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Extraction Procedures Reshape the Detectable Fumonisin Chemical Space of Fusarium verticillioides across Culture Media

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Abstract

Fusarium verticillioides produces fumonisins and numerous secondary metabolites, yet extraction procedures may strongly shape their analytical detection. We combined targeted and untargeted LC–HRMS approaches to compare ethyl acetate, acidified ACN/H_2O, and sequential ACN/H_2O extraction across four culture media. Extraction-dependent metabolite profiles varied markedly with both culture medium and metabolite structure. Acidified ACN/H_2O procedures generated the most consistent fumonisin B1 (FB1) signals, whereas ethyl acetate yielded weak FB1 responses but revealed complementary subsets of metabolites, including fusarin A, fusaric acid, bikaverin-related compounds, and several fumonisin-related features. Structurally related fumonisins displayed heterogeneous solvent-dependent responses, and sequential extraction provided no systematic advantage over single ACN/H_2O extraction. These findings demonstrate that extraction procedures reshape the detectable chemical space of F. verticillioides and support objective-driven solvent selection for fungal metabolomics and mycotoxin profiling.

1. Introduction

Mycotoxin contamination represents a persistent global food safety challenge, and *Fusarium verticillioides* is a major fungal contaminant of maize and other cereal crops worldwide. This pathogen produces, among others, fumonisins, a class of structurally diverse mycotoxins that have been associated with adverse health outcomes, including esophageal cancer and neural tube defects for fumonisin B1, while experimental studies have also documented hepatotoxic, nephrotoxic and immunotoxic effects (Azman et al., 2020 ; Gao et al., 2023 ; Ji et al., 2019). Given their toxicological importance and widespread occurrence, accurate detection and comprehensive profiling of *F. verticillioides* mycotoxins are fundamental to effective food safety monitoring and regulatory enforcement. The fumonisin family is characterized by a 20-carbon backbone, bearing a variable number of hydroxyl groups and esterified with tricarballylic acid (TCA) or cis-acotinic acid (AA) moieties (Bartok et al., 2010). Beyond fumonisins, *F. verticillioides* produces several other families of secondary metabolites, including fusarins, bikaverin and its biosynthetic precursors, and fusaric acid (Kleigrewe et al., 2012 ; dos Santos et al., 2021 ; Benito-Fuertes et al., 2026). Although these compounds are not all classified as major mycotoxins, several have reported biological or toxicological activities and may contribute to the broader chemical burden associated with fungal contamination. In this context, multi-mycotoxin detection strategies have become essential, as food and feed are often co-contaminated with multiple mycotoxin classes that may have additive or synergistic toxic effects (Kovalsky et al., 2016 ; Lee et al., 2025). In addition, modified and structurally related mycotoxin forms remain analytically challenging because they may not be adequately captured by conventional targeted methods, potentially contributing to an incomplete characterization of fungal metabolite profiles (Lapris et al., 2024). Acetonitrile–water mixtures, often acidified with formic acid, are among the most widely used extraction systems for multi-mycotoxin LC–MS analysis (Azman et al., 2020 ; Mackay et al., 2022 ; Shanakhat et al., 2018 ; Škrbić et al., 2014). However, emerging evidence from studies using liquid chromatography–tandem mass spectrometry (LC–MS/MS) and high-resolution mass spectrometry (LC–HRMS) for the screening of modified, emerging and non-regulated mycotoxins highlights significant limitations of conventional extraction procedures. These limitations are particularly relevant for the comprehensive recovery and detection of structurally diverse and co-occurring toxins, including Alternaria toxins, enniatins, beauvericin and modified fumonisin derivatives.

Recent suspect- and non-target-screening strategies based on LC–HRMS have enabled the tentative identification and preliminary categorization of previously overlooked parent and modified mycotoxins in complex food matrices (Righetti et al., 2016 ; Puntscher et al., 2018 ; Steiner et al., 2022 ; Zhang et al., 2023 ; Bessaire et al., 2024). Assuming that a standardized protocol provides equivalent analytical coverage across structurally diverse and co-occurring metabolites may lead to incomplete detection and, in quantitative applications, to an underestimation of contamination (Qu et al., 2024 ; Zhao et al., 2020 ; Andrade et al., 2013).

To address this knowledge gap, we combined targeted and untargeted metabolomic approaches to evaluate how different extraction procedures shape the LC–HRMS profiles obtained from *F. verticillioides* grown on contrasting culture media. Our experimental design compared three distinct extraction protocols—ethyl acetate (EtOAc), ACN/H_2O/FA (v:v:v 80/19.9/0.1), and a sequential ACN/H_2O/FA double extraction (v:v:v, first 80/19.9/0.1, then 20/79.9/0.1)—across four culture media: Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Czapek's Agar (CZA), and Malt Extract Agar (MEA). CMA was selected as a maize-based medium known to promote fumonisin production. In contrast, PDA and MEA are widely used rich media differing in nutrient composition whereas CZA is considered a more defined and nutritionally limited medium. This design enabled extraction-dependent analytical responses to be compared across contrasting nutritional environments that generate distinct fungal metabolite repertoires. By combining LC–HRMS profiling with multivariate and univariate statistical analyses, we characterized how extraction procedure and culture medium jointly shape the detectable metabolite profile of *F. verticillioides*.

This metabolomic investigation examines how solvent composition, metabolite structure, and culture-medium composition jointly influence the chemical profiles detected by LC–HRMS. Rather than seeking a universally optimal extraction protocol, the study provides a controlled experimental basis for comparing solvent-dependent analytical responses within different culture media. Because the observed signals may reflect a combination of metabolite production, extractability, matrix effects, and ionization efficiency, these findings should be considered a first step toward objective-driven extraction strategies requiring further validation in naturally contaminated food and feed matrices.

2. Materials and Methods

2.1. Extraction solvents and mycotoxin standards.

All solvents used for chromatography were of HPLC grade. Ultrapure water (18.2 MΩ·cm) was obtained using a Milli-Q RG system (Millipore, France). Three extraction systems were evaluated: solvent 1 (S1), 100% ethyl acetate (EtOAc; Fisher Chemical); solvent 2 (S2), acetonitrile/water/formic acid (ACN/H_2O/FA, 80/19.9/0.1, v/v/v), prepared from acetonitrile (Fisher Chemical), demineralized water and formic acid (> 98%; Sigma-Aldrich, St. Louis, MO, USA); Solvent 3 (S3) consisted of two successive extractions, first with 30 mL of ACN/H_2O/FA (80/19.9/0.1, v/v/v), followed by 30 mL of ACN/H_2O/FA (20/79.9/0.1, v/v/v). Mycotoxin standards of fumonisin B1 (FB1) and fumonisin B2 (FB2) were purchased from Libios (Vindry-sur-Turdine, France).

