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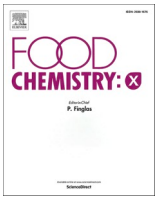

Strategy to improve the confidence level of qualitative screening by high resolution mass spectrometry: A case study of mycotoxins in maize

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ABSTRACT

Targeted, suspect and non-targeted screening by high-resolution mass spectrometry (HRMS) is developing rapidly. In this study, a qualitative screening method was established using HPLC-HRMS on data dependent acquisition for the analysis of mycotoxins in maize. To ensure the sensitivity and applicability of the method, 41 mycotoxin standards were applied for method optimization. A quantitative structure-retention relationships (QSRR) model was developed for retention time prediction and projection using machine learning, providing supplementary evidence for molecule annotation. The predicted errors were all below 0.5 min, contributing to improve the confidence level of suspect and non-targeted screening for mycotoxins. Thresholds affecting the accuracy of screening results were also investigated systematically. Performance metrics including Accuracy, F1 score, Matthew's correlation coefficient (MCC) were introduced to evaluate the qualitative screening method. The developed method was applied in the qualitative screening of collected maize samples, where 11 mycotoxins were screened at high confidence level.

1. Introduction

High resolution mass spectrometry (HRMS) allows for the identification of known or unknown compounds with high sensitivity, and its application is gradually increasing in the screening of contaminants in environment and food (Beccari et al., 2016; Pereira et al., 2021; Su et al., 2023; Yang et al., 2024). Compared with traditional methods, screening methods based on high-resolution mass spectrometry emphasize more on qualitative than quantitative analysis. Although there was no harmonized criteria, False positive rates and false negative rates were usually applied for method validation (Angeles et al., 2021; Fisher et al., 2022; Vergeynst et al., 2015). To improve the accuracy, more and more studies focused on the instrument parameters and threshold of the screening method.

Kern et al. developed a six-step funnel procedure to identify transformation products (TPs) of organic contaminants in natural waters using HRMS, where exact mass, peak intensity, retention time, isotope fit, ionization efficiency, and MS/MS fragments were applied step by step to reduce false positive rates (Kern et al., 2009). And it was revealed that the setting of the screening threshold had effect on the screening results. Turnispeed et al. developed a screening method for veterinary drugs in milk by HRMS. False detection occurred because of the unproper threshold (Turnipseed et al., 2014). A comparison of false negative and false positive rates with different mass accuracy windows was performed, and the results showed that a wider mass accuracy window may lead to an increase in the false positive rate and a narrower mass window may lead to an increase in the false negative rate. Ates et al. established a screening method for plant and fungal metabolites in wheat, maize and animal feed samples. The mass accuracy was set according to the document SANCO/10684/2009, and the standard of compound identification was set (Ates et al., 2014). The method was validated by matrix spiked samples. Ergocornine was not detected in all samples because the intensity threshold didn't meet the low peak intensity of Ergocornine. Klitgaard compared the thresholds of retention time, isotope fit, mass accuracy in broad, medium and narrow levels (Klitgaard et al., 2014). It was proposed to set a wide range on the isotope fit and mass accuracy to avoid false negative results. However, false positive may occur in this case. Eyken et al. investigated the

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influence of 7 parameterson the non-targeted identification of trace contaminants in honey, including match tolerance mass, expansion values for chromatogram extraction, isotope abundance score, peak filter absolute height, spectra to include average scans, exclude TOF spectra if above, and post-processing filter absolute height (von Eyken & Bayen, 2019). It was revealed that the expansion window for chromatogram extraction and the average scans included in the spectra influenced significantly the identification process results. Schymanski et al. proposed identification confidence levels including Exact mass of interest, unequivocal molecular formula, tentative candidates, probable structure and confirmed structure which contributed much to communicate identification confidence of screening results by HRMS (Schymanski et al., 2014). Charbonnet et al. contextualize the guidelines and define sublevels and identification criteria specific to the study of *per*- and polyfluoroalkyl substances (Charbonnet et al., 2022). The confidence levels were applied in non-targeted screening in various fields (Angeles et al., 2021; Kiefer et al., 2019; Man et al., 2022; Wang et al., 2022). However, the investigation of factors affecting the accuracy of screening results was still relatively limited.

Mycotoxins were toxic metabolites produced by fungi during growth and reproduction (Deng et al., 2023; Yang et al., 2021; Zheng et al., 2022). More than 400 species of mycotoxins have been identified, and some mycotoxins were classified as carcinogens by the International Agency for Research on Cancer (IARC), and mycotoxins in cereals and their derivative products were regulated and restricted in many countries and regions around the world, including China, the European Union and the United States. Screening methods by HRMS were widely used to provide a comprehensive information of mycotoxin contamination and contamination levels in foods, as well as the discovery of unknown mycotoxins and their metabolites (Li et al., 2023; Mao et al., 2018; Wu et al., 2023).

