Application Note

Food & Beverage Testing

Determination of Multiclass Multiresidue Mycotoxins in Dry Corn Kernels and Soybeans

Using Agilent Captiva EMR Mycotoxins passthrough cleanup and LC/MS/MS detection

Authors

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Abstract

This application note presents the development and validation of a method for the analysis of multiclass multiresidue mycotoxins in dry corn kernels and soybeans. The method uses QuEChERS extraction followed by enhanced matrix removal (EMR) mixed-mode passthrough cleanup using the Agilent Captiva EMR Mycotoxins cartridge, then LC/MS/MS detection. The method features simplified and efficient sample preparation and sensitive detection on LC/MS/MS. Captiva EMR Mycotoxins cartridges were developed and optimized specifically for mycotoxins analysis in seeded dry feed and other complex processed food matrices. The method was compared with traditional solvent extraction method and another commercial workflow for multiclass multiresidue mycotoxins analysis.

Introduction

Mycotoxins are toxic compounds generated by various fungal species that can grow on many agricultural commodities and processed food and feed, either in the field or during storage.[1] Consumption of foods or feeds contaminated with mycotoxins can cause serious public health concerns. Many regulatory agencies have established maximum residue limits (MRLs) for mycotoxins in foods and feeds to closely monitor and control dietary exposure.[2-4]

Current analytical methods for mycotoxins are mostly based on LC/MS/MS detection, due to high sensitivity and selectivity provided by LC/MS/MS detection. Common mycotoxins include many highly sensitive compounds that tend to be lost during typical sample preparation techniques. Therefore, for multiclass multiresidue mycotoxins analysis in various food and feed sample matrices, the sample preparation method mainly relies on direct solvent/water mixture extraction with the use of a large panel of isotopically labeled internal standards (ISTD), called stable isotope dilution assay (SIDA).[5] This simple and reliable method has been cross-validated in multiple labs and is widely used in many mycotoxins testing labs. However, the method heavily relies on the use of isotopic ISTD for sample matrix correction and instrument detection selectivity. Complex food or feed samples are extracted without further cleanup of matrix coextractives. The injection of sample crude extract into an LC/MS/MS can cause significant flow path and MS source contamination and carryover issues, and result in more frequent instrument downtime for cleaning and maintenance.

QuEChERS extraction followed by dispersive SPE (dSPE) cleanup has also been used in food sample preparation for mycotoxins analysis.[6,7] However, dSPE cleanup does not provide efficient matrix removal, and also causes the loss of sensitive mycotoxins such as fumonisins. Captiva EMR–Lipid passthrough cleanup was used after QuEChERS extraction for mycotoxins analysis in fatty food matrices[8,9], demonstrating acceptable mycotoxins recovery and efficient fatty matrices cleanup. But for more complex matrices, comprehensive matrix cleanup is necessary to remove matrix co-extractives other than lipids and fats.

Agilent Captiva EMR Mycotoxins cartridges were developed and optimized specifically for multiclass multiresidue mycotoxins analysis in food and feed, providing comprehensive mixed-mode passthrough cleanup after QuEChERS extraction without compromise on target recovery. The objective of this study was to develop and validate a method for multiclass multiresidue mycotoxins analysis in dry corn and soybeans using QuEChERS extraction followed by Captiva EMR Mycotoxins passthrough cleanup and Agilent 6475 triple quadrupole LC/MS detection and quantitation.

Experimental

Chemicals and reagents

Native mycotoxins stock solutions, including Cannabis Mycotoxins Mix (part number TOX-CBS-MIX1), aflatoxin M1 (part number TOX-UNI-AFLAM1) and M2 (part number TOX-UNI-AFLAM2), deoxynivalenol (part number TOX-UNI-DON), fumonisin B1 (part number TOX-UNI-FUMOB1), B2 (part number TOX-UNI-FUMOB2), HT-2 (part number TOX-UNI-HT2), T-2 (part number TOX-UNI-T2), and zearalenone (part number TOX-UNI-ZON) were from Agilent Technologies. Other native mycotoxin standard stock solutions and stable labelled ISTD stock solutions were purchased from Romer Labs (Newark, DE, U.S.). Methanol (MeOH), acetonitrile (ACN) and isopropyl alcohol (IPA) were from VWR International (Radnor, PA, U.S.). Formic acid, ammonium formate, and ammonium fluoride were procured from MilliporeSigma (Burlington, MA, U.S.).

Solutions and standards

Two mycotoxin spiking solutions, I and II, were prepared by diluting the individual standard stock solutions or mycotoxins spiking solution I with 1:1 ACN:water. Due to the various concentrations of different stock solutions and the adjustment for individual standard spiking volumes based on their responses on LC/MS/MS, the concentrations of targets in the combined standard spiking solutions vary. These two standard spiking solutions were then used to prepare the calibration curve standard solutions in 1:1 ACN:water, covering the broad dynamic range (500x) from instrument limit of quantitation (LOQ_i) to instrument high limit of quantitation ($HLOQ_i$). For prespiked quality control (QC) samples, a standard solution was spiked into a pre-weighed sample at four levels, including method limit of quantitation 1 (LOQ_m1) and 2 (LOQ_m2), mid- and high-level. The individual target concentrations in each standard spiking solution; calibration standard solutions at LOQ_i (cal. STD 1) and $HLOQ_i$ (cal. STD 9); and four levels of prespiked QC samples are listed in Table 1.

The isotopic ISTD spiking solution was prepared by diluting the individual ISTD stock solutions with 1:1 ACN:water at the concentration of 1,000 ng/mL for DON-$^{13}C_{15}$, 8 ng/mL for AB1-$^{13}C_{24}$, 1,000 ng/mL for 3-ADON-$^{13}C_{17}$, 500 ng/mL for

Table 1. Mycotoxin standard solutions and QC samples.

