Application Note

Food & Beverage Testing

Determination of Multiclass Multiresidue Mycotoxins in Pet Food

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

Introduction

People often see their companion animals as members of the family that have deep impacts on their owners' lives. Given this emotional connection and a rising awareness of the link between pet health and pet food safety, mycotoxin contamination in pet food becomes a prevalent issue.[1] Dry pet food is by far the most preferred and consumed pet food option, containing nutritional balanced ingredients such as meat, fish, cereals, and vegetables.[2] Mycotoxin contamination in pet food can originate in the raw ingredients, as well as be generated during product manufacturing and storage. The most problematic mycotoxins in pet foods include aflatoxins (AF), deoxynivalenol (DON), fumonisins (FUM), ochratoxin A (OTA), and zearalenone (ZON).[3,4] Monitoring and control of mycotoxins contamination in pet food is thus important, calling for reliable analytical method for the determination of mycotoxins in pet food.

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 and repeatability. In a previous study, this method was successfully demonstrated for mycotoxins analysis in dry corn and soybeans.[5] The objective of this study was to validate the method for determination of multiresidue mycotoxins in pet food.

Experimental

Chemicals and reagents

Native mycotoxins stock solutions were purchased from Agilent Technologies, including Cannabis Mycotoxins Mix (part number TOX-CBS-MIX1); aflatoxin M1 (part number TOX-UNI-AFLAM1) and M2 (part number TOX-UNI-AFLAM2); deoxynicalenol (part number TOX-UNI-DON); fumonisin B1 (part number TOX-UNI-FUMOB1) and 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). 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 (Radnor, PA, U.S.). Formic acid, ammonium formate, and ammonium fluoride were procured from MilliporeSigma (Burlington, MA, U.S.).

Solutions and standards

The preparation of standard solutions and QC samples is described in the previous application note.[5]

Equipment and material

The study was performed using an Agilent 1290 Infinity II LC system coupled to an Agilent 6475 triple quadrupole LC/MS system (G6475AA). Agilent MassHunter Workstation software 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 (part number 959758-902) and an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1200-bar pressure limit, UHPLC guard (part number 821725-901). Other equipment used for sample preparation is described in the previous application note.[5]

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, 6 mL cartridges, 600 mg (part number 5610-2234)

LC/MS/MS instrument conditions

The LC/MS/MS instrument method was same as the one detailed in previous study.[5]

Sample preparation

Commercially available dog food and cat food 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 mycotoxins standard spiking solution to all prespiked QC samples appropriately. The samples were vortexed for 10 to 15 seconds after spiking. Samples were then ready for the sample preparation procedure described in Figure 1. The entire sample preparation procedure introduced a 10x dilution factor.

