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Interference-free analysis of aflatoxin B_1 and G_1 in various foodstuffs using trilinear component modeling of excitation-emission matrix fluorescence data enhanced through photochemical derivatization

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Abstract

A novel 'dilute-and-shoot' analytical strategy coupling self-weighted alternating normalized residue fitting (SWANRF) algorithm with two-dimensional fluorescence detection enhanced through photochemical derivatization (PD) was proposed in the present work for rapid, simultaneous and accurate quantitative analysis of aflatoxin B_1 and G_1 in various foodstuffs (including cereals, honey, and edible oil). By coupling the predominant second-order advantage of SWANRF algorithm with the ultra-sensitivity of fluorescence detection enhanced through off-line photochemical derivatization, the specific quantitative information of both analytes could be successfully extracted out from heavily interferential matrices without complicated multi-step purification and chromatographic separation procedure. Consequently, the whole analytical time and expense were significantly decreased, the accurate recoveries (with relative standard deviations, RSDs) (93.5±6.6%-102.8±4.0% for AFB_1, and 96.4±3.6%-107.2±6.0% for AFG_1) and extremely low limits of detection (LODs) (0.12-0.21 ng mL^{-1} for AFB_1, and 0.27-0.75 ng mL^{-1} for AFG_1) were obtained for analytical foodstuffs matrices. In addition, all quantitative results of this proposed strategy were carefully compared with standard IAC-LC-ESI^+-MS method for further confirmation, which proved that SWANRF-EEMs was excellent and promising as an alternative analytical strategy for routine analysis of multiplex aflatoxins, and theoretical basis for developing portable detecting device.

Keywords: Aflatoxins; Foodstuffs matrices; Second-order calibration; Excitation-

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emission matrix fluorescence; SWANRF algorithm

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1. Introduction

Aflatoxins are naturally occurring, small (usually MW≤400) and highly toxic mycotoxins produced as secondary metabolites by filamentous fungi (e.g. fungi A. spergillusflavus, A. parasiticus and A. nomius)[1]. At least fourteen different types of aflatoxins have been found occurring in various foodstuffs matrices (including cereals, honey, edible oil, even milk and egg). Hereinto, AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2 lead the most fatal harm to the health of human, especially liver and enteric canal. The former four aflatoxins mainly derive from vegetable sources (e.g. cereals and oilseeds), the latter two aflatoxins are mainly generated and enriched through a hydroxylation reaction to AFB_1 in animal liver, and then enter human body when intaking milk and eggs[2]. Even at extremely low concentration levels, they maybe contribute to the risk of liver cancer. Many studies have shown that the median lethal dose (LD50) of AFB_1 in rats is low to 0.48 $mg\,kg^{-1}$ [3, 4]. Many authoritative institutions, e.g. the Scientific Committee for Food have seriously warned that natural occurring aflatoxins are acutely carcinogenic, mutagenic, and immunosuppressive to almost all animal species, and the International Agency for Research on Cancer (IARC) has also classified aflatoxins into carcinogenic substances of group I (IARC, 2012).

For this reason and toxicity, all countries and international organizaitons have established a series of fairly strict limit standards via regulations and even legislations, e.g. the European Union in the Commission Regulation (EC, NO.1881/2006) has specified the maximum residue limits (MRLs) of aflatoxins in cereals: 2-8 $\mu g\,kg^{-1}$ for

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AFB_1, and 4-15 $\mu g\, kg^{-1}$ for total aflatoxins (AFT). In addition, the U.S. food and Drug Administration (FDA 225-96-2001) has also established the guideline criteria: 20 $\mu g\, kg^{-1}$ level for total aflatoxins in raw peanut, maize, nuts, etc., and 10 $\mu g\, kg^{-1}$ level in cooking oils. In China, the current national standard (GB2761-2005) has set the limit of AFB_1 at below 20 $\mu g\, kg^{-1}$ in corn, peanut and its by-products, and 10 $\mu g\, kg^{-1}$ in rice, cooking oil (except peanut oil).

For the purpose of the innovations of analytical strategies of aflatoxins, in recent years, many new and efficient detection methods have been developed and improved for the quantitative determination of aflatoxins, such as thin-layer chromatography (TIC)[5], enzyme-linked immunosorbent assay (ELISA)[6, 7], liquid chromatography hyphenated to mass spectrometry (LC-MS)[8, 9] or tandem mass spectrometry (LC-MS^2)[10-12], high performance liquid chromatography coupled with diode array detector (HPLC-DAD)[13-15] or fluorescence detector (HPLC-FLD)[16-20]. Among these available analytical methods, HPLC-FLD method is currently the most widely used due to its great versatility and high sensitivity for analysis of complex matrices (e.g. peanut, maize, edible oils). However, because of heavy solvent-quenching effect, the natural molecular fluorescence of AFB_1 and AFG_1 are extremely weak that cannot be directly analyzed, especially at extremely low concentration levels. In order to improve signal-to-noise ratio (SNR) of analytical method, fluorescence enhancement procedures that significantly prevent solvent-quenching effect on the natural molecular fluorescence of AFB_1 and AFG_1, e.g. pre-column derivatization with trifluoroacetic acid[21, 22] and post-column derivatization with bromine reagent (directly added or electrochemically

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produced) or iodine[23, 24], are required. Both of pre- and post-column derivatization could highly enhance the fluorescence, however, there are still several defects cannot be overcome, e.g. need of time-consuming purification, harmful reagents-consuming derivatization, N_2 blow drying process and heating condition (75 ℃), poor stability of derivative results. In recent studies, β-cyclodextrin as a new non-toxic and pollution-free reagent is often used as a fluorescence sensitization for increasing the sensitivity of methodology by blocking the external solvent-quenching effect by accommodating the molecules of aflatoxins into circular cavity and partitioned the surrounding solvent, but the enhanced fluorescence easily influenced by pH value and the solvent ratio[25, 26]. As an alternative apinoid derivative method, photochemical derivation (PD) technique characterized by a number of advantages including simplicity, linearity of response, reproducibility, without derivatization reagents and electrochemical cells, widely attracts the attention of researchers. In PD procedure, the molecular structures of AFB_1 and AFG_1 are respectively converted to two more stable forms AFB_{2a} and AFG_{2a} through their reactions with hydroxyl radical produced from the solvent (e.g. water and methanol) under ultraviolet radiation, in comparison to AFB_1 and AFG_1, the fluorescence signals of AFB_{2a} and AFG_{2a} allow a significant enhancement and will no longer be easily quenched by solvents. Referring to the previous literatures [27-30], high performance liquid chromatography coupled with fluorescence detection enhanced through photochemical derivatization (HPLC-PD-FLD) can obtain more accurate quantitative results and extremely low limits of detection and quantitation in many cases. However, there are still same problems hindering the further promotion of this

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method. On the one hand, on-line post-column PD procedure is often preformed in a special photochemical reactor connected to chromatographic column system for about 30 min, so it is hard to maintain sharp peak shapes for all analytes while the pump running. On the other hand, in consideration of the complexity of matrices (e.g. maize, flour, honey and edible oil), the multi-step purification for aflatoxins is needed before being injected into chromatographic column, for instance, the preliminary extraction procedure is often performed in methanol-water solution, and usually incorporated with a liquid-liquid extraction step with chloroform, then an immunoafinity column (IAC) or solid-phase extraction (SPE) procedure is followed for the purpose of further purification[31]. Due to the specificity of selectively bonding aflatoxins to monoclonal antibodies, the efficiency of IAC purification are more satisfactory comparing with non-specific SPE. Whereas the disadvantage of IAC purification cannot be ignored, such as the massive consumption of pretreatment time and organic solvents, the need of higher levels of expertise and the use of expensive and disposable IA-column. Moreover, no matter which multi-step purifications being applied for the analysis of aflatoxins, it is inevitable to lead to the loss of components of interest and increase analytical errors which sometimes were the major source of errors in the whole experiment.

In recent years, the development of new analytical methods with less extraction and clean-up steps, more rapid and accurate determination was still a hot issue for the analysis of aflatoxins at extremely low concentration levels (few ppb). Therefore, chemometrics techniques also carried out unremitting exploration and obtained good

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applications in many cases, e.g. Vosougha et al. developed a strategy for simultaneous and rapid determination of four aflatoxins (i.e. AFB_1, AFB_2, AFG_1, AFG_2) in pistachio nuts using HPLC-DAD method coupled with parallel factor analysis (PARAFAC) [14, 15], which showed that the method coupling SPE-HPLC-DAD with parallel factor analysis could be applied for quantitative analysis of multiple aflatoxins in complex foodstuffs matrices. A.S. Luna et al. also proposed a novel strategy for simultaneous determination of AFB_2 and AFG_2 in peanuts using three-dimensional fluorescence detection coupled with parallel factor analysis (PARAFAC) and IAC purification [32], which proved that chromatographic separation and multi-step purification could be absolutely replaced by chemometrics-assisted two-dimensional fluorescence analysis, however, there were still some obvious insufficiencies in this study, e.g. fluorescence enhancement when AFB_1 and AFG_1 were analyzed and more sufficient use of second-order method even in the present of unknown interferences using chemometrics-assisted three-dimensional fluorescence analysis and without IAC purification, needed to be further explored.

Therefore, in the present paper, for the purpose of further exploit of second-order advantage of chemomatrics techniques in determination of aflatoxins at extremely low concentration levels (often few ng g^{-1}), a novel 'dilute-and-shoot' analytical strategy coupling self-weighted alternating normalized residue fitting (SWANRF) algorithm with excitation-emission matrix (EEM) fluorescence detection enhanced through photochemical derivatization (PD) was proposed for rapid, simultaneous and accurate determination of AFB_1 and AFG_1 in various foodstuffs matrices (including maize,

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flour, honey and edible oil). The conception of 'dilute-and-shoot' analysis is defined as a strategy based on direct analysis of the dilution mixtures obtained from direct dilution of crude matrices (e.g. honey and edible oil) or a single extraction, sometimes following by a simple N_2 drying procedure and then dilution (e.g. cereals). using the predominant second-order advantage of SWANRF algorithm, the specific quantitative information of components of interest can be successfully extracted from heavily interferential environment by decomposing excitation-emission matrix fluorescent data (EEMs) [33]. Due to simple sample pretreatment, the loss of analytes of interest caused by multi-step purification can be significantly reduced, and the consumption of expense and time can also be decreased considerably using 'dilute-and-shoot' analysis [34].

The mainly aim of this work was further exploring the predominant second-order advantage of SWANRF algorithm for the quantitative analysis of AFB_1 and AFG_1 at extremely low concentration levels, and developing a sensitive excitation-emission matrix (EEM) fluorescence detection enhanced through photochemical derivatization (PD) to resolve the solvent-quenching effect on natural molecular fluorescence signals of AFB_1 and AFG_1. The entire experimental schematic diagram was illustrated as **Graphic abstract**. Finally, quantitative results of SWANRF-EEMs method were carefully validated by standard IAC-LC-ESI^+-MS method in SIM mode to assess the feasibility of the proposed strategy.

2. Theory

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2.1. Trilinear component model

When a single measurement runs, a $I \times J$ data matrix can be obtained for each analytical sample (here, I is the number of excitation wavelength points and J is the number of emission wavelength points, respectively). After K samples (including calibration set and prediction set) being measured, all two-dimensional data matrixes obtained can be sequentially stacked along the direction of sample arrangement to compose a three-way array $\mathbf{X}$ with the size of $I \times J \times K$ (K is the number of all analytical samples). According to the theory of trilinear component model, the array $\mathbf{X}$ ($I \times J \times K$) can be mathematically represented as follow[35]:

$$x_{ijk} = \sum_{n=1}^{N} a_{in}\, b_{jn} c_{kn} + e_{ijk}; \quad for\ i = 1, \dots, I; j = 1, \dots, J; k = 1, \dots, K \qquad (1)$$

Where N denotes the total number of detectable components including the calibrated components of interest, uncalibrated and backgrounds as well as unknown interferences. x_{ijk} represents the element of three-way array $\mathbf{X}$ ($I \times J \times K$) obtained at ith retention time, jth detection wavelength and kth sample. a_{in}, b_{jn}, c_{kn} are the elements of normalized excitation spectra matrix $\mathbf{A}$ ($I \times N$), normalized emission spectra matrix $\mathbf{B}$ ($J \times N$) and relative concentration matrix $\mathbf{C}$ ($K \times N$), respectively. e_{ijk} is the element of three-way residue array $\mathbf{E}$ ($I \times J \times K$).

2.2. SWANRF algorithm

Self-weighted alternating normalized residue fitting (SWANRF) algorithm for trilinear decomposition of a three-way array partially reextract valid information from the residue array and further remove invalid information to the residue array based on the truncated least squares method. Particularly, it can deal with high collinear

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problems and achieve very smooth resolved profiles in the present of heavy overlaps and unknown interferences.

As illustrated in **Figure 2** and **Figure 4** (**I**, **II**, **III**, **IV-A**, and **I**, **II**, **III**, **IV-B**), the data obtained had subtle collinear problems and in the present of heavy overlaps and unknown interferences. Therefore, in this work, SWANRF algorithm was selected for the trilinear decomposition of three-way array obtained. The concrete theory and model of second-order calibration based on SWANRF algorithm had been discussed in detail in relevant reference[33], so it was not described in details here.

2.3. Software

In all case, recorded datasets are analyzed in MATLAB environment (Mathworks, Inc) and the programs are developed in the laboratory on Dell computer (Intel$^{(R)}$ Core$^{(TM)}$ 2, 1G RAM, DELL, China) using Windows XP (Microsoft) software. A routine for self-weighted alternating normalized residue fitting (SWANRF) algorithm was written in our laboratory beforehand.

3. Experimental

3.1. Chemicals and apparatuses

Analytical standards of aflatoxin B_1 (AFB_1, ≥98%) and aflatoxin G_1 (AFG_1, ≥98%) were purchased from *J&K* Scientific Ltd. (Shanghai, China). Referring to the previous literature [27], the detail structural information of AFB_1 and AFG_1 was specified in **Table 1**. All standards were stored in darkness at -20℃ until utilized. Methanol (CH_3OH, HPLC-grade) used for extraction and mobile phase was obtained

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from Oceanpak (Sweden). *n*-hexane (C_6H_{14}, AR) and isopropanol (C_3H_7OH, AR) for dissolving edible oil samples were obtained from Sigma-Aldrich (Shanghai, China). Milli-Q water (18.2 MΩ. cm) employed in the whole experiment was obtained daily from water passed through a Milli-Q water purification system (Millipore Ltd., Bedford, USA).

Insert Table 1 here

In this work, a Hitachi (Japan) F-7000 fluorescence spectrometer equipped with an xenon lamp, a UV cross-linking instrument (Shanghai Bilon instrument Co., Ltd, China), a 3K30 ultracentrifuge equipped with cooling system (Sigma, USA), a Milli-Q water purification system (Millipore, USA), a grinder (Zhejiang Yiligongmao, China) and a ultrasonic instrument (Kunshan Hechuang ultrasonic Co., Ltd, China) were used.

3.2. Standard solutions

Stock solutions of AFB_1 and AFG_1 at the concentration level of 40 μg mL^{-1} were prepared through dissolving the corresponding standards with methanol into brown volumetric flasks and stored in the refrigerator at 4 ℃ for the whole experiment. The calibration set was partitioned into ten concentration levels referring to the orthogonal table (as presented in **Table 2**), the lowest concentration levels of calibration curves presented in **Table 3** were designed below the allowable maximum residue limits (MRLs) set by the Commission Regulation (EU). Calibration samples were obtained by further diluted appropriate volumes of stock solutions (SWANRF-EEMs: 0.95-19.00 ng mL^{-1} for AFB_1, and 1.90-38.00 ng mL^{-1} for AFG_1; IAC-LC-ESI^+-MS: 1.90-

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38.00 ng mL^{-1} for both AFB_1 and AFG_1) into methanol-water solvent (50:50, v/v), which was prepared immediately before utilized. Note that all solutions must be filtered through 0.22 μm nylon filters before utilized in IAC-LC-ESI^+-MS method.

Insert Table 2 here

3.3. Sample pretreatment

Due to extra selectivity from predominant second-order advantage of SWANRF algorithm, complex foodstuffs matrices just follow simple pretreatments including extraction, dissolution or N_2 drying procedure, and then dilution for detection. In this work, mixed solvent of methanol-water (80:20, v/v) was selected to extract out both AFB_1 and AFG_1 from solid matrices (e.g. cereals) and directly dissolve water-soluble matrices (e.g. honey), and mixed solvent of *n*-hexane-isopropanol (25:75, v/v) was efficient to directly dissolve oil-soluble matrices (e.g. edible oil).

3.3.1. Maize and flour

Aflatoxin-free maize and flour samples were randomly purchased in supermarket (Changsha). The pretreatment procedure can summarized as following steps: Firstly, 500.0 g of sample was ground and passed through an 80 mesh screen to obtain fine powders. And then, six portions weighted 5.0 g were moved into 50.0 mL centrifuge tubes, five of which were spiked with appropriate volumes of standard solutions at final concentration levels: 1.90 to 12.54 ng g^{-1} for AFB_1, and 3.80 to 19.00 ng g^{-1} for AFG_1, respectively. The remaining portion was regarded as unspiked sample. Secondly, 25.0 mL mixed solvent of methanol-water (80:20, v/v) was transferred into each centrifuge tube, and the mixtures were vigorously shocked and sonicated for 30

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min, next, the centrifuge tubes were centrifuged for 15 min at 4000 rpm, an aliquot of 10.0 mL supernatant and 6.0 mL ultrapure water were transferred into another 50.0 mL tube together, and then vigorously shaken and centrifuged for 5 min at 4000 rpm again. Thirdly, the secondary supernatant was collected again and followed by a simple N_2 drying procedure at 60℃, and reconstructed with 2.0 mL mixed solvent of methanol-water (80:20, v/v). Finally, the resulting solutions were stored in brown volumetric flasks and kept in the refrigerator at 4 ℃ for the following photochemical derivatization.

3.3.2. Honey

Six spiked honey samples hefted 5.0 g were directly homogenized into 10.0 mL of methanol-water (80:20, v/v) by ultrasonic-assisted extraction for 15 min in 50.0 mL centrifuge tubes, and then statically kept in ice bath environment for 10 min. Next, all mixtures were passed through 0.45 μm membranes to remove the insoluble gels. The resulting solutions were feasible for the following photochemical derivatization.

3.3.3. Edible oil

Aflatoxin-free rapeseed oil was one of the main kinds of edible oils in China and prepared for this survey. Firstly, similarly, six portions weighted 5.0 g of oil samples (five spiked and one unspiked samples) were synthesized, and then dissolved by an aliquot of 10.0 mL of the mixed solvent of *n*-hexane-isopropanol (1:3, v/v) into 50.0 mL centrifuge tubes. The mixed solutions were directly processed by photochemical derivatization.

3.3.4. Incubated samples

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Differing from aflatoxin-free samples, naturally contaminated samples often become a more intractable problem for quantitative determination of aflatoxins. In order to further investigate the availability and stability of this proposed method for analysis of aflatoxins in mycotoxin-contaminated environment occurring in various foodstuffs matrices, an artificial incubation was arranged for analytical foodstuffs matrices as following steps: Firstly, three parallel portions hefted 5.0 g of each sample were weighed, and then evenly spread out on the glass culture dishes. Secondly, these culture dishes were placed into a thermotank system set at 26 ℃ and 50% RH for 15 days, 30 days and 45 days, respectively. Finally, all incubated samples were extracted and disposed as the corresponding foodstuffs matrices, respectively. In addition, the quantitative results obtained from SWANRF-EEMs method were further validated by standard IAC-LC-ESI^+-MS method.

3.4. Photochemical derivatization (PD)

Prior to fluorescence detection, in order to significantly improve methodological sensitivity, fluorescence enhancement procedures must be performed to enhance the molecular fluorescence signals of AFB_1 and AFG_1 which are easily influenced by solvent-quenching effect. In previous literatures, many available sensitizing methods had been developed for fluorescence enhancement, i.e. pre-column trifluoroacetic acid (TFA), post-column bromine reagent and photochemical derivatization. Among these methods, photochemical derivatization (PD) was considered as the most simple and apinoid method. The condition needed for photochemical derivatization was just the UV-irradiation at 315 nm in a simple UV-lamp device. The steps of photochemical

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derivatization procedure were as follows: Firstly, 2.0 mL of supernatant obtained from pretreatment was transferred into a quartz cuvette. Secondly, all quartz cuvettes were abreast placed into UV cross-linking instrument, the ultraviolet wavelength was set at 315 nm and 100 J of energy would be inputted into these solutions in a period of 5 min (In order to avoid the potential side reactions between analytes and matrices, weak energy (315 nm) was selected). Thirdly, the second step was repeated four times. As presented in **Figure 1**, the resulting molecular fluorescence signals of AFB_1 and AFG_1 were drastically enhanced over 20 times during 15 min and remained stable until 30 min, in addition, the peak shapes and positions of both analytes were unchanged and shifted during the whole procedure of photochemical derivatization. Besides, due to following the first-order reaction mechanism, the reaction rate of photochemical derivatization was irrelevant to the initial concentration of AFB_1 and AFG_1, therefore all sample sets (including calibration set and predication set) could be derived synchronously, which was conducive to high-throughput and real-time monitoring of aflatoxin contamination in various foodstuffs.

Insert Figure 1 here

3.5. Data acquisition (DAQ)

At a single run on F-7000 fluorescence spectrometer, as shown in **Figure 2**, an excitation-emission fluorescence matrix with the size of 56×76 for each sample could be obtained in the excitation wavelength range from 300 to 410 nm (step 2 nm) and in the emission wavelength range from 380 to 530 nm (step 2 nm)，scanning rate was set at 12,000 nm min^{-1}. 1.8 min scanning time was cost on each sample and the

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whole data acquisition procedure was finished in 1.5 hour. After all samples being detected, three-way array **X** was established by stacking all EEMs along the direction of sample arrangement for calibration samples and prediction samples. Finally, the SWANRF algorithm was applied for the decomposition of the three-way array **X**.

Insert Figure 2 here

3.6. IAC-LC-ESI$^+$-MS analysis

Due to the prominent advantage of no need of any derivatization procedures and high selectivity and sensitivity, standard IAC-LC-ESI$^+$-MS method was carried out for further validating the quantitative results of this proposed method. LC-separation of aflatoxins was performed on an Eclipse XDB-C18 column (150×2.1 mm i.d., 3.5 μm; Agilent, CA, USA) at room temperature (20 ℃). AFB_1 and AFG_1 were eluted out at a isocratic flow rate of 0.4 mL min^{-1} with mobile phases: 0.1% formic acid-acetonitrile (solvent A): 0.1% formic acid-water (solvent B), 60:40 (v/v) in 6 min.

An Agilent 1290/G6460 triple-quadruple mass spectrometer (Agilent, CA, USA) equipped with electrospray ion source in positive ion mode (ESI$^+$) was applied for selected ion monitoring (SIM) detection. Selected quantitative ion (m/z) was 313.1 for AFB_1, and 329.2 for AFG_1, respectively. Main MS parameters were optimized and finally set as follows: nebulizer gas (GS1), 40psi; capillary temperature, 350 ℃; ionspray voltage (IS), 4000 V; fragment voltage, 200 V. Nitrogen gas was used as the nebulizer, heater, curtain and collision gas at flow rate: 8 L min^{-1}.

However, similar to all chromatographic methods, supernates and mixed solutions obtained from the pretreatment above must be further purificated before

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injected into column system. The steps were as following: First, 25.0 mL of the supernate and mixed solutions were diluted with 25.0 mL of water. Second, the solutions were passed through an Afla Test-P^{TM} immunoaffinity column (IAC) at a flow rate of approximately a drop s^{-1}. The IAC was then washed with 20.0 mL of water at a flow rate of 3-4 drops s^{-1} until 2-3 mL air passed the column. Finally, AFs was eluted out with 2.0 mL of methanol and collected into clean brown vials. The eluent was evaporated to dryness under nitrogen stream at 50 ℃ and the residual was reconstituted with methanol-water (60:40, v/v) to 5 mL for IAC-LC-ESI^{+}-MS analysis.

3.7. Safety considerations

Aflatoxins are highly carcinogenic, mutagenic, teratogenic compounds, and may cause fatal risk to experimental operator even at extremely low concentration. In this work, the omission of multi-step purification and derivatization procedures greatly reduced the possibility of exposure to mycotoxins, however, extreme care should be taken while handling of these compounds, gloves and other protective clothes must be worn as safety precautions in the whole experiment.

4. Results and discussion

4.1. Elimination of non-trilinear factors

The three-way array **X** obtained could be exactly decomposed into three matrixes (i.e. normalized excitation spectra matrix (**A**), normalized emission spectra matrix (**B**), and corresponding relative concentration matrix (**C**)) using SWANRF algorithm. However, the prerequisite that analytical three-way array should be conformed to the

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trilinear structure could not be always met because the fluorescent landscape often contains light scattering effects, such as first- and second-order Rayleigh scattering and Raman scattering (As illustrated in **Figure 2**), which would definitely destroy the trilinear structure of original data array. In order to overcome this problem, one strategy is as A.S. Luna et al. reported [32], the spectral range was cut down to avoid the present of Rayleigh scattering and Raman scattering. But sometimes the shrunken information may not be enough efficient to distinguish components of interest from complex matrices, especially when overlaps was heavy and scatterings penetrated the fluorescent landscape. Therefore, in this paper, a novel method was proposed to deal with three-way fluorescence data including scattering. As illustrated in **Figure 3**, the gray regions (**Figure 3 (b)**) representing for Rayleigh and Raman scattering were removed from raw data (**Figure 3 (a)**), and missing values were renewedly repaired using one-dimensional interpolations. In this convenient method, accurate assessment of scattering regions was required. Overlarge widths will cause some uncertainty in the interpolated area, whereas too narrow widths will bias the solution because scatter will be included. Approximately 1.5 times of visually assessed scattering area was reasonable for completely removing the scattering values. Herein, ±15nm, ±5nm and ±0nm (no second-order Rayleigh) were set for first-order Rayleigh, Raman scattering and second-order Rayleigh, respectively. The typical interpolation result was shown in **Figure 3(c)**, which indicated that the interpolation provided a visually sound result even with quite wide gaps.

Insert Figure 3 here

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The theory of interpolation method has been discussed in details in the relevant references[36, 37], so it is not described here. Dedicated program written for interpolation of Rayleigh scattering and Raman scattering area at EEM landscape is available at www.models.kvl.dk.

4.2. Decomposition and recovery study using SWANRF-EEMs method

With the purpose of accurately decomposing the three-way array **X** (56×76×18) composing of 56 excitation wavelength points, 76 emission wavelengths points and 18 samples (including ten calibration samples, three unspiked samples and five spiked samples), the number of components, before SWANRF algorithm was applied, should be carefully ascertained through so-called core-consistency analysis[38]. It consisted in studying the structural model based on the data and the estimated parameters of gradually augmented models. If the number of components estimated was *N*, which could be taken into account that *N* different signals (corresponding to AFB_1, AFG_1 and *N*-2 interferences) were presented in the selected region. In this work, the three-way array **X** was decomposed by the SWANRF method with the estimated number of components *N*= 4, 5, 4 and 4 for maize, flour, honey and edible oil, respectively.

Insert Figure 4 here

Insert Table 3 here

Figure 4 displayed actual spectral profiles (dash-line), resolved spectral profiles (symbolized solid-line for analytes and symbolized dash-line for interferences) and relative concentration profiles corresponding to the decomposed results of SWANRF algorithm. It was clear that in this figure that heavy overlaps simultaneously occurred

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in both emission and excitation spectra among analytes and unknown interferences, and the response intensities of these interferences was higher than interesting analytes, which severely hindered direct fluorescence spectrophotometric analysis of AFB_1 and AFG_1. Therefore, chemometrics-assisted EEMs analysis was intended for overcoming heavily overlapping problems.

Finally, satisfactory prediction results were obtained by SWANRF-EEMs for all four kinds of foodstuffs matrices (i.e. maize, flour, honey and edible oil). Resolved spectral profiles were consistent well with actual pure fluorescence spectral profiles measured AFB_1 and AFG_1 alone, as shown in **Figure 4 (I**, **II**, **III**, **IV-A** and **I**, **II**, **III**, **IV-B**). In addition, the content information of AFB_1 and AFG_1 in five spiked samples for four kinds of foodstuffs matrices was obtained by the corresponding regression from concentration matrix **C**. As shown in **Table 4**, the accurate average recoveries: AFB_1: 93.5±6.6%-102.8±4.0% and AFG_1: 96.4±3.6%-107.2±6.0%, were obtained, respectively. Additionally, **Table 3** presented that linearity of analytical concentrations within the studied concentration range was excellent, with correlation coefficients (R^2) 0.9995 for AFB_1 and 0.9996 for AFG_1, respectively. Present study demonstrates that the established model could meet the simultaneous determination of AFB_1 and AFG_1 at sufficiently low concentration levels.

Insert Table 4 here

4.3. Analytical figures of merit

Figures of merit (FOMs) are regularly employed for surveying the effectiveness of a method. The most relevant figure of merit is the sensitivity (SEN), which may be

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defined as the change in response for given change in analyte concentration. The limit of detection (LOD) represents the lowest quantity of a substance that can be distinguished from the absence of that substance (a background value) within a stated confidence limit. The limit of quantification (LOQ) is the limit at which we can reasonably tell the difference between two different values. Those FOM parameters can be estimated by the following mathematical equations[39]:

$$\mathrm{SEN}_n = l_n\{[(\mathbf{A}_{\mathrm{cal}}^{\mathrm{T}}(\mathbf{I}-\mathbf{A}_{\mathrm{unx}}\mathbf{A}_{\mathrm{unx}}^{+})\mathbf{A}_{\mathrm{cal}}) * (\mathbf{B}_{\mathrm{cal}}^{\mathbf{T}}(\mathbf{I}-\mathbf{B}_{\mathrm{unx}}\mathbf{B}_{\mathrm{unx}}^{+})\mathbf{B}_{\mathrm{cal}})]^{-1}\}_{nn}^{-1/2} \quad (2)$$

$$\mathrm{LOD}_n = 3.3(\mathrm{SEN}_n^{-2}\sigma_x^2 + h_0\mathrm{SEN}_n^{-2}\sigma_x^2 + h_0\sigma_{y\mathrm{cal}}^2)^{1/2} \quad (3)$$

$$\mathrm{LOQ}_n = 10(\mathrm{SEN}_n^{-2}\sigma_x^2 + h_0\mathrm{SEN}_n^{-2}\sigma_x^2 + h_0\sigma_{y\mathrm{cal}}^2)^{1/2} \quad (4)$$

Where **A** and **B** denote the obtained normalized matrixes from the decomposition of SWANRF algorithm, the subscript *n* identifies a particular analyte of interest, l_n is the total response signal for *n*th component at unit concentration. σ_x represents the standard deviation of predicted concentrations in three different unspiked samples. h_0 denotes the Hadamard value for the leverage in the blank sample. The mathematical theory has been described in previous literature [39], so it is not explained here in detail.

Insert Table 5 here

All FOM parameters aforementioned were collected in **Table 5**. In consideration that the permissive content in actual environment and the complexity of the matrices, the LODs and SENs of two analytical aflatoxins in the four matrices were acceptable. The statistical parameters indicated that the proposed analytical strategy can be used as an alternative method for routine analysis of AFB_1 and AFG_1 in various foodstuffs.

4.4. IAC-LC-ESI$^+$-MS confirmation

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For further validation of the practicability and reliability of this proposed method in actual contamination environments of aflatoxins, analytical samples for each kind of foodstuffs matrices were measured by SWANRF-EEMs method and standard IAC-LC-ESI$^+$-MS method, respectively. As illustrated in **Figure 5**, the mass spectra in full scan mode (**Figure 5 (a)**) and total ion current chromatograms (TIC) (**Figure 5(b)**) of AFB_1 and AFG_1 demonstrated that two targeted analytes and background interferences were absolutely separated in SIM mode. Finally, the actual contents of incubated samples were obtained through the regression from calibration set.

Insert Figure 5 here

Firstly, the accuracy of this proposed method in prediction samples was carefully investigated for validation purposes, five validation samples with same concentration levels of AFB_1 and AFG_1 as the prediction samples were prepared using mixed solvent of methanol-water (80:20, v/v) for LC-ESI$^+$-MS analysis. The accuracy of recoveries obtained from both methods (SWANRF-EEMs method and LC-ESI$^+$-MS method for prediction and validation samples, respectively) were compared using a significance test based on the elliptic joint confidence region (EJCR) [40]. As illustrated in **Figure 6,** by taking the critical value for the Snedecor-Fisher statistic at $F_{2,3}$=9.55 (P=95% confidence level), the idealistic point (0,1) lay inside the EJCR, which meant that the intercept maybe considered to be zero and simultaneously the slope to be consistent. Although, the areas of EJCR in SWANRF-EEMs was slightly bigger than the one in LC-ESI$^+$-MS, the distribution range of whole data points were very narrow which indicates that the bias was hardly absent. Therefore, the accuracy of recoveries

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obtained from both methods were considered to be no significant different.

Insert Figure 6 here

The quantitative results of incubated sample using SWANRF-EEMs method and standard IAC-LC-ESI$^+$-MS method were shown in **Table 6** together, contaminations of aflatoxins was not detected in all four foodstuffs matrices using both methods after 15 days incubation, and then detected after 30 days incubation and 45 days incubation in maize, flour and edible oil. However, honey samples were exempt from contamination of aflatoxins till 45 days incubation, which maybe because of the function of antioxidant ingredients in honey.

Insert Table 6 here

Then, comparing the recoveries obtain from SWANRF-EEMs method with one obtained from intricate IAC-LC-ESI$^+$-MS method in which the prediction samples was processed with strictly pretreatment (IAC clean-up), there were some nuances between the quantitative results obtained from these two methods, it is easy to deduce from the significant test that the unconformities maybe mainly caused by the error of complex IA-column purification in IAC-LC-ESI$^+$-MS method rather than the error of algorithm itself, and could be ignored in the determination of aflatoxins at extremely low concentration levels. Therefore, the quantitative results in all four foodstuffs matrixes could generally be deemed to be coincident. The present study indicated that the proposed SWANRF-EEMs method can be an alternative strategy for rapid, accurate, and simultaneously determination of AFB_1 and AFG_1 in complex foodstuffs matrixes without tanglesome multi-step purification and chromatographic separation.

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5. Conclusions

In this paper, a simple, rapid, efficient and eco-friendly method for simultaneous determination of AFB_1 and AFG_1 in various foodstuffs matrices (including maize, flour, honey and edible oil) was developed using dilute-and-shoot analysis coupling with second-order calibration method with excitation-emission matrix fluorescence enhanced through photochemical derivatization. Due to the predominant second-order advantage of SWANRF algorithm, qualitative and quantitative information of multiple aflatoxins could be successfully extracted out from heavily interferential environment where overlapping peaks and unknown interferences exists. The simple, fast, sensitive two-dimensional fluorescence detection enhanced through apinoid photochemical derivatization provided high sensitivity and selectivity for simultanous determination of AFB_1 and AFG_1 at extremely low concentration levels (few ppb). No exogenous derivatization reagents, multi-step purification and chromatographic separation were required, which significantly decreased experimental time and expenses. Therefore, the proposed strategy was promising as an alternative for rapid, accurate and sensitive analysis of multiple aflatoxin-contaminations in various foodstuffs matrices and even theoretical basis for the development of portable detecting instrument.

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2012CB910602) for financial supports.

Appendix A. Supporting information

The original SIM quantitative reports exported from the Agilent MassHunter quantitative analysis software (version B.04.00) in supporting information (PDF): (1) Quantitative Analysis Calibration Report; (2) Quantitative Analysis Summary Report. This information is available free of charge on the Internet.

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References

1. D. Bhatnagar and S. Garcia, *Guide to Foodborne Pathogens, Second Edition*, 2013, 257-272.
2. S. Z. Iqbal, S. Nisar, M. R. Asi and S. Jinap, *Food Control*, 2014, 43, 98-103.
3. G. N. Wogan, *Bacteriol. Rev.*, 1966, 30, 460-470.
4. H. S. Hussein and J. M. Brasel, *Toxicology*, 2001, 167, 101-134.
5. D. Larionova, I. Y. Goryacheva, C. Van Peteghem and S. De Saeger, *World Mycotoxin Journal*, 2011, 4, 113-117.
6. S. M. Siahi, M. Ansarin, A. Tahavori, F. Ghaderi and M. Nemati, *Advanced pharmaceutical bulletin*, 2011, 2, 123-126.
7. H. Franco, A. Vega, S. Reyes, J. De Leon and A. Bonilla, *Archivos latinoamericanos de nutricion*, 2014, 64, 42-49.
8. Y. Nonaka, K. Saito, N. Hanioka, S. Narimatsu and H. Kataoka, *Journal of Chromatography A*, 2009, 1216, 4416-4422.
9. K. Benesova, S. Belakova, R. Mikulikova and Z. Svoboda, *Food Control*, 2012, 25, 626-630.
10. M. R. Khan, Z. A. Alothman, A. A. Ghfar and S. M. Wabaidur, *Journal of separation science*, 2013, 36, 572-577.
11. G. H. Tan and R. C. Wong, *Food Control*, 2013, 31, 35-44.
12. W. Yong, L. Rong, Z. XianChen, Z. PengJie, C. DingHu, J. YueMing, D. XueWu, L. TaoTao, H. WeiWen and Y. Fang, *Journal of Food Safety and Quality*, 2014, 5, 2457-2461.
13. S. M. Herzallah, *Food chemistry*, 2009, 114, 1141-1146.
14. M. Vosough, M. Bayat and A. Salemi, *Analytica chimica acta*, 2010, 663, 11-18.
15. M. Vosough and A. Salemi, *Food chemistry*, 2011, 127, 827-833.
16. M. Muscarella, M. Iammarino, D. Nardiello, S. Lo Magro, C. Palermo, D. Centonze and D. Palermo, *Food Additives and Contaminants*, 2009, 26, 1402-1410.
17. P. D. Andrade, J. L. G. da Silva and E. D. Caldas, *Journal of Chromatography A*, 2013, 1304, 61-68.
18. M. Hashemi and Z. Taherimaslak, *Analytical Methods*, 2014, 6, 7663-7673.
19. M. Hashemi, Z. Taherimaslak and S. Rashidi, *Journal of Chromatography B*, 2014, 960, 200-208.
20. M. Quinto, G. Spadaccino, C. Palermo and D. Centonze, *Journal of Chromatography A*, 2009, 1216, 8636-8641.
21. W. S. Khayoon, B. Saad, T. P. Lee and B. Salleh, *Food chemistry*, 2012, 133, 489-496.
22. A. Medina and N. Magan, *Journal of Chromatography B*, 2012, 889, 138-143.
23. G. Liu, Z. Zhu, J. Cheng and H. Z. Senyuva, *Journal of AOAC International*, 2012, 95, 122-128.
24. R. Ghali, I. Belouaer, S. Hdiri, H. Ghorbel, K. Maaroufi and A. Hedilli, *Journal of Food Composition and Analysis*, 2009, 22, 751-755.
25. M. Aghamohammadi, J. Hashemi, G. A. Kram and N. Alizadeh, *Analytica chimica acta*, 2007, 582, 288-294.
26. L. MA, M. ZHANG, Y.-h. ZHANG, Y.-y. HU and Y. ZHOU, *Food Science*, 2012, 12, 029.
27. H. Joshua, *J. Chromatogr. A*, 1993, 654, 247-254.
28. W.-J. Kong, J.-Y. Li, F. Qiu, J.-H. Wei, X.-H. Xiao, Y. Zheng and M.-H. Yang, *Analytica chimica acta*, 2013, 799, 68-76.
29. W.-J. Kong, S.-Y. Liu, F. Qiu, X.-H. Xiao and M.-H. Yang, *Analyst*, 2013, 138, 2729-2739.

View Article Online
DOI: 10.1039/C5RA26549E

30. W. GuiLing, Z. YanYan, L. LinLin and Z. WeiWei, *Journal of Food Safety and Quality*, 2014, 5, 833-839.
31. N. W. Turner, S. Subrahmanyam and S. A. Piletsky, *Analytica chimica acta*, 2009, 632, 168-180.
32. A. Luna, R. Luiz, I. Lima, P. Março, P. Valderrama, R. Boqué and J. Ferré, *Analytica chimica acta*, 2013, 778, 9-14.
33. J.-F. Nie, H.-L. Wu, S.-R. Zhang, Y.-J. Yu and R.-Q. Yu, *Analytical Methods*, 2010, 2, 1918-1926.
34. S. Michael, K. Rudolf and S. Rainer, *Analytical & Bioanalytical Chemistry*, 2007, 389, 1505-1523.
35. H. L. Wu, M. Shibukawa and K. Oguma, *J. Chemom.*, 1998, 12, 1-26.
36. Y. Li, H.-L. Wu, Y.-J. Yu, S.-R. Zhang, Y. Chen, D.-Z. Tu, C.-C. Nie, H. Xu and R.-Q. Yu, *Analytical Methods*, 2012, 4, 3987-3996.
37. M. Bahram, R. Bro, C. Stedmon and A. Afkhami, *Journal of Chemometrics*, 2006, 20, 99-105.
38. R. Bro and H. A. Kiers, *J. Chemom.*, 2003, 17, 274-286.
39. A. C. Olivieri, *Chem. Rev.*, 2014, 114, 5358-5378.
40. A. G. González, M. A. Herrador and A. G. Asuero, *Talanta*, 1999, 48, 729-736.

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CAPTIONS AND LEGENDS TO FIGURES AND TABLES

Table 1 The names, abbreviations, structural formulas, toxicity, the maximum residue limits and mass-to-charge ratios of four aflatoxins

Table 2 The designed concentration in calibration set divided into ten levels for SWANRF-EEMs method and the validation using IAC-LC-ESI$^+$-MS method

Table 3 The concentration ranges of calibration sets, regression equations and correlation coefficients (R^2) related to SWANRF-EEMs method and IAC-LC-ESI$^+$-MS method, respectively

Table 4 Recovery studies of AFB_1 and AFG_1 in maize, flour, honey and edible oil using SWANRF-EEMs method

Table 5 Analytical figures of merit using SWANRF-EEMs method in maize, flour, honey and edible oil samples, respectively

Table 6 The comparison of quantitative results of AFB_1 and AFG_1 using SWANRF-EEMs method and IAC-LC-ESI$^+$-MS method in incubated samples, respectively

Figure 1 (**a**) The fluorescence enhancement spectral profiles (recorded at λ_{ex}=365 nm) of AFB_1 and (**b**) AFG_1 using photochemical derivation under UV-irradiation set at λ=315 nm.

Figure 2 3-D plots of the excitation-emission matrix fluorescence: (**a**) 5th calibration sample; (**b**) maize sample; (**c**) flour sample; (**d**) honey sample and (**e**) edible oil sample in the range of excitation wavelength from 300 nm to 410 nm and emission wavelength from 380 nm to 530 nm at an interval 2 nm.

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Figure 3 The elimination of non-trilinear factors (including Rayleigh scattering and Raman scattering) in excitation-emission matrix fluorescence data: (**a**) Original data with obvious Rayleigh scattering and Raman scattering; (**b**) Gapped data with the scatterings in the gray regions being removed; (**c**) Prothetic data fitted by an one-dimensional interpolation.

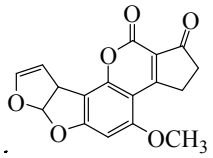
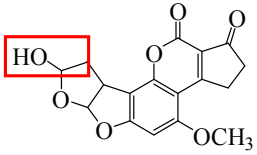
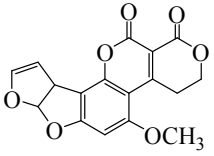
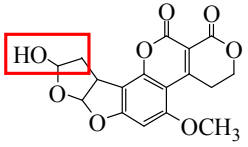
Figure 4 The profiles of normalized excitation spectra (**I**, **II**, **III**, **IV-A**), normalized emission spectra (**I**, **II**, **III**, **IV-B**) and corresponding relative concentration (**I**, **II**, **III**, **IV-C**) obtained from the SWANRF-EEMs method, and the actual spectral profiles of AFB_1 (red dash-line) and AFG_1 (green dash-line) in maize sample (**I**), flour sample (**II**), honey sample (**III**) and edible oil sample (**IV**), respectively.

Figure 5 (**a**) The mass spectrograms in full scan mode of AFB_1 and AFG_1; (**b**) the total ion chromatograms (TIC) recorded at m/z 312.27 for AFB_1 and 328.27 for AFG_1 in SIM mode of 5th calibration sample, 3th incubated sample of maize sample, flour sample, honey sample and edible oil sample.

Figure 6 The elliptic joint confidence region (EJCR) for the significance test between the accuracy of recoveries obtained from SWANRF-EEMs method and standard IAC-LC-ESI^+-MS method, respectively.

Table 1

The names, abbreviations, structural formulas, toxicity, the maximum residue limits and mass-to-charge ratios of four aflatoxins

Compound	Abbreviation	MW	Structural Formula	Toxicity	MRLs[a]	$[M-H]^+(m/z)$[b]
Aflatoxin B_1	AFB_1	312.2		oral-rat:LD50: 4.80 mg kg^{-1} oral-mice: LD50: 9.00 mg kg^{-1}	$\leq 2-6$ μg kg^{-1} (EC) ≤ 20 μg kg^{-1} (USA. China)	313.2
Aflatoxin B_{2a}	AFB_{2a}	330.2		Far weaken	≤ 20 μg kg^{-1} in total aflatoxins (AFT)	NO Given
Aflatoxin G_1	AFG_1	328.2		abdominal-rat: LD50: 14.90 mg kg^{-1} oral-duck: LD50: 2.45 mg kg^{-1}	≤ 10 μg kg^{-1} (EC) ≤ 20 μg kg^{-1} (USA. China)	329.2
Aflatoxin G_{2a}	AFG_{2a}	346.2		Far weaken	≤ 20 μg kg^{-1} in total aflatoxins (AFT)	NO Given

[a] The abbreviation of the maximum residue limits.

[b] The mass-to-charge of selected quantitative ion in IAC-LC-ESI^+-MS.

Table 2

The designed concentration in calibration set divided into ten levels for SWANRF-EEMs and the validation using IAC-LC-ESI$^+$-MS

Sample NO.	Analytical concentration (ng mL^{-1})			
	SWANRF-EEMs		IAC-LC-ESI$^+$-MS	
	AFB_1	AFG_1	AFB_1	AFG_1
C01	19.00	1.90	1.90	1.90
C02	17.10	7.60	7.60	7.60
C03	15.20	11.40	11.40	11.40
C04	13.30	15.20	15.20	15.20
C05	11.40	19.00	19.00	19.00
C06	9.50	22.80	22.80	22.80
C07	7.60	26.60	26.60	26.60
C08	5.70	30.40	30.40	30.40
C09	3.80	34.20	24.20	34.20
C10	0.95	38.00	38.00	38.00

Table 3

The concentration ranges of calibration sets, regression equations and correlation coefficients (R^2) related to SWANRF-EEMs method and IAC-LC-ESI$^+$-MS method, respectively

Method	Analyte	c (ng mL^{-1})	Regression equation[a]	R^2
SWANRF-EEMs	AFB_1	0.95-19.00	$y = 1.28 \times 10^3 x + 6.19 \times 10^3$	0.9995
	AFG_1	1.90-38.00	$y = 5.25 \times 10^2 x + 7.22 \times 10^2$	0.9996
IAC-LC-ESI$^+$-MS	AFB_1	1.90-38.00	$y = 1.29 \times 10^3 x + 1.36 \times 10^3$	0.9970
	AFG_1	1.90-38.00	$y = 6.89 \times 10^2 x + 5.02 \times 10^2$	0.9983

[a] x is concentration (ng mL^{-1}) and y is corresponding response intensity (a. u. in SWANRF-EEMs method and mAu in IAC-LC-ESI$^+$-MS method, respectively)

Table 4

Recovery studies of AFB_1 and AFG_1 in maize, flour, honey and edible oil using SWANRF-EEMs method

Sample	Spiked concentration	Maize sample	Flour sample	Honey sample	Edible oil sample
$AFB_1(ng\,g^{-1})$	Taken	Found	Found	Found	Found
P01	1.90	2.13(111.9)[a]	1.59(83.6)	1.73(90.9)	2.20(116.0)
P02	4.56	4.74(103.8)	4.34(95.2)	3.96(86.8)	4.53(99.7)
P03	7.22	7.52(104.2)	6.88(95.2)	7.48(103.6)	7.10(98.4)
P04	9.88	9.47(95.9)	9.25(93.6)	9.17(92.8)	9.07(91.8)
P05	12.54	12.33(98.3)	13.62(108.6)	11.72(93.4)	11.75(93.7)
Average recovery ± S.D. (%)		102.8 ± 4.0	95.3 ± 9.3	93.5 ± 6.6	99.9 ± 9.5

Sample	Spiked concentration	Maize sample	Flour sample	Honey sample	Edible oil sample
$AFG_1(ng\,g^{-1})$	Taken	Found	Found	Found	Found
P01	19.00	18.86(99.3)	17.66(93.0)	19.29(101.5)	20.03(105.4)
P02	15.20	15.84(104.2)	14.57(95.9)	16.11(106.0)	16.46(108.3)
P03	11.40	12.34(108.2)	11.44(100.3)	11.84(103.8)	13.41(117.6)
P04	7.60	6.87(90.4)	7.08(93.2)	7.32(96.3)	7.69(101.1)
P05	3.80	4.52(118.9)	3.78(99.4)	4.38(115.4)	3.93(103.4)
Average recovery ± S.D. (%)		104.2 ± 10.1	96.4 ± 3.6	104.6 ± 6.7	107.2 ± 6.0

[a] Recovery (%) was in parentheses and calculated as $(c_p\text{-}c_o)/\,c_s \times 100$, c_p, c_o is the predicted concentration in spiked and unspiked prediction sample and c_s is the actual spiked concentration.

Table 5

Analytical figures of merit using SWANRF-EEMs method in maize, flour, honey and edible oil samples, respectively

Figures of merit	Maize sample		Flour sample		Honey sample		Edible oil sample	
	AFB_1	AFG_1	AFB_1	AFG_1	AFB_1	AFG_1	AFB_1	AFG_1
$SEN/mL\ ng^{-1}$	80.45	8.57	48.98	9.43	47.21	11.49	50.14	10.99
$LOD/ng\ mL^{-1}$	0.21	0.27	0.18	0.75	0.16	0.28	0.12	0.70
$LQD/ng\ mL^{-1}$	0.63	0.82	0.55	2.29	0.48	0.86	0.35	2.14

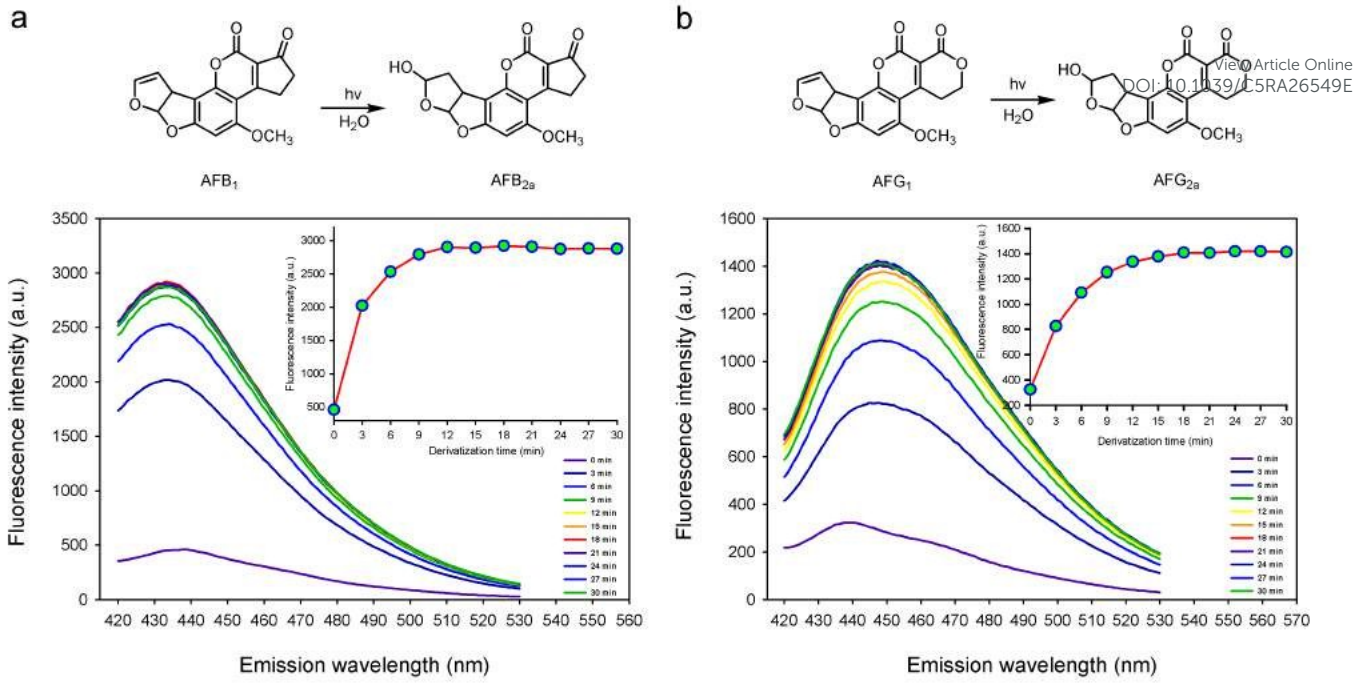
Table 6

The comparison of quantitative results of AFB_1 and AFG_1 using SWANRF-EEMs method and IAC-LC-ESI^+-MS method in incubated samples, respectively

Incubated time	Maize sample				Flour sample			
	SWANRF-EEMs		IAC-LC-ESI^+-MS		SWANRF-EEMs		IAC-LC-ESI^+-MS	
	AFB_1 (ng g^{-1})	AFG_1 (ng g^{-1})	AFB_1 (ng g^{-1})	AFG_1 (ng g^{-1})	AFB_1 (ng g^{-1})	AFG_1 (ng g^{-1})	AFB_1 (ng g^{-1})	AFG_1 (ng g^{-1})
15 days	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]
30 days	2.40	2.30	3.27	5.16	2.88	2.89	1.73	2.99
45 days	7.43	9.90	8.45	13.69	5.92	4.86	6.65	5.65

Incubated time	Honey sample				Edible oil sample			
	SWANRF-EEMs		IAC-LC-ESI^+-MS		SWANRF-EEMs		IAC-LC-ESI^+-MS	
	AFB_1 (ng g^{-1})	AFG_1 (ng g^{-1})	AFB_1 (ng g^{-1})	AFG_1 (ng g^{-1})	AFB_1 (ng g^{-1})	AFG_1 (ng g^{-1})	AFB_1 (ng g^{-1})	AFG_1 (ng g^{-1})
15 days	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.70
30 days	nd[a]	nd[a]	nd[a]	nd[a]	1.67	2.56	1.64	1.69
45 days	nd[a]	nd[a]	nd[a]	nd[a]	3.42	4.94	2.67	2.78

[a] nd is the abbreviation of not detected.



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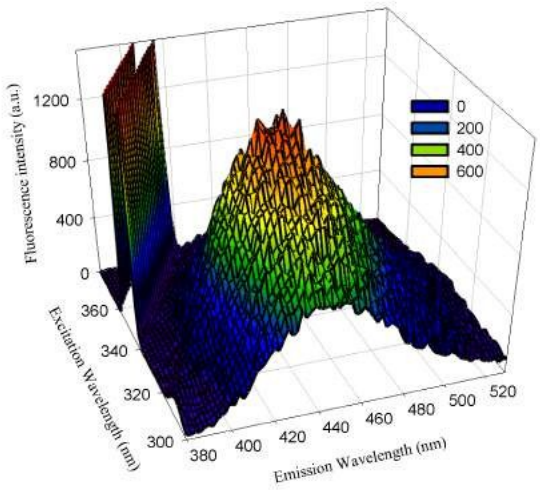

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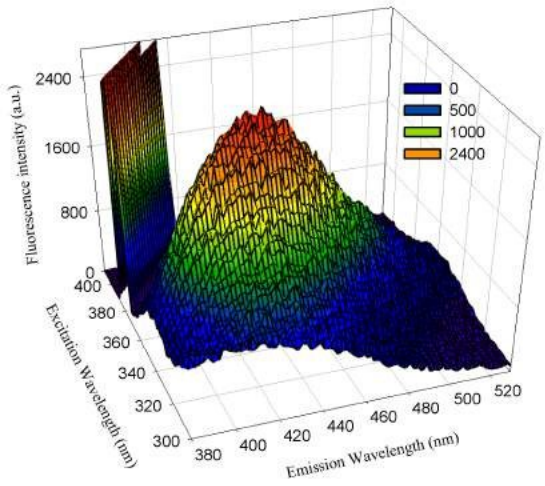

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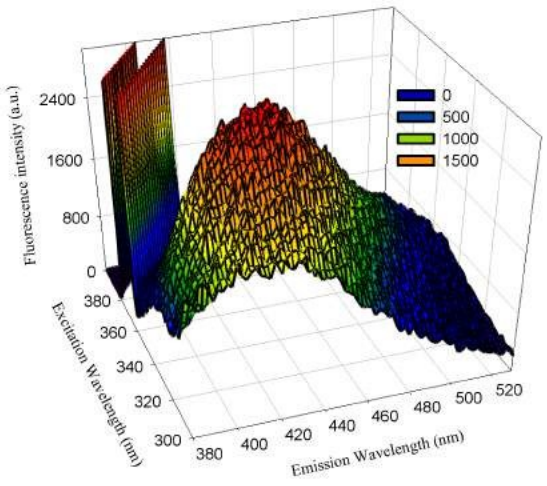

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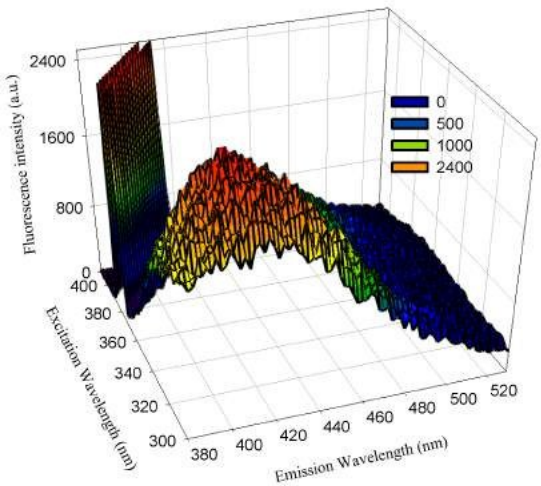

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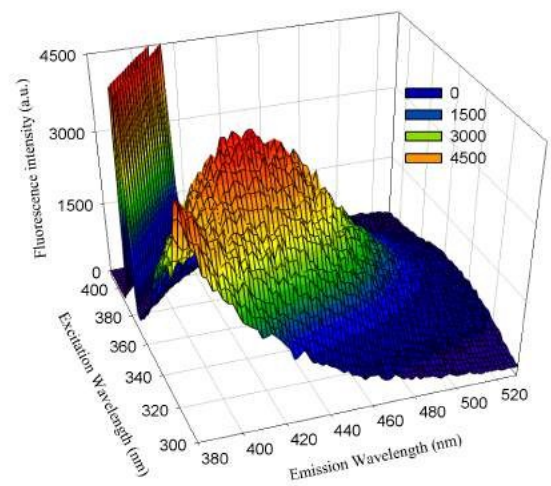

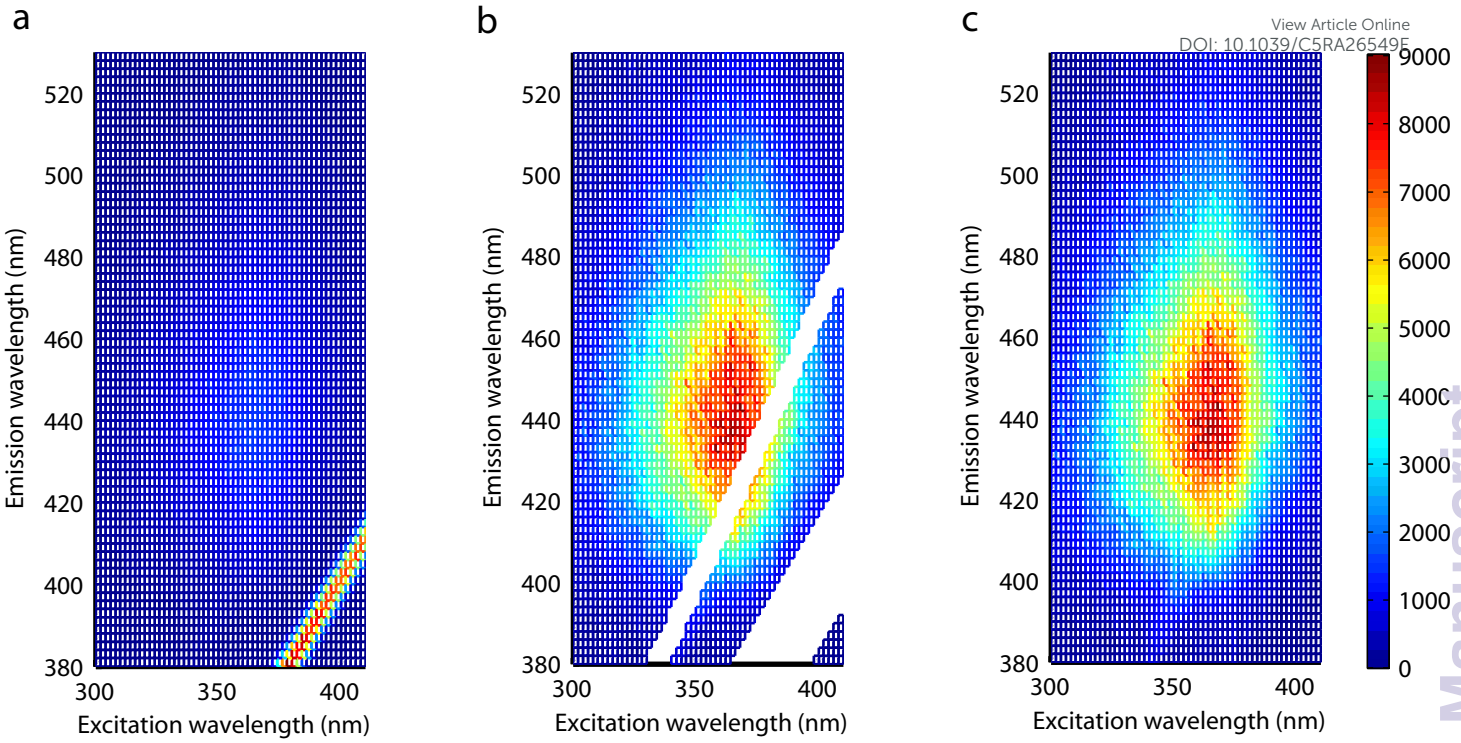

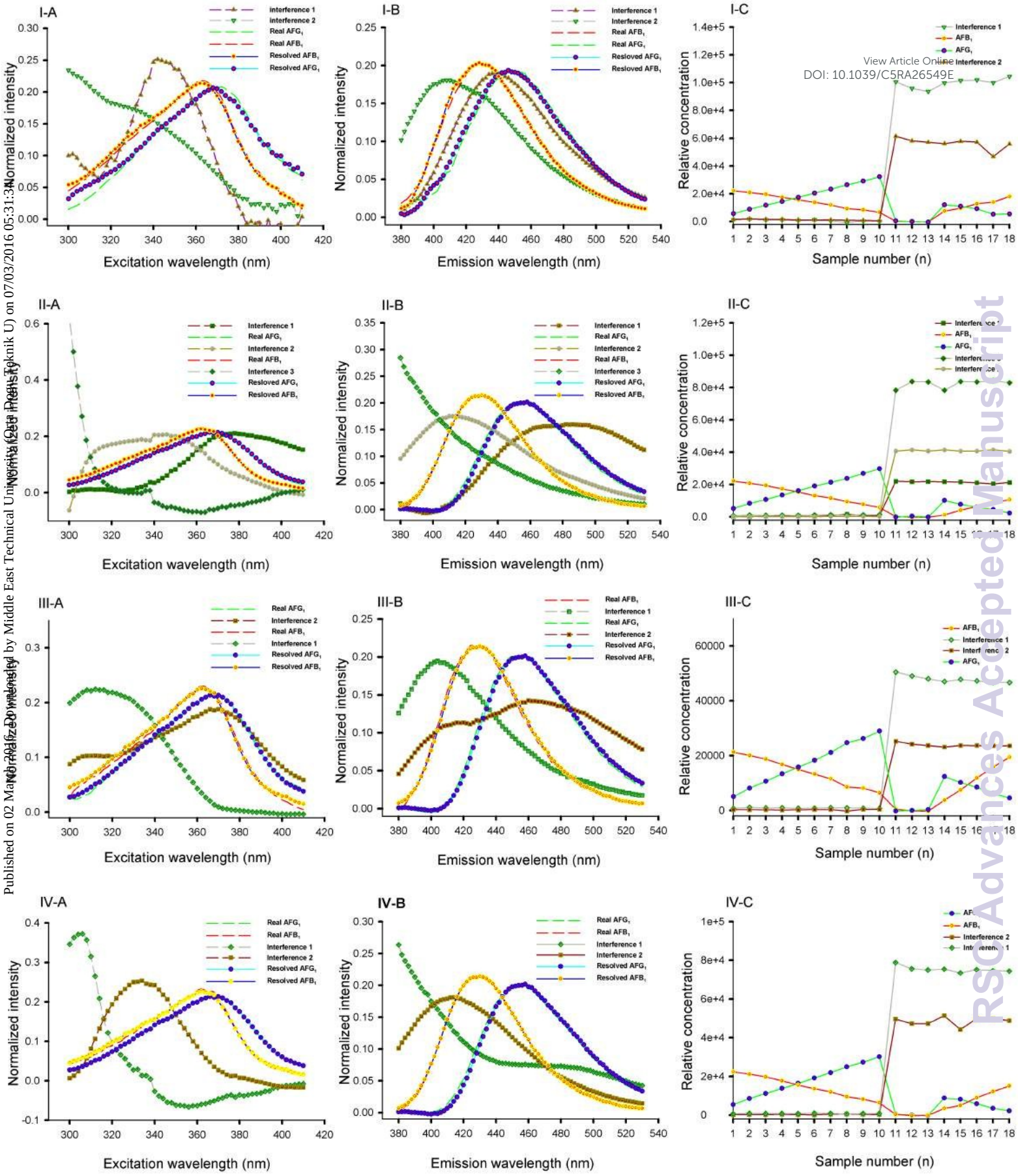

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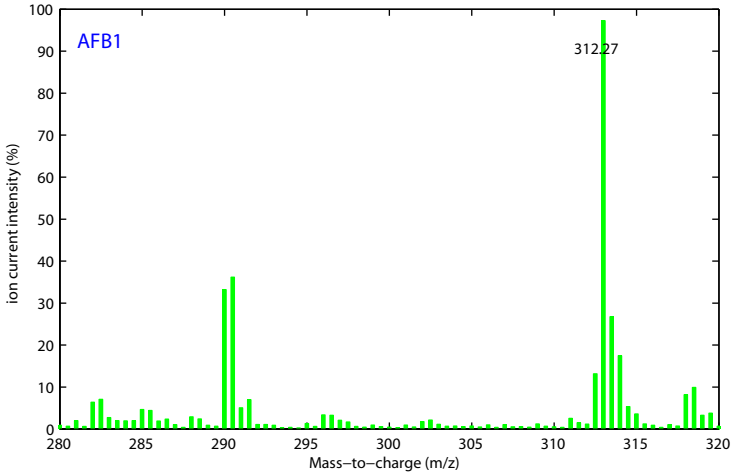

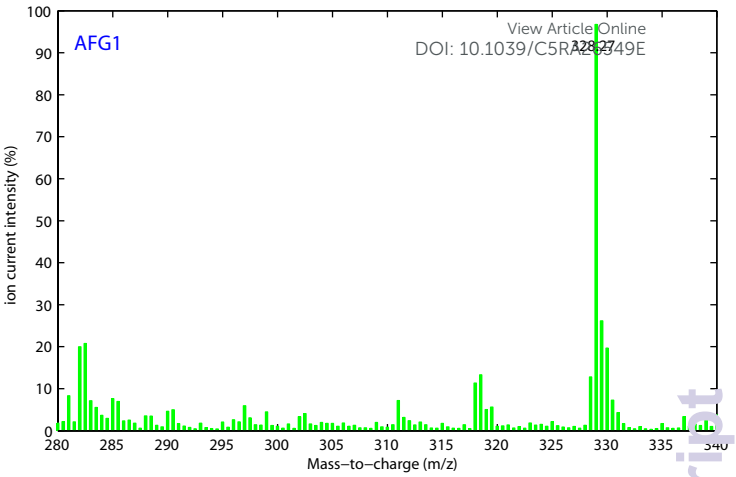

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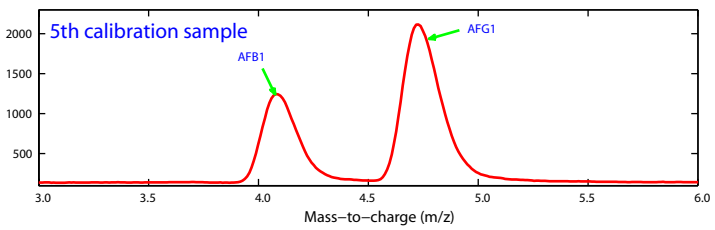
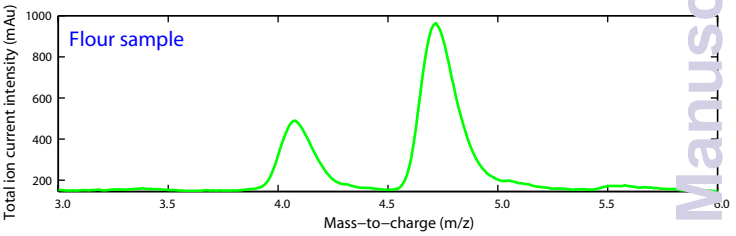
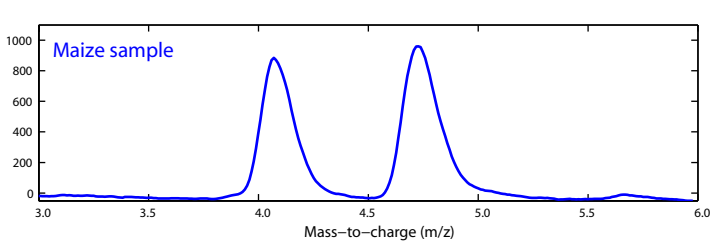
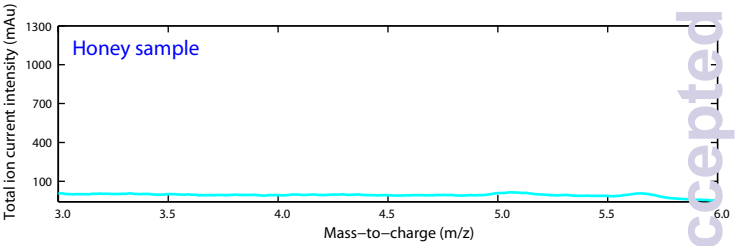
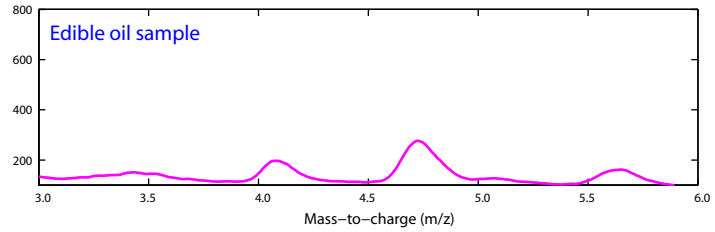
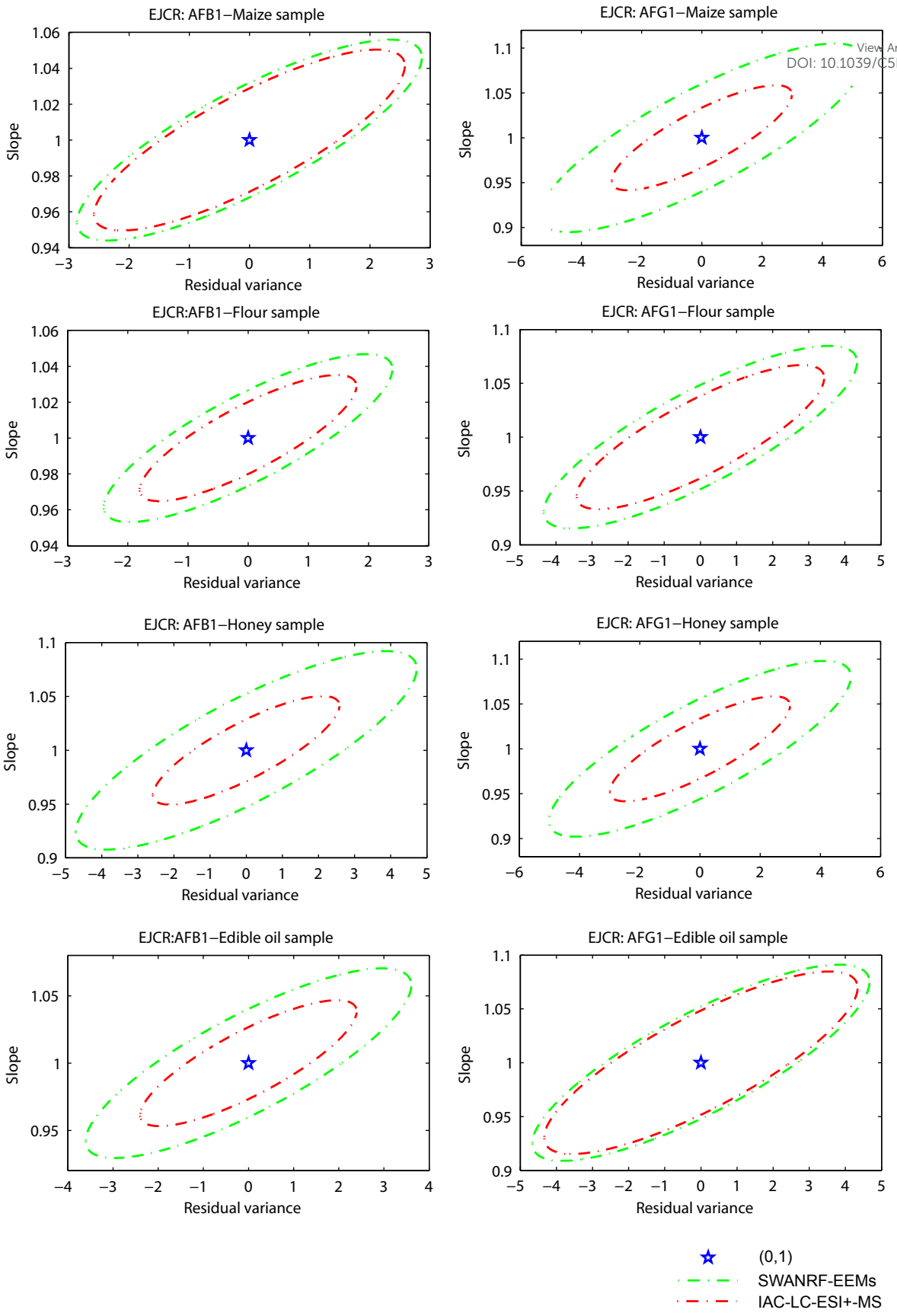




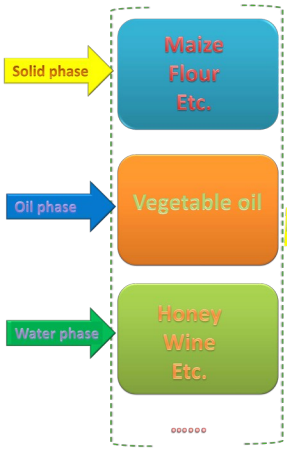




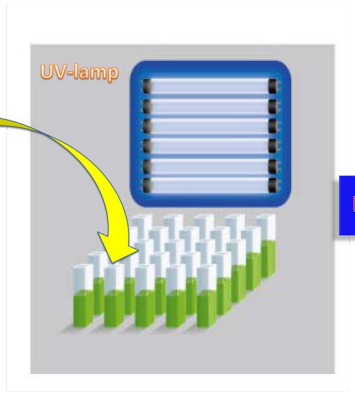




Experimental operation part



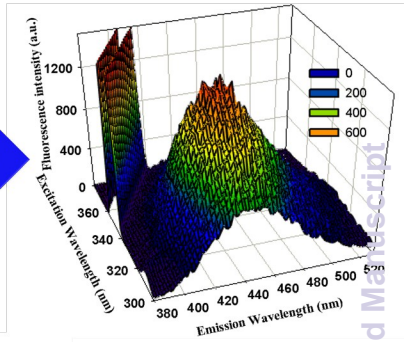
Simple sample pretreatment



Photochemical derivatization

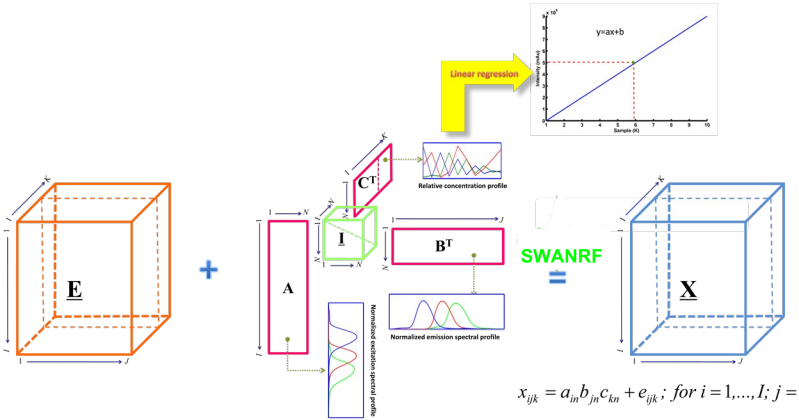
EEM detection

single sample



Stack samples

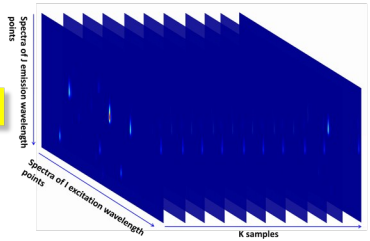
Data analysis part



$$x_{ijk} = a_{in} b_{jn} c_{kn} + e_{ijk}\,;\ for\ i = 1,...,I;\ j = 1,...,J;\ k = 1,...,K$$

Matlab DAQ

Multiple samples



Simple sample pretreament | Simple experimental instrument | Ultra-high sensitivity | Ultra-high accuracy