Target (Abbreviation)	Standard Solutions (ng/mL)				Prespiked QC Samples (ng/g)			
	STD Spiking I	STD Spiking II	$HLOQ_i$	LOQ_i	QC-High	QC-Mid	$QC\text{-}LOQ_m2$	$QC\text{-}LOQ_m1$
Deoxynivalenol (DON)	3,750	150	75	0.15	375	75	15	1.5
Fusarenon X (FS-X)	3,750	150	75	0.15	375	75	15	1.5
Neosolaniol (NEO)	2,500	100	50	0.1	250	50	10	1
Aflatoxin M2 (AM2)	375	15	7.5	0.015	37.5	7.5	1.5	0.15
3-Acetyl deoxynivalenol (3-ADON)	2,500	100	50	0.1	250	50	10	1
15-Acetyl deoxynivalenol (15-ADON)	3,750	150	75	0.15	375	75	15	1.5
Aflatoxin G2 (AG2)	25	1	0.5	0.001	2.5	0.5	0.1	0.01
Aflatoxin M1 (AM1)	375	15	7.5	0.015	37.5	7.5	1.5	0.15
Aflatoxin G1 (AG1)	100	4	2	0.004	10	2	0.4	0.04
Aflatoxin B2 (AB2)	25	1	0.5	0.001	2.5	0.5	0.1	0.01
Aflatoxin B1 (AB1)	100	4	2	0.004	10	2	0.4	0.04
Diacetoxyscirpenol (DAS)	2,500	100	50	0.1	100	20	4	0.4
HT-2	3,750	150	75	0.15	375	75	15	1.5
Fumonisin B1 (FB1)	1,000	40	20	0.04	100	20	4	0.4
T2	1,000	40	20	0.04	100	20	4	0.4
Fumonisin B3 (FB3)	1,000	40	20	0.04	100	20	4	0.4
Ochratoxin A (OTA)	1,000	40	20	0.04	100	20	4	0.4
Zearalenone (ZON)	937.5	37.5	18.75	0.0375	93.75	18.75	3.75	0.375
Sterigmatocystin (STC)	250	10	10	0.01	25	5	1	0.1
Cyclopiazonic acid (CPA)	500	20	20	0.02	50	10	2	0.2
Fumonisin B2 (FB2)	1,000	40	40	0.04	100	20	4	0.4

T2-$^{13}C_{24}$, and 2,000 ng/mL for FB1-$^{13}C_{34}$. All standards were stored at 4 °C and used for no more than two weeks.

The ACN with 2% formic acid extraction solvent was prepared by adding 5 mL of formic acid into 245 mL of ACN and stored at room temperature. The water with 1% formic acid was prepared by adding 1 mL of formic acid into 100 mL of water. The LC mobile phase A was water with 10 mM ammonium formate, 0.05 mM ammonium fluoride, and 0.1% formic acid, and mobile phase B was MeOH.

Equipment and materials

The study was performed using an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II binary pump (G4220A), an Agilent 1290 Infinity II high-performance autosampler (G4226A), and an Agilent 1290 Infinity II thermostatted column compartment (G1316C). The LC system was coupled to an Agilent 6475 triple quadrupole LC/MS system (G6475AA). Agilent MassHunter Workstation software version 12.0 was used for data acquisition and analysis.

Chromatographic separation was performed using an Agilent ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm column (part number 959758-902) and an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1,200-bar pressure limit, UHPLC guard column (part number 821725-901).

Other equipment used for sample preparation included:

- Centra CL3R centrifuge (Thermo IEC, MA, U.S.)
- Geno/Grinder (Metuchen, NJ, U.S.)
- Multi Reax test tube shaker (Heidolph, Schwabach, Germany)
- Pipettes and repeater (Eppendorf, NY, U.S.)
- Agilent positive pressure manifold 48 processor (PPM-48; part number 5191-4101)
- Ultrasonic cleaning bath (VWR, PA, U.S.)

The sample preparation and other consumables used included:

- Agilent Bond Elut QuEChERS EN extraction kit, EN 15662 method, buffered salts, ceramic homogenizers

(part number 5982-5650CH)

– Captiva EMR Mycotoxins cartridges, 3 mL cartridges, 300 mg (part number 5610-2233)

LC/MS/MS instrument conditions

Tables 2, 3, and 4 show the LC pump conditions, LC multisampler conditions, and MS acquisition conditions.

LC pump conditions

Table 2. LC pump conditions for LC/MS/MS.

Parameter	Setting			
Mobile Phase A	Water with 10 mM ammonium formate, 0.05 mM ammonium fluoride, and 0.1% formic acid			
Mobile Phase B	MeOH			
Gradient	Time (min)	A%	B%	Flow (mL/min)
	0.00	75	25	0.25
	0.50	75	25	0.25
	7.00	0	100	0.25
	8.00	75	25	0.25
	8.50	0	100	0.25
Stop Time	11.0 min			
Post Time	2.5 min			

LC multisampler conditions

Table 3. LC multisampler program for LC/MS/MS

Parameter	Setting				
Injection Program	Draw 3.00 µL of water Draw 8.00 µL of sample Wash needle Draw 5.00 µL of water Mix 3.00 µL from air three times Inject				
Multiwash	Step	Solvent	Time (s)	Seat backflush	Needle wash
	1	IPA	10	Enabled	Enabled
	2	ACN	10	Enabled	Enabled
	3	Water	10	Enabled	Enabled
	Start cond.	Water	NA	Enabled	Enabled

LC column compartment

Isothermal temperature 40 ± 0.8 °C

Mass spectrometer parameter settings

The ESI source settings included drying gas at 150 °C, 11 L/min; sheath gas at 350 °C, 11 L/min; nebulizer gas at 40 psi; capillary voltage at 2,000 V (positive) and 3,000 V (negative); and nozzle voltage at 0 V (positive) and 1,500 V (negative).

Table 4. MS acquisition conditions for mycotoxin targets and ISTDs.

Target	RT (min)	Precursor Ion (*m/z*)	Fragmentor (V)	Product Ion (*m/z*)	Collision Energy (V)	Collision Cell Accelerator (V)	Polarity
DON-$^{13}C_{15}$	2.42	312.2	110	263.1 123.9	4 76	5	Positive
DON	2.45	297.1	90	231.1 203.0	10 10	5	Positive
FS-X	3.12	355.0	166	277.0 247.0	10 10	5	Positive
NEO	3.33	400.1	166	305.0 215.1	10 18	5	Positive
AM2	3.9	331.3	143	285.2 273.1	20 25	4	Positive
3-ADON-$^{13}C_{17}$	3.93	356.2	113	83.0 44.9	80 76	5	Positive
3-ADON	3.99	339.1	166	279.0 231.1	10 10	5	Positive
15-ADON	4.01	356.0	166	321.1 260.9	10 10	5	Positive
AG2	4.16	331.1	160	313.0 245.0	30 34	4	Positive
AM1	4.19	329.1	143	273.1 259.0	21 25	4	Positive
AG1	4.41	329.1	150	243.0 199.6	28 48	4	Positive
AB2	4.6	315.1	160	287.0 259.0	28 32	4	Positive
AB1-$^{13}C_{24}$	4.77	330.1	175	301.1 255.0	26 40	4	Positive
AB1	4.81	313.1	160	285.0 241.0	24 44	2	Positive
DAS	5.13	384.2	166	307.1 247.0	8 12	4	Positive
HT-2	5.81	442.2	100	263.0 214.9	12 12	4	Positive