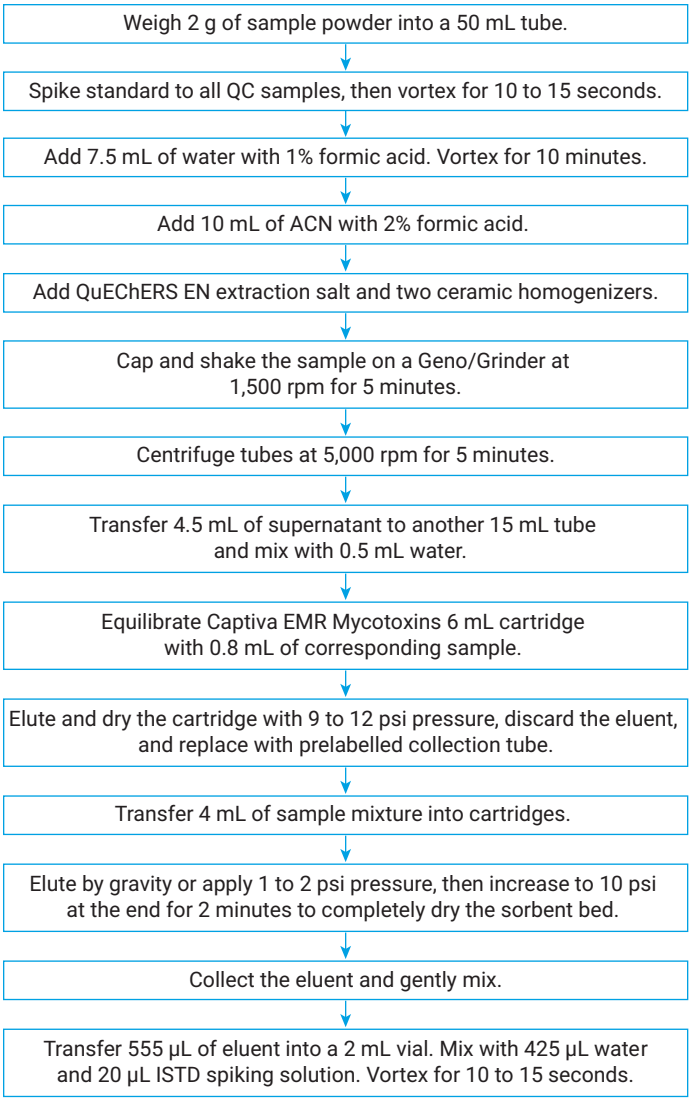

Figure 1. Sample preparation procedure for mycotoxins analysis in pet food powder.

Method performance evaluation

The mixed-mode passthrough cleanup using Agilent Captiva EMR Mycotoxins cartridges was evaluated in terms 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 stable isotopic dilution assay (SIDA)[5], using 1:1 ACN:water extraction followed with syringe filter filtration. Method quantitation was based on the use of neat calibration curves with isotopic ISTDs spiked.

Results and discussion

EMR mixed-mode passthrough cleanup

Captiva EMR cartridges provide the comprehensive matrix removal after traditional QuEChERS extraction through a mixed-mode passthrough cleanup. The method provides a simplified yet efficient matrix cleaning to remove matrix interferences, including carbohydrates, organic acids, pigments, fats and lipids, and other hydrophobic and hydrophilic matrix co-extractives. The Captiva EMR Mycotoxins cartridges were developed for multiclass multiresidue mycotoxins analysis in complex dry seed or processed food or feed matrices. The cartridge 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 a special dispersive SPE (dSPE) for mycotoxins, the EMR mixed-mode passthrough cleanup demonstrated a simplified matrix cleanup procedure and improved sensitive mycotoxins recovery in the previous study.[5]

Compared to the SIDA method[6] using 1:1 ACN:water for sample extraction followed by syringe filter filtration, the newly developed method provided significantly cleaner final samples for LC/MS/MS injection with the comparable procedure simplicity. Figure 2 shows the comparison of samples prepared by the two different methods for final sample cleanliness. Results show that the use of QuEChERS extraction followed with 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 3 shows the method procedure comparison of three methods, demonstrating that the newly developed method uses a comparable or simplified procedure compared to the traditional SIDA method or another method developed by a competitor.

