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Trace-Level Determination of Aflatoxins B_1, B_2, G_1 and G_2 in Peanuts and Raisins Using a Simplified QuEChERS–HPLC–FLD Method

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ABSTRACT

Aflatoxins are highly toxic and carcinogenic mycotoxins that contaminate peanuts and dried fruits during storage. A simplified QuEChERS extraction, performed without dispersive solid-phase extraction cleanup, was developed and validated for the determination of aflatoxins B_1, B_2, G_1 and G_2 by high-performance liquid chromatography with fluorescence detection. Separation was achieved on a reversed-phase C18 column with acetonitrile/methanol/water (55:18:27, v/v/v), giving baseline resolution of all four analytes within 16 min. Calibration was linear from 0.05 to 4.00 ng mL^{-1} with coefficients of determination of 0.9998–0.9999. Limits of detection were 0.2–0.4 μg kg^{-1} and limits of quantification 0.3–0.6 μg kg^{-1}. Mean recoveries from fortified blank matrices ranged from 84.2 to 96.9%, with repeatability, expressed as relative standard deviation, of 3.4–5.4%. The method was applied to 15 peanut and 15 raisin samples from commercial markets in the Syrdarya region of Uzbekistan. Total aflatoxins reached 6.27 μg kg^{-1} in peanuts (mean 2.84 μg kg^{-1}) and 3.29 μg kg^{-1} in raisins (mean 1.67 μg kg^{-1}), and at least one peanut sample exceeded the European Union maximum level of 4 μg kg^{-1} for groundnuts intended for direct human consumption. Omitting the cleanup step shortened sample preparation and lowered cost without measurable loss of accuracy, precision or selectivity.

KEYWORDS

aflatoxins; QuEChERS; HPLC–FLD; peanuts; raisins

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INTRODUCTION

Food safety is a critical global public health priority because of the increasing occurrence of toxic contaminants in agricultural commodities and processed foods. Among these contaminants, mycotoxins are of major concern owing to their widespread occurrence, chemical stability and severe toxicological effects. Mycotoxins are toxic secondary metabolites produced by filamentous fungi belonging primarily to the genera *Aspergillus*, *Penicillium* and *Fusarium*, which colonise a wide range of commodities during the pre-harvest, harvest and post-harvest stages.[1,2] Estimates of the global extent of contamination vary considerably: the frequently cited FAO figure of 25% of food crops has been critically re-examined, and the proportion of crops carrying detectable mycotoxin levels may be substantially higher.[3] Climate change, globalisation of food supply chains and inadequate storage infrastructure further favour fungal growth and mycotoxin production, particularly in regions with warm and humid climates.[4,5]

Aflatoxins are the most toxic and carcinogenic of the mycotoxins and are produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi frequently contaminate cereals, nuts, dried fruits, oilseeds and spices, particularly at elevated temperature (>25°C) and relative humidity (>70%).[1,6] Aflatoxins occur in several structurally related forms, including aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), of which AFB_1 is the most toxic and most prevalent.[7] Because of their chemical stability, aflatoxins resist conventional processing, including thermal treatment, drying and fermentation, and persist throughout the supply chain.[8]

AFB_1 has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer.[7] Following ingestion, AFB_1 is activated in hepatocytes by cytochrome P450 enzymes, principally CYP1A2 and CYP3A4, to the highly reactive AFB_1-8,9-epoxide. This electrophilic metabolite forms covalent adducts with DNA, particularly at guanine residues, causing mutations in tumour suppressor genes, such as *TP53*, and contributing to the development of hepatocellular carcinoma.[2,9,10] Chronic exposure is strongly associated with liver cancer, with synergistic effects in individuals infected with hepatitis B virus.[11,12] Aflatoxins are also immunotoxic, hepatotoxic, teratogenic and growth-retarding, particularly in infants and children.[5,13]

