Automated Extraction of Mycotoxins from Crop Samples using Disposable Pipette Extraction (DPX) and Analysis by LC/MS/MS

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Abstract

The automated cleanup of mycotoxin residues from corn sample extracts using disposable pipette extraction (DPX) is described. DPX is a solid-phase extraction (SPE) technique that is based on loosely contained sorbent inside a pipette tip fitted with a screen. This device provides faster extraction because only minimal conditioning steps are needed which limits solvent use. A weak anion exchange sorbent (DPX-WAX) was found to provide selective extraction of 6 different mycotoxins from crop samples. Recovery of the analytes of interest averaged 84 % with a relative standard deviation of 6 %.

Keywords

DPX, LC/MS/MS, Sample Preparation, Mycotoxin, Aflatoxin, Food Safety

Introduction

The analysis of mycotoxins in foods and animal feeds is critical in food safety due to their high level of toxicity and also because of the increasing legal requirements concerning their content in different products. In complex matrices such as foods, grains, and animal feeds, the extraction and cleanup methods are crucial in order to obtain accurate and precise results. The major challenge is to determine mycotoxin concentrations at trace levels in the presence of large quantities of coextracting sample matrix.

In this work, automated cleanup and determination of 6 different mycotoxins is described. A dispersive solid phase extraction (dSPE) technique referred to as disposable pipette extraction (DPX) is used to remove matrix interferences and provide a cleaned sample for subsequent LC/MS/MS analysis. DPX is based on sorbent loosely contained inside pipette tips. DPX differs from other SPE approaches in that sample solutions are dynamically mixed with the sorbent within the pipette tip. The extraction efficiency is dependent on the equilibration time between solutions and sorbent, rather than flow rates through a packed bed. The mixing step often eliminates the need for sorbent conditioning and allows the use of smaller volumes of elution solvent, which means that less solvent is typically needed for DPX (< 1 mL) than for traditional SPE (~ 2-10 mL). A schematic of a DPX extraction is shown in Figure 1.

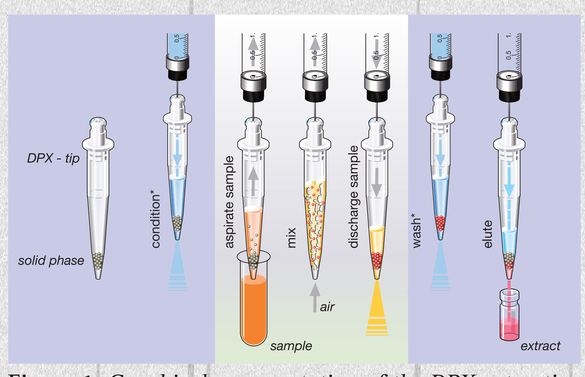

Figure 1. *Graphical representation of the DPX extraction process.*

Experimental

Materials. 1 mL ampoules of the following mycotoxins were obtained from Romer Labs for this study: Mix 1 (Aflatoxins B1,B2, G1 and G2), Deoxynivalenol (DON) and Zearalenone (ZEN). Tyson Foods standards were purchased from Sigma Aldrich for method validation. Mycotoxin free corn samples were supplied by Tyson Foods.

High concentration calibration standards and intermediate QC corn samples were prepared by making appropriate dilutions of the combined intermediate analyte stock solution.

DPX-WAX-1 mL (20 mg, 10-20 µm) tips were obtained from DPX Labs, LLC (Columbia, SC). All solvents used were reagent grade.

Instrumentation. All automated DPX PrepSequences were performed using a dual-head MPS XL multi-purpose sampler with the GERSTEL DPX Option as shown in Figure 1. All analyses were performed using Waters Acquity UPLC binary pumps coupled to a Micromass Quattro (MS/MS) Mass Spectrometer with an electrospray source. Chromatographic separations were performed with a Restek UltraAqueous C18 column (50 mm x 2.1 mm 5 µm). The sample injection volume was 10 µL.

Sample preparation. 5 g of dried shelled corn was weighed and homogenized with 20 mL of acetonitrile:water mixture (84:16) and fortified with the mixture of mycotoxin standards. After homogenization, approximately 10 mL of the corn suspension was centrifuged and the clear supernatant was stored at -2 °C.

250 µL aliquots of the clear corn extract were transferred to 2 mL shell vials for automated DPX extraction on the GERSTEL MPS XL autosampler (Figure 2).