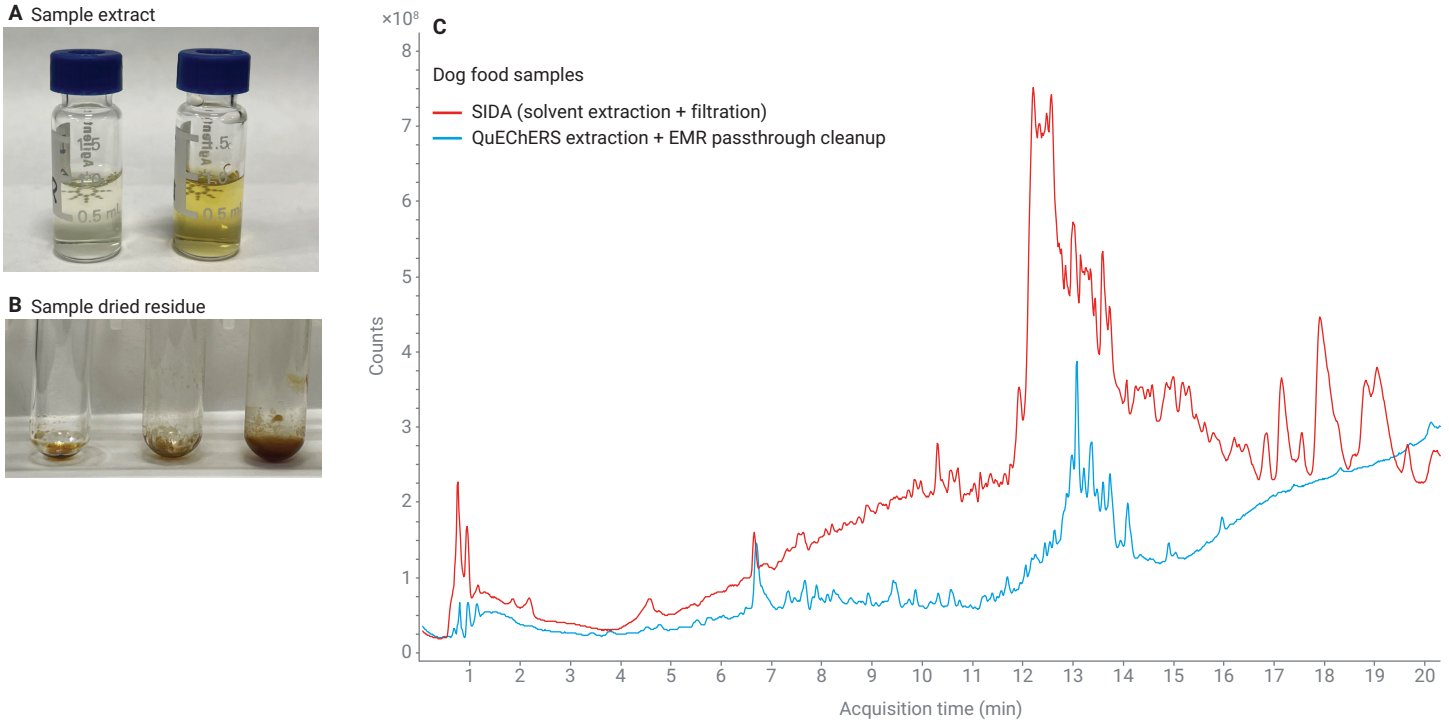

Figure 2. Dog food matrix cleanliness comparison between samples prepared by the SIDA method and the newly developed method. (A) Final sample extract using SIDA method (right) and EMR method (left); (B) sample dried residue for sample prepared by SIDA method (right), QuEChERS extraction (middle) and QuEChERS extraction followed with EMR passthrough cleanup (left); (C) LC/Q-TOF scan chromatography background for samples prepared by SIDA method (red) and EMR method (blue).