Target	RT (min)	Precursor Ion (*m/z*)	Fragmentor (V)	Product Ion (*m/z*)	Collision Energy (V)	Collision Cell Accelerator (V)	Polarity
FB1-$^{13}C_{34}$	5.84	756.5	180	738.7 374.4	36 37	4	Positive
FB1	5.87	722.4	180	704.3 352.3	32 40	4	Positive
T2-$^{13}C_{24}$	6.19	508.4	110	322.2 229.1	10 20	4	Positive
T2	6.23	484.2	110	305.1 215.0	12 20	4	Positive
FB3	6.25	706.4	180	336.2 318.2	40 44	4	Positive
OTA	6.35	404.1	120	358.1 238.9	14 26	3	Positive
ZON	6.43	317.1	190	187.0 175.0	26 26	3	Negative
STC	6.48	325.1	165	310.0 281.0	26 44	4	Positive
CPA	6.61	337.1	130	196.0 182.0	24 20	5	Positive
FB2	6.61	706.4	180	688.4 354.6	32 40	4	Positive

Figure 1 shows the individual target chromatogram at LOQ_i level using the developed LC/MS/MS method.

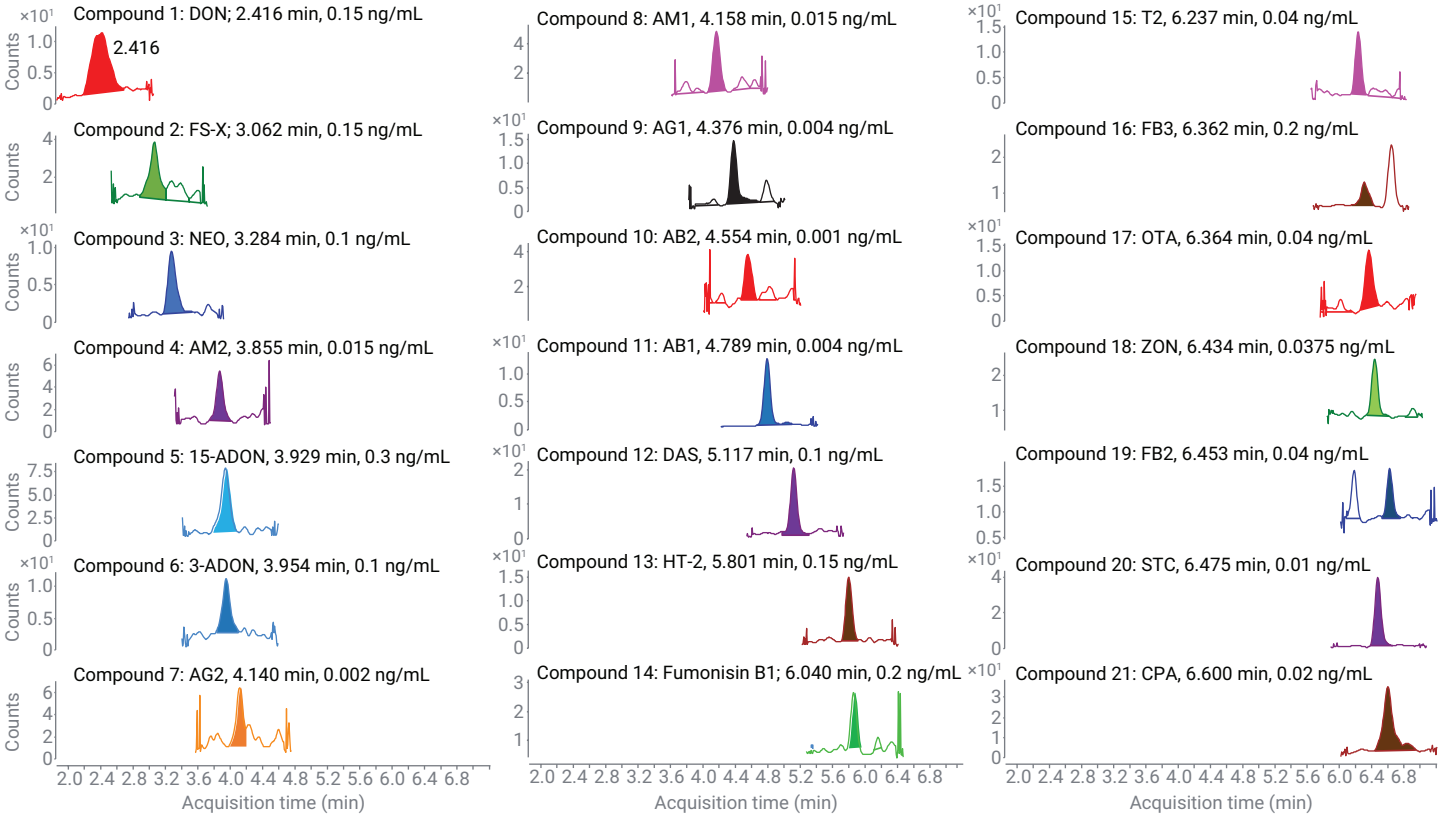

Figure 1. Mycotoxin MRM chromatograms at LOQ_i level.

Sample preparation

Dry corn kernel and soybean samples were purchased from local grocery stores. The dry samples were then ground to a fine powder using a mechanical grinder.

Two grams of sample powder were weighed into a 50 mL centrifuge tube for extraction, and then spiked with mycotoxin standard spiking solution to all prespiked QC samples appropriately. The sample was vortexed for 10 to 15 seconds after spiking. Samples were then ready for the sample preparation described in Figure 2. The entire sample preparation procedure introduced a 10x dilution factor.