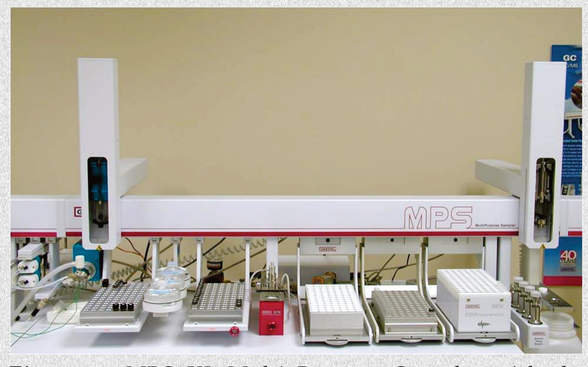

Figure 2. *MPS XL Multi-Purpose Sampler with the GERSTEL DPX option for extraction of mycotoxins.*

The DPX extraction consisted of the following steps:

Automated DPX Prep Sequence

1. Using a DPX tip, aspirate 250 µL of sample from test tube and equilibrate for 30 s.
2. Dispense cleaned solution into the original vial
3. Add 250 µL of acetonitrile (with 4 % formic acid) to the top of the tip and elute into original vial.
4. Using the 250 µL syringe on the MPS, transfer eluent from original vial to a clean capped 2 mL vial and place under stream of nitrogen at 60°C until dryness in the autosampler drying station.
5. Reconstitute in 250 µL of 90:10 (Mobile Phase A:Mobile Phase B) solution for LC/MS/MS analysis.

The PrepSequence generated for the automated DPX extraction method is displayed in Figure 3.

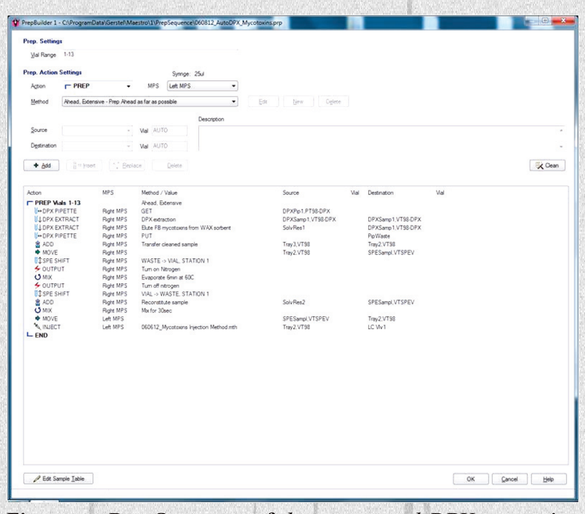

Figure 3. *PrepSequence of the automated DPX extraction method for mycotoxins.*

Analysis conditions LC.		
Pump:	flowrate = 0.3 mL/min	
Mobile Phase:	A - Water/MeOH/acetic acid (94:5:1) containing 5 mM ammonium acetate B – MeOH/water/acetic acid (97:2:1) containing 5 mM ammonium acetate	
Column:	UltraAqueous C18 (Restek) 50 mm x 2.1 mm x 5 µm	
Gradient:	Initial	5 % B
	2.33	65 % B
	3.67	75 % B
	4.33	95 % B
	5.0	95 % B
	5.33	60 % B
	7.33	40 % B
	7.67	5 % B
	8.33	5 % B
Injection volume:	10 µL	
Column temperature:	25°C	

Analysis conditions MS.	
Operation:	electrospray positive mode
Gas temperature:	350°C
Gas flow (N_2):	12 L/min
Nebulizer pressure:	35 psi
Capillary voltage:	4000 V

Table 1. *Mycotoxin MS/MS parameters.*

Analyte	Retention Time [min]	Q1 [m/z]	Q3 (1st Trans.) [m/z]	Q3 (2nd Trans.) [m/z]	Cone [V]	CE [V]
DON	0.71	297.00	249.00	231.00	11.00	15.00
AF-G2	3.00	331.00	313.10	245.30	46.00	27.00
AF-G1	3.06	328.80	311.20	243.40	43.00	23.00
AF-B2	3.25	315.00	287.30	259.40	50.00	27.00
AF-B1	3.31	313.00	285.30	241.40	47.00	27.50
ZEN	4.26	319.20	185.40	283.30	25.00	19.00

Results and Discussion

Six important mycotoxins were successfully analyzed from corn sample extracts using the MPS with DPX option described in this work. Relative recoveries were determined by comparing results from spiked samples (at least 5 replicates) with "matrix-matched" samples prepared by adding the neat standard mixture of mycotoxin standards directly to the eluent of the extracted blank matrix. Percent recoveries and relative standard deviations are shown in Table 2. It should be noted that using an internal standard would significantly improve the reproducibility. In this preliminary study no internal standards were used.