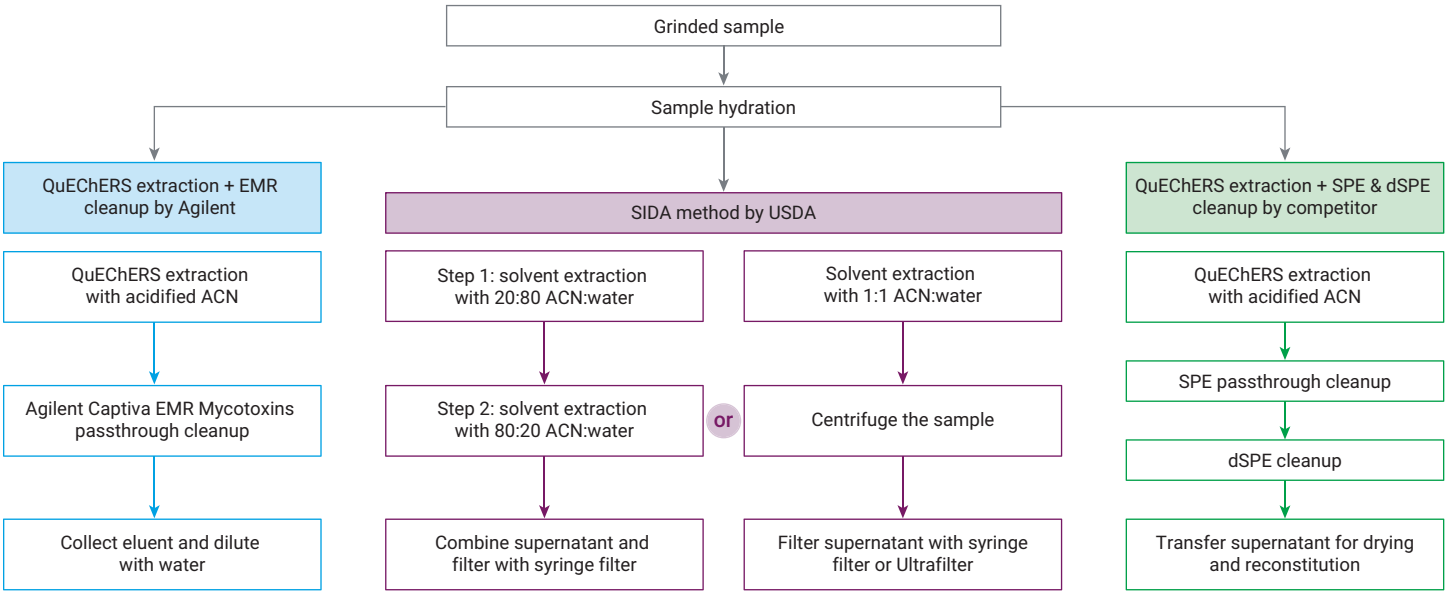

Figure 3. Methods procedure comparison.

Method recovery and repeatability

The developed method was evaluated for target recovery and repeatability using the pre-spiked QC-LOQ$_m$2, QC-mid and QC-high cat food samples. The recovery was calculated based on the target's response (peak area) in corresponding prespiked and postspiked samples. Results shown in Figure 4 demonstrate that target recovery ranged from 79 to 134% with %RSD from 1.1 to 14.4% for all mycotoxins in cat food at three spiking levels with replicates of four using the developed method.

Entire method validation

Method calibration

The use of neat standard calibration curves with isotopic ISTDs versus matrix-matched calibration curves for target quantitation was discussed in the previous study.[5] 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. Overall, all of the targets except 15-ADON, AG2, FB1, and FB3 generated 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$_i$.