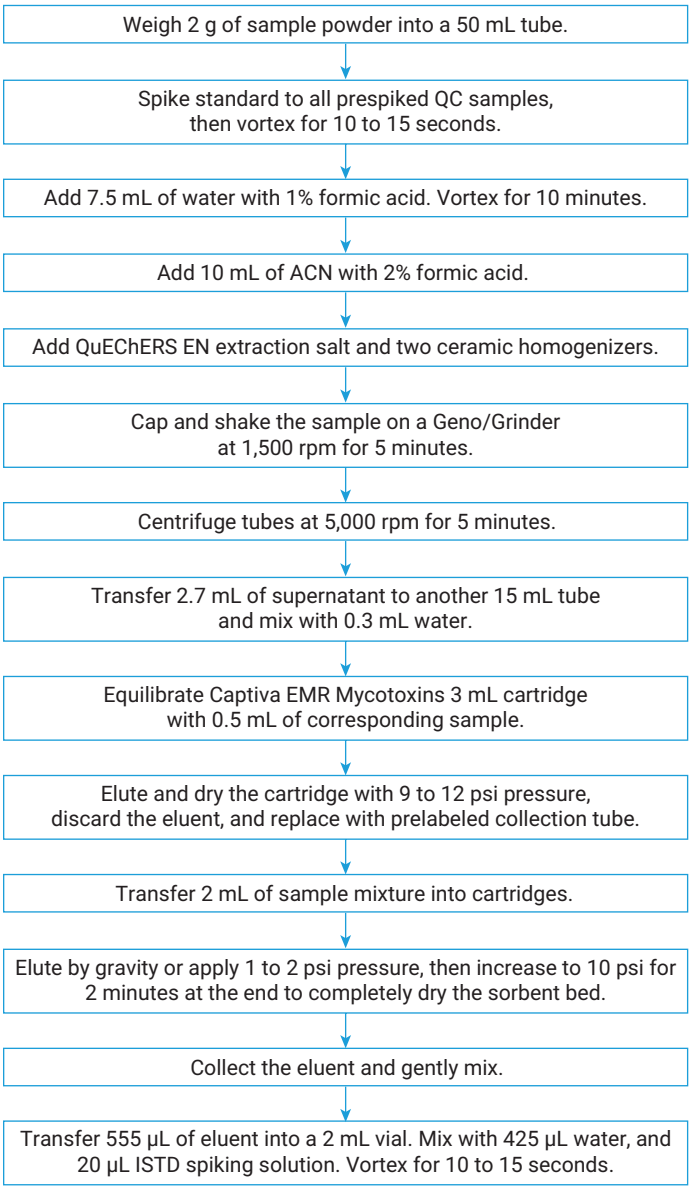

Figure 2. Sample preparation procedure for mycotoxins analysis in corn and soybean powder.

Method performance evaluation

The EMR mixed-mode passthrough cleanup using Agilent Captiva EMR Mycotoxins cartridges was evaluated in terms of target recovery and repeatability, and matrix removal. Target recovery and repeatability were studied using prespiked QCs versus matrix-matched postspiked QCs at corresponding levels. Matrix removal was investigated by comparing the chromatographic background for samples prepared by different methods. Results were compared with the other two current methods, including the stable isotopic dilution assay (SIDA)[5], and QuEChERS extraction followed with SPE and dSPE cleanup. Method quantitation was based on the use of neat calibration curves with isotopic ISTDs spiked. Considering the high cost of isotopic ISTDs, five isotopic ISTDs were used and spiked at the end of sample preparation. The entire method was then validated through calibration study, method LOQ, accuracy, and precision. Due to the different requirements for target LOQs, four prespiked QC level samples were prepared in replicates of four at each level. In addition, the matrix blanks were prepared in replicates of five for quantitation of targets in the matrix control. This is important for the target accuracy evaluation, as matrix positive contribution is unavoidable. The prespiked QC sample concentrations are listed in Table 1.

Results and discussion

EMR mixed-mode passthrough cleanup

Agilent Captiva EMR cartridges provide comprehensive matrix removal after traditional QuEChERS extraction through a mixed-mode passthrough cleanup, which is a simplified yet efficient matrix cleaning procedure to remove matrix interferences including carbohydrates, organic acids, pigments, fats and lipids, and other hydrophobic and hydrophilic matrix co-extractives. Captiva EMR Mycotoxins cartridges were developed for multiclass multiresidue mycotoxins analysis in complex dry seeded or processed food or feed matrices. The cartridge formula was specifically optimized to prevent the loss of highly sensitive mycotoxin compounds such as fumonisins and aflatoxins during sample cleanup.

Compared to another matrix cleanup method after QuEChERS extraction, which uses a typical commercial SPE cartridge plus special dSPE for mycotoxins analysis, the EMR mixed-mode passthrough cleanup provided simplified matrix cleanup procedure with fewer cleanup steps (one cleanup versus two cleanups) and a simpler cleanup procedure (removing steps such as uncapping and capping of dSPE tubes, vortexing, centrifugation, and multiple sample transfers). It provides the equivalent matrix removal efficiency but improved sensitive mycotoxins recovery, including fumonisins, OTA, and CPA. Figure 3 shows the mycotoxin recovery during post-QuEChERS extraction sample cleanup. Comparing to the other method using sequential SPE plus dSPE cleanups, the EMR mixed-mode passthrough cleanup significantly improved the recovery of sensitive mycotoxins. Especially for FB1, FB2, and FB3. The use of the other cleanup method caused almost complete loss for these targets, while the EMR passthrough cleanup provided > 90% recovery.