In this study, mycotoxins in maize was conducted as a case study of qualitative screening. 41 mycotoxins with standards were selected from the following aspects. On the one hand, as a case study for mycotoxins in maize, the target mycotoxins conclude mycotoxins with high detection rates in mycotoxins including Aflatoxins, Fumonisins, ZEN, DON, Ochratoxins and T-2 Toxin. On the other hand, to expand the method for wider range of mycotoxins, chemical diversity was taken into consideration, ensuring coverage of *m/z* 171–722 and polarity ranges (logP -1.41 to 6.31) by the selected 41 mycotoxins (Fig. S1). Instrument parameters affecting the accuracy of screening results including ion source parameters, mass spectrometry scan mode, mass spectrometry screening parameters optimization, were systematically investigated by using ultra performance liquid chromatography tandem Orbitrap high resolution mass spectrometry. A QSRR model using machine learning was developed for retention time prediction. Screening parameters including peak area threshold override, mass tolerance, retention time window override and isotope fit threshold were also optimized. The accuracy of the method was evaluated by performance metrics including true positive rate (TPR), false negative rate (FNR), true negative rate (TNR), false positive rate (FPR), precision, false discovery rate (FDR), Accuracy, F1 score, and Matthew's correlation coefficient (MCC). The screening detection limits (SDLs) of the method were also investigated. Moreover, the developed method was applied in collected maize samples.

2. Materials and methods

2.1. Materials and regents

Methanol and acetonitrile (HPLC grade) were purchased from Supelco (America). Formic acid (LC/MS grade) and ammonium acetate (HPLC grade) were purchased from Fisher (America). Water was prepared with Milli pore (MA, USA). Standards of mycotoxins were obtained or purchased from National Institute of Metrology (Beijing, China), Romer Labs Inc. (Getzersdorf, Austria), Fermentek Ltd. (Jerusalem, Israel), Toronto Research Chemicals (Toronto, Canada), Merck (Darmstadt, Germany), PriboLab (Qingdao, China), First Standard (Tianjin, China), J&K Scientific (Beijing, China), TOKU-E (Tokyo, Japan). Specific information of 41 mycotoxins was listed in Table 1 and Table S1.

2.2. Sample pretreatment

The sample pretreatment method was performed according to our previous method (Liu et al., 2021). Briefly, 5 g of maize sample was weighed into a 50 mL centrifuge tube. 20 mL of extraction solution (acetonitrile:water:formic acid = 80:19.9:0.1, v:v:v) was added and soaked overnight. The mixture was extracted by shaking for 1 h and centrifuged for 5 min, after which the supernatant was collected in another clean centrifuge tube. The residue was mixed with 20 mL of extraction Solution (acetonitrile:water:formic acid, 20:79.9:0.1, v:v:v). After shaking for 30 min, the supernatants were mixed and centrifuged at 4 °C for 5 min. The supernatant was filtered through a 0.20 μm PTFE filter before injection.

2.3. Instrument method

A Vanquish high-performance liquid chromatography couple with Q Extractive Plus Orbitrap high resolution mass spectrometry (Thermo Fisher Scientific, Bremen, Germany) was used for analysis. An Acquity UPLC HSS T3 column (2.1 mm × 50 mm, 1.8 μm, Waters, America) was applied. Binary mobile phases were composed of water with 0.1 % formic acid and 2 mM CH_3COONH_4 (A) and methanol with 0.1 % formic acid and 2 mM CH_3COONH_4 (B). The flow rate was set as 0.3 mL min^{-1}. The elution gradient was illustrated in Table S2. The injection volume was 5 μL.

The screening was on Full Mass-ddMS2 screening mode. The HESI probe was operated in positive and negative mode. Specific parameters were listed in Table S3.

2.4. Compound database and screening work flow

In this study, an in-house compound database was built using TraceFinder 4.1 software. The database contains information of mycotoxins including name, CAS number, molecular formula, exact mass, fragment ions and retention time. Different adduct ions and fragments ions at 5 different CEs were all took into consideration. Information including the most abundant adduct ions and fragments was listed in Table S4. A list of 134 mycotoxins was also built for suspect screening. The information was illustrated in Table S5.

For targeted screening of mycotoxins with reference standards, verification was conducted through a five-dimensional confirmation protocol encompassing peak area thresholds, mass accuracy, RT alignment, isotopic fit, and fragment ions; For suspect