving rise to more complex chromatograms.

Sample transfer from the fiber to the column is not a crucial step in gas chromatography (GC) because problems arising from slow desorption kinetics can be simply counteracted by increasing the injector temperature and/or the desorption time and

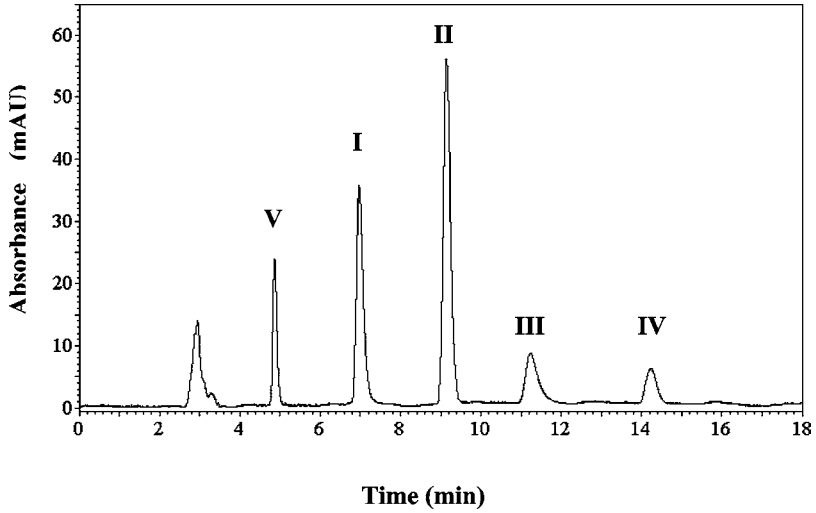

Figure 4. HPLC-UV/DAD chromatograms obtained by direct injection of a standard solution of the target analytes (5 ng/20 μL injected) using the optimized mobile phase, i.e., an acetonitrile/methanol/ammonium acetate buffer (50 mM, pH 7) mixture (78:2:20, v/v/v). The chromatogram is displayed according to the "Spectrum Max Plot" option of the ChromQuest software; i.e., each peak is plotted at the wavelength λ where maximum absorbance occurs in the "Abs-time-λ" data matrix. Symbol legend is as in **Figure 1**.

Table 2. Relative Extraction Efficiencies (Percent) of the Target Analytes by Each Fiber[a]

	fiber		
mycotoxin	PA	PDMS/DVB	CW/TPR
penicillic acid	ne[b]	ne	ne
cyclopiazonic acid	42.9	62.2	100
mycophenolic acid	49.8	64.7	100
tenuazonic acid	ne	100	24.8
ochratoxin A	19.7	86.5	100

[a] Extraction efficiencies were calculated as peak area ratio and normalized to the highest value observed for the given analyte. [b] Not extracted.

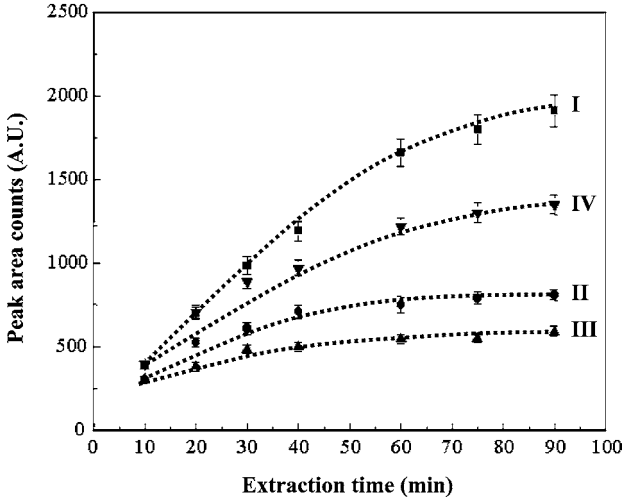

Figure 5. Extraction profiles obtained (three replicates for each point) at 50 °C using the PDMS/DVB fiber. Chromatographic conditions were as in **Figure 4**; fiber desorption was in dynamic mode. Symbol legend is as in **Figure 1**.