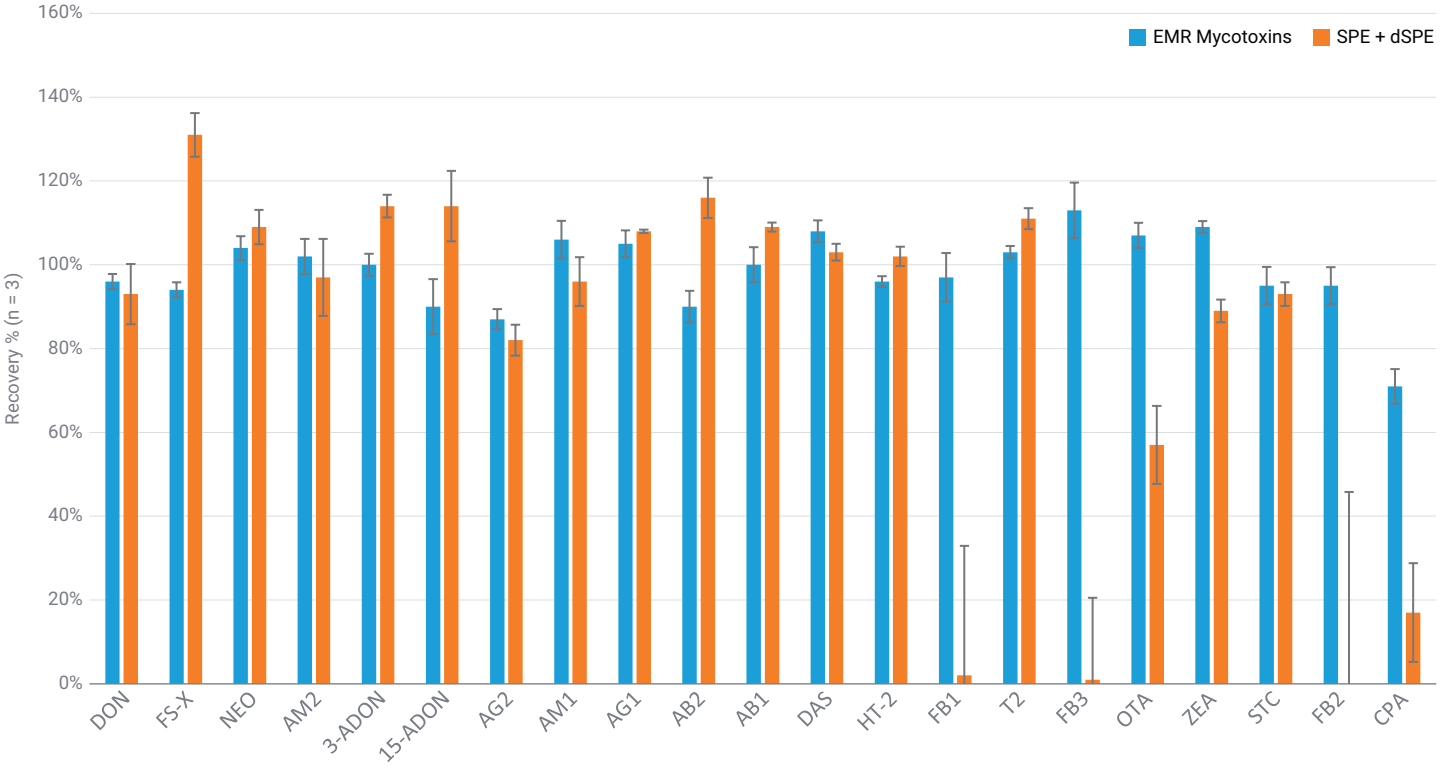

Figure 3. Post-QuEChERS extraction cleanup method comparison for mycotoxin recovery, Agilent Captiva EMR Mycotoxins passthrough cleanup (blue) versus SPE plus dSPE cleanup (orange).

Compared to the SIDA method using 1:1 ACN:water for sample extraction followed with syringe filter filtration, the QuEChERS extraction followed by EMR passthrough cleanup provided significantly cleaner final samples for LC/MS/MS injection. Figure 4 shows the comparison of samples prepared by the two different methods for final sample cleanliness. Fermented corn was used in this evaluation since it is a more challenging matrix than common corn kernels. Results show that the use of QuEChERS extraction followed by EMR passthrough cleanup removed more than 90% of matrix co-extractives, which significantly reduced the matrix co-extractives getting into the LC/MS/MS detection system when samples were injected.

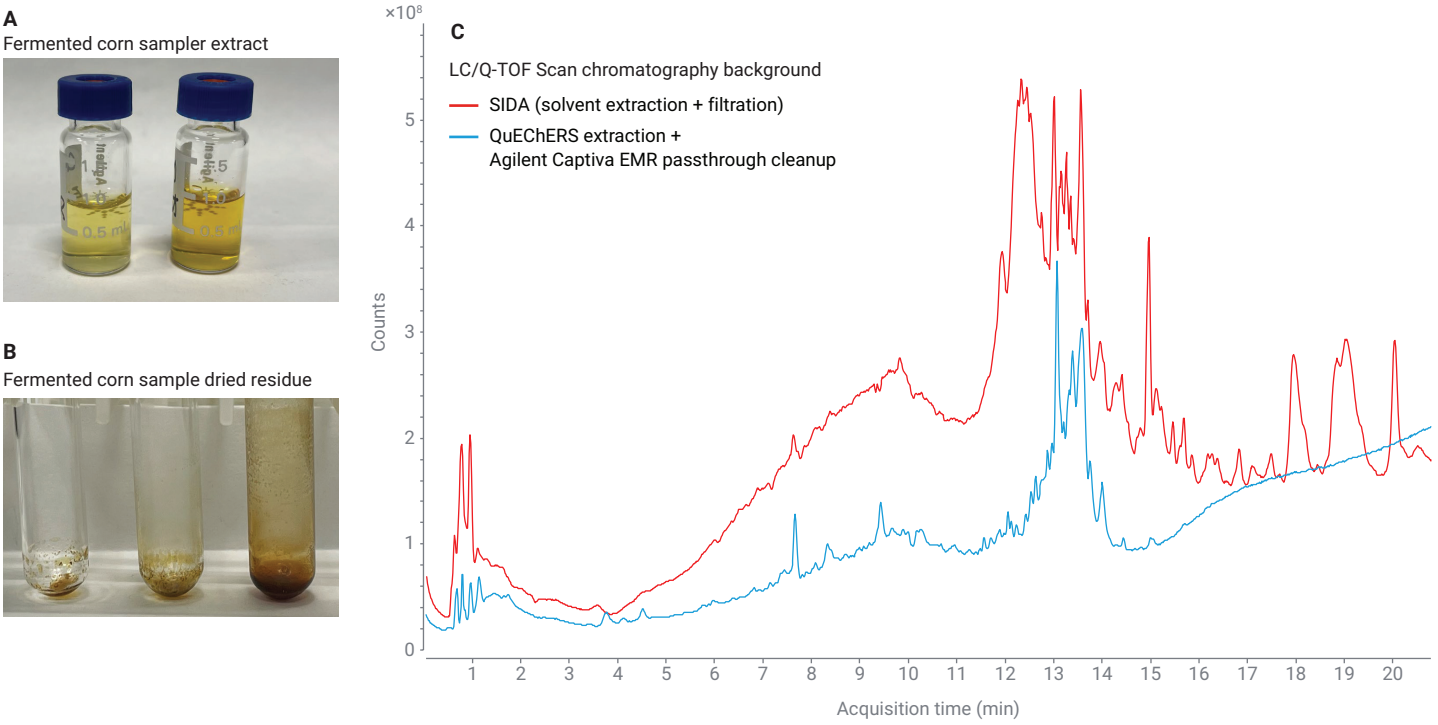

Figure 4. Fermented corn powder matrix cleanliness comparison between the SIDA method and a method using QuEChERS extraction followed by Agilent Captiva EMR Mycotoxins cartridge passthrough cleanup. (A) Final sample extract using the SIDA method (right) and the new method (left); (B) dried residue for sample prepared by the SIDA method (right), QuEChERS extraction (middle), and QuEChERS extraction followed with EMR passthrough cleanup (left); (C) LC/Q-TOF scan chromatography background for sample prepared by the SIDA method (red) and the EMR method (blue).