Peanuts, tree nuts and dried fruits are especially susceptible to aflatoxin contamination because of their nutrient composition, hygroscopic behaviour and extended storage periods.[6,14] Contamination may arise from pre-harvest infection, improper drying, mechanical damage or inadequate storage, and temperature, water activity, oxygen availability and storage duration strongly influence fungal proliferation and toxin biosynthesis.[4,6] In the European Union, maximum levels for AFB_1 and for the sum of the four congeners are laid down in Commission Regulation (EU) 2023/915, which repealed Regulation (EC) No 1881/2006; for groundnuts intended for direct human consumption the limits are 2 μg kg^{-1} for AFB_1 and 4 μg kg^{-1} for total aflatoxins.[15] Complementary standards and codes of practice have been issued by other bodies.[8,9] Reliable trace-level determination is therefore essential for regulatory compliance, consumer protection and international trade.

Accurate determination of aflatoxins in complex food matrices is analytically demanding because of their low concentrations, close structural similarity and interference from co-extracted pigments, lipids and phenolic compounds.[16,17] Thin-layer chromatography and enzyme-linked immunosorbent assays are limited in sensitivity, specificity and quantitative accuracy, which has driven the adoption of chromatographic techniques.[16,17] Liquid chromatography coupled to tandem mass spectrometry offers excellent sensitivity and selectivity, but requires costly instrumentation, skilled personnel and demanding maintenance, which restricts its routine use in many laboratories.[18] High-performance liquid chromatography with fluorescence detection (HPLC–FLD) has therefore remained a reliable and cost-effective alternative.[16] Aflatoxins fluoresce, enabling sensitive detection after

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separation, and derivatisation can further enhance the response of AFB_1 and AFG_1, whose native fluorescence is strongly quenched in aqueous mobile phases.[16,17]

Sample preparation has advanced in parallel. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach, originally developed for pesticide residues, has been adapted to other contaminant classes because of its simplicity, efficiency and low solvent consumption,[13,19] and several studies have applied it to mycotoxin determination in complex foods.[18,20] In its original form the method includes a dispersive solid-phase extraction (d-SPE) cleanup step, which adds sorbent cost, sample-handling time and a further source of analyte loss. Whether this step is indispensable for aflatoxin analysis in peanut and dried-fruit matrices has not been established, and information on the analytical performance of simplified QuEChERS workflows combined with HPLC–FLD under real market conditions remains scarce.

The present study therefore had two objectives: to develop and validate a simplified QuEChERS–HPLC–FLD method, without d-SPE cleanup, for the quantitative determination of AFB_1, AFB_2, AFG_1 and AFG_2; and to evaluate its performance on peanut and raisin samples obtained from commercial markets in the Syrdarya region of Uzbekistan, a region for which occurrence data are largely absent. The novelty of the work lies in demonstrating, with quantifiable advantages in preparation time, reagent consumption and cost per sample, that regulatory-level sensitivity can be retained when the cleanup step is removed for these two matrices.

EXPERIMENTAL

Chemicals and reference standards

Certified reference standards of AFB_1, AFB_2, AFG_1 and AFG_2 (certified purity ≥98%) were obtained from LGC Standards (Teddington, UK). Individual stock solutions (1 μg mL^{-1}) were prepared in HPLC-grade acetonitrile and stored in amber glass vials at −20 °C / 4 °C to limit photodegradation. Working solutions were prepared freshly by serial dilution with the mobile phase immediately before analysis. HPLC-grade acetonitrile and methanol were supplied by ACI Labscan (Bangkok, Thailand). Analytical-grade sodium chloride and anhydrous magnesium sulfate were purchased from PanReac AppliChem (Darmstadt, Germany). Ultrapure water (resistivity ≥18.2 MΩ cm) was produced with a Milli-Q Advantage A10 system (MilliporeSigma, Burlington, MA, USA). All solvents were selected to minimise background fluorescence.

Instrumentation

Samples were homogenised with an IKA A11 basic analytical mill (IKA-Werke GmbH & Co. KG, Staufen, Germany) and centrifuged in an Eppendorf 5810R refrigerated centrifuge fitted with an A-4-62 swing-bucket rotor (Eppendorf AG, Hamburg, Germany). Chromatographic analyses were performed on a Shimadzu LC-20AT system with an RF-20A / RF-20Axs fluorescence detector and a Shim-pack GIST C18 analytical column (250 × 4.6 mm, 5 μm; Shimadzu Corporation, Kyoto, Japan).