2.2. Fungal material and culture conditions

The *Fusarium verticillioides* strain INRA63 (MycSA-INRAE, Bordeaux, France) was cultivated on malt extract agar (MEA) to prepare the conidial inoculum. Four culture media were used for extraction assays: MEA, potato dextrose agar (PDA; Difco™), Czapek agar (CZA; Difco™) and cornmeal dextrose agar (CMA). For the extraction assays, *F. verticillioides* was cultured in Petri dishes containing 25 mL of MEA, PDA, CZA, or CMA and incubated for 14 days at 27°C and 80% relative humidity in darkness. Relative humidity was set at 80% to support stable fungal growth while preventing excessive desiccation or condensation. These controlled conditions were selected to ensure reproducible secondary metabolite production rather than to mimic natural or food-related environments. For each condition, three independent biological replicates were performed, corresponding to *F. verticillioides* cultures grown in separate Petri dishes under identical conditions.

Experimental variability was controlled by using predefined culture and extraction conditions, independent biological replicates, pooled QC samples, and identical UHPLC–HRMS acquisition parameters across samples.

2.3. Chemical extraction of metabolites.

For each medium and extraction condition, metabolites were extracted with 30 mL of solvent. For S1 and S2, samples were agitated for 30 min and sonicated for 30 min, after which 1 mL of supernatant was collected and centrifuged at 12,000 x g for 10 min. For S3, a first extraction was performed with ACN/H_2O/FA (80/19.9/0.1, v/v/v), followed by 30 min agitation and 30 min sonication. A 500 µL aliquot of supernatant was collected. After complete removal of the first extraction supernatant, the residual culture material was subjected to a second extraction with ACN/H_2O/FA (20:79.9:0.1, v/v/v) under identical conditions. A further 500 µL aliquot was collected and combined with the first aliquot before centrifugation. A pooled quality control (QC) sample was prepared for each medium by combining 10 µL of each extract. This QC sample was injected every 10 samples throughout the UHPLC-HRMS sequence to monitor instrument stability and analytical reproducibility.

2.4. LC–HRMS Profiling.

UHPLC–HRMS analyses were performed using an Ultimate 3000 UHPLC system (Dionex) coupled to an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK). Chromatographic separation followed the previously established protocol described by Groppi et al. (2024, 2025). A Zorbax Eclipse XDB-C18 column (2.1 × 150 mm, 3.5 µm; Agilent Technologies, Santa Clara, CA, USA) was used. The mobile phases consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The gradient was run at 0.3 mL/min as follows: 95% A to 95% B from 0 to 10 min, hold at 95% B from 10 to 12.5 min, increase to 100% B for 1 min, then return to 95% A from 13.5 to 14 min and hold until 17 min. The column temperature was set at 40°C, the autosampler at 15°C, and the injection volume at 5 µL. Mass spectrometry data were acquired in positive electrospray ionization mode (ESI+) at 15,000 resolving power (FWHM at *m/z* 400), over an *m/z* range of 100–2000. Spray voltage was set at 3.5 kV and capillary temperature at 300°C. Each full MS scan was followed by data-dependent MS/MS acquisition of the three most intense ions using stepped collision-induced dissociation (CID) at 35 arbitrary units. Dynamic exclusion was set to 6 s.

2.5. Data Processing.

LC–MS/MS data were processed using the MS-CleanR workflow according to previously established metabolomic procedures from our group (Groppi et al. 2025). Positive-ionization (ESI+) datasets were first processed with MS-DIAL v5.1 in centroid mode. MS1 and MS2 tolerances were set to 0.01 and 0.2 Da, respectively. Signals were considered between 0 and 17 min and from 100 to 1500 Da, and the minimum peak height was set to 1×10^6. Peak alignment was performed using a QC reference file with retention time and MS1 tolerances of 0.1 min and 0.025 Da, respectively. The aligned feature tables were then cleaned with MS-CleanR using default settings except for the maximum relative standard deviation (RSD), which was set to 40. All filters were activated, the minimum blank ratio was set to 0.8, and the relative mass defect (RMD) range was restricted to 50–3000. The maximum mass and retention time differences were set to 0.005 Da and 0.025 min, respectively. Feature clustering was performed using Pearson correlation with a minimum correlation threshold of 0.8 and $\alpha = 0.05$. Two features per cluster were retained: the most intense one and the one showing the highest connectivity to other ions. The filtered dataset was annotated with MS-Finder v3.52 using MS1 and MS2 tolerances of 5 and 15 ppm, respectively. Formula generation included C, H, O, N, and Cl as permitted elements. Annotation levels were assigned according to the framework proposed by Schymanski et al. (2014). Level 1 (confirmed annotation) corresponded to confirmation with authentic standards analyzed under identical UHPLC-HRMS conditions, based on retention time (RT tolerance of ± 0.1 min), accurate mass (mass error < 5 ppm) and MS/MS fragmentation pattern. Level 2 (probable structure) corresponded to spectral similarity matches with public MS/MS libraries (MassBank, GNPS, MS-Dial) using Metgem. Level 3 annotations (tentative candidates) were assigned based on database matching using MS-Finder, supported by curated Dictionary of Natural Products databases: 3.1. *F. verticillioides* metabolites, 3.2. *Fusarium* spp. metabolites, 3.3. Mycotoxins and 3.4. MS-Finder generic databases (ChEBI, NPA, NANPDB, COCONUT, KNApSAcK, PubChem, UNPD and T3DB). All features passing the MS-CleanR filtering criteria were retained for untargeted statistical analyses. Annotation efforts were subsequently focused on Fusarium-derived secondary metabolites and mycotoxin-related features, which were manually curated using accurate mass and MS/MS evidence. Features showing diagnostic MS/MS fragmentation consistent with published experimental spectra were assigned a probable structure when the available evidence was sufficient, whereas isomeric structures that could not be distinguished unambiguously were retained as tentative annotations. All annotated features (Rt, *m/z*, annotation level and peak area) were exported as .csv files and integrated into an internal database.

2.6. Visualization of selected metabolites

For targeted visualization of representative metabolites, four canonical features corresponding to FB1, fusarin A, fusaric acid and bikaverin were extracted from the processed dataset using medium-specific feature identifiers. For each culture medium and extraction solvent, peak areas were summarized as mean ± SD and plotted together with individual sample values. This section is therefore better interpreted as a relative signal comparison rather than absolute quantification.

Because the study was designed as a comparative metabolomics experiment rather than as a fully validated quantitative method, extraction-dependent responses were assessed using relative LC–HRMS signal intensities. Accordingly, the results are described in terms of relative signal, detectability, or analytical response rather than absolute extraction recovery. Full method validation, including spike-recovery

experiments, linearity, precision, matrix effects, and LOD/LOQ determination, will be required before quantitative application to food or feed matrices.

