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Inkjet-Based Dispersive Liquid−Liquid Microextraction Method Coupled with UHPLC−MS/MS for the Determination of Aflatoxins in Wheat

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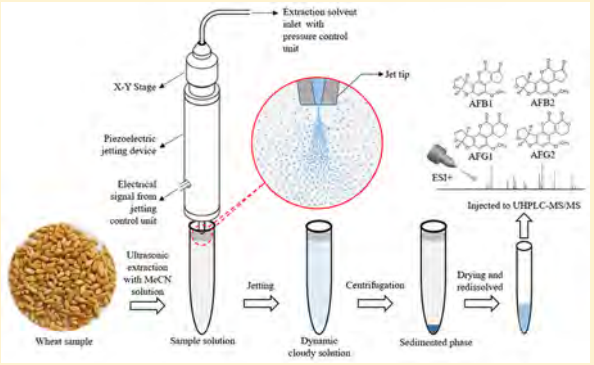
Supporting Information

ABSTRACT: A novel method was developed for determination of aflatoxin B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2) in wheat using inkjet-based dispersive liquid−liquid microextraction (DLLME) coupled with ultrahigh-pressure liquid chromatography−tandem mass spectrometry. A drop-on-demand jetting device was used to form a cloudy solution in traditional DLLME by injecting extraction solvent (10 μL) as ultrafine droplets (~20 μm diameter) at high frequency into sample solution. The method was validated using wheat as a representative matrix, which was pretreated with acetonitrile/water solution. Good linearity was observed over the studied range (0.06−6 μg/kg), and the limits of quantification (0.06−0.18 μg/kg) were below the maximum level established by the European Union for cereal. Satisfactory recoveries, ranging from 83.2% to 93.0% with relative standard deviations below 4.6%, were obtained for all compounds. The method, which is convenient and reliable and has low solvent consumption, represents a new direction for the development of traditional DLLME technology.



Mycotoxins are secondary metabolites produced by filamentous fungi and are some of the most common natural contaminants of plants.[1,2] According to the United Nations Food and Agriculture Organization, up to 25% of crops in different parts of the world are contaminated with mycotoxins that include the highly toxic AFB1, AFB2, AFG1, and AFG2 produced by *Aspergillus flavus* and other parasitic *Aspergillus* fungi.[3] AFB1 is the most toxic and is classified as a human carcinogen (group 1) by the International Agency for Research on Cancer.[4] Contamination by aflatoxins (AFs) can occur as a result of poor storage and processing conditions as well as during growth of crops that include cereals, oilseeds, spices, nuts, and their derivatives.[5−8]

Governments and international organizations have set strict limits on aflatoxin contamination. The European Union (EU) fixed maximum levels for AFs (2 μg/kg for AFB1 and 4 μg/kg for the sum of AFB1, AFB2, AFG1, and AFG2 in cereals and all products derived from cereals, including processed cereal products) by means of Commission Regulation No. 1881/2006[9] and subsequent amendments.[10] Sampling and analysis methods for official control of mycotoxins are specified in Regulation No. 401/2006.[11]

Quantitative analytical methods for determination of AFs are mainly based on sample treatments involving solvent extraction followed by a cleanup step to remove interference, with subsequent quantification by liquid chromatography using fluorescence or mass spectrometry (MS) detection.[12,13]

Cereals are very complex solid matrixes that present challenges for sample treatment. Some necessary steps of pretreatment, such as solvent extraction under optimized conditions, can effectively transfer the AFs from solid matrix to liquid matrix (as the sample solution) so as to facilitate further treatment. AFs must be efficiently extracted from samples to reduce matrix effects and permit quantification at the very low concentration levels required.[14] A number of sample treatments have been proposed for extraction of AFs from cereals, such as immunoaffinity columns,[15,16] solid phase extraction,[17,18] and liquid−liquid extraction.[13,19,20] Recently, liquid-phase microextraction techniques have become increasingly popular since they have lower reagent consumption (more environmentally friendly) and have a high preconcentration factor. Of these techniques, dispersive liquid−liquid microextraction (DLLME) is being increasingly used.

Since first introduced by Rezaee et al. in 2006, DLLME has become an outstanding example of the many solvent-minimized extraction methods developed in the past 20