Table 2. *Percent recovery and percent RSD for all mycotoxins analyzed.*

Analyte	Recovery [%]	RSD [%]
DON	89.30 %	3.87 %
AF-G2	88.83 %	2.71 %
AF-G1	81.69 %	2.60 %
AF-B2	81.14 %	2.15 %
AF-B1	82.84 %	2.30 %
ZEN	78.85 %	5.89 %

The approach taken in this study was to develop a sample matrix cleanup method using a DPX-WAX tip to bind and eliminate matrix interferences (e.g., fatty acids) in the sample. The WAX sorbent also has reversed phase characteristics, which can selectively extract some the mycotoxins of interest Therefore, the addition of acetonitrile to the top of the tip and elution into original vial increased the recoveries for all the analytes.

A representative calibration curve for ZEN is shown in Figure 4. Regression analysis for this mycotoxin using this method resulted in an R^2 value of > 0.99, limits of quantitation (LOQ) of 4.3 ng/g with % RSD of 4.3 % respectively. Table 3 summarizes all the LOQs from the DPX-LC/MS/MS mycotoxin method. Figure 5 shows LC/MS/MS chromatograms from a low QC sample of all the myxotoxins extracted with DPX-WAX.

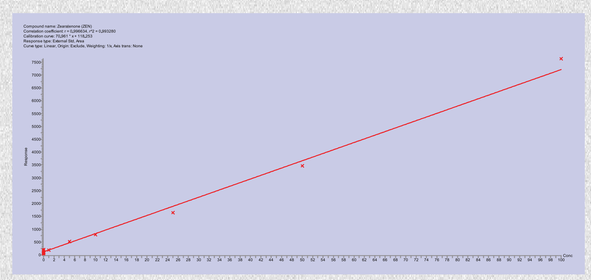

Figure 4. *Calibration curve for Zearalenone (ZEN).*

Table 3. *Limits of quantitation (LOQ) for the DPX-LC/MS/MS mycotoxin method.*

Analyte	LOQ [ng/g]	RSD [%]
DON	4.81	3.99 %
AF-G2	0.54	5.42 %
AF-G1	0.56	5.71 %
AF-B2	0.41	4.06 %
AF-B1	0.23	2.51 %
ZEN	4.29	4.31 %

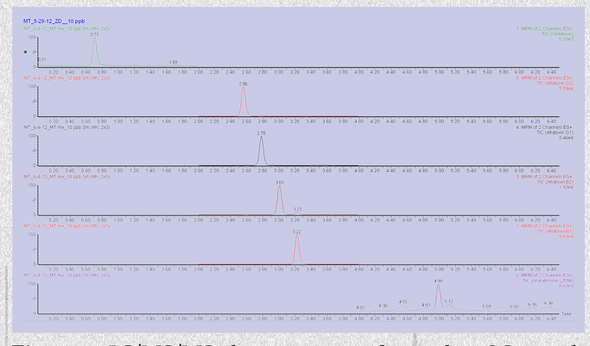

Figure 5. *LC/MS/MS chromatograms from a low QC sample of mycotoxins extracted at a low QC sample.*

The total cycle time per sample for the DPX extraction and sample evaporation was 14 minutes, enabling "just in time" sample preparation using the MAESTRO software PrepAhead function. Using this automated procedure for extraction and analysis over 100 samples can be processed per day.

Future work will involve the inclusion of other mycotoxins of interest to the analysis panel: Fumonisins FB1 & FB2, Nivalenol (NIV), Fusarenon X (FUS X), 3-Acetyldeoxynivalenol (3-AcDON), Diacetoxyscirpenol (DAS), HT-2 toxin and T-2 toxin. For example, FB-1 and FB-2 are acidic mycotoxins that would be retained by the WAX sorbent, and can selectively be eluted with acetonitrile (4 % formic acid).

Conclusions

A fast, reproducible automated cleanup method for the determination of mycotoxins in corn extracts has been developed using disposable pipette extraction and LC/MS/MS. The described DPX extraction requires only small volumes of sample and solvent (approximately 0.5 mL). High recovery (> 75 %) and good reproducibility (% RSD < 6 %) were achieved for all mycotoxins analyzed providing LOQs for the mycotoxins ranging between 0.23 ng/g to 4.81 ng/g. Future work will involve the expansion of the method to include other mycotoxins and applicability to other food and feed matrices and to different LC/MS/MS platforms.