refocusing the injection band on the GC column head (25). In the case of SPME interfaced to HPLC, analyte transfer represents more of a problem. Dynamic desorption mode, which ensures quantitative recoveries, causes a significant increase of both peak width and peak asymmetry compared to conventional loop injection (20 μL), deteriorating the chromatographic efficiency and resolution. Thus, a static desorption technique was evaluated as a possible alternative. The fiber was soaked in the static mobile phase contained in the desorption chamber (60 μL volume) for a variable period of time before injection into the HPLC column. The best conditions were reached after 5 min of static desorption followed by 4 s of injection time into the mobile phase stream. The peak shape observed for conventional loop injection was now quite well preserved, especially for tenuazonic acid and ochratoxin A. However, under these conditions, complete sample transfer could not be necessarily achieved and fiber memory effects could be observed; indeed, analyte carry-overs of 23% for cyclopiazonic acid, 25% for mycophenolic acid, 19% for tenuazonic acid, and 21% for ochratoxin A could be estimated.

The response of the developed SPME-HPLC procedure was linear in the ranges of 5−300 ng/mL for cyclopiazonic and mycophenolic acids, 15−500 ng/mL for tenuazonic acid, and 10−500 ng/mL for ochratoxin A, with correlation coefficients always >0.998 and intercepts not significantly different from zero at the 95% confidence level. The estimated limit of detection (LOD) and limit of quantitation (LOQ) obtained on standard solutions, calculated as 3- and 10-fold the standard deviation of the intercept of the calibration curve (*23*) obtained by unweighted linear regression, ranged from 3 to 12 ng/mL and from 7 ng/mL (cyclopiazonic acid) to 29 ng/mL (tenuazonic acid), respectively.

The within-day and day-to-day precision of the method, estimated by an ANOVA test (three replicates for 9 days), ranged from 1.1 to 3.7% and from 2.1 to 6.7%, respectively.

The applicability of the developed method to real food samples was demonstrated on cornflakes. Average percent recoveries ± standard deviation ($n = 3$), calculated on samples spiked at the 200 ng/g level with the target analytes as described under Materials and Methods, were 74 ± 4 for mycophenolic acid, 94 ± 7 for cyclopiazonic acid, 95 ± 9 for ochratoxin A, and 103 ± 9 for tenuazonic acid. **Figure 6** shows the SPME-HPLC-UV chromatogram for a cornflake sample naturally contaminated with cyclopiazonic and tenuazonic acids. Whereas tenuazonic acid eluted in a "clean" region of the chromatogram, cyclopiazonic acid eluted as a shoulder; this, however, did not prevent (see insets in **Figure 6**) confirmation of peak identity

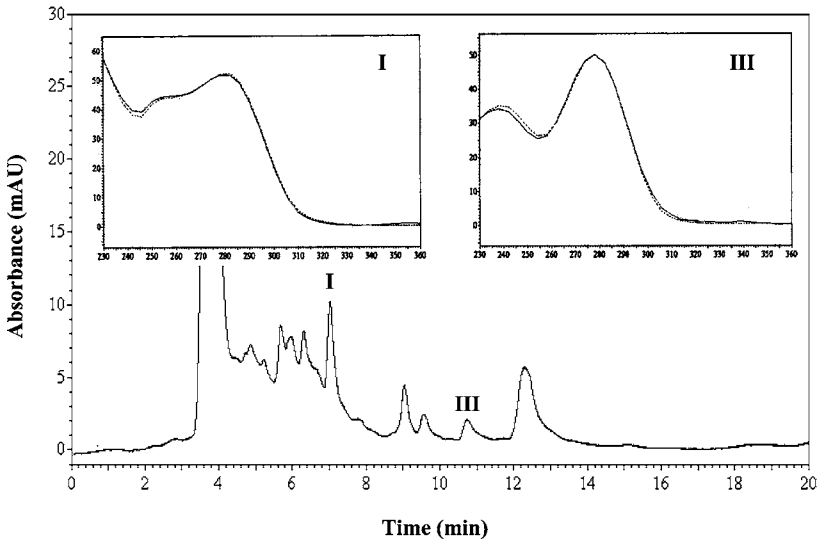

Figure 6. SPME-HPLC-UV chromatogram relevant to the analysis of a cornflakes sample naturally contaminated with CPA and TA. Detection wavelength was 282 nm. Chromatographic conditions were as in **Figure 3**; fiber desorption was in static mode (see Materials and Methods). The inset shows the overlay of normalized UV spectra for CPA and TA in the sample (solid line) with those of authentic standard (dotted line). Symbol legend is as in **Figure 1**.