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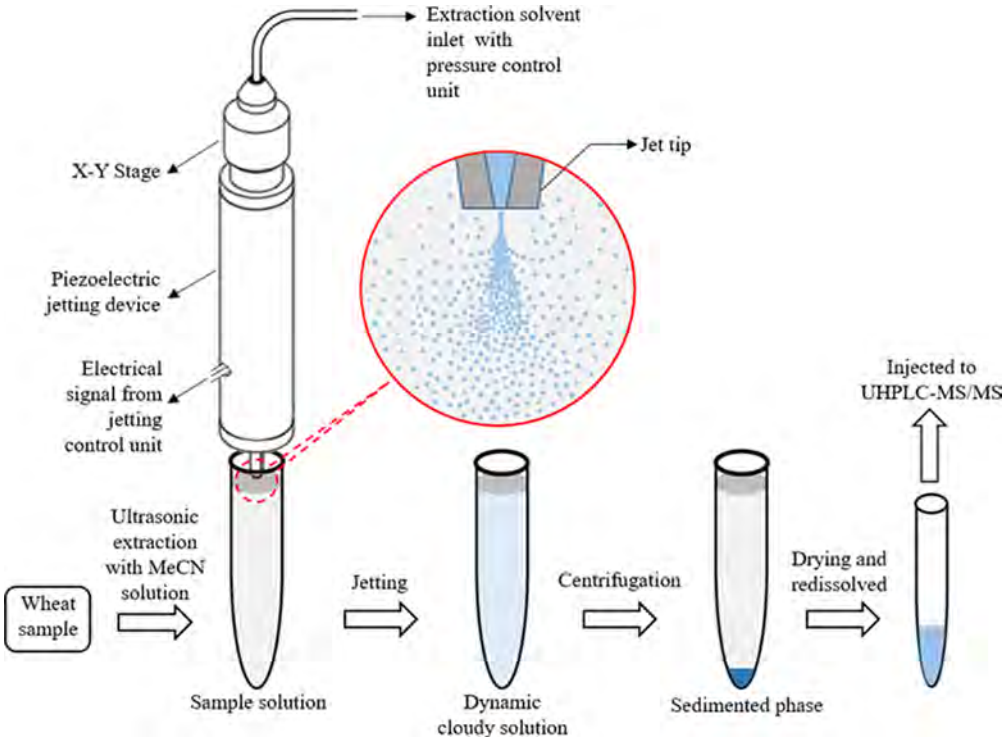

Figure 1. Overall scheme of the inkjet-DLLME setup.

years.[21−26] In DLLME, by rapid injection into the aqueous sample solution together with a water-miscible dispersive solvent, the extraction solvent becomes highly dispersed in the form of fine droplets into which the analytes can be rapidly extracted. With advantages that include simplicity, reduced solvent consumption, and high enrichment factors, DLLME has been widely adopted in food analysis, including several applications for determination of AFs in cereals.[27−30]

In the most basic versions of DLLME, a mixture of extraction solvent and dispersive solvent is injected rapidly into the sample solution manually via a microsyringe. The extraction solvent is dispersed into the sample solution with the aid of a dispersive solvent as fine droplets by shear force. The droplets generated manually in conventional DLLME means that their size cannot be controlled, resulting in insufficient surface contact between the extraction and aqueous phase and the need for an additional mixing process. The limitation mentioned above leads to the extraction efficiency that cannot be maximized and has relatively low precision in the conventional DLLME method. With regard to the fine mixing of both extraction and aqueous phase, several approaches have recently been reported which utilized kinetic energy instead of dispersive solvent using, such as vigorous-injection assisted,[31] vortex-assisted liquid−liquid microextraction,[32,33] ultrasound-assisted emulsication microextraction,[34,35] and air-assisted liquid−liquid microextraction.[36,37]

Inkjet printing is recognized as an important industrial technology that enables precise control of droplet volume at the nanoliter to picoliter level by adjusting the drive voltage and applying a pulse waveform on the piezoelectric actuator. It is currently one of the most promising methods for efficient microdroplet generation.[38−41] Based on these features, the application of inkjet printing to microscale high-throughput analysis could not only satisfy the requirements for speed and low reagent consumption but also facilitate more precise sample injection and automation. Yang et al. reported that inkjet printing technology can be applied for the preparation of monodisperse polymer particles.[42] Chen et al. reported a novel chemiluminescence diagnosis system for high-throughput human IgA detection by inkjet nanoinjection on a multi-capillary glass plate.[43] Zeng et al. reported a highly accurate sample injection system for capillary electrophoresis based on an inkjet microchip capable of reproducing exact volumes at the picoliter level.[44] To the best of our knowledge, generating ultrafine droplets with an inkjet device for application in DLLME has not been reported.

In the present work, a novel DLLME method based on drop-on-demand inkjet technology (inkjet-DLLME) was established. In this method, the droplets (picoliter level per drop) of extraction solvent were injected into the sample solution automatically, accurately, and controllably via the jetting device at high frequency. A dynamic cloudy solution was formed, consisting of ultrafine droplets of extraction solvent dispersed entirely into sample solution. Extraction equilibrium was established in a short time because of the extensive surface contact between droplets of the extraction solvent and the sample solution. Compared with the conventional DLLME method, the inkjet-DLLME method is more convenient and reliable and has low solvent consumption.

The proposed inkjet-DLLME method was combined with ultrahigh-pressure liquid chromatography−tandem mass spectrometry (UHPLC−MS/MS) for determination of four major AFs in wheat. Also, to identify the best extraction conditions, several parameters affecting the efficiency of inkjet-DLLME, such as the nature and volume of extraction solvent, salt addition, pH, and jetting device parameters, were studied.