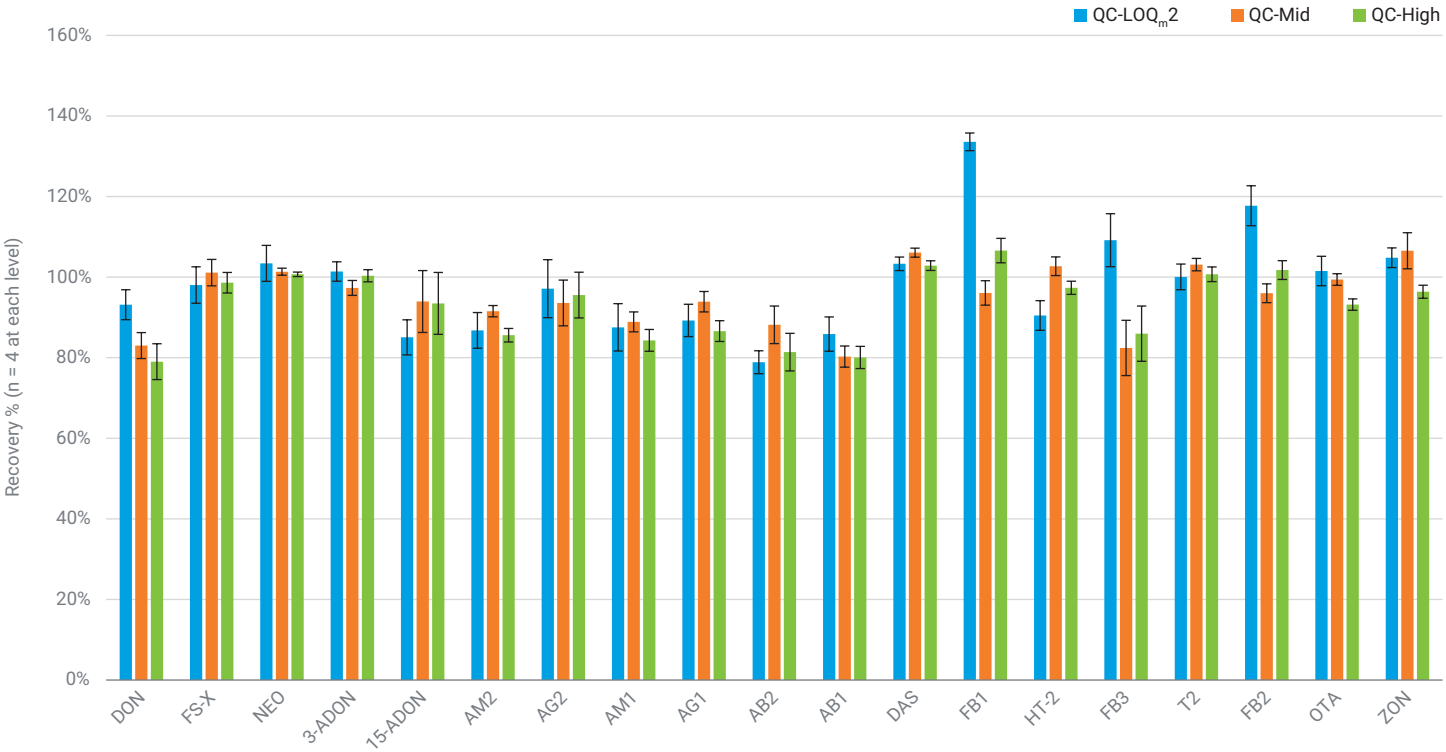

Figure 4. Mycotoxins recovery in cat food extracted by QuEChERS extraction followed with mixed-mode passthrough cleanup using the Agilent Captiva EMR Mycotoxins cartridge.

Method validation for target quantitation

Table 1 shows the method validation results for mycotoxins analysis in dog food and cat food, including the method LOQ (LOQ_m), the ISTD used, and the quantitation accuracy and precision (RSD) for reportable prespiked QC sample quantitation.

Two factors that impacted 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 had to use noncorresponding isotopic ISTDs. For the five targets with a 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 recovery and ≤ 25% for RSD. The recovery results show that all the failures were on the targets without corresponding isotopic ISTDs. For the dog food matrix, the five isotopic ISTDs covered all targets except FS-X and FB3, generating acceptable quantitation results. For the cat food matrix, the five isotopic ISTDs covered all targets except OTA, generating acceptable quantitation results. The method delivered < 20% RSD for all targets in both matrices, demonstrating excellent method repeatability. For the few targets that failed to meet acceptable accuracy criteria, using their corresponding isotopic ISTD can certainly address the quantitation. Note that high-level positive detection of DON, 3-ADON, and a few fumonisins in pet food caused higher method LOQs and fewer prespiked QC levels for reporting.

Table 1. Method lowest reportable LOQs (calculated) and validated LOQs for mycotoxins in pet food. (Continued on next page).

Mycotoxin	Neat Calibration Curve Dynamic Range (ng/mL)	Dog Food					Cat Food				
				Prespiked QC (n = 4 at Each Level)					Prespiked QC (n = 4 at Each Level)		
		ISTD	LOQ_m (ng/g)	Concentration (ng/g)	Average Accuracy (%)	RSD (%)	ISTD	LOQ_m (ng/g)	Concentration (ng/g)	Average Recovery (%)	RSD (%)
DON	0.15 to 75	DON-$^{13}C_{15}$	75	75*	112	4	DON-$^{13}C_{15}$	375	375*	102	9
				–	–	–			–	–	–
				375	99	4			–	–	–
FS-X	0.15 to 75	DON-$^{13}C_{15}$	75	75*	210	7	DON-$^{13}C_{15}$	15	15	90	6
				–	–	–			75	98	6
				375	224	16			375	116	1
NEO	0.1 to 50	3-ADON-$^{13}C_{17}$	10	10	80	2	AB1-$^{13}C_{24}$	1	1	94	3
				50	89	10			10	93	16
				250	80	6			250	90	1
AM2	0.015 to 7.5	AB1-$^{13}C_{24}$	1.5	1.5	91	8	AB1-$^{13}C_{24}$	1.5	1.5	94	17
				7.5	66	6			7.5	64	8
				37.5	61	7			37.5	66	5
3-ADON	0.1 to 50	3-ADON-$^{13}C_{17}$	10	10	109	6	3-ADON-$^{13}C_{17}$	10	10	91	5
				50	91	4			50	94	8
				250	80	5			250	87	2
15-ADON	0.3 to 75	3-ADON-$^{13}C_{17}$	375	375*	76	12	3-ADON-$^{13}C_{17}$	75	75*	90	3
				–	–	–			–	–	–
				–	–	–			375	95	19
AG2	0.002 to 0.5	AB1-$^{13}C_{24}$	0.1	0.1	84	16	AB1-$^{13}C_{24}$	0.1	0.1	101	9
				0.5	74	11			0.5	107	6
				2.5	75	5			2.5	97	5
AM1	0.015 to 7.5	AB1-$^{13}C_{24}$	1.5	1.5	65	19	AB1-$^{13}C_{24}$	1.5	1.5	83	8
				7.5	65	3			7.5	80	7
				37.5	67	7			37.5	79	1

Table 1. Method lowest reportable LOQs (calculated) and validated LOQs for mycotoxins in pet food. (Continued).