Sample collection and preparation

Thirty samples, comprising 15 peanut (*Arachis hypogaea* L.) and 15 raisin (*Vitis vinifera* L.) samples, were collected from retail markets, storage facilities and distribution centres in several districts of the Syrdarya region, Uzbekistan, during the 2025 harvest and post-harvest storage period. These commodities were selected because they are among the food products most susceptible to fungal colonisation and subsequent aflatoxin contamination.[6,14] Individual samples were taken from different commercial batches, and sampling was performed with reference to the sampling plan laid down in the Commission Implementing Regulation (EU) 2023/2782, Annex I.24

Samples were transported to the laboratory in sterile polyethylene containers within 6 h of collection and stored at 4°C. Each sample was homogenised in the IKA A11 mill to a uniform particle size; careful homogenisation is critical because aflatoxins are distributed heterogeneously within contaminated lots and inadequate sample preparation is a major source of analytical variability.[21] Homogenised material was transferred to airtight, light-protected containers, stored at 4°C and analysed within 48 h.

Simplified QuEChERS extraction

Aflatoxins were extracted by a simplified QuEChERS procedure in which the d-SPE cleanup step of the original protocol was omitted.[13,19] A 10.0 g portion of homogenised sample was weighed into a 50 mL polypropylene centrifuge tube and 20 mL of acetonitrile/water (40:60, v/v) was added. The mixture was vortexed vigorously for 3 min to ensure solvent penetration and analyte extraction. Sodium chloride (1.0 g) and anhydrous magnesium sulfate (4.0 g) were then added to induce phase separation, sodium chloride raising the ionic strength of the medium and magnesium sulfate acting as a dehydrating agent.

The tubes were centrifuged at 3000 rpm for 3 min to complete phase separation. The upper organic phase was transferred to amber glass vials, a 1.0 mL aliquot was evaporated to dryness under a gentle stream of high-purity nitrogen at ambient temperature, and the residue was reconstituted in 1.0 mL of mobile phase and filtered through a 0.22 μm PTFE syringe filter before injection. The comparatively high water content of the extraction mixture was chosen to improve wetting and penetration of the dried, low-moisture matrices, the aflatoxins being subsequently transferred into the acetonitrile phase during salting-out partitioning; the composition was selected on the basis of preliminary trials reported in the Supplementary Material.

HPLC–FLD conditions

Separation was carried out on the Shim-pack GIST C18 column with an isocratic mobile phase of acetonitrile/methanol/water (55:18:27, v/v/v) at 1.0 mL min^{-1}, a column temperature of 40°C and an injection volume of 10 μL. Fluorescence detection used excitation at 360 nm and emission at 440 nm.[16,17] Data acquisition, peak integration and quantification were performed with the Shimadzu chromatographic data system. The conditions are summarised in Table 1.

Method validation

The method was validated with reference to Commission Implementing Regulation (EU) 2023/2782, ISO 21748:2017 and ICH Q2(R2).[18,21,22] Linearity was assessed from external calibration curves prepared in mobile phase from 0.05 to 4.00 ng mL^{-1} at eight concentration levels. Limits of detection and quantification were determined from signal-to-noise ratios of 3:1 and 10:1, respectively. Accuracy and repeatability were assessed by recovery experiments using peanut and raisin matrices fortified at 1.0 and 5.0 μg kg^{-1}, with

Table 1. Chromatographic conditions used for the HPLC–FLD determination of aflatoxins B_1, B_2, G_1 and G_2.

Parameter	Value
Analytical column	Shim-pack GIST C18, 250 × 4.6 mm, 5 μm
Mobile phase	Acetonitrile/methanol/water (55:18:27, v/v/v), isocratic
Flow rate/mL min^{-1}	1.0
Column temperature/°C	40
Injection volume/μL	10
Excitation wavelength/nm	360
Emission wavelength/nm	440
Run time/min	16

six replicate determinations at each level. Repeatability was expressed as the relative standard deviation (RSD) of replicate determinations. Selectivity was evaluated by examining blank matrix extracts for interfering peaks at the retention times of the target aflatoxins.