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EXPERIMENTAL SECTION

Chemicals and Materials. Acetonitrile (MeCN), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), and formic acid were purchased from Merck KGaA (Darmstadt, Germany). All solvents used were HPLC grade. Ultrapure water was produced using a Milli-Q system (Millipore Corporation, Billerica, MA). AFs were obtained from Sigma-Aldrich (St. Louis, MO). All standards had purity >99%. AFB1, AFB2, AFG1, and AFG2 stock solutions were prepared in MeCN. The daily standard working solutions at different concentrations were obtained by diluting the stock solutions with MeCN. A mixed working standard solution was prepared by diluting individual stock solutions to obtain a solution containing 50 μg/L of each standard. All standard solutions were stored at −20 °C in the dark.

Sample Preparation. The wheat samples, from material intended for human consumption, were obtained from a farm in Baoji district, Shaanxi province, China. After harvest each June, the wheat was stored in small barns without special protection. Five batches were collected from samples stored for 36, 24, 12, and 0 months after harvest. The samples were ground with a household grinder and passed through a 100-mesh sieve before use.

Samples (1 ± 0.05 g) were weighed into a centrifuge tube (polypropylene, 10 mL) and homogenized in a MeCN/water mixture (3 mL, 70%, v/v) by sonication for 5 min. The mixture was centrifuged at 3200*g* for 5 min, and the sediment was extracted again using the same method. A sample of the combined extracts (2 mL) was diluted to 20 mL with ultrapure water, treated with NaCl to 10% w/v, filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane (Merck KGaA, Darmstadt, Germany) and stored in the dark at 4 °C.

Inkjet-DLLME Procedure. The inkjet-DLLME system (MicroFab Technologies, Plano, TX) consisted of a piezoelectric jetting device (MJ-AT-01) connected to a JetDrive V control unit (CT-M5-01) with JetServer software. A pressure control unit (CT-PT-21) was used to provide stable pressure conditions. A CCD camera with microscope lens and an LED (CM-VS-02) synchronized with the piezoelectric pulse to provide lighting were used to capture droplet formation at the jet tip. A schematic of the inkjet-DLLME setup is shown in Figure 1.

To carry out the inkjet-DLLME procedure, sample solutions (200 μL) were placed into conical tubes. The position of the jetting device was adjusted with an *X*−*Y* stage holder to enable contact of the nozzle with the sample solution such that the extraction solvent droplet could be freely generated at the top of the sample solution.

Subsequently, a bipolar voltage waveform was applied to the jetting device at voltages of 15 and −5 V, with rise and fall times of 3 μs, a dwell time of 5 μs, an echo time of 9 μs, and a frequency of 50 000 Hz. Chloroform (10 μL), as the extraction solvent for DLLME, was ejected in the form of fine droplets (~20 μm in diameter) from the piezoelectric jetting device into the sample solution during 36.5 s.

The mixture was then centrifuged at 5700*g* for 5 min and the lower phase was transferred to an autosampler vial with a 100 μL insert. After drying under a gentle nitrogen stream, the residue was dissolved in MeCN (20 μL) before injection into the UHPLC−MS/MS system.

UHPLC−MS/MS Conditions. UHPLC−MS/MS analysis was performed using a Nexera UHPLC system coupled to an LCMS-8040 tandem quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). LabSolutions LCMS software (Shimadzu Corporation) was used to control the instruments and process the data. The Nexera UHPLC system used in the analysis consisted of a system controller (CBM-20A), two pumps ($LC\text{-}20AD_{XR}$), an autosampler ($SIL\text{-}20A_{XR}$), a column heater (CTO-20AC), and a degasser ($DGU\text{-}20A_3$).

The LC conditions were optimized as follows: solvent A was 1% formic acid in water (v/v) and solvent B was 1% formic acid in MeCN (v/v). The gradient profile was 30% B (0−0.5 min), 30%−45% B (0.5−4.0 min), 45%−100% B (4.0−5.0 min), 100% B (5.0−6.0 min), 100%−30% B (6.0−6.5 min), and 30% B (6.5−10.0 min).

The flow rate was set at 0.2 mL/min, and the column temperature was 40 °C. The chromatographic separation was carried out on a Shim-pack XR-ODS III column (75 mm × 2.0 mm, 1.6 μm, Shimadzu Corporation). The injection volume was 10 μL.

The mass spectrometer was operated in positive ion mode using an electrospray ionization (ESI) source. The operating parameters were optimized as follows: nebulizer gas flow, 3 L/min (N_2, purity >99.999%); drying gas flow, 15 L/min (N_2, purity >99.999%); collision gas pressure, 230 kPa (He, purity >99.999%); desolvation line temperature, 250 °C; heat block temperature, 400 °C; interface voltage, 4.5 kV. The other parameters were tuned automatically. The multiple reaction monitoring (MRM) parameters for the four AFs, such as voltage potential (Q1, Q3) and collision energy (CE), are summarized in Table S1.

RESULTS AND DISCUSSION

Optimization of the inkjet-DLLME conditions was performed using a blank wheat sample analyzed by UHPLC−MS/MS using one variable at a time. All optimization experiments were performed in triplicate.