Method accuracy and precision

Recovery and repeatability of the developed method were evaluated for target recovery and repeatability using the prespiked QC-LOQ$_m$2, QC-mid, and QC-high corn samples. The mycotoxin standard was prespiked in the corn sample, then went through the sample preparation procedure. Similarly, the corn matrix blanks were prepared until the last step for dilution with water prior to LC/MS/MS detection.

The mycotoxin standard was then postspiked in the dilution step appropriately to correspond with targets' theoretical concentrations in prespiked QC samples. Both prespiked and postspiked samples were then run on LC/MS/MS, and the target responses were used to calculate the target's recovery and repeatability. Results shown in Figure 5 demonstrate that target recovery ranged from 80 to 111% with RSD% from 0.4 to 12.5% for all mycotoxins at three spiking levels at replicates of four by using the developed method.

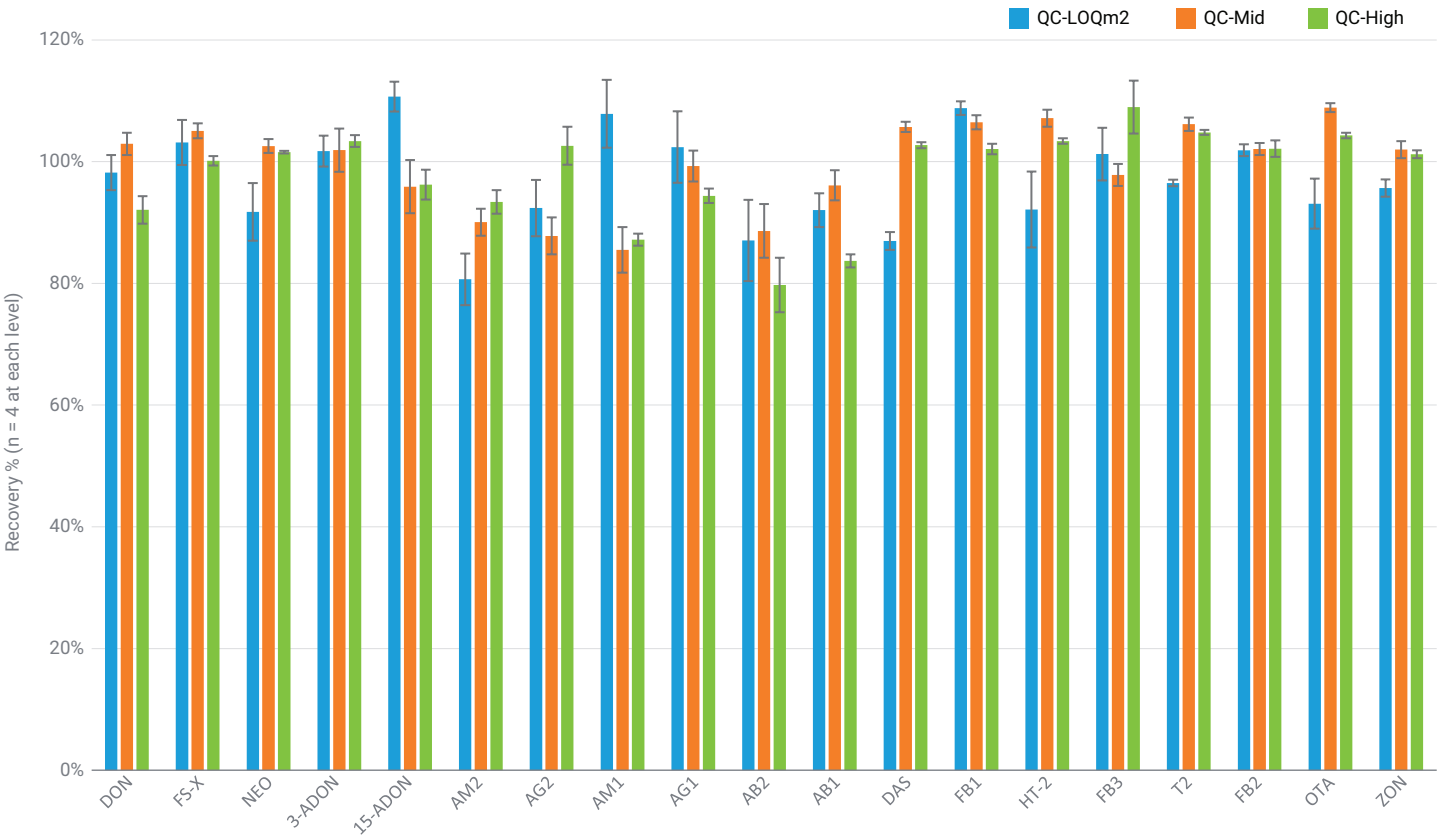

Figure 5. Mycotoxin recovery in dry corn extracted by QuEChERS extraction followed with EMR mixed-mode passthrough cleanup using Agilent Captiva EMR Mycotoxins cartridge.

Entire method validation

Method calibration: The method quantitation is based on back calculation of each analyte's response against the standard calibration curve for the analyte's concentration in sample. It is usually recommended to use the matrix-matched calibration curve for correction of the sample matrix effect in many food safety tests. The other option is the use of the neat calibration curve with the isotopic ISTD for the target's quantitation in the sample matrix. The use of neat calibration standards can make it easier for same analysis in multiple food matrices, saving the time and effort needed to prepare matrix-matched calibration curves in each sample matrix. The use of prespiked ISTDs, especially the isotopic ISTDs, can track the deviations on targets caused by matrix effect and recovery during sample preparation. When the method demonstrates excellent target recovery and repeatability, the postspiked isotopic ISTD after sample extraction can be used, which allows the use of less ISTDs, especially considering the high cost of isotopic ISTDs. However, the latter option requires more isotopic ISTDs use, both from the number of corresponding isotopic ISTDs to targets and spiking concentration and volume. The more corresponding isotopic ISTDs are used for the targets, the better the quantitation results will be. But in many case, not every target can have the commercially available corresponding isotopic ISTD, and the high cost of isotopic ISTDs has to be considered carefully and practically, especially for multiresidue targets analysis. In many cases, compromise has to be taken for targets quantitation without corresponding isotopic ISTD.