Statistical analysis

Calibration and validation calculations were based on replicate chromatographic measurements. Recovery and repeatability are reported as mean recovery and relative standard deviation (RSD), respectively. For the market-sample survey, aflatoxin occurrence is summarised descriptively by concentration range and arithmetic mean for each commodity. Because individual-sample raw values required for inferential statistical testing were not included in the present manuscript dataset, no between-commodity significance test is reported.

RESULTS AND DISCUSSION

Chromatographic separation and selectivity

The optimised conditions gave baseline separation of all four aflatoxins within a 16 min run. A representative chromatogram of a mixed standard solution is shown in Figure 1. The elution order was AFG_2, AFG_1, AFB_2 and AFB_1, consistent with the decreasing polarity of the analytes on a reversed-phase C18 stationary phase.

The analytes eluted at 9.83, 11.22, 12.68 and 15.35 min for AFG_2, AFG_1, AFB_2 and AFB_1, respectively (Table 2). Retention times were stable throughout the analytical sequence, and no interfering peaks were observed at these retention times in blank matrix extracts. Adequate resolution is particularly important for structurally related aflatoxins, because incomplete separation compromises both identification and quantitative accuracy.[16,17] The absence of interfering peaks indicates that solvent partitioning alone reduced matrix-derived interference sufficiently for reliable determination, despite the omission of the d-SPE cleanup step.

Analytical performance

Calibration curves for all four aflatoxins were linear across the working range, with coefficients of determination between 0.9998 and 0.9999 (Table 2, Figure 2), indicating a highly proportional relationship between fluorescence response and analyte concentration. The relative standard deviation of the response factors ranged from 2.48 to 3.91%, confirming a stable detector response across the calibration range. Limits of detection of 0.2–0.4 μg kg^{-1} and limits of quantification of 0.3–0.6 μg kg^{-1} were obtained; both lie well below the maximum

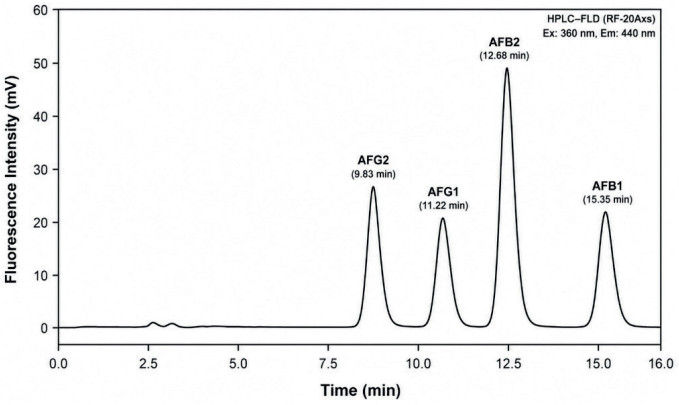


Figure 1. Representative HPLC–FLD chromatogram of a mixed aflatoxin standard solution obtained under the conditions given in Table 1, showing the separation of AFG2, AFG1, AFB2 and AFB1 within 16 min.