Effect of Extraction Solvent. A mixture of MeCN/water effectively extracts AFs from solid samples.[45,46] Therefore, the selected AFs were extracted from wheat samples using a mixture of MeCN/water (70:30 v/v) and then diluted with ultrapure water. The final sample solution for inkjet-DLLME process contained 7% (v/v) MeCN, which could readily act as a dispersive solvent due to its miscibility with most extraction solvent.

In conventional DLLME procedures, the extraction solvent should have certain characteristics, such as high extraction capability for the target analyte and low solubility in water. In the proposed method, droplets of the extraction solvent were injected at the top of the sample solution via the jet tip. Therefore, the extraction solvents with a density higher than water were preferred. In addition, driven by the jetting device, droplets of high-density extraction solvent tend to form a turbulent state in the sample solution, resulting in more sufficient contact with the sample solution. Therefore, the extraction efficiencies of 5 μL of three solvents, CH_2Cl_2, $CHCl_3$, and CCl_4 were investigated using the spiked sample (0.1 μg/L of the four AFs), respectively. From the results, shown in Figure 2A, $CHCl_3$ had the best extraction efficiency. Thus, $CHCl_3$ was chosen as the extraction solvent. To examine the effect of the extraction solvent volume, different amounts of $CHCl_3$ (3, 5, 10, 20, and 30 μL) were investigated using the same spiked sample. The extraction recovery increased as the volume of $CHCl_3$ was increased up to 10 μL and then remained constant over the range of 10−30 μL (Figure 2B).

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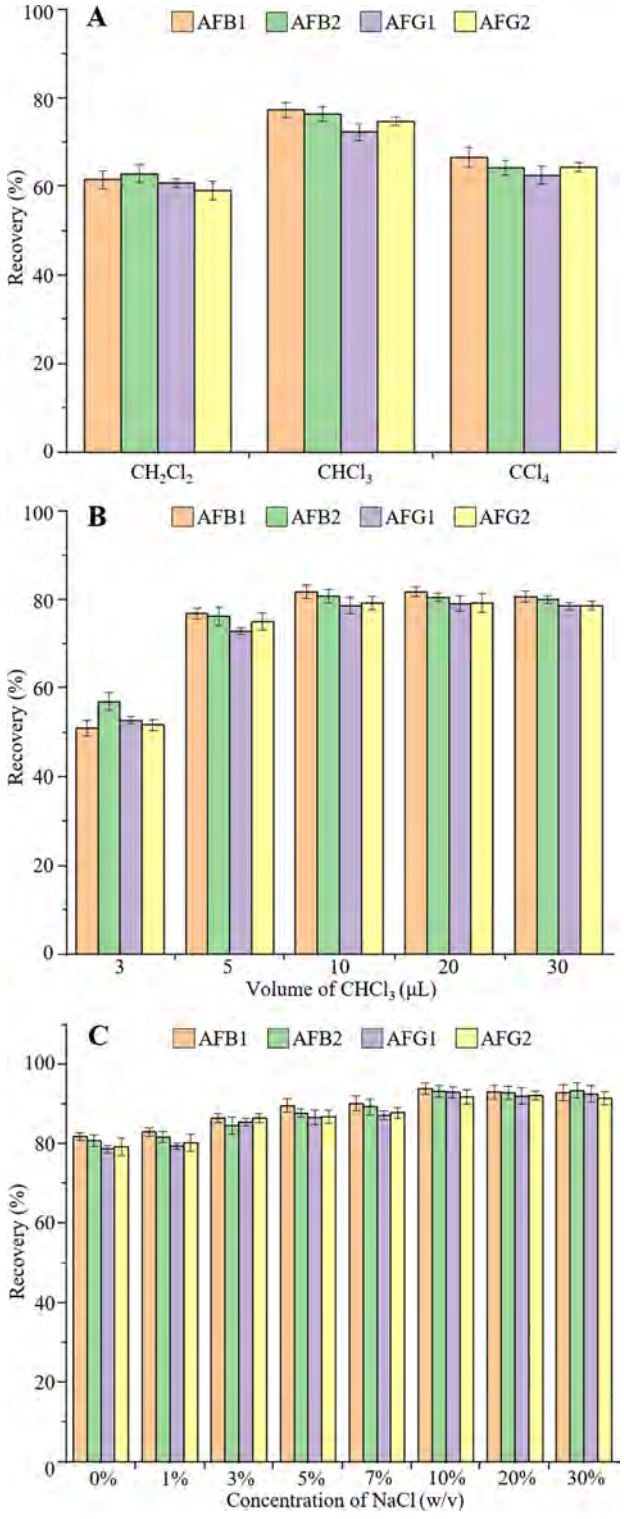

Figure 2. Effect of extraction solvent on extraction efficiency of four AFs. (A) Extraction efficiency of CH_2Cl_2, $CHCl_3$, and CCl_4 in 5 μL. (B) Effect of volume of $CHCl_3$ on extraction efficiency. (C) Effect of concentration of NaCl on extraction efficiency. Error bar represents ± SD, n = 3.