2.7. Multivariate and univariate statistical analyses

All statistical analyses were performed in R using feature tables derived from the processed LC–MS data. For each culture medium, the data were reshaped into sample × feature matrices, and QC samples were either retained for quality assessment or removed for biological interpretation. Prior to statistical analysis, intensities were square-root transformed [sqrt(x + 1)] and features with zero variance were removed. PCA was then performed separately for each culture medium on centered and scaled data and was used exclusively as an exploratory tool to visualize sample structure. To test whether extraction solvent affected the overall metabolomic structure within each medium, PERMANOVA was applied separately for each culture medium using Euclidean distances computed from centered and scaled sqrt-transformed data. Significance was assessed with 9,999 permutations. Homogeneity of multivariate dispersion was evaluated for the same datasets using betadisper, followed by permutation testing. Both PCA and PERMANOVA/dispersion analyses were generated with and without QC samples.

Univariate analyses were performed feature by feature within each culture medium after QC removal using a one-factor design (y ~ extraction). After square-root transformation and removal of zero-variance variables, residual normality and homoscedasticity were assessed using the Shapiro–Wilk and Levene tests ($\alpha = 0.05$). Features fulfilling both assumptions were analysed by one-way ANOVA; otherwise, Kruskal–Wallis tests were used. P values from the global tests were adjusted using the Benjamini–Hochberg (BH) procedure. Pairwise post hoc comparisons were computed using BH-adjusted estimated marginal means contrasts after ANOVA and BH-adjusted pairwise Wilcoxon tests after Kruskal–Wallis analysis. Given the limited number of biological replicates per condition, feature-level inferential analyses were considered exploratory, and effect sizes and consistency across complementary analyses were emphasized alongside adjusted P values.

To support global visualization of abundance patterns, heatmaps were generated separately for each medium from feature × sample matrices after removal of all-zero features, log10(area + 1) transformation and row-wise z-score scaling. Additional summary heatmaps were built from the top 10 features contributing to PC1 and PC2 in each medium; these were displayed as group means for each medium × extraction combination, again after row-wise z-score scaling, and were annotated according to feature annotation level and source medium.

Integrative volcano-style plots were constructed separately for each culture medium by combining pairwise post hoc results with PCA loadings. For each medium, log2 fold changes were calculated from mean log2-transformed intensities after addition of a pseudocount defined as half of the smallest positive value observed in that medium. Two contrasts relative to S2 were displayed (S1 vs S2 and S3 vs S2). Pairwise P values were corrected by the BH procedure within each contrast and plotted as $-\log10(q)$. Point size was weighted according to PCA importance, calculated as $\sqrt{(PC1^2 + PC2^2)}$, and features were highlighted using $q < 0.05$ and $|\log2FC| \geq 1$ thresholds.

In a FAIR approach, a run log was generated for each statistical workflow and is provided in the Supplementary Information.

2.8 Molecular network construction and visualization

The .MGF file generated through the MS-DIAL/MS-CleanR/MS-FINDER workflow, together with the corresponding ESI^+ metadata table, was imported into MetGem v1.4.3 (Olivon et al. 2018 ; Elie et al., 2019). The resulting molecular network (MN) was visualized and manually curated in Cytoscape v3.8.2 version. For cosine score computing, the *m/z* tolerance was set to 0.2 Da and spectra sharing a minimum of 4 matching peaks were kept. MS/MS spectra were filtered and fragment ions below m/z 50 were removed. All peaks in the ± 17 Da range around the precursor *m/z* were removed. The MN was created with a cosine score above 0.7. Further edges between two nodes were kept if each of the nodes appeared in each other's respective top 10 most similar nodes. Edge width depends on the cosine score between two related molecules. The nodes are filled with a pie chart representing normalized area mean value of each molecule detected across the different culture conditions.

3. Results and discussion

3.1. Global LC–HRMS profiles reveal marked solvent-dependent differences across culture media

Raw LC–HRMS chromatographic profiles acquired in positive ionization mode showed clear differences among extraction solvents across the four culture media (CMA, CZA, MEA and PDA) (Fig. 1). Overall, extracts obtained with solvents 2 and 3 displayed broader and more intense chromatographic profiles than those obtained with solvent 1, although the magnitude of these differences varied depending on the culture medium. In most cases, solvents 2 and 3 produced comparable overall profiles, while solvent 1 yielded fewer and lower-intensity signals.

These findings are consistent with previous studies on solvent optimization for mycotoxin extraction. Acetonitrile-based extraction systems have been reported to provide high recoveries for polar mycotoxins, particularly when combined with acidified aqueous phases (Trebstein et al., 2009). The observed differences in chromatographic complexity between solvent systems are also in line with reports indicating that the analytical profiles obtained from fungal cultures can vary substantially with solvent polarity and matrix composition (Spanjer et al., 2008).

3.2. Extraction solvent shapes the metabolomic structure in a medium-dependent manner

Principal component analysis (PCA) performed separately for each culture medium revealed a clear solvent-dependent structuring of the LC–HRMS datasets, although the strength and geometry of this structuring varied among media (Fig. 2). In CMA, the three extraction systems occupied distinct regions of the PCA score space, with S1 clearly distinct from the two ACN-based extraction procedures and an additional separation between S2 and S3. A comparable exploratory pattern was observed in CZA, where the three solvent systems also occupied distinct regions of the ordination space. In MEA, S1 was strongly separated from S2 and S3, whereas the latter remained closer to each other despite incomplete overlap. In PDA,

solvent-dependent organization was also visible, although the separation between S2 and S3 was less marked than in the other media.

The application of PCA to assess extraction-related differences is well established in metabolomics studies. Similar multivariate approaches have been used to evaluate extraction protocols in multi-mycotoxin analysis, showing that PCA effectively reveals systematic biases introduced by different extraction conditions (Sulyok et al., 2007). The medium-dependent variation observed here is also consistent with previous reports demonstrating that culture medium composition significantly influences both metabolite production and analytical extraction profiles in fungal isolates (Van Pamel et al., 2011).

These exploratory patterns were supported by PERMANOVA, which detected a significant effect of extraction solvent within each culture medium after QC removal (Table 1). The corresponding tests of multivariate dispersion were not significant in any of the no-QC datasets. The PERMANOVA effect size was highest in CMA and lower in CZA, MEA and PDA, indicating that extraction solvent significantly influences the global metabolomic structure of *F. verticillioides* extracts, but that the magnitude and configuration of this effect depend on the culture medium. The use of PERMANOVA to assess multivariate differences in metabolomics datasets is well established (Anderson, 2001). The variation in effect size across media suggests that the analytical consequences of changing the extraction procedure depend on the culture medium (Nielsen and Smedsgaard, 2003). More broadly, the results indicate that the observed LC–HRMS profiles reflect interactions among fungal metabolite production, solvent-dependent extractability, matrix composition, and ionization response Sulyok et al., 2006, 2020 and 2024 ; Desai et al, 2026).