Mycotoxin	Neat Calibration Curve Dynamic Range (ng/mL)	Dog Food					Cat Food				
		ISTD	LOQ_m (ng/g)	Prespiked QC (n = 4 at Each Level)			ISTD	LOQ_m (ng/g)	Prespiked QC (n = 4 at Each Level)		
				Concentration (ng/g)	Average Accuracy (%)	RSD (%)			Concentration (ng/g)	Average Recovery (%)	RSD (%)
AG1	0.004 to 2	AB1-$^{13}C_{24}$	0.4	0.4	92	20	AB1-$^{13}C_{24}$	0.4	0.4	114	12
				2	81	3			2	113	4
				10	86	3			10	114	1
AB2	0.001 to 0.5	3-ADON-$^{13}C_{17}$	0.1	0.1	116	11	3-ADON-$^{13}C_{17}$	0.5	0.5*	85	8
				0.5	85	10			–	–	–
				2.5	86	6			2.5	83	3
AB1	0.004 to 2	AB1-$^{13}C_{24}$	0.2	0.2	83	12	AB1-$^{13}C_{24}$	0.04	0.04	93	11
				2	85	4			0.4	84	9
				10	94	3			10	99	3
DAS	0.1 to 50	T2-$^{13}C_{24}$	1	1	115	10	T2-$^{13}C_{24}$	1	1	84	6
				10	115	3			50	137	2
				250	113	2			250	130	2
HT-2	0.15 to 75	T2-$^{13}C_{24}$	15	15	78	4	T2-$^{13}C_{24}$	15	15	101	20
				75	71	7			75	94	3
				375	74	3			375	95	1
FB1	0.2 to 20	FB1-$^{13}C_{34}$	100	100*	83	6	FB1-$^{13}C_{34}$	20	20*	69	5
				–	–	–			–	–	–
				–	–	–			100	73	2
T2	0.04 to 20	T2-$^{13}C_{24}$	0.4	0.4	88	10	T2-$^{13}C_{24}$	4	4	102	7
				4	105	6			20	105	3
				100	99	4			100	106	3
FB3	0.2 to 20	FB1-$^{13}C_{34}$	20	20*	39	12	FB1-$^{13}C_{34}$	0.4	0.4	104	10
				–	–	–			4	114	11
				100	49	7			100	108	2
OTA	0.04 to 20	None	4	4	116	5	None	0.4	0.4	170	6
				20	96	7			4	192	4
				100	99	4			100	168	1
ZON	0.0375 to 18.75	AB1-$^{13}C_{24}$	0.375	0.375	104	5	AB1-$^{13}C_{24}$	3.75	3.75	81	3
				18.75	114	4			18.75	114	11
				93.75	126	5			93.75	108	2
STC	0.01 to 5	AB1-$^{13}C_{24}$	0.1	0.1	96	8	AB1-$^{13}C_{24}$	1	1	91	5
				1	85	7			5	79	3
				25	95	8			25	75	1
CPA	0.02 to 10	3-ADON-$^{13}C_{17}$	2	2	128	7	AB1-$^{13}C_{24}$	0.2	0.2	79	15
				10	126	1			2	75	7
				50	116	8			50	71	2
FB2	0.04 to 20	FB1-$^{13}C_{34}$	20	20*	73	5	FB1-$^{13}C_{34}$	4	4	128	25
				–	–	–			20	85	7
				100	74	4			100	84	4

* Higher LOQ_m and fewer reporting levels due to positive detection of the target in the 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 mixed-mode passthrough cleanup with the Agilent Captiva EMR Mycotoxins cartridge and LC/MS/MS detection was developed and validated for 21 mycotoxins in pet food. The method demonstrated the significant improvement over the SIDA method in terms of matrix removal, excellent recovery and repeatability, and acceptable final quantitation accuracy and precision. The method also features a simplified procedure, saving time and effort, and thus improving overall lab productivity.

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