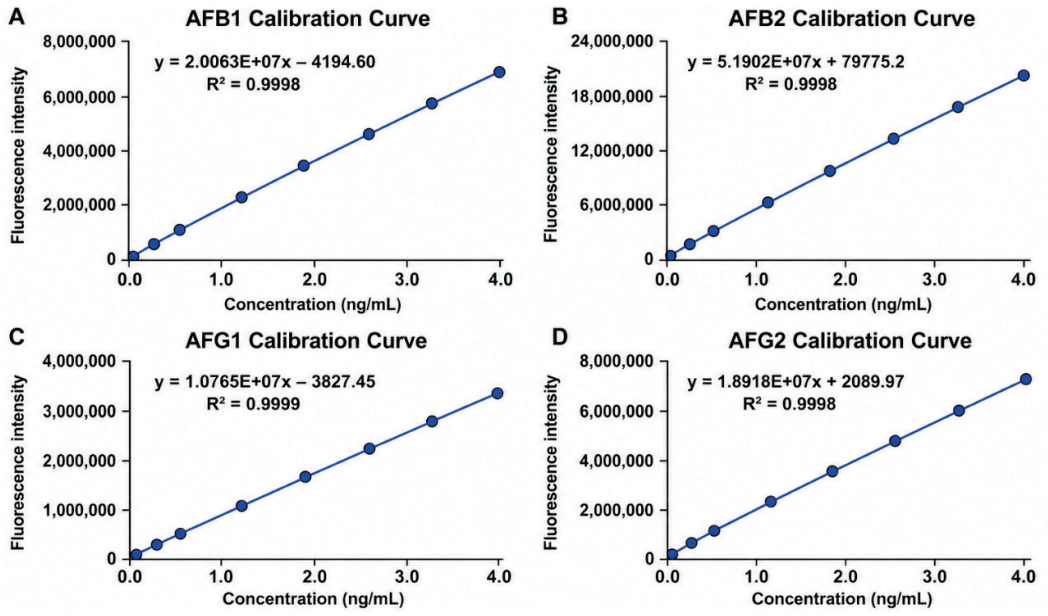


Figure 2. External calibration curves for (A) AFB_1, (B) AFB_2, (C) AFG_1 and (D) AFG_2 obtained by the proposed simplified QuEChERS–HPLC–FLD method from 0.05 to 4.00 ng mL^{-1} at eight concentration levels, each injected n times. Coefficients of determination ranged from 0.9998 to 0.9999.

Table 2. Retention, calibration and sensitivity parameters obtained for aflatoxins B_1, B_2, G_1 and G_2 by the proposed simplified QuEChERS–HPLC–FLD method. Each calibration level was injected n times.

Analyte	t_R/min	Calibration equation[a]	R^2	RF	RSD RF/%	LOD/μg kg^{-1}	LOQ/μg kg^{-1}
AFB_1	15.35	$y = 2.0063 \times 10^7x - 4194.60$	0.9998	2.021×10^7	3.23	0.2	0.3
AFB_2	12.68	$y = 5.1902 \times 10^7x + 79775.2$	0.9998	5.348×10^7	3.91	0.3	0.4
AFG_1	11.22	$y = 1.0765 \times 10^7x - 3827.45$	0.9999	1.080×10^7	2.48	0.2	0.3
AFG_2	9.83	$y = 1.8918 \times 10^7x + 2089.97$	0.9998	1.909×10^7	2.60	0.4	0.6

[a] y = peak area; x = concentration/ng mL^{-1}. t_R, retention time; RF, response factor; LOD, limit of detection; LOQ, limit of quantification.

levels laid down in Regulation (EU) 2023/915 and are therefore fit for regulatory monitoring.[15]

Mean recoveries from fortified blank matrices ranged from 84.2 to 96.9% with repeatability (RSD) of 3.4–5.4% (Table 3), values that satisfy the performance criteria for aflatoxins at these concentrations.[21] Recovery was consistent across both matrices and both fortification levels, indicating that analyte losses during partitioning, solvent exchange and filtration were small and reproducible.

Comparison with previously reported methods

Table 4 compares the analytical characteristics of the proposed method with those of established chromatographic procedures for aflatoxin determination in nuts and dried fruits. The internationally standardised approach, based on immunoaffinity column cleanup with post-column derivatisation, achieves a limit of quantification of 8 μg kg^{-1} for total aflatoxins in cereals, nuts and derived products,[23] which is more than an order of magnitude higher than the 0.3–0.6 μg kg^{-1} obtained here, although laboratory implementations of the same principle reach considerably lower limits.[24] Immunoaffinity cleanup, however, relies on single-use antibody columns that dominate the consumable cost of the analysis and add a conditioning, loading, washing and elution sequence to the workflow. QuEChERS-based extraction with fluorescence detection avoids that expense and has been applied successfully to nuts and cereals,[25] but is normally combined with a dispersive solid-phase extraction cleanup. The present workflow removes that step as well, and the sensitivity, recovery and precision obtained show that, for peanut and raisin matrices, doing so does not compromise the quality of the determination.