by UV spectra overlay (note that ochratoxin A and mycophenolic acid when detected at their absorption maximum wavelengths of 332 and 250 nm, respectively, did not suffer from significant interference from matrix components). Estimated concentrations (standard addition method) were 72 ± 9 and 25 ± 6 ng/g for cyclopiazonic and tenuazonic acids, respectively.

In conclusion, an SPME-HPLC-UV method for the simultaneous determination of acidic mycotoxins produced by *Aspergillus*, *Penicillium*, and *Alternaria* has been developed for the first time. The HPLC separation of the target mycotoxins could be achieved, by a mixed mechanism, on a propylamino-bonded silica gel stationary phase using a mobile phase composed of an acetonitrile/methanol/ammonium acetate buffer (50 mM, pH 7) (78:2:20, v/v/v). A PDMS/DVB coated fiber ensured good extraction efficiency, whereas the static desorption mode ensured the best compromise in terms of analyte transfer efficiency and preservation of chromatographic efficiency. The potential of the described procedure was demonstrated by the ability to detect cornflake samples naturally contaminated by cyclopiazonic and tenuazonic acids of ca. 70 and 25 ng/g, respectively. To the best of our knowledge, this is probably the first example of such a commodity contaminated by these two mycotoxins; co-occurrence of the same mycotoxins was also recently reported for two samples of tomato (puree pulp) products (*24*). Different lots of only one cornflake brand were analyzed, and all of them were found to be contaminated at levels similar to those above-reported. These data are too limited to infer any sound conclusion about natural occurrence and risk assessment for human or animal health. On the other hand, in view of the limited toxicity of these mycotoxins, neither legal limits nor maximum tolerable daily intake levels have been yet established.

Finally, it is worth noting the very simple sample pretreatment and the minute amount of organic solvent required. The method could be potentially applied, with no or little modifications, to the determination of the target mycotoxins in other food commodities such as milk and cheese. The chromatographic conditions are fully compatible, as already demonstrated for cyclopiazonic acid determination in milk (26), by liquid chromatography with electrospray ionization mass spectrometry (LC-ESI-MS). Work is in progress in these directions.

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LITERATURE CITED

(1) Dutton, M. F.; Westlake, K. Occurrence of mycotoxins in cereals and animal feedstuffs in Natal, South Africa. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 839−842.

(2) Chamberlain, W. J.; Bacon, C. W.; Norred, W. P.; Voss, K. A. Levels of fumonisin B1 in corn naturally contaminated with aflatoxins. *Food Chem. Toxicol.* **1993**, *31*, 995−998.

(3) Chu, F. S.; Li, G. Y. Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Appl. Environ. Microbiol.* **1994**, *60*, 847−852.

(4) Wang, D. S.; Liang, Y. X.; Chau, N. T.; Dieu, L. D.; Tanaka, T.; Ueno, Y. Natural co-occurrence of *Fusarium* toxins and aflatoxin B1 in corn for feed in North Vietnam. *Nat. Toxins* **1995**, *3*, 445−449.

(5) Yamashita, A.; Yoshizawa, T.; Aiura, Y.; Sanchez, P. C.; Dizon, E. Y.; Arim, R. H.; Sandjono, O. O. *Fusarium* mycotoxins (fumonisins, nivalenol, and zearalenone) and aflatoxins in corn from Southeast Asia. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1804−1807.

(6) Patel, S.; Hazel, C. M.; Winterton, A. G. M.; Mortby, E. Survey of ethnic foods for mycotoxins. *Food Addit. Contam.* **1996**, *13*, 833−841.

(7) Sweeney, M. J.; Dobson, A. D. W. Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. *Int. J. Food Microbiol.* **1998**, *43*, 141−158.