Therefore, 10 μL of $CHCl_3$ was used in subsequent experiments.

Effects of Ionic Strength and pH. Salting-out involves the addition of electrolytes to an aqueous phase to increase the distribution ratio of a particular solute. Accordingly, the effect of salt on extraction efficiency was investigated by adding different amounts of NaCl (0%, 1%, 3%, 5%, 7%, 10%, 15%, and 20%, w/v) into the spiked sample solution (0.10 μg/L of the four AFs). As illustrated in Figure 2C, by increasing the concentration of NaCl over the range of 0%−10% w/v, the recovery increased and then remained almost constant. The observed trend can be explained by the decreased solubility of analytes in the sample solution due to the salting-out effect. Therefore, further experiments were carried out in the presence of 10% w/v NaCl.

Generally, the pH value of a sample can influence the ratio of ionic to molecular forms of the analytes. To increase the extraction efficiency of AFs, a neutral environment is necessary and the optimum pH of the sample solutions should be between 5 and 7.[47−49] In this study, the pH values of the aqueous samples were measured as 6.7−7.1, so there was no need for pH adjustment.

Effect of Extraction Time. In the process of inkjet-DLLME, the extraction solvent is ejected in the form of fine droplets driven by the jetting device into the sample solution during dozens of seconds. According to the experimental results under the selected conditions, the droplets of extraction solvent caused significant turbulence in the sample solution when the inkjet frequency was greater than 5000 Hz. As a result, the mixture formed a dynamic cloudy solution (see Supporting Information video 1). The transition of the AFs from the aqueous phase to the extraction solvent is therefore fast. Subsequently, equilibrium conditions are achieved quickly after injection of the extraction solvent into the sample solution.

Effect of Jetting Device Parameters. In this method, the jetting device produced uniform and controllable fine droplets of extraction solvent and formed a dynamic cloudy solution in the sample solution. The size and frequency of the extraction droplets generated by the jetting device (which controls the amount of extraction solvent in unit time) were therefore the crucial parameters to be investigated.

The jetting device used in this work is essentially a drop-on-demand piezoelectric inkjet. The size of the extraction droplet can be adjusted by controlling the pulse waveform, drive voltage, line pressure, and orifice size of the jetting device. Droplets of $CHCl_3$ having different diameters (20, 40, 60, and 80 μm) in the sample solution are shown in Figure 3, and the parameters of the jetting device are shown in Table S2. To avoid the optical distortion caused by a conical tube, a square quartz capillary tube (600 μm × 600 μm, i.d., Chengteng Equipment Corporation, Beijing, China) was used as the vessel for observation.

The relationship between volume of $CHCl_3$ and the duration of jetting at different droplet diameters was obtained using a weighing method at room temperature (25 °C), as shown in Figure 4A. The graph was linear, with correlation coefficient (R^2) of 0.9994, 0.9995, 0.9997, and 0.9998, for droplet diameters of 20, 40, 60, and 80 μm, respectively. Good linearity indicated that the size of the droplets was controllable.

The extraction efficiency of $CHCl_3$ (10 μL) at different droplet diameters (20, 40, 60, and 80 μm) for the spiked sample (0.10 μg/L of the four AFs) was investigated. The results, shown in Figure 4B, indicated that the extraction efficiency at 20 μm was best. The number of droplets (theoretical value) at different diameters (20, 40, 60, and 80 μm) generated via the jetting device can be reached over 1.8×10^6, 3.0×10^5, 8.7×10^4, and 3.7×10^4, respectively. Among

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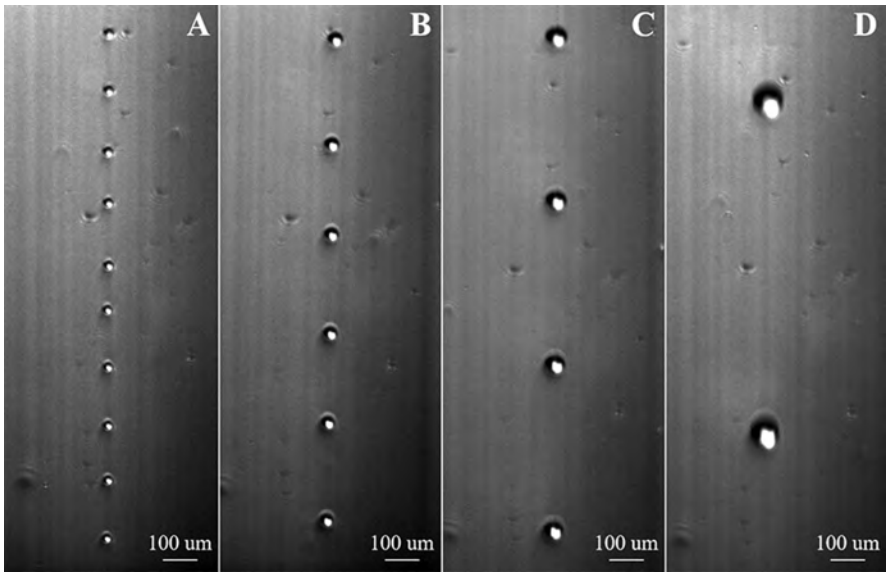

Figure 3. Droplets of $CHCl_3$ at different diameters in the sample solution: (A) droplet diameter at ~20 μm, (B) droplet diameter at ~40 μm, (C) droplet diameter at ~60 μm, and (D) droplet diameter at ~80 μm. All droplets were generated at a frequency of 1 Hz.