In this study, the method quantitation was based on neat calibration curves using isotopic ISTDs. Given the high cost of isotopic ISTD stock solutions, five isotopic ISTDs were chosen, considering the different mycotoxin classes and the retention time distribution. Figure 6 shows the representative mycotoxins' calibration curve within the 500x dynamic range, demonstrating excellent linearity. Overall, all the targets

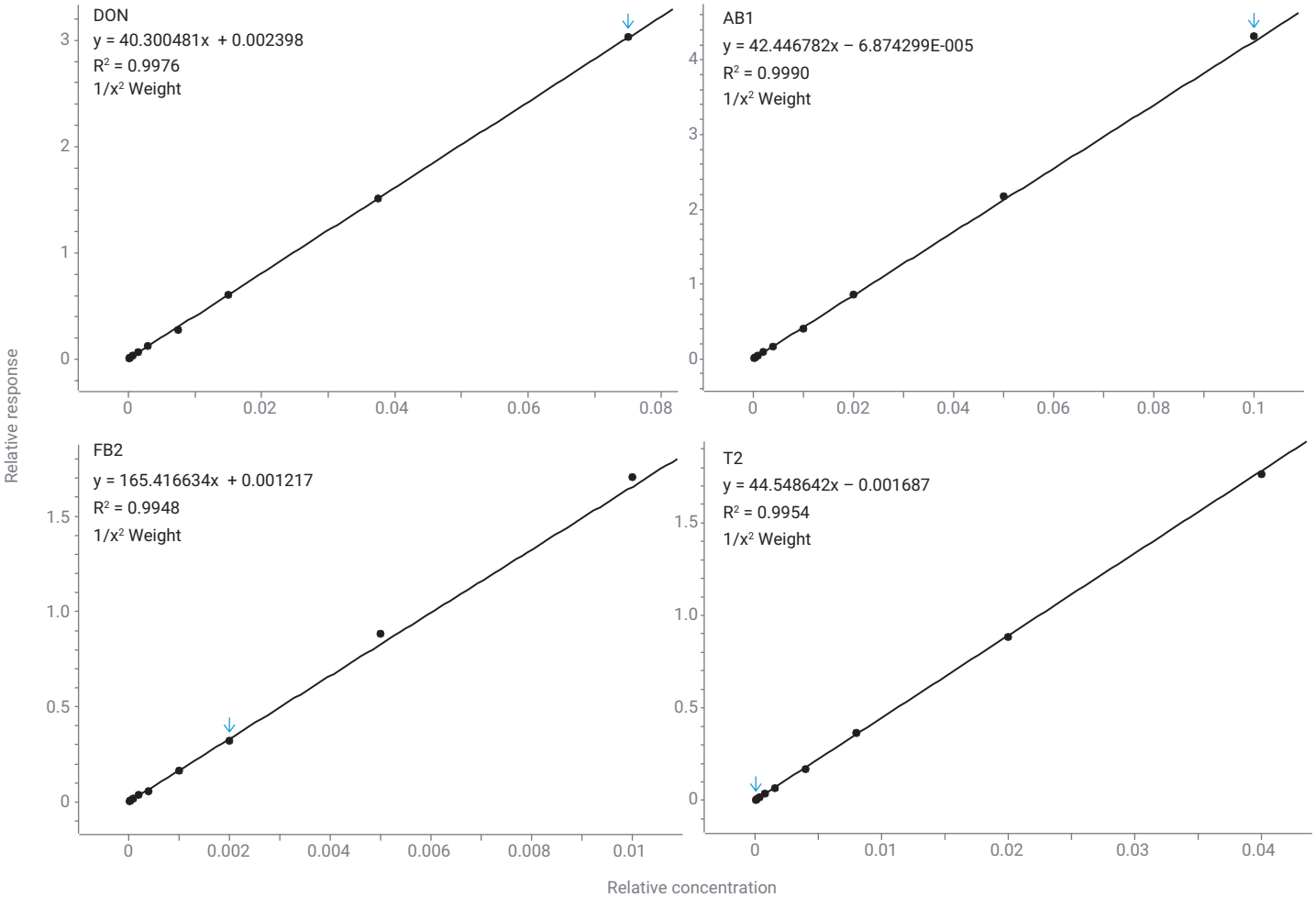

Figure 6. Calibration curves (neat) for representative mycotoxins using isotopic ISTDs.

except 15-ADON, AG2, FB1, and FB3 provided excellent linearity within the 500x dynamic range from LOQ_i to $HLOQ_i$, using linear regression and $1/x^2$ weight and generating $R^2 > 0.99$. For 15-ADON and AG2, the LOQ_i was raised due to sensitivity and resulted in the 250x dynamic range from LOQ_i. For FB1 and FB3, the LOQ_i was further increased and resulted in the 100x dynamic range from LOQ_i.

Method validation for target quantitation: Table 5 shows the method validation results for mycotoxin quantitative analysis in dry corn and soybean samples, including the method LOQ (LOQ_m), the ISTD used, calibration curve dynamic range, accuracy, and precision for the reported two to three levels of prespiked QC sample quantitation.

Two factors that affected the method quantitation results are the targets without corresponding isotopic ISTD and the positive detection of targets in the matrix blank. Since only five targets have the corresponding isotopic ISTD, the remaining 16 targets have to use the noncorresponding isotopic ISTD. The use of noncorresponding ISTD to target can vary by sample matrix, since the correction on target using noncorresponding ISTD can be different in different sample matrix. Therefore, it is important to always include the calibration curve standards in the sample testing batch, as the change of ISTD for quantitation needs to include the re-calculation of calibration standards, even though the neat calibration standards do not need to run for each sample analysis batch.

For the five targets with the corresponding isotopic ISTD, the acceptance criteria are 70 to 120% for accuracy and ≤ 20% for RSD. For the remaining targets without the corresponding isotopic ISTD, the acceptance criteria are 65 to 135% for accuracy and ≤ 25% for RSD. The accuracy results show that all the failures were on the targets without corresponding isotopic ISTD. For the corn matrix, the five isotopic ISTDs covered all targets except AB2, generating acceptable recovery results. For the soybean matrix, the five isotopic ISTDs covered all targets except HT-2, FB3, and OTA, generating acceptable accuracy results. However, the method delivered < 20% RSD for all targets in both matrices, demonstrating the excellent method repeatability. For the few targets that failed to meet accuracy acceptance criteria, using their corresponding isotopic ISTD can certainly improve the quantitation accuracy.

Table 5. Method validation results for the quantitative analysis of mycotoxins in dry corn kernels and soybeans.