(8) Takeda, Y.; Isohata, E.; Amano, R.; Uchiyama, M. Simultaneous extraction and fractionation and thin layer chromatographic determination of 14 mycotoxins in grains. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 573−578.

(9) Lee, K. Y.; Poole, C. F.; Zlatkis, A. Simultaneous multi-mycotoxin determination by high performance thin-layer chromatography. *Anal. Chem.* **1980**, *52*, 837−842.

(10) Scott, P. M.; Weber, D.; Kanhere, S. R. Gas chromatography−mass spectrometry of *Alternaria* mycotoxins. *J. Chromatogr. A* **1997**, *765*, 255−263.

(11) Sobolev, V. S.; Horn, B. W.; Dorner, J. W.; Cole, R. J. Liquid chromatographic determination of major secondary metabolites produced by *Aspergillus* species from section Flavi. *J. AOAC Int.* **1998**, *81*, 57−60.

(12) Lin, L.; Zhang, J.; Wang, P.; Wang, Y.; Chen, J. Thin-layer chromatography of mycotoxins and comparison with other chromatographic methods. *J. Chromatogr. A* **1998**, *815*, 3−20.
(13) da Motta, S.; Valente Soares, L. M. Simultaneous determination of tenuazonic and cyclopiazonic acids in tomato products. *Food Chem.* **2000**, *71*, 111−116.
(14) Rundberget, T.; Wilkins, A. Determination of *Penicillium* mycotoxins in foods and feeds using liquid chromatography−mass spectrometry. *J. Chromatogr. A* **2002**, *964*, 189−197.
(15) Beltran, J.; Lopez, F. J.; Hernandez, F. Solid-phase microextraction in pesticide residue analysis. *J Chromatogr. A* **2000**, *885*, 389−404.
(16) Ulrich, S. Solid-phase microextraction in biomedical analysis. *J. Chromatogr. A* **2000**, *902*, 167−194.
(17) Kataoka, H.; Lord, L.; Pawliszyn, J. Applications of solid-phase microextraction in food analysis. *J Chromatogr. A* **2000**, *880*, 35−62.
(18) Zambonin, C. G. Coupling solid-phase microextraction to liquid chromatography. A review. *Anal. Bioanal. Chem.* **2003**, *375*, 73−80.
(19) Zambonin, C. G.; Monaci, L.; Aresta, A. Determination of cyclopiazonic acid in cheese samples using solid-phase microextraction and high performance liquid chromatography. *Food Chem*. **2001**, *75*, 249−254.
(20) Zambonin, C. G.; Monaci, L.; Aresta, A. Solid-phase microextraction-high performance liquid chromatography and diode array detection for the determination of mycophenolic acid in cheese. *Food Chem*. **2002**, *75*, 249−254.
(21) Monaci, L.; Aresta, A.; Palmisano, F.; Visconti, A.; Zambonin, C. G. Amino-bonded silica as stationary phase for liquid chromatographic determination of cyclopiazonic acid in fungal extracts. *J Chromatogr. A* **2002**, *955*, 79−86.
(22) Foley, J. P.; Dorsey, J. G. Equations for calculation of chromatographic figures of merit for ideal and skewed peaks. *Anal. Chem.* **1983**, *55*, 730−737.
(23) Long, G. L.; Winefordner, J. D. Limit of detection. A closer look at the IUPAC definition. *Anal. Chem*. **1983**, *55*, 712A−724A.
(24) Motta, S. D.; Valente Soares, L. M. Survey of Brazilian tomato products for alternariol, alternariol monomethyl ether, tenuazonic acid and cyclopiazonic acid. *Food Addit. Contam.* **2001**, *18*, 630−634.
(25) Lord, H.; Pawliszyn, J. Evolution of solid-phase microextraction technology. *J. Chromatogr.* **2000**, *885*, 153−193.
(26) Losito, I.; Monaci, L.; Aresta, A.; Zambonin, C. G. LC-ion trap electrospray MS-MS for the determination of cyclopiazonic acid in milk samples. *Analyst* **2002**, *127*, 499−502.

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