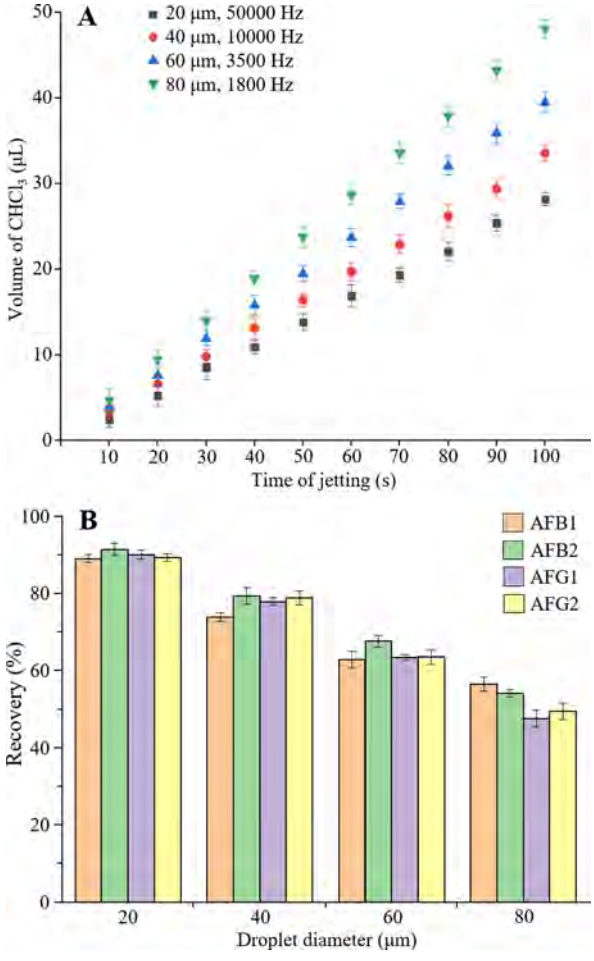

Figure 4. Effect of droplet size at different diameters on extraction efficiency of four AFs: (A) relationship between the volume of $CHCl_3$ and the jetting time and (B) extraction efficiency of the $CHCl_3$ (10 μL) at different droplet diameters for the spiked sample. Error bar represents ± SD, n = 3.

them, the droplets with a diameter of 20 μm showed a larger surface area and more sufficient contact with the sample solution, thus achieving a relatively high recovery efficiency. The jetting times (frequency) for each droplet diameter were 36.5 s (50 000 Hz), 30.5 s (10 000 Hz), 17.5 s (5 000 Hz), and 7.5 s (5 000 Hz), respectively.

Analytical Performance. A series of standard mixture solutions of four AFs were added to blank sample at different concentration levels, extracted by inkjet-DLLME and analyzed by UHPLC−MS/MS under the optimized conditions. The MRM chromatograms of the four AFs from the UHPLC−MS/MS are shown in Figure 5, corresponding to the blank wheat sample matrix spiked with the four AFs at 0.10 μg/L. The established method was found to be highly specific, since no interfering peaks were observed in the blank wheat sample at the retention times of the analytes.

As shown by the results in Table 1, the method was linear over the ranges of 0.06−6.0, 0.06−6.0, 0.12−6.0, and 0.18−1.0 μg/kg for AFB1, AFB2, AFG1, and AFG2, respectively, with a correlation coefficient (R^2) between 0.9976 and 0.9993. The limit of detection (LOD) and limit of quantification (LOQ) were defined as the concentrations of AFs, which gave signal-to-noise ratios of 3:1 and 10:1, respectively. The observed LODs and LOQs were 0.018−0.06 and 0.06−0.18 μg/kg, respectively. The reproducibility of the proposed method was assessed as the intra- and interday precisions of blank samples spiked with AFs at 0.10 μg/L. The intraday precisions were determined using five parallel spiked samples within 1 day, and the interday precisions were determined using four parallel spiked samples over 4 consecutive days. The observed relative standard deviations (RSDs) were in the range of 2.1−3.1% for intraday precision (n = 5) and 2.6−3.7% for interday precision (n = 4), which indicated that the method had excellent precision and could be used for routine analysis of AFs in wheat.