Table 1
Summary of PERMANOVA and multivariate dispersion tests performed separately for each culture medium after QC removal. Significant PERMANOVA effects were detected in all media, while dispersion tests remained non-significant, supporting interpretation of the observed multivariate differences.

Medium	n samples	n features	R^2 (%)	F PERMANOVA	p PERMANOVA	p dispersion	Interpretation
CMA	9	274	79.7	11.769	0.004 **	0.128 ns	Multivariate difference detected without heterogeneity of dispersion: robust interpretation
CZA	9	227	61	4.692	0.014 *	0.769 ns	Multivariate difference detected without heterogeneity of dispersion: robust interpretation
MEA	9	280	62.9	5.082	0.004 **	0.136 ns	Multivariate difference detected without heterogeneity of dispersion: robust interpretation
PDA	15	191	52.5	6.63	< 0.001 ***	0.735 ns	Multivariate difference detected without heterogeneity of dispersion: robust interpretation

The main information provided by these global profiles is not simply that ACN-based extracts produced stronger signals, but that each medium generated a different solvent-dependent chemical background. These global profiles reflect two successive sources of variation: the metabolite repertoire produced by *F. verticillioides* on each culture medium and the subsequent ability of each extraction procedure to solubilize and render this repertoire detectable by LC–HRMS.

3.3. Acidified ACN–water systems yield consistently higher FB1 signals across culture media

To complement the global multivariate analyses, four representative metabolites were examined individually across the four culture media and three extraction procedures: fumonisin B1 (FB1), fusarin A, fusaric acid, and bikaverin (Fig. 3). These metabolite-level profiles showed that the solvent-dependent differences

observed at the whole-dataset level were also apparent for individual compounds, although their direction and magnitude depended on both the metabolite and culture medium. FB1 displayed the clearest and most consistent pattern. In CMA, MEA, and PDA, S2 and S3 produced markedly higher FB1 signals than S1. FB1 was not detected in CZA with any extraction procedure; this absence is therefore consistent with limited FB1 production under the tested culture conditions rather than with a solvent-specific analytical effect. Overall, the two ACN-based procedures yielded comparable FB1 responses, with no consistent advantage of S3 over S2.

This behavior is consistent with the physicochemical properties of fumonisins, which are highly polar molecules bearing multiple hydroxyl groups, tricarballylic acid moieties and protonable amine functions (Rheeder et al., 2002). Their high polarity and ionizable functional groups may favor their detection following extraction with acidified aqueous organic mixtures. Therefore, the higher FB1 signals observed with acidified ACN–water may result from a combination of enhanced solubilization, reduced analyte–matrix interactions and favorable ESI+ response (Gelderblom et al., 1988). these mechanisms were not separately evaluated in the present study.

The suitability of acetonitrile-based mixtures for fumonisin extraction is well established, with high recoveries reported in maize and other cereal matrices (Şenyuva et al., 2008 ; Vega et al., 2016). In the present study, S2 and S3 generated comparable FB1 signals, suggesting that the additional extraction step used in S3 did not provide a systematic analytical advantage. This suggests that the observed analytical response is not governed solely by solvent composition, but also by metabolite accessibility and matrix interactions.

3.4. Solvent-dependent responses of fusarin A, fusaric acid, and bikaverin vary among culture media

In contrast to FB1, fusarin A, fusaric acid, and bikaverin displayed more variable and culture-medium-dependent responses (Fig. 3). Fusarin A generated higher signals with S1 in CMA, CZA, and MEA, whereas its highest signal in PDA was observed with S3, with S2 showing intermediate values. Fusaric acid displayed a contrasting pattern: in CZA, the highest signals were obtained with S2 and S3, whereas in MEA it was more abundant in S1 extracts. In CMA and PDA, fusaric acid remained low or undetectable under all extraction conditions. Bikaverin showed similarly contrasting responses, with high signals in S1 extracts from CMA and MEA, but markedly higher responses with S3, and to a lesser extent S2, in PDA.

These results indicate that ethyl acetate should not be reduced to a broadly inefficient extraction solvent; a more accurate interpretation is that it is selective rather than broadly efficient. Ethyl acetate is a moderately polar organic solvent that may preferentially solubilize relatively lipophilic or weakly ionized metabolites (Biselli and Hummert, 2005). The higher signals observed for bikaverin and related features in several S1 extracts are therefore consistent with their aromatic and comparatively low-polarity structures. Several fusarin-related metabolites exhibit relatively hydrophobic and conjugated structural features that may favor partitioning into organic phases (Gelderblom et al., 1984). In the case of fusaric acid, its extraction behavior likely reflects its amphiphilic nature, with solvent preference depending on its ionization state and the physicochemical properties of the surrounding matrix. The selective behavior of ethyl acetate has also been reported in multi-mycotoxin analysis, where it preferentially extracts less polar *Fusarium* metabolites while

showing lower efficiency for highly polar compounds such as fumonisins (Royer et al., 2004) This selectivity can be advantageous for targeted analysis of specific metabolite classes but may limit comprehensive metabolomic coverage.

Taken together, these targeted comparisons show that solvent-dependent analytical responses cannot be generalized across metabolites or culture media. While the ACN-based procedures consistently produced higher FB1 signals, other metabolites displayed extraction-dependent patterns that varied with the culture medium. These findings are consistent with a solvent × medium × metabolite interaction rather than with a single universally optimal extraction system (Krska et al., 2008).

3.5. Medium-specific feature subsets drive solvent discrimination

To identify the variables underlying the PCA-based separation, a summary heatmap was generated from the top features contributing to PC1 and PC2 in each culture medium (Fig. 4). This integrative visualization showed that the variables driving sample discrimination were largely medium-specific rather than shared across all conditions. Features selected from each medium tended to display their highest relative abundances within the corresponding medium block, indicating that the principal sources of variance differed according to the growth substrate. Within these medium-specific feature sets, clear extraction-dependent abundance shifts were also observed. Several annotated ions contributed to these patterns, together with numerous unannotated features.

The contrasting responses observed across media suggest that solvent-dependent analytical differences arise from interactions among metabolite chemistry, culture-medium composition, carbon source availability, possibly medium-dependent pH or ionic properties, extractability, and ionization behavior and may alter both fungal metabolism and the accessibility of metabolites to a given solvent system. The same metabolite did not necessarily respond similarly across all media, and conversely, a given solvent did not enrich the same feature families in all contexts (Smedsgaard, 1997). This may help explain, for example, why FB1 was not detected in CZA extracts under the tested conditions, whereas fusaric acid signals were comparatively prominent, or why bikaverin and fusarin-related signals shifted toward different solvents depending on the medium. This is consistent with previous studies in *Fusarium* showing that extraction solvent polarity substantially affects both the number of detected UHPLC–HRMS features and their distribution across the chemical polarity range (Behner et al., 2025). In addition, the non-detection of certain metabolites in specific media may reflect medium-dependent regulation of biosynthesis rather than extraction inefficiency, highlighting the need to distinguish between metabolite production, extractability, and analytical detectability (Groppi et al., 2024 ; Frisvad and Filtenborg, 1983).

3.6. Cross-methodological analyses reveal selective extraction biases and heterogeneous behavior within the fumonisin family

To combine effect size, statistical significance and multivariate contribution in a single representation, volcano-style plots were generated separately for each culture medium by combining pairwise univariate

comparisons with PCA loadings (Fig. 5).

Across media, the distribution of significant features differed markedly between contrasts and culture conditions. In CMA, the S1 vs S2 comparison revealed a broad set of fumonisin-related features among the most discriminant signals, but these did not shift uniformly in one direction. Several features tentatively annotated as fumonisin-related compounds, including iso-fumonisin B1 (m/z 722.3962; Rt 7.826), fumonisin C4 (m/z 676.3908; Rt 6.785), fumonisin B4 2AA (m/z 686.3747; Rt 7.391), and fumonisin A2 (m/z 748.4118; Rt 7.421), were located on the positive side of the plot, indicating higher relative signal intensity in S1 than in S2. In contrast, other fumonisin-related derivatives, such as fumonisin C1 (m/z 708.3818; Rt 5.762) and fumonisin B1 AA,TCA (m/z 720.3807; Rt 5.897), were shifted toward negative log2FC values, indicating higher abundance in S2. The heterogeneous behavior within the fumonisin family has been previously documented. Falavigna et al. (2012). demonstrated using LC/ESI-MS/MS that different fumonisin analogues exhibit distinct extraction patterns, with structural modifications (hydroxylation, esterification, partial hydrolysis) significantly affecting polarity and extraction efficiency. This structural diversity within the fumonisin family necessitates careful consideration of extraction conditions depending on the analytical target.

These opposite trends suggest that extraction differences in CMA were compound-dependent rather than consistent across the whole fumonisin family. By contrast, the corresponding S3 vs S2 panel for CMA contained fewer strongly significant annotated features, suggesting more limited differences between the two ACN-based procedures. In CZA, the most prominent signal in the S1 vs S2 comparison was the positive displacement of fusarin A (*m/z* 416.2070; Rt 7.918) and its $[M + Na]^+$ adduct (*m/z* 438.1888; Rt 8.365), indicating a higher relative signal in S1 than in S2. The S3 vs S2 contrast showed very few highly discriminant features. In MEA, the S1 vs S2 comparison again highlighted several fumonisin-related derivatives among the most discriminant signals, including fumonisin B2,3 AA,TCA (*m/z* 704.3859; Rt 5.809) and fumonisin B1 AA,TCA (*m/z* 720.3810; Rt 5.883), most of them shifted toward negative log2FC values and therefore showed higher relative signals in S2 than in S1. In PDA, the integrative plot identified fumonisin A1 (*m/z* 764.4071; Rt 5.568), fumonisin C1 (*m/z* 708.3807; Rt 5.772), and fumonisin B1 AA,TCA (*m/z* 720.3807; Rt 5.897) among the main discriminant features in the S1 vs S2 comparison, with most of these signals located on the negative side of the plot, indicating higher relative signal intensity in S2. The S3 vs S2 panel in PDA was characterized by fewer strongly significant ions and a narrower spread of effect sizes. Importantly, the features showing the strongest univariate shifts were not always those contributing most strongly to the multivariate structure, and conversely, some highly discriminant PCA features showed only moderate fold changes. This indicates that differences between extraction procedures involve both changes in relative signal intensity and changes in feature detectability, neither of which is fully captured by a single analytical metric. Within the fumonisin family, features annotated as fumonisins or fumonisin-derived forms (e.g., hydrolysed, acetylated, or TCA-conjugated variants) did not respond uniformly to solvent changes and, in several cases, displayed opposite trends depending on the derivative considered. Structural variations among fumonisin analogs (hydroxylation, esterification, partial hydrolysis) modulate polarity and charge, resulting in differential partitioning between solvent systems. Several putatively less polar or structurally modified analogues generated higher relative signals in S1, whereas highly polar compounds such as FB1 were more readily detected following extraction with acidified ACN–water mixtures. This diversity indicates

that regulated fumonisins (e.g., FB1, FB2) are not necessarily co-extracted or co-detected alongside their modified forms, and that analytical outcomes may differ substantially depending on whether the objective is targeted quantification or broader metabolomic profiling.

Moreover, although features annotated as FB2- and FB3-related compounds showed higher relative signals in S1 based on feature-level analyses, this trend was not readily visible in the raw chromatographic overlays (Fig. 1), which are dominated by highly abundant compounds such as FB1. One possible explanation is that the less complex chromatographic background observed in S1 extracts reduced ion suppression and increased the relative detectability of some lower-abundance fumonisin-related features. However, matrix effects were not directly measured in this study.

3.7. Molecular networking supports the organization of solvent-responsive features into structurally coherent clusters

In this study, a spectral similarity molecular network was used as a complementary structure-based visualization approach rather than solely as an annotation tool. It showed that several solvent-responsive features grouped into structurally coherent molecular families.

Consistent with the targeted, multivariate and integrative analyses, exploratory molecular network visualization further supported the grouping of solvent-responsive features into chemically coherent clusters, including a major fumonisin-related cluster and smaller clusters associated with bikaverin and fusaric acid (Fig. 6). This network was constructed based on detected features in *F. verticillioides* grown on CMA and extracted with the three solvent systems, where nodes correspond to individual compounds and edges reflect cosine similarity scores derived from MS/MS fragmentation patterns.

Three major molecular families were identified within the network based on spectral similarity and available annotations. The first corresponds to fumonisins and related compounds, including FB1 and FB2 standards, as well as previously characterized analogs such as FB3, FB4, FA1, FA3 and FC-series compounds. This cluster also includes partially hydrolyzed and ester-modified fumonisins. Within this group, extraction-dependent responses differed markedly, with several analogues showing higher signals in S1, whereas others, including FB1, generated higher relative responses following extraction with the acidified ACN–water systems S2 and S3. The second cluster comprises bikaverin and its precursors, and the third includes fusaric acid and its derivatives. In both cases, metabolites were predominantly detected using S1, consistent with the comparatively low polarity of these compounds and their favorable detection in ethyl acetate extracts.

Zooming into the fumonisin cluster revealed several structurally distinct subgroups. Canonical fumonisin-related features displayed heterogeneous solvent-dependent profiles. Partially hydrolyzed fumonisin-related features generally showed higher relative signals in S1, whereas FB1 and some related features produced higher responses with S2 and S3. Ester-modified fumonisin-related features similarly displayed mixed behavior, with several showing higher signals in S1 and others responding more strongly to the acidified ACN–water procedures. In addition, several nodes within the fumonisin cluster were putatively assigned to FB1-related analogues based on MS-DIAL and GNPS library information; many of these showed higher

relative signals in S1, indicating that ethyl acetate revealed a complementary subset of fumonisin-related features within the CMA dataset.

Overall, the molecular network highlights that solvent-dependent analytical responses are strongly influenced by structural variability within the fumonisin family, with ethyl acetate yielding detectable signals for a complementary subset of fumonisin-related analogs, while acidified ACN–water procedures produced higher responses for highly polar compounds such as FB1 in the CMA dataset. These patterns further indicate that the observed analytical response depends not only on metabolite structure and extractability, but also on the chemical background co-extracted from the culture medium and its potential influence on ionization.

3.8. Analytical implications for fungal metabolomics and mycotoxin profiling

From an analytical perspective, the present results support the continued use of ACN/H_2O/FA-based systems when the main objective is fumonisin-focused profiling, but they also show that such systems do not maximize all metabolites across all conditions. The targeted and untargeted approaches converged toward the same interpretation: S2 produced the most consistent FB1 signals and favorable responses for several fumonisin-related features, whereas S3 rarely provided a sufficiently clear improvement to offset its greater procedural complexity.

Matrix effects remain a significant challenge in UHPLC-HRMS mycotoxin analysis, particularly when using electrospray ionization. Fabregat-Cabello et al. (2016) demonstrated that matrix-induced ion suppression can reach up to -89% in complex matrices, emphasizing the need for appropriate compensation strategies. The authors recommended post-extraction addition, matrix-matched calibration, or isotope-labeled internal standards for quantitative correction, approaches that should be considered in future applications of the extraction protocols evaluated here.

Under the present conditions, this suggests that the additional extraction step of S3 may often be unnecessary when the analytical priority is consistent detection of FB1 and related polar fumonisins. At the same time, the broader metabolomic results show that focusing exclusively on a single targeted compound would overlook important solvent-specific biases affecting other metabolite classes. This is precisely where a metabolomics framework becomes valuable: rather than validating one solvent against one analyte only, it reveals how extraction protocols reshape the detectable chemical space as a whole. For future applications, solvent selection should therefore be guided by the analytical objective—targeted fumonisin quantification, broader secondary-metabolite profiling, or selective detection of specific compound families—rather than by the assumption that a single protocol will be optimal in every context.

3.9. Limitations and future directions

This study has several limitations that should be considered when interpreting the results. The experiments were performed on culture media rather than on real food or feed matrices. Although the controlled design enabled extraction procedures to be compared within each culture medium, it did not fully disentangle differences in fungal metabolite production, extractability, matrix-dependent ionization, and analytical detectability. Consequently, the ranking of extraction procedures observed here cannot be assumed to apply

directly to naturally contaminated cereals, processed foods, or feed matrices. In addition, S3 involved twice the total extraction volume and two agitation–sonication cycles, whereas S1 and S2 involved a single 30-mL extraction. Comparisons between S2 and S3 therefore reflect both solvent composition and differences in total solvent volume and processing intensity. Also, the targeted comparisons remain semi-quantitative and do not replace a full analytical validation including recovery, matrix effects, linearity and LOD/LOQ determination. Despite extensive annotation efforts, a substantial fraction of the solvent-responsive features remained only putatively annotated or unannotated, which limits biochemical interpretation. Future work should therefore extend this framework to real agricultural matrices such as maize, cereals or feed, where solvent–matrix interactions are likely to be even more complex. Furthermore, the limited number of biological replicates per condition also reduces the precision of feature-level statistical inference; accordingly, the univariate analyses should be considered exploratory. A full validation of the most promising extraction conditions should also be performed, especially for fumonisins and related analogs. More broadly, applying the same strategy to additional *Fusarium* metabolites and mycotoxin families would help determine whether the matrix-dependent extraction patterns identified here reflect a general principle of fungal metabolomics or are specific to the metabolite spectrum produced by *F. verticillioides* under the tested conditions.

This study demonstrates that the LC–HRMS profiles obtained from *F. verticillioides* cultures depend jointly on the extraction procedure, culture medium, and metabolite considered. Under the tested *in vitro* conditions, acidified ACN–water procedures generated the most consistent FB1 signals, whereas ethyl acetate yielded weak FB1 responses but revealed complementary subsets of detectable metabolites, including fumonisin-related features, fusarin A, fusaric acid, and bikaverin-related compounds. Structurally related fumonisin features did not respond uniformly to changes in extraction conditions, indicating that the behavior of FB1 alone cannot represent the broader fumonisin chemical space. The sequential procedure did not provide a consistent analytical advantage over the single ACN/H_2O/FA extraction despite its greater solvent volume and procedural complexity. Overall, these findings support culture-medium-aware and objective-driven selection of extraction conditions for fungal metabolomics. Full quantitative validation in naturally contaminated food and feed matrices remains necessary before these procedures can be applied to routine monitoring or risk-assessment workflows.

Abbreviations

ACN, acetonitrile; CMA, corn meal agar; CZA, Czapek agar; ESI, electrospray ionization; EtOAc, ethyl acetate; FA, formic acid; FB1, fumonisin B1; FB2, fumonisin B2; HRMS, high-resolution mass spectrometry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MEA, malt extract agar; MN, molecular network; PCA, principal component analysis; PDA, potato dextrose agar; PERMANOVA, permutational multivariate analysis of variance; QC, quality control; RSD, relative standard deviation; UHPLC, ultrahigh-performance liquid chromatography.

Declarations

CRediT authorship contribution statement

Emie Groppi: Writing – original draft, review & editing, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization.

Terance Mobarak: Writing – review & editing, Data curation, Data processing, Data visualization

Alice Gadea: Writing – review & editing, Methodology, Conceptualization, Supervision,

Marieke Vansteelandt: Writing – review & editing, Methodology, Visualization, Investigation

Valérie Cristofoli: Methodology, Visualization, Investigation

Mohamed Haddad: Writing – original draft, review & editing, Validation, Formal analysis, Data curation, Conceptualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.Figure S1–S8: Global heatmaps of all detected features for each culture medium, including analyses performed with and without quality-control samples (JPG).Runlog S1: Statistical workflow for principal component analysis, PERMANOVA, and dispersion testing (TXT).Runlog S2: Statistical workflow for heatmap generation and identification of top PCA features (TXT).Runlog S3: Statistical workflow for one-factor univariate analysis (TXT).Runlog S4: Statistical workflow for integrative pseudo-volcano analysis (TXT).Runlog S5: Statistical workflow for global heatmap generation (TXT).Table S1: Normalized metabolite peak-area data annotated by culture medium (XLSX).Table S2: PCA outputs by culture medium, including analyses performed with and without quality-control samples (XLSX).Table S3: PERMANOVA and dispersion-test outputs by culture medium (XLSX).Table S4: One-factor univariate analysis results, including analyses performed with and without quality-control samples (XLSX).

References

1. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x
2. Andrade PD, Gomes da Silva JL, Caldas ED (2013) Simultaneous analysis of aflatoxins B1, B2, G1, G2, M1 and ochratoxin A in breast milk by high-performance liquid chromatography/fluorescence after

liquid-liquid extraction with low temperature purification (LLE-LTP). J Chromatogr A 1304:61–68. https://doi.org/10.1016/j.chroma.2013.06.049

3. Azman N, Zainudin NAIM, Ibrahim WNW (2020) Fumonisin B1 production by Fusarium species and mycotoxigenic effect on larval zebrafish. Trop Life Sci Res 31(3):91–107. https://doi.org/10.21315/tlsr2020.31.3.7
4. Bartók T, Tölgyesi L, Szekeres A, Varga M, Bartha R, Szécsi A, Bartók M, Mesterházy A (2010) Detection and characterization of twenty-eight isomers of fumonisin B1 (FB1) mycotoxin in a solid rice culture infected with Fusarium verticillioides by reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight and ion trap mass spectrometry. Rapid Commun Mass Spectrom 24(1):35–42. https://doi.org/10.1002/rcm.4353
5. Behner A, Palicova J, Tobolkova AH, Prusova N, Stranska M (2025) Pulsed electric field induces significant changes in the metabolome of Fusarium species and decreases their viability and toxigenicity. Toxins 17(1):33. https://doi.org/10.3390/toxins17010033
6. Benito-Fuertes M, Rodríguez-Garraus A, Gascón-Corella S, Azqueta A, Lizarraga E, González-Peñas E, Vettorazzi A (2026) Genotoxicity and mutagenicity of emerging mycotoxins: a systematic review. J Toxicol Environ Health B Crit Rev 29(3):236–273. https://doi.org/10.1080/10937404.2025.2566495
7. Bessaire T, Savoy M-C, Ernest M, Christinat N, Badoud F, Desmarchelier A, Carrères B, Chan W-C, Wang X, Delatour T (2024) Enhanced surveillance of > 1100 pesticides and natural toxins in food: harnessing the capabilities of LC-HRMS for reliable identification and quantification. Foods 13(19):3040. https://doi.org/10.3390/foods13193040
8. Biselli S, Hummert C (2005) Development of a multicomponent method for Fusarium toxins using LC-MS/MS and its application during a survey for the content of T-2 toxin and deoxynivalenol in various feed and food samples. Food Addit Contam 22(8):752–760. https://doi.org/10.1080/02652030500158617
9. Desai R, Preet G, Astakala RV, Romero-Otero A, Sanchez P, Mackenzie TA, Larsen TO, Ebel R, Jaspars M (2026) An exploratory LC-HRMS metabolomics study of culture medium-dependent metabolic variation and bioactivity in ten fungal strains. Int J Mol Sci 27(9):3866. https://doi.org/10.3390/ijms27093866
10. dos Santos MC, Bicas JL (2021) Natural blue pigments and bikaverin. Microbiol Res 244:126653. https://doi.org/10.1016/j.micres.2020.126653
11. Elie N, Santerre C, Touboul D (2019) Molecular networking in mass spectrometry-based metabolomics. Anal Chem 91(18):11489–11492. https://doi.org/10.1021/acs.analchem.9b02802
12. Fabregat-Cabello N, Zomer P, Sancho JV, Roig-Navarro AF, Mol HGJ (2016) Comparison of approaches to deal with matrix effects in LC-MS/MS based determinations of mycotoxins in food and feed. World Mycotoxin J 9(2):149–161. https://doi.org/10.3920/WMJ2014.1872
13. Falavigna C, Cirlini M, Galaverna G, Dall'Asta C (2012) Masked fumonisins in processed food: cooccurrence of hidden and bound forms and their stability under digestive conditions. World Mycotoxin J 5:325–334
14. Frisvad JC, Filtenborg O (1983) Classification of terverticillate penicillia based on profiles of mycotoxins and other secondary metabolites. Appl Environ Microbiol 46(6):1301–1310. https://doi.org/10.1128/aem.46.6.1301-1310.1983

15. Gao Z, Luo K, Zhu Q, Peng J, Liu C, Wang X, Li S, Zhang H (2023) The natural occurrence, toxicity mechanisms and management strategies of fumonisin B1: a review. Environ Pollut 320:121065. https://doi.org/10.1016/j.envpol.2023.121065
16. Gelderblom WC et al (1984) Fusarin C, a mutagen produced by Fusarium moniliforme. Appl Environ Microbiol 48(1):123–128
17. Gelderblom WC, Jaskiewicz K, Marasas WF, Thiel PG, Horak RM, Vleggaar R, Kriek NP (1988) Fumonisins—novel mycotoxins with cancer-promoting activity produced by Fusarium moniliforme. Appl Environ Microbiol 54(7):1806–1811. https://doi.org/10.1128/aem.54.7.1806-1811.1988
18. Groppi E, Gadea A, Monge C, Cristofoli V, Vansteelandt M, Haddad M (2024) Untargeted metabolomics to investigate the influence of epigenetic modifiers on the metabolism of Fusarium verticillioides. Int J Microbiol 2024:1763495. https://doi.org/10.1155/2024/1763495
19. Groppi E, Haddad M, Cristofoli V, Vansteelandt M, Gadea A (2025) Unveiling the substrate-dependent dynamics of mycotoxin production in Fusarium verticillioides using an OSMAC-metabolomics approach. Chem Biodivers 22(1):e202401747. https://doi.org/10.1002/cbdv.202401747
20. Ji F, He D, Olaniran AO et al (2019) Occurrence, toxicity, production and detection of Fusarium mycotoxin: a review. Food Prod Process Nutr 1:6. https://doi.org/10.1186/s43014-019-0007-2
21. Kleigrewe K, Aydin F, Hogrefe K, Piecuch P, Bergander K, Würthwein EU, Humpf HU (2012) Structure elucidation of new fusarins revealing insights in the rearrangement mechanisms of the Fusarium mycotoxin fusarin C. J Agric Food Chem 60(21):5497–5505. https://doi.org/10.1021/jf3009469
22. Kovalsky P, Kos G, Nährer K, Schwab C, Jenkins T, Schatzmayr G, Sulyok M, Krska R (2016) Co-occurrence of regulated, masked and emerging mycotoxins and secondary metabolites in finished feed and maize. Toxins 8(12):363. https://doi.org/10.3390/toxins8120363
23. Krska R, Schubert-Ullrich P, Molinelli A, Sulyok M, MacDonald S, Crews C (2008) Mycotoxin analysis: an update. Food Addit Contam Part Chem Anal Control Expo Risk Assess 25(2):152–163. https://doi.org/10.1080/02652030701765723
24. Lapris M, Errico M, Rocchetti G, Gallo A (2024) The potential of multi-screening methods and omics technologies to detect both regulated and emerging mycotoxins in different matrices. Foods 13:1746. https://doi.org/10.3390/foods13111746
25. Lee SY, Woo SY, Park SB, Zheng Y, Chun HS (2025) Multi-mycotoxin occurrence and risk assessment in Korean foods by LC–MS/MS. Food Res Int 221:117500. https://doi.org/10.1016/j.foodres.2025.117500
26. Mackay N, Marley E, Leeman D, Poplawski C, Donnelly C (2022) Analysis of multiple mycotoxins in animal feed by LC–MS/MS using immunoaffinity cleanup. J AOAC Int 105(5):1330–1340. https://doi.org/10.1093/jaoacint/qsac035
27. Nielsen KF, Smedsgaard J (2003) Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. J Chromatogr A 1002(1–2):111–136. https://doi.org/10.1016/S0021-9673(03)00490-4
28. Olivon F, Elie N, Grelier G, Roussi F, Litaudon M, Touboul D (2018) MetGem software for the generation of molecular networks based on the t-SNE algorithm. Anal Chem 90(23):13900–13908. https://doi.org/10.1021/acs.analchem.8b03099

29. Puntscher H, Kütt ML, Skrinjar P, Mikula H, Podlech J, Fröhlich J, Marko D, Warth B (2018) Tracking emerging mycotoxins in food: development of an LC-MS/MS method for free and modified Alternaria toxins. Anal Bioanal Chem 410(18):4481–4494. https://doi.org/10.1007/s00216-018-1105-8
30. Qu B, Yu J, Wang B (2024) Fusarium mycotoxins: the major food contaminants. mLife 3(2):12112. https://doi.org/10.1002/mlf2.12112
31. Rheeder JP, Marasas WF, Vismer HF (2002) Production of fumonisin analogs by Fusarium species. Appl Environ Microbiol 68(5):2101–2105. https://doi.org/10.1128/AEM.68.5.2101-2105.2002
32. Righetti L, Paglia G, Galaverna G, Dall'Asta C (2016) Recent advances and future challenges in modified mycotoxin analysis: why HRMS has become a key instrument in food contaminant research. Toxins 8(12):361. https://doi.org/10.3390/toxins8120361
33. Royer D, Humpf H, Guy PA (2004) Quantitative analysis of Fusarium mycotoxins in maize using accelerated solvent extraction before liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. Food Addit Contam 21:678–692
34. Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP, Hollender J (2014) Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ Sci Technol 48(4):2097–2098. https://doi.org/10.1021/es5002105
35. Şenyuva H, Gilbert J, Samson R, Özcan S, Öztürkoğlu Ş, Önal D (2008) Occurrence of fungi and their mycotoxins in individual Turkish dried figs. World Mycotoxin J 1(1):79–86. https://doi.org/10.3920/WMJ2008.x009
36. Shanakhat H, Sorrentino A, Raiola A, Romano A, Masi P, Cavella S (2018) Current methods for mycotoxins analysis and innovative strategies for their reduction in cereals. J Sci Food Agric 98(11):4003–4013. https://doi.org/10.1002/jsfa.8933
37. Škrbić B, Živančev J, Godula M (2014) Multimycotoxin analysis of crude extracts of nuts by UHPLC-MS/MS. J Food Compos Anal 34:171–177
38. Smedsgaard J (1997) Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures. J Chromatogr A 760(2):264–270. https://doi.org/10.1016/S0021-9673(96)00803-5
39. Spanjer MC, Rensen PM, Scholten JM (2008) LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. Food Addit Contam Part Chem Anal Control Expo Risk Assess 25(4):472–489. https://doi.org/10.1080/02652030701552964
40. Steiner D, Humpel A, Stamminger E, Schoeberl A, Pachschwoell G, Sloboda A, Swoboda C, Rigg J, Zhang D, Wang Y, Davis J, Sulyok M, Krska R, Quinn B, Greer B, Elliott CT, Dzuman Z, Hajslova J, Gschaider A et al (2022) An interlaboratory comparison study of regulated and emerging mycotoxins using liquid chromatography mass spectrometry: challenges and future directions of routine multi-mycotoxin analysis including emerging mycotoxins. Toxins 14(6):405. https://doi.org/10.3390/toxins14060405
41. Sulyok M, Berthiller F, Krska R, Schuhmacher R (2006) Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. Rapid Commun Mass Spectrom 20(18):2649–2659. https://doi.org/10.1002/rcm.2640

42. Sulyok M, Krska R, Schuhmacher R (2007) A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. Anal Bioanal Chem 389(5):1505–1523. https://doi.org/10.1007/s00216-007-1542-2
43. Sulyok M, Stadler D, Steiner D et al (2020) Validation of an LC-MS/MS-based dilute-and-shoot approach for the quantification of > 500 mycotoxins. Anal Bioanal Chem 412:2607–2620. https://doi.org/10.1007/s00216-020-02489-9
44. Sulyok M, Suman M, Krska R (2024) Quantification of 700 mycotoxins and secondary metabolites in grain products. npj Sci Food 8:49. https://doi.org/10.1038/s41538-024-00294-7
45. Trebstein A, Lauber U, Humpf HU (2009) Analysis of Fusarium toxins via HPLC-MS/MS multimethods: matrix effects and strategies for compensation. Mycotoxin Res 25:201–213. https://doi.org/10.1007/s12550-009-0029-8
46. Van Pamel E, Vlaemynck G, Heyndrickx M, Herman L, Verbeken A, Daeseleire E (2011) Mycotoxin production by pure fungal isolates analysed by means of an UHPLC-MS/MS multi-mycotoxin method with possible pitfalls and solutions for patulin-producing isolates. Mycotoxin Res 27(1):37–47. https://doi.org/10.1007/s12550-010-0073-4
47. Vega VA (2016) A quick assay for the quantitation of fumonisin B1 and B2 in maize samples by liquid chromatography and mass spectrometry. J AOAC Int 99(3):717–720. https://doi.org/10.5740/jaoacint.15-0266
48. Zhang Y, Chen T, Chen D, Liang W, Lu X, Zhao C, Xu G (2023) Suspect and nontarget screening of mycotoxins and their modified forms in wheat products based on ultrahigh-performance liquid chromatography-high resolution mass spectrometry. J Chromatogr A 1708:464370. https://doi.org/10.1016/j.chroma.2023.464370
49. Zhao Y, Yuan YC, Bai XL, Liu YM, Wu GF, Yang FS, Liao X (2020) Multi-mycotoxins analysis in liquid milk by UHPLC-Q-Exactive HRMS. Food Chem 305:125429. https://doi.org/10.1016/j.foodchem.2019.125429