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Microfluidic chip-based nano-liquid chromatography tandem mass spectrometry for quantification of aflatoxins in peanut products

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ABSTRACT

Aflatoxins (AFs), a group of mycotoxins, are generally produced by fungi *Aspergillus* species. The naturally occurring AFs including AFB1, AFB2, AFG1, and AFG2 have been clarified as group 1 human carcinogen by International Agency for Research on Cancer. Developing a sensitive analytical method has become an important issue to accurately quantify trace amount of AFs in foodstuffs. In this study, we employed a microfluidic chip-based nano LC (chip-nanoLC) coupled to triple quadrupole mass spectrometer (QqQ-MS) system for the quantitative determination of AFs in peanuts and related products. Gradient elution and multiple reaction monitoring were utilized for chromatographic separation and MS measurements. Solvent extraction followed by immunoaffinity solid-phase extraction was employed to isolate analytes and reduce matrix effect from sample prior to chip-nanoLC/QqQ-MS analysis. Good recoveries were found to be in the range of 90.8%–100.4%. The linear range was 0.048–16 ng g^{-1} for AFB1, AFB2, AFG1, AFG2 and AFM1. Limits of detection were estimated as 0.004–0.008 ng g^{-1}. Good intra-day/inter-day precision (2.3%–9.5%/2.3%–6.6%) and accuracy (96.1%–105.7%/95.5%–104.9%) were obtained. The applicability of this newly developed chip-nanoLC/QqQ-MS method was demonstrated by determining the AFs in various peanut products purchased from local markets.

1. Introduction

Aflatoxins (AFs), a group of mycotoxins, are generally produced by fungi *Aspergillus* species and were reported to be hepatocellular carcinoma risk factors when aflatoxin contamination occurred in foodstuffs [1–3]. The naturally occurring AFs (including AFB1, AFB2, AFG1, and AFG2) have been classified as group 1 human carcinogens by WHO (world health organization) International Agency for Research on Cancer (IARC) since 1993 [4]. AFM1 and AMF2 have been known as the hydroxylated metabolites of AFB1 and AFB2, respectively. The occurrence of AFs has been reported in animal feed [5], food products including peanuts [6] and milk [7], as well as traditional Chinese medicines [8]. Due to the high toxicity and widespread occurrence of AFs, the action levels or maximum permitted levels of individual and/or total AFs in various commodities and foodstuffs have been set in many countries for food safety as well as general public health concerns. For example, US Food and Drug Administration (FDA) has established the action levels of 20 ng g^{-1} for total AFs in peanuts and peanut products as well as 0.5 ng g^{-1} for AFM1 in milk [9]. In Europe, the European Commission set the maximum permitted levels at 2 ng g^{-1} and 4 ng g^{-1} (five times lower than the limits set by US FDA) for AFB1 and total AFs, respectively, in foodstuffs intended for direct human consumption [10]. According to the regulations set by Taiwan Department of Health (DOH), the maximum permitted level for total AFs (AFB1, AFB2, AFG1, and AFG2) was 15 ng g^{-1} in peanut products. Therefore, developing a sensitive analytical method has become an important task to accurately quantify AFs in food products.

In order to enhance sensitivity for quantification, sample pretreatment was usually required to remove complex matrix components prior to determining trace amount of AFs in samples. Solvent extraction [11], liquid–liquid extraction (LLE) [12], solid-phase extraction (SPE) [6,13], and immunoaffinity SPE [14,15] have been utilized for sample pretreatment prior to quantitative determination of AFs in various sample matrixes. A number of analytical methods, including immunoassay and chromatography-based methods, have been developed for screening and/or quantitative determination of AFs in different sample matrixes [16–18]. Although immunoassay methods offered rapid screening for the occurrence of AFs in samples, the lack of simultaneous determination of individual AFs urged the need to develop a sensitive analytical method for simultaneous determination.

Liquid chromatography (LC) techniques have been extensively applied for simultaneous determination of AFs with a variety of detection methods including UV [19], fluorescence [14], and mass

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spectrometry (MS) [6,20,21]. Li et al. [14] employed solvent extraction and immunoaffinity SPE for removing matrix components from samples prior to chromatographic separation with fluorescence detection (FLD). This LC–FLD method offered a LOD (limit of detection) of 0.15 ng g^{-1} for determining AFs in peanut butter and sesame paste. Ren's group [6] applied a home-made mixed SPE cartridge, which contained silica gel, neutral alumina, and kieselguhr, for isolating AFs from complex sample matrix. The extracted analytes were further separated by ultra-high-performance liquid chromatography (UHPLC) coupled to tandem MS for simultaneous determination. The authors reported the LOD values of target AFs ranging from 0.09 ng g^{-1} to 0.21 ng g^{-1} for quantifying AFs in peanuts and peanut butter.

Due to the enhanced sensitivity, microfluidic chip-based devices have been widely utilized in the field of bioanalysis. A recent review [22] discussed several commercial chip-based LC-MS systems employed for the determination of small molecules in bioanalytical applications. Among these, a fully integrated microfluidic chip-nanoLC interface (chip-nanoLC) provided the advantages of reducing matrix interference and online sample pre-concentration by using a 2-column design on the chip [23,24]. Chip-nanoLC MS system has been utilized for analyzing proteins and peptides in proteomic research [25,26], oligosaccharides and N-linked glycans in glycomics [27,28]. Our group successfully applied chip-nanoLC-tadem MS to determine drug metabolites and biomarkers in clinical human urine for clinical diagnosis [29,30]. The sensitivities of chip-nanoLC-tandem MS were enhanced by 3–10 folds compared to previous reported LC methods. In addition, chip-nanoLC MS system was also used to determine various drugs in serum for pharmaceutical application [31]. Good agreement between the results from routine LC-MS and chip-nanoLC MS was obtained. However, no application has been done for quantifying toxins such as AFs in agricultural products for food safety.

In this study, we employed a chip-nanoLC/triple quadrupole MS (chip-nanoLC/QqQ-MS) system to quantitatively determine AFs (AFB1, AFB2, AFG1, AFG2 and AFM1) in peanut powder, peanut butter, and peanut samples. The occurrence of AFB1, AFB2, AFG1, and AFG2 were commonly reported in various sample matrixes [5–8,32]. AFM1 has been mostly detected in mil or dairy products [7,33,34]. Only a few publications reported the occurrence of AFM1 in some peanut products [6,32]. To our knowledge, there was only one report for the determination of AFM2 in peanut products [6]. The authors utilized a home-made mixed cartridge for sample pretreatment. Therefore, five AFs were determined in this study. The optimal conditions for MS detection, chip-nanoLC analysis, and sample pretreatment procedures were investigated prior to quantitative evaluation. The quantitative features in terms of linearity, method precision and accuracy, LOD, and limit of quantitation (LOQ) were validated under the optimal conditions for the proposed method. The applicability of this newly developed chip-nanoLC/QqQ-MS method was also demonstrated by quantifying the AFs in peanut butter, peanut powder, and peanut samples purchased from local markets. To our knowledge, this is the first investigation using chip-based LC-MS to determine toxins for food safety application.

2. Experimental

2.1. Materials and reagents

Peanut products (peanuts, peanut butter, and peanut powders) were purchased from various local markets in Taipei city, Taiwan. Peanut powder and peanut butter samples were used directly, while peanuts were ground into powders prior to extraction. HPLC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from Mallinckrodt Baker, Inc. (Paris, KY, USA). Deionized water was obtained from a Milli-Q Integral 5 water purification system (Millipore, Bedford, MA, USA). AF standards (AFB1, AFB2, AFG1, AFG2, and AFM1) were purchased from Fermentek (Jerusalem, Israel). AFB2-$^{13}C_{17}$, as internal standard (ISTD), ammonium acetate (NH_4OAc), and NaCl were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of the ISTD (500 ng mL^{-1}) and AFs (1000 μg mL^{-1} each) were prepared individually in MeOH and stored at −20 °C. A mixture of 5 AFs (1000 ng mL^{-1} each) was prepared in MeOH as the working solution. Each calibration standard containing five AFs was prepared by spiking the working solution into peanut extract at a desired concentration. All calibration standards contained 0.032 ng g^{-1} of ISTD. Vicam AflaTest immunoaffinity cartridges from Waters (Milford, MA, USA) were applied for selective SPE of AFs.

2.2. Instrumentation and conditions

Agilent 1100 capillary HPLC pump and online degasser were utilized for sample loading. An Agilent 1200 nano-HPLC system, containing a quaternary pump and an online degasser, was used to provide nano-flow mobile phase for chromatographic separation. An Agilent G1377A micro well-plate auto-sampler was used for sample injection. Two Agilent nanoLC-chips, consisting of SB-C18 or SB-C8 Zorbax 300A stationary phase (5 μm), were examined for this study. Both chips contained a 500 nL enrichment column and an analytical column (150 mm × 75 μm). SB-C8 chip was then chosen for optimization, method validation and sample analysis.

Under the enrichment mode [29,30], the capillary pump was used for sample loading (injection volume of 8 μL) and pre-concentrating the target analytes on the enrichment column. $MeOH_{(aq)}$ (2%, v/v) was used as loading solvent at a flow rate of 4 μL min^{-1}. In addition, 10 μL of the loading solvent was applied to remove un-retained matrix components from the system. The μ-valve was then switched to the analysis mode [29,30]. Once the μ-valve was switched to the analysis mode, 90% $MeOH_{(aq)}$ delivered by the capillary pump was applied for 5 min to wash the loading flow path; afterward, 2% $MeOH_{(aq)}$ was employed to equilibrate until next run. A nano-LC gradient elution was utilized for the chromatographic separation of AFs. The flow rate was set at 300 nL min^{-1}. The mobile phases used for nano-LC separation included (A) 0.1 mM $NH_4OAc_{(aq)}$ and (B) ACN:MeOH (25:75, v/v) containing 1 mM NH_4OAc. Starting from 40% B, the solvent was increased to 50% B in 2 min and then increased to 80% B in another 3 min followed by an immediate change to 90% B for additional 5 min. Afterward, the μ-valve was switched back to the enrichment mode and the separation column was equilibrated for 15 min using 40% B.

MS experiments were performed using Agilent 6410 series Triple Quad LC/MS mass spectrometer (Agilent, Waldbronn, Germany). The nanospray source on chip- nanoLC was operated under the positive mode with the spray voltage set at 1950 V. A heated gas mixture (N_2: air/3:7; 4 L min^{-1} at 325 °C) was introduced to evaporate solvent from the ionization chamber and obtain stable responses. System control, data acquisition, and data processing were carried out using Agilent Mass Hunter Workstation (version B.03.01). Multiple reaction monitoring (MRM) was performed to quantitatively determine AFs.

2.3. Extraction procedures

A 2.5 g of sample (peanut powder, peanut butter, or ground peanut) spiked with ISTD (0.032 ng g^{-1}) was mixed with 0.5 g NaCl and 10 mL of 60% $MeOH_{(ag)}$ in a polypropylene tube. The mixed sample was then homogenized at 15,000 rpm for 2 min

utilizing a Polytron PT2100 homogenizer (Kinematica; Lucerne, Switzerland). The homogenized sample was centrifuged for 15 min at 15,000 rpm using a Hitachi CF15RXII high-speed micro-centrifuge with a T15A42 rotor (Tokyo, Japan). Five mL of supernatant was transferred to a new polypropylene tube and diluted with 15 mL Milli-Q water before passing through a 1.6 μm Whatman glass microfiber filter paper (GE Healthcare; Buckinghamshire, UK). The filtered solution (20 mL) was applied to a pre-conditioned immunoaffinity cartridge (IAC) by 1 mL of Milli-Q water at 1–2 drops/s. After sample loading, the cartridge was washed with Milli-Q water twice (10 mL each) and the elution was carried out using 1 mL MeOH at 1–2 drops/s. The extracted analytes were dried under N_2 gas, reconstituted in 2 mL of 10% $MeOH_{(ag)}$ and centrifuged at 10,000 rpm for 5 min. Then 8 μL of the sample solution was auto-injected into the chip-nanoLC/QqQ-MS system for determination. The extraction recovery (R_e) was calculated using the following equation: $R_e = A_s/A_{w,0} \times 100\%$, where A_s = peak area of AFs-spiked peanut sample after extraction and $A_{w,0}$ = peak area of AFs-spiked MeOH–water (10:90) solution without extraction.

2.4. Method validation process

The following experiments were performed to validate the proposed method for quantitative determination of AFs. Seven different AFs-spiked ground peanut samples (0.048, 0.08, 0.16, 0.80, 1.60, 8.00, 16.00 ng g^{-1}, each contained 0.032 ng g^{-1} of ISTD) were prepared and used as matrix-matched calibration standards. A calibration curve of peak area ratio (AF/ISTD) versus AF concentration was constructed for each target AF to evaluate the linearity of the proposed method. The LOD was defined as the signal-to-noise ratio of 3. The precision and accuracy, including intra-day and inter-day experiments, were performed and evaluated using three different concentrations (0.08, 0.80, and 8.00 ng g^{-1}, each contained 0.032 ng g^{-1} of ISTD) of AFs-spiked ground peanut samples. The precisions were estimated by calculating the relative standard deviation (RSD) of measurements.

3. Results and discussion

3.1. Optimization of mass spectrometry detection and chip-nanoLC conditions

Under the positive mode, the $[M+H]^+$ ion was obtained as the base ion for target AFs and ISTD using the experimental conditions mentioned in Section 2.2 and Table 1. The MRM parameters and characteristic transitions of each analyte and ISTD were summarized in Table 1.

Chromatographic separation of AFs on chip-nanoLC analysis was first investigated using two Agilent nanoLC-chips, consisting of either Zorbax SB-C18 or SB-C8 stationary phase and the results were shown in Fig. 1. While ACN was used as the organic solvent for chip-nanoLC analysis, the SB-C8 chip (Fig. 1B) exhibited faster separation with better resolution compared with the SB-C18 chip (Fig. 1A). Although the resolution was improved (as shown in Fig. 1C) when MeOH was used as the organic solvent for chip-nanoLC analysis, the peaks became much broader compared to the results shown in Fig. 1B. In order to improve resolution and minimize peak broadening of chromatographic separation, various compositions of ACN–MeOH mixtures were examined as organic mobile phase for nanoLC separation. According to the experimental results, the best chromatographic separation of AFs was achieved when ACN–MeOH (25:75, v/v) was applied. However, the variations of the MS signals were higher than 20%. In order to obtain more stable measurements, various concentrations of

Table 1
MRM parameters for QqQ-MS under positive mode.

	Transition	Fragmentor (V)	Collision energy (V)
AFB1	313→241	181	44
	→269		36
	→285[a]		24
AFB2	315→243	186	44
	→259[a]		32
	→287		28
AFG1	329→200	164	44
	→243[a]		28
	→311		20
AFG2	331→189	154	44
	→245		32
	→313[a]		24
AFM1	329→259	171	24
	→273[a]		24
	→301		16
AFB2-$^{13}C_{17}$ (ISTD)	332→257	134	44
	→273		32
	→303[a]		28

[a] Quantitative ion.

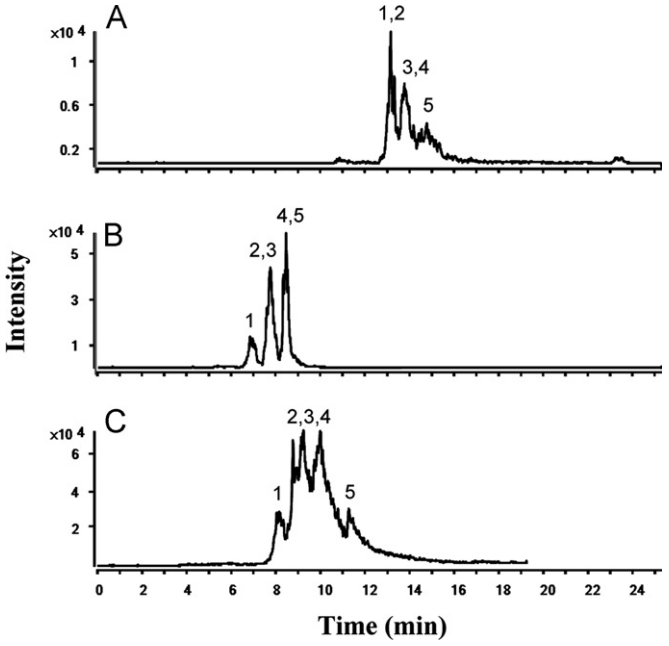

Fig. 1. Results of chip-nanoLC/QqQ-MS analysis for AFs with (A) Zorbax 300A SB-C18 chip, (B) Zorbax 300A SB-C8 chip, and (C) Zorbax 300A SB-C8 chip using the following conditions: for (A) and (B), Milli-Q water and ACN were used as gradient mobile phases; for (C), Milli-Q water and MeOH were used as gradient mobile phases. The gradient for separating AFs was described in Section 2.2. Peak identification: (1) AFM1; (2) AFG2; (3) AFG1; (4) AFB2 and (5) AFB1.

NH_4OAc (0, 0.5, 1.0, 2.0 mM) were added into the two mobile phase solutions (A and B) delivered by nano pump. The results are shown in Fig. 2. Compared with the results obtained without adding NH_4OAc, about 5% to 10% decrease on MS signals were observed when 0.5 mM and 1.0 mM of NH_4OAc were added into the mobile phase, respectively. More than 25% decrease on MS signals was observed while the concentrations of NH_4OAc reached 2.0 mM. Although approximately 10% decrease on MS responses was detected, the mobile phase containing 1.0 mM NH_4OAc provided the smallest MS signal variations. Therefore, solutions containing 1.0 mM NH_4OAc were used as the mobile phases of chip-nanoLC/QqQ-MS analysis.

In typical chip-nanoLC analysis [29,30], the analytes were retained and concentrated in the enrichment column under the

enrichment mode. Additional mobile phase delivered by capillary pump was then applied to remove un-retained components from the system. The enrichment column provides the functions of analayte pre-concentration and matrix reduction. Afterward, the μ-valve was switched to the analysis mode for chip-nanoLC separation. Meanwhile, a gradient program (summarized in Table 1) for capillary flow was employed to wash the loading flow path and minimize carry-over effect. After complete nanoLC elution of analytes, the μ-switch valve was switched back to the enrichment mode for equilibrating the enrichment column by capillary flow till the next run. Therefore, further investigations on chromatographic separation of AFs were carried out using the gradient elution. Adequate separation of AFs, as shown in Fig. 3A, was achieved by using the gradient program summarized in Table 1.

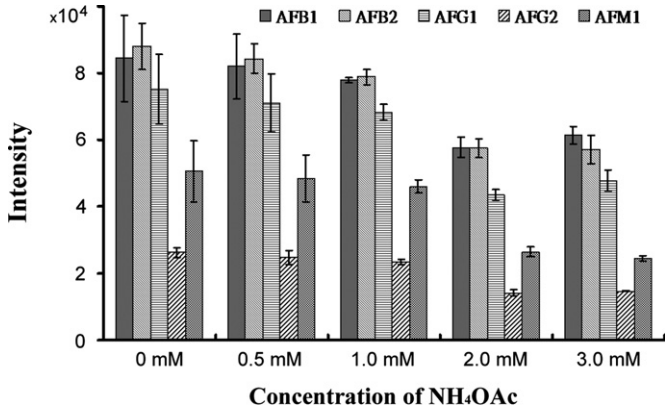

Fig. 2. Effect of salt addition on chip-nanoLC/QqQ-MS analysis. Experimental conditions are described in Section 2.2.

3.2. Sample pretreatment

The proper MeOH content in the loading solution for immunoaffinity SPE was first investigated. In this study, AFs-spiked (1 ng mL^{-1} each) MeOH–water solutions at various MeOH contents (10, 15, 20%; v/v) were used as loading samples for immunoaffinity SPE and the extraction recoveries were determined. The results showed the extraction recoveries gradually decreased as the MeOH content increased from 10% to 20%. The highest extraction recovery of SPE was found when AFs was spiked in 10% $MeOH_{(aq)}$. AFs-spiked ground peanut samples were then utilized to examine the effectiveness of solvent extraction/SPE sample pretreatment procedure prior to chip-nanoLC/QqQ-MS

Table 2
Extraction recovery[a] of AF-spiked ground peanut samples.

	Spiked concentration (ng g^{-1})		
	0.08	0.80	8.00
AFB1	95.9 ± 3.9	98.7 ± 1.2	92.9 ± 8.1
AFB2	93.4 ± 3.4	94.6 ± 5.2	96.4 ± 6.3
AFG1	91.2 ± 2.9	92.0 ± 2.6	90.9 ± 6.8
AFG2	91.5 ± 9.3	93.5 ± 2.2	94.0 ± 4.0
AFM1	90.8 ± 8.8	92.2 ± 4.2	100.4 ± 5.3

[a] The recovery (%) was expressed as "mean ± SD" (n=3).

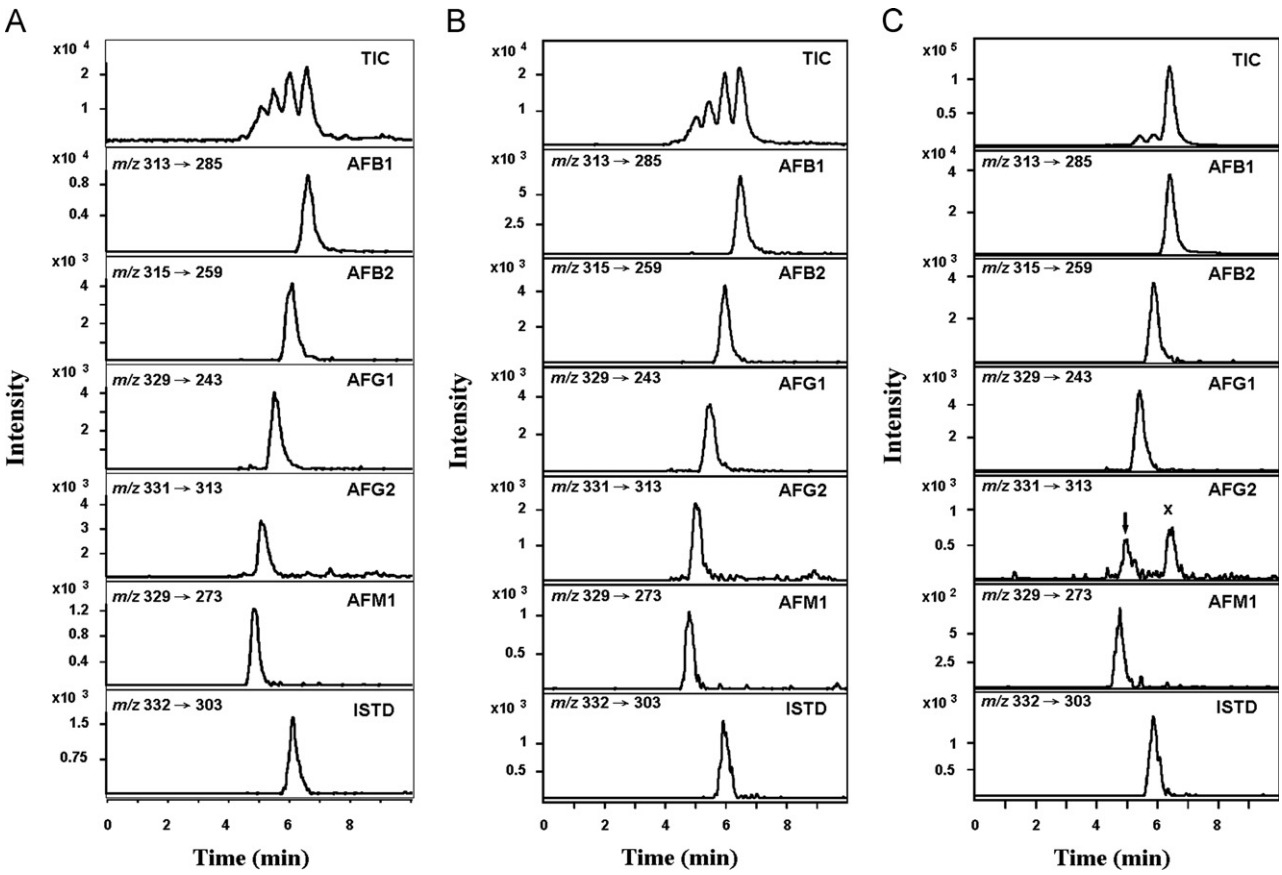

Fig. 3. Representative results of chip-nanoLC/QqQ-MS analysis for (A) AFs standard (0.1 ng mL^{-1} each, ISTD: 0.032 ng g^{-1}) in 10% MeOH–water solution, (B) AFs-spiked (0.16 ng g^{-1} each, ISTD: 0.032 ng g^{-1}) ground peanut extract, and (C) peanut butter extract (B-5 in Table 5; containing 0.032 ng g^{-1} of ISTD) using Zorbax 300A SB-C8 chip. Experimental conditions are described in Section 2.2 for chip-nanoLC/QqQ-MS and Section 2.3 for sample preparation.

analysis. However, noticeable ion-suppression effect, about 20% decrease in MS signals, was detected when the solvent extracted sample was diluted to 10% $MeOH_{(aq)}$ for SPE loading. It might be caused by the non-specific binding of matrix components onto the sorbents. Less than 10% decrease in MS signals was determined when the solution of ground peanut extract was diluted to 15% $MeOH_{(aq)}$. Therefore, the supernatant (60% MeOH/H_2O mixture) obtained from solvent extraction of ground peanut sample was diluted with Milli-Q water to contain 15% MeOH before loading into IAC. The extraction recovery was examined using ground peanut samples spiked with 5 AFs at 3 different concentration levels (0.08, 0.80, 8.00 ng g^{-1} each) from the working solution (1000 ng mL^{-1} each). As shown in Table 2, minute matrix effect was observed and good recoveries were found to be in the range of 90.8-100.4% using the proposed sample pre-treatment method.

3.3. Quantitative evaluation and method validation

Linearity, LOD, LOQ, as well as precision and accuracy were examined using AFs-spiked ground peanut samples under optimal conditions. For quantitative measurements, peak area ratio of analyte/ISTD was utilized. As shown in Table 3, good linearity was obtained in the range of 0.048–16.00 ng g^{-1} (each contained 0.032 ng g^{-1} of ISTD) with a regression coefficient ≥0.996 for each target AF. LOD, LOQ (the lowest concentration in the linear range), precisions and accuracy were also determined and summarized in Table 3. The intra-day and inter-day precisions were in the ranges of 2.3–9.5% and 2.3–6.6%, respectively. The ranges of intra-day and inter-day accuracy were 96.1–105.7% and 95.5–104.9%, respectively. Based on these results, the proposed chip-nanoLC/QqQ-MS method was proved to be suitable for quantitative determination of AFs for food safety application. The comparison of the proposed chip-nanoLC/QqQ-MS method with other LC-MS/MS techniques for simultaneous determination of AFs is summarized in Table 4. Cervino et al. [20] employed similar procedures (solvent extraction followed by immunoaffinity SPE) for sample pretreatment and reported LOD values of 0.09–0.51 ng g^{-1} utilizing conventional HPLC-MS/MS to determine AFs in food samples. In this study, the proposed chip-nanoLC/QqQ-MS method offered LOD values of 0.004–0.008 ng g^{-1}, which were about 10 times lower than the conventional HPLC-MS/MS method.

3.4. Application: occurrence of AFs in peanut powder, peanut butter, and peanut samples

The newly developed chip-nanoLC/QqQ-MS method was utilized to determine the occurrence of AFs in peanut powder, peanut butter, and peanut samples purchased from local markets. Fig. 3B and C shows the representative chromatograms of a AFs-spiked blank peanut sample and a peanut butter sample, respectively. In this peanut butter sample, all five AFs were identified and quantified based on the three MRM transitions of individual analytes shown in Table 1. An unidentified peak (marked with "X") shown in the extracted ion chromatogram of transition 331→313; however, it was not detected on the other two characteristic transitions of AFG2. Table 5 summarizes the identified AFs and the corresponding concentrations found in peanut products. No detectable AFs were found in all four peanut samples. AFB1 and AFB2 were the frequently found AF in peanut powder and peanut butter samples with the mean/median values of 3.969/2.632 ng g^{-1} and 0.943/0.956 ng g^{-1}, respectively. Nevertheless, the amount of AFs detected in these samples were all below the maximum permitted level or the action level set by either Taiwan DOH or US FDA.

4. Conclusions

In this study, the chip-nanoLC/QqQ-MS method showed good linearity in sub- to low ng g^{-1} range and offered good intra-day/

Table 3
Numerical results for the calibration, LOQ, LOD, intra-day/inter-day precision and accuracy of AFs in spiked ground peanut samples using the proposed chip-nanoLC/QqQ-MS method.

	Calibration curve[a]	Regression (R^2)	LOQ (ng g^{-1})	LOD (ng g^{-1})	Intra-day[b] ($n=5$)		Inter-day[b] ($n=5$)	
					Precision (RSD, %)	Accuracy (%)	Precision (RSD, %)	Accuracy (%)
AFB1	$y=30.97x+0.43$	0.996	0.048	0.004	4.4–4.0	97.6–105.6	2.9–5.4	99.0–104.9
AFB2	$y=18.41x+0.47$	0.999	0.048	0.006	2.3–9.5	98.8–105.1	2.8–6.5	98.7–103.8
AFG1	$y=18.79x+0.39$	0.999	0.048	0.007	3.1–4.8	98.4–102.6	2.3–6.3	98.5–103.5
AFG2	$y=12.84x+0.04$	0.997	0.048	0.007	4.1–6.3	96.1–105.7	3.8–5.8	95.5–102.6
AFM1	$y=4.06x+0.05$	0.996	0.048	0.008	3.9–8.0	97.5–103.5	2.7–6.6	96.5–102.2

[a] y: Peak area ratio (AF/ISTD), x: concentration (0.048, 0.08, 0.16, 0.80, 1.60, 8.00, 16.00 ng g^{-1} each; ISTD: 0.032 ng g^{-1}); $n=3$.
[b] The intra-day/inter-day precision and accuracy were determined at three different spiked concentration (0.08, 0.80, 8.00 ng g^{-1} each).

Table 4
Comparison of the proposed chip-nanoLC-MS/MS with other LC-MS/MS techniques for quantitative determination of aflatoxins.

	Chip-nanoLC/QqQ-MS	**UHPLC-MS/MS [6]**	**HPLC-MS/MS [13]**	**HPLC-MS/MS [20]**
Sample matrix	Peanut powder, peanuts, and peanut butter	Peanuts and peanut butter	Sweet pepper	Almonds, peanuts, peanut butter, etc.
Sample pretreatment[a]	Solvent extraction and immunoaffinity SPE	Solvent extraction and home-made mixed SPE (silica+alumina +kieselguhr)	Solvent extraction and SAX, NH_2-, and C_{18}-SPE in series[b]	Solvent extraction and immunoaffinity SPE
Linear range (ng g^{-1})	0.048–16	0.4–20	1–6	0.4–40
LOD (ng g^{-1})	0.004–0.008	0.09–0.21	0.8–1.4	0.09–0.51
Precision (RSD, %)	Intra-day: 2.3–9.5 Inter-day: 2.3–6.6	Intra-day: 0.8–9.1 Inter-day: 1.7–10.9	Intra-day: 10–16 Inter-day: 18–24	3.6–14
Recovery (%)	90.8–100.4	74.7–86.8	92–100	89–106

[a] The process performed prior to LC-MS/MS analysis.
[b] SAX: strong anion-exchange.

Table 5
Quantitative measurement of AFs in peanut products using the proposed chip-nanoLC/QqQ-MS method.

Sample[a]	Measured concentration[b], ng g^{-1}				
	AFB1	AFB2	AFG1	AFG2	AFM1
A-1	9.480 ± 0.080	2.011 ± 0.097	< LOQ	< LOD	1.232 ± 0.094
A-2	7.322 ± 0.673	1.574 ± 0.111	< LOD	< LOD	0.793 ± 0.031
B-1	2.632 ± 0.178	0.391 ± 0.021	0.068 ± 0.009	< LOQ	0.292 ± 0.027
B-2	7.122 ± 0.221	1.522 ± 0.033	< LOD	< LOD	0.815 ± 0.045
B-3	< LOD	< LOD	< LOD	< LOD	< LOD
B-4	0.072 ± 0.006	< LOD	< LOD	< LOD	< LOD
B-5	0.924 ± 0.030	0.108 ± 0.014	0.165 ± 0.018	< LOQ	0.091 ± 0.003
B-6	0.230 ± 0.006	0.051 ± 0.002	< LOD	< LOD	< LOD
C-1	< LOD	< LOD	< LOD	< LOD	< LOD
C-2	< LOD	< LOD	< LOD	< LOD	< LOD
C-3	< LOD	< LOD	< LOD	< LOD	< LOD
C-4	< LOD	< LOD	< LOD	< LOD	< LOD

[a] A: peanut powders; B: peanut butter; C: peanuts.
[b] The measured concentration was expressed as "mean ± SD" (n=3).

inter-day precision and accuracy for quantitative determination of AFs. Moreover, no obvious carry-over was observed using the proposed method for determining AFs in peanut products. To our knowledge, this is the first reported chip-nanoLC/tandem MS assay for determining toxins in food products. Based on these facts, the proposed chip-nanoLC/QqQ-MS method has been illustrated as a sensitive analytical technique for analyzing aflatoxins (B1, B2, G1, G2 and M1) in peanuts and peanut products.

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