Effect of Matrix. To assess the matrix effect, a blank wheat sample solution and blank solvent (MeCN/water, 7%, v/v) were spiked at 0.05, 0.1, and 0.5 μg/L of each AF standard and subjected to the procedure. The relative recoveries, calculated from the ratio of the peak areas of the AFs in the spiked sample

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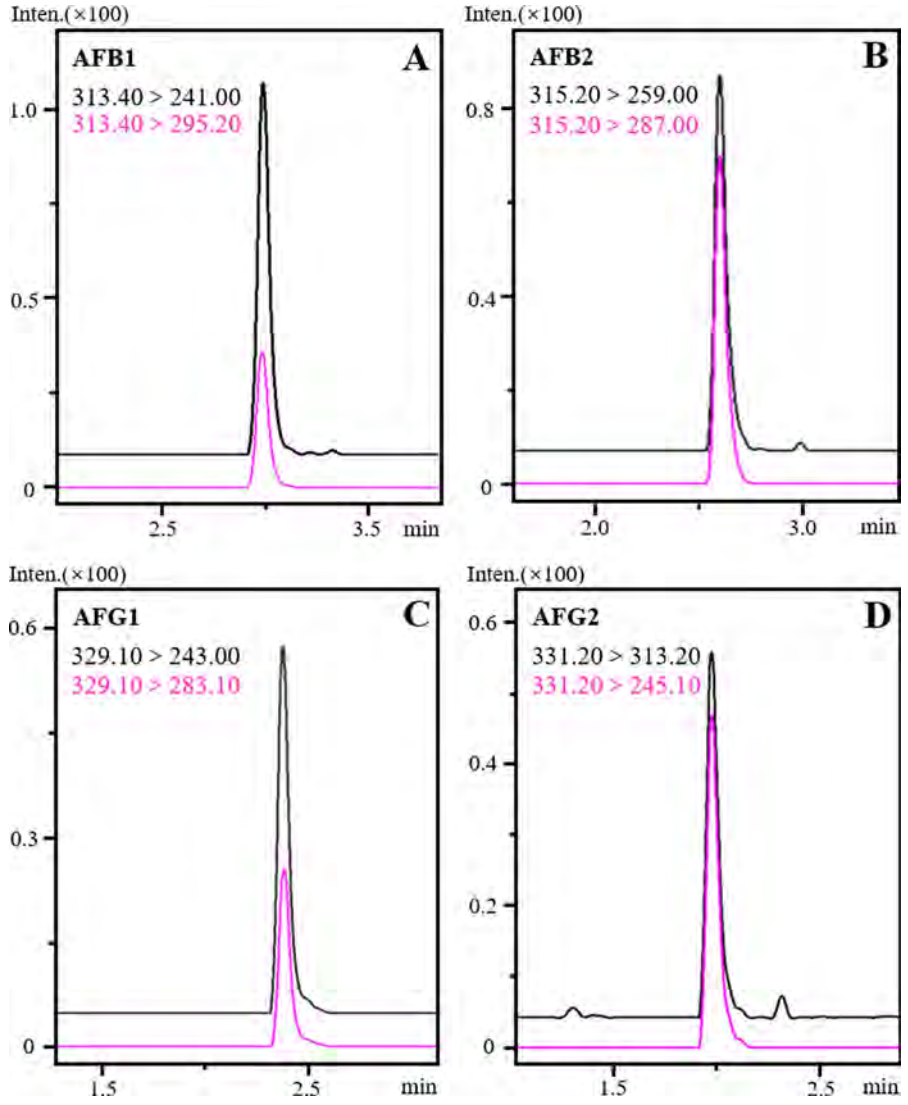

Figure 5. MRM chromatograms of AFs in spiked wheat sample after extraction by inkjet-DLLME: (A) AFB1 (spiked at 0.10 μg/L), (B) AFB2 (spiked at 0.10 μg/L), (C) AFG1 (spiked at 0.10 μg/L), and (D) AFG2 (spiked at 0.10 μg/L).

Table 1. Linearity, Limit of Detection, Relative Recovery, and Precision of the Inkjet-DLLME Method Combined with UHPLC−MS/MS

					relative recovery (%) ± RSD, (%, n = 9)			precision (RSD, %)	
analyte	linear range (μg/kg)	R^2	LOD (μg/kg)	LOQ (μg/kg)	spiked 0.05 μg/L	spiked 0.1 μg/L	spiked 0.5 μg/L	intraday (n = 5)	interday (n = 4)
AFB1	0.06−6.0	0.9993	0.018	0.06	83.2 ± 3.2	87.4 ± 4.1	90.2 ± 4.1	2.7	3.3
AFB2	0.06−6.0	0.9991	0.018	0.06	84.6 ± 4.3	87.9 ± 4.6	93.0 ± 4.1	3.1	3.7
AFG1	0.12−6.0	0.9987	0.036	0.12	86.2 ± 3.3	87.7 ± 2.3	93.0 ± 2.2	2.1	2.6
AFG2	0.18−6.0	0.9976	0.06	0.18	84.1 ± 2.7	90.6 ± 3.2	92.8 ± 2.5	2.2	2.8

extract and spiked solvent extract at the same concentrations, are shown in Table 1. The relative recoveries were 83.2% to 93.0% with RSDs < 4.6% (n = 9), indicating an acceptable matrix effect and the applicability of the proposed method to trace-level analysis of AFs in wheat samples.

Analysis of AFs in Wheat Samples. The optimized method was applied to the analysis of wheat for human consumption. A total of 20 wheat samples were collected that had been stored for 0−36 months after harvesting. Table 2 shows that the amount of AFs in wheat samples increased as the storage time increased. Special attention should be paid to AFs above the maximum levels in cereal set by the EU regulations that were detected in wheat samples. This indicated that wheat stored for longer than 12 months without special storage conditions had potential food safety hazards. Furthermore, AFs were also found in wheat harvested in the same year, confirming previous reports that aflatoxin may be produced after contamination by molds during growth of the crop.

Comparison with Conventional Methods. The proposed inkjet-DLLME method was compared to methods based on conventional DLLME for preconcentration of AFs in

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Table 2. Analysis of Four AFs in Wheat Samples[a]

		analyte (μg/kg)				
SN[b]	ST[c]	AFB1	AFB2	AFG1	AFG2	amount
1	36	2.875	2.112	1.010	1.731	7.728
2		1.660	1.562	1.602	0.653	5.477
3		1.447	0.237	<LOQ	<LOQ	1.684
4		0.499	0.192	0.676	0.310	1.677
5		0.313	0.179	N.D	0.227	0.719
6	24	2.631	0.915	0.673	0.463	4.682
7		1.203	1.013	0.397	0.463	3.076
8		1.112	0.414	<LOQ	N.D.	1.526
9		0.516	0.165	<LOQ	0.185	0.866
10		0.674	0.071	N.D.	N.D.	0.475
11	12	1.125	0.625	0.972	0.882	3.604
12		0.744	0.666	<LOQ	0.334	1.744
13		0.247	<LOQ	<LOQ	N.D.	0.247
14		0.109	0.095	<LOQ	<LOQ	0.204
15		<LOQ	N.D.	<LOQ	<LOQ	N.D.
16	0	0.671	0.190	0.452	<LOQ	1.313
17		0.091	N.D.	<LOQ	<LOQ	0.091
18		<LOQ	N.D.	N.D.	N.D.	N.D.
19		<LOQ	N.D.	N.D.	N.D.	N.D.
20		N.D.	N.D.	N.D.	N.D.	N.D.

[a]<LOQ, below of linear range; N.D., not detected. [b]Sample number. [c]Storage time (month).

various samples before analysis by HPLC. As summarized in Table 3, the inkjet-DLLME coupled to the UHPLC−MS/MS method is superior in terms of analytical performance, precision, and solvent consumption. Moreover, there is no requirement for additional mixing in this method, significantly simplifying operation compared with conventional DLLME.

CONCLUSION

In this study, a novel method was developed for the automatic formation of a cloudy solution in DLLME by injecting the extraction solvent as ultrafine droplets into the sample solution via a drop-on-demand jetting device. Building on the advantages of traditional DLLME, the major improvements of the proposed inkjet-DLLME method were convenience, reliability, and low solvent consumption. The evaluation data indicated satisfactory analytical performance of the proposed inkjet-DLLME method coupled with UHPLC−MS/MS for quantitative determination of AFs at trace levels in wheat. The presented method represents a new direction for development of traditional DLLME technology.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b05344.

Mass spectrometry MRM parameters of four AFs and parameters of the jetting device for generation the droplets with different diameters; (PDF)

Video of the formation of a cloudy solution when the inkjet frequency was greater than 5000 Hz (AVI)

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[§]M.S. and D.X. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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Table 3. Comparison of Inkjet-DLLME with Other DLLME for the Determination of AFs

ES[a]	ES[b] (vol, μL)	DS[c] (vol, μL)	LOD (ppb)	LOQ (ppb)	R[d] (%)	RSD (%)	AM[e]	ref
MeCN/water	$CHCl_3$ (620)	MeCN (950)	0.45−0.57	1.50−1.91	85−98	<9.8	UHPLC−MS/MS	50
not used	CH_2Cl_2 (800)	MeCN (866)	0.05−0.50	0.10−1.00	84−111	<20	HPLC−MS/MS	51
MeOH/water	$CHCl_3$ (220)	MeOH (800)	0.01−0.17	0.04−0.57	67−92	<18	HPLC−FLD	52
MeOH/water	$CHCl_3$ (1000)	MeCN (1000)	0.03−0.07	0.10−0.27	90−112	<11.9	HPLC−FLD	53
MeCN/water	$CHCl_3$ (10)	MeCN[f] (14)	0.018−0.06	0.06−0.18	83−93	<4.6	UHPLC−MS/MS	this work

[a]Extraction solution in pretreatment. [b]Extraction solvent in DLLME. [c]Dispersive solvent. [d]Recovery. [e]Analytical method; HPLC−FLD, high-pressure liquid chromatography with fluorescence detection. [f]MeCN from sample solution.

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Anal. Chem. 2019, 91, 3027−3034

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