Table 3. Recovery and repeatability of the proposed method in blank peanut and raisin matrices fortified at two concentration levels.

Analyte	Matrix	Spiking level / μg kg^{-1}	Recovery / %	RSD / %	*n*
AFB1	Peanut	1.0	92.3	4.2	6
AFB1	Peanut	5.0	94.1	3.8	6
AFB1	Raisin	1.0	88.7	4.5	6
AFB1	Raisin	5.0	90.2	4.0	6
AFB2	Peanut	1.0	89.5	5.1	6
AFB2	Peanut	5.0	91.8	4.6	6
AFB2	Raisin	1.0	85.9	5.4	6
AFB2	Raisin	5.0	87.3	4.9	6
AFG1	Peanut	1.0	93.6	3.7	6
AFG1	Peanut	5.0	95.2	3.4	6
AFG1	Raisin	1.0	89.1	4.1	6
AFG1	Raisin	5.0	91.4	3.9	6
AFG2	Peanut	1.0	86.2	4.8	6
AFG2	Peanut	5.0	88.7	4.3	6
AFG2	Raisin	1.0	84.5	5.2	6
AFG2	Raisin	5.0	86.0	4.7	6

Occurrence of aflatoxins in peanuts and raisins

The validated method was applied to 30 market samples. Total aflatoxin concentrations in peanuts ranged from below the limit of quantification (<LOQ) to 6.27 μg kg^{-1}, with a mean of 2.84 μg kg^{-1}, whereas raisins ranged from <LOQ to 3.29 μg kg^{-1}, with a mean of 1.67 μg kg^{-1}. The overall mean across all samples was 2.25 μg kg^{-1} (Table 5, Figure 3). Individual congener concentrations are provided in the Supplementary Material.

Peanuts were more contaminated than raisins, with a mean concentration approximately 1.7-fold higher, and the highest peanut result (6.27 μg kg^{-1}) was about 1.9-fold above the highest raisin result

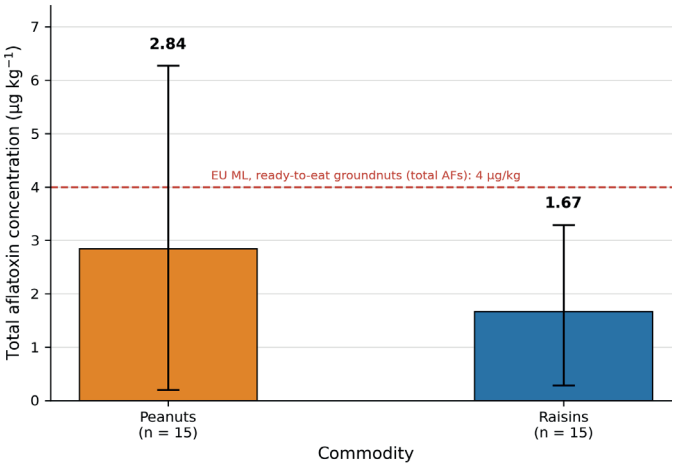


Figure 3. Mean total aflatoxin concentrations in peanut and raisin samples (*n* = 15 per commodity) determined by the proposed simplified QuEChERS–HPLC–FLD method. Error bars show the observed minimum–maximum range; the dashed line marks the European Union maximum level of 4 μg kg^{-1} for total aflatoxins in groundnuts intended for direct human consumption.

Table 4. Comparison of the proposed method with previously reported methods for the determination of aflatoxins in nuts and dried fruits.