		Dry Corn Kernels					Dry Soybeans				
				Prespiked QC (n = 4 at Each Level)					Prespiked QC (n = 4 at Each Level)		
Mycotoxin	Neat Calibration Curve Dynamic Range (ng/mL)	ISTD	LOQ_m (ng/g)	Conc. (ng/g)	Avg. Accu. (%)	RSD (%)	ISTD	LOQ_m (ng/g)	Conc. (ng/g)	Avg. Accu. (%)	RSD (%)
DON	0.15 to 75	DON-$^{13}C_{15}$	75	75*	99	8	DON-$^{13}C_{15}$	15	15	101	10
				–	–	–			75	104	2
				375	102	5			375	100	3
FS-X	0.15 to 75	DON-$^{13}C_{15}$	75	75*	95	5	DON-$^{13}C_{15}$	75	75*	91	6
				–	–	–			–	–	–
				375	114	1			375	118	3
NEO	0.1 to 50	AB1-$^{13}C_{24}$	50	50*	85	3	AB1-$^{13}C_{24}$	1	1	100	11
				–	–	–			10	98	5
				250	87	1			250	105	2
AM2	0.015 to 7.5	AB1-$^{13}C_{24}$	1.5	1.5	99	5	AB1-$^{13}C_{24}$	1.5	1.5	102	4
				7.5	90	7			7.5	109	4
				37.5	97	1			37.5	116	2
3-ADON	0.1 to 50	3-ADON-$^{13}C_{17}$	50	50*	110	3	3-ADON-$^{13}C_{17}$	10	10	76	6
				–	–	–			50	96	1
				250	96	3			250	93	4
15-ADON	0.3 to 75	3-ADON-$^{13}C_{17}$	75	75*	97	6	3-ADON-$^{13}C_{17}$	75	75*	87	8
				–	–	–			–	–	–
				375	92	10			375	84	12
AG2	0.002 to 0.5	AB1-$^{13}C_{24}$	0.1	0.1	92	2	AB1-$^{13}C_{24}$	0.1	0.1	93	4
				0.5	88	5			0.5	88	5
				2.5	105	1			2.5	73	4

Mycotoxin	Neat Calibration Curve Dynamic Range (ng/mL)	Dry Corn Kernels					Dry Soybeans				
		ISTD	LOQ_m (ng/g)	Prespiked QC (n = 4 at Each Level)			ISTD	LOQ_m (ng/g)	Prespiked QC (n = 4 at Each Level)		
				Conc. (ng/g)	Avg. Accu. (%)	RSD (%)			Conc. (ng/g)	Avg. Accu. (%)	RSD (%)
AM1	0.015 to 7.5	AB1-$^{13}C_{24}$	1.5	1.5	110	6	AB1-$^{13}C_{24}$	1.5	1.5	86	16
				7.5	99	8			7.5	80	8
				37.5	104	2			37.5	83	3
AG1	0.004 to 2	AB1-$^{13}C_{24}$	0.4	0.4	106	2	AB1-$^{13}C_{24}$	0.4	0.4	114	12
				2	89	3			2	113	4
				10	98	1			10	114	1
AB2	0.001 to 0.5	3-ADON-$^{13}C_{17}$	0.1	0.1	42	11	AB1-$^{13}C_{24}$	0.01	0.01	110	10
				0.5	54	7			0.1	88	12
				2.5	48	11			2.5	98	4
AB1	0.004 to 2	AB1-$^{13}C_{24}$	0.04	0.04	100	13	AB1-$^{13}C_{24}$	0.04	0.04	100	7
				0.4	103	5			0.4	89	5
				10	88	1			10	87	1
DAS	0.1 to 50	T2-$^{13}C_{24}$	50	50*	110	3	T2-$^{13}C_{24}$	1	1	115	4
				–	–	–			10	99	7
				250	108	1			250	116	3
HT-2	0.15 to 75	T2-$^{13}C_{24}$	1.5	1.5	97	11	T2-$^{13}C_{24}$	15	15	193	4
				15	90	5			75	193	4
				375	79	4			375	205	2
FB1	0.2 to 20	FB1-$^{13}C_{34}$	20	20*	120	3	FB1-$^{13}C_{34}$	0.4	0.4	85	9
				–	–	–			20	72	5
				100	106	5			100	73	3
T2	0.04 to 20	T2-$^{13}C_{24}$	20	20 *	92	2	T2-$^{13}C_{24}$	0.4	0.4	80	16
				–	–	–			4	87	14
				100	96	1			100	97	2
FB3	0.2 to20	FB1-$^{13}C_{34}$	20	20*	95	2	T2-$^{13}C_{24}$	20	20	167	7
				–	–	–			–	–	–
				100	96	1			100	157	5
OTA	0.04 to 20	FB1-$^{13}C_{34}$	0.4	0.4	112	18	T2-$^{13}C_{24}$	0.4	0.4	170	6
				4	115	9			4	192	4
				100	95	4			100	168	1
ZON	0.0375 to 18.75	T2-$^{13}C_{24}$	3.75	3.75	122	6	AB1-$^{13}C_{24}$	0.375	0.375	122	11
				18.75	83	5			3.75	120	9
				93.75	83	3			93.75	116	2
STC	0.01 to 5	AB1-$^{13}C_{24}$	0.1	0.1	80	6	3-ADON-$^{13}C_{17}$	0.1	0.1	83	5
				1	105	4			1	94	6
				25	109	2			25	90	2
CPA	0.02 to 10	AB1-$^{13}C_{24}$	2	2	89	2	3-ADON-$^{13}C_{17}$	2	2	113	8
				10	92	4			10	117	5
				50	98	2			50	108	4
FB2	0.04 to 20	FB1-$^{13}C_{34}$	4	4	121	10	FB1-$^{13}C_{34}$	4	4	82	14
				20	86	4			20	94	4
				100	86	4			100	92	2

* Raised LOQ_m and fewer reporting levels due to the positive detection of the target in matrix blank.
Results in red indicate outliers due to the missing corresponding isotopic ISTD for the target.

Conclusion

A simplified, rapid, and reliable method using QuEChERS extraction followed by EMR mixed-mode passthrough cleanup with the Agilent Captiva EMR Mycotoxins cartridge and LC/MS/MS detection was developed and validated for 21 mycotoxins in dry corn kernels and soybeans. The method demonstrated a significant improvement over the SIDA method in terms of matrix removal and better sensitive mycotoxins recovery compared to the other matrix cleanup using other commercial SPE and dSPE products being suggested for multiresidule mycotoxins analysis. The method also features simplified sample cleanup, saving time and effort, and thus improving overall lab productivity.

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