Matrix	Extraction and cleanup	Detection	LOD (LOQ) / μg kg^{-1}	Recovery/%	Reference
Cereals, nuts and derived products	Immunoaffinity column	HPLC–FLD, post-column derivatisation	(8, total AFs)		23
Peanut kernels	Immunoaffinity column	HPLC–FLD	0.06–0.15 (0.20–0.50)		24
Nuts, cereals and dried foods	QuEChERS, methanol/acetonitrile (60:40, v/v)	HPLC–FLD		71.8–107.7	25
Peanuts, raisins	QuEChERS, no d-SPE cleanup	HPLC–FLD	0.2–0.4 (0.3–0.6)	84.2–96.9	This work

Table 5. Occurrence of total aflatoxins in peanut and raisin samples collected from commercial markets in the Syrdarya region, Uzbekistan.

Commodity	*n*	Positive samples/%	Range/μg kg^{-1}	Mean/μg kg^{-1}	Samples above ML[a]
Peanuts	15	Not reported	<LOQ–6.27	2.84	≥1
Raisins	15	Not reported	<LOQ–3.29	1.67	0
All samples	30	Not reported	<LOQ–6.27	2.25	≥1

[a] ML, European Union maximum level for total aflatoxins: 4 μg kg^{-1} for groundnuts intended for direct human consumption and 10 μg kg^{-1} for ready-to-eat dried fruit.[15] LOQ, limit of quantification.

(3.29 µg kg^{-1}). The difference is consistent with the differing matrix composition, water activity and susceptibility of the two commodities to Aspergillus colonisation, and with the influence of temperature, moisture and storage duration on aflatoxin biosynthesis.[4,6,14] Considerable variability was observed between individual samples of the same commodity, which is characteristic of the heterogeneous nature of fungal colonisation and reinforces the importance of representative sampling and thorough homogenization.[21]

From a food-safety perspective, the highest peanut result (6.27 µg kg^{-1}) exceeded the European Union maximum level of 4 µg kg^{-1} for total aflatoxins in groundnuts intended for direct human consumption.[15] Thus, at least one of the analysed peanut samples exceeded this regulatory threshold. Because chronic dietary exposure to aflatoxins, and to AFB1 in particular, is associated with hepatocellular carcinoma,[7,11,12] this finding supports the need for systematic surveillance of peanuts on local markets. Concentrations of the individual congeners in all 30 samples are given in the Supplementary Material.

Methodological significance of omitting the d-SPE cleanup step

A schematic overview of the complete workflow is shown in Figure 4.

The performance obtained here indicates that, for peanut and raisin matrices, solvent partitioning combined with selective chromatographic separation and fluorescence detection can provide acceptable analytical performance without the d-SPE cleanup step. Dispersive solid-phase extraction is conventionally used in QuEChERS workflows to reduce matrix-derived interference, and the present results suggest that its necessity depends on the matrix–analyte combination rather than being universal.[13,19] In the present workflow, omitting d-SPE removed an additional cleanup operation and its associated sorbent consumption, while recovery, repeatability and chromatographic selectivity remained within the performance demonstrated in this study. Quantitative claims regarding time or cost savings should be made only when supported by recorded comparative data.

This should not be taken as evidence that cleanup is dispensable for all food matrices. Rather, it illustrates that the extent of sample preparation can be adapted to a specific matrix–analyte combination and then verified experimentally through recovery, precision, selectivity and sensitivity assessments.[13,18,19,21,22] Pigment-rich, high-lipid or high-sugar matrices may still require cleanup, and the conclusion drawn here is confined to the two commodities studied.

CONCLUSION

A simplified QuEChERS–HPLC–FLD method was developed and validated for the determination of AFB_1, AFB_2, AFG_1 and AFG_2 in peanuts and raisins. The method gave baseline separation within 16 min, coefficients of determination between 0.9998 and 0.9999, limits of detection of 0.2–0.4 µg kg^{-1}, limits of quantification of 0.3–0.6 µg kg^{-1} and mean recoveries of 84.2–96.9%, without the dispersive solid-phase extraction cleanup step of the original QuEChERS protocol. Removing that step shortened sample preparation and reduced reagent consumption and cost without measurable loss of analytical performance for these two matrices.

Application to 30 market samples from the Syrdarya region confirmed natural aflatoxin contamination in both commodities, with peanuts approximately 1.7-fold more contaminated than raisins on average and at least one peanut sample exceeding the European Union maximum level for total aflatoxins in groundnuts intended for direct human consumption. These findings indicate a need for systematic monitoring of peanuts on local markets. Further work should confirm positive findings by mass spectrometry, extend validation to additional matrices and evaluate integration of the workflow with automated, higher-throughput systems.

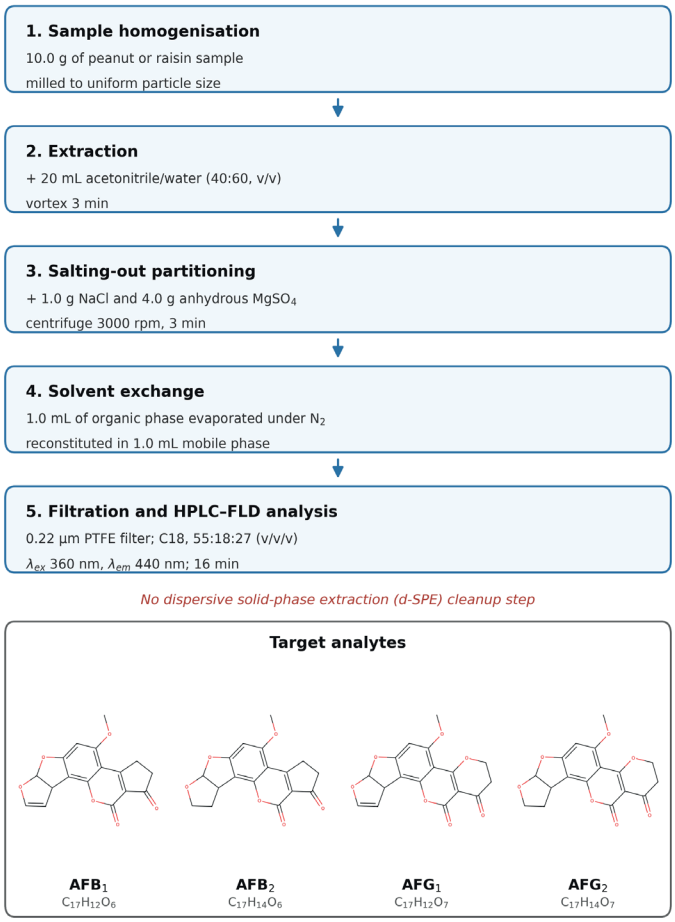


Figure 4. Schematic overview of the simplified QuEChERS–HPLC–FLD workflow, comprising sample homogenisation, extraction and salting-out partitioning, centrifugation, solvent exchange, membrane filtration and chromatographic determination. Structures of the four target analytes are shown below the workflow.

SUPPLEMENTARY MATERIAL

Supplementary material accompanying this article contains representative HPLC–FLD chromatograms of the analysed peanut and raisin samples, raw calibration data for all four analytes, replicate data from the recovery and repeatability experiments, the concentrations of AFB_1, AFB_2, AFG_1 and AFG_2 in each of the 30 samples, and retention-time reproducibility data.

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AUTHOR CONTRIBUTIONS

K.H.K.: conceptualisation, writing – original draft, writing – review and editing, supervision, project administration. A.A.T.: conceptualisation, methodology, investigation, formal analysis, validation. T.A.D.: methodology, validation, investigation. U.M.S.: formal analysis, data curation. Sh.K.T.: methodology, investigation. Kh.A.E.: investigation. All authors have read and agreed to the submitted version of the manuscript.

DECLARATION OF COMPETING INTERESTS

The authors declare no competing financial or personal interests. This study did not involve human participants or animals; all procedures were conducted in accordance with institutional and international guidelines for food safety analysis.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript the authors used an AI-assisted language tool to improve the English language, grammar, readability and organisation of the text. The tool was not used to generate experimental data, perform statistical or analytical calculations, interpret experimental results or formulate scientific conclusions. All scientific content, data, interpretations and conclusions were reviewed and verified by the authors, who take full responsibility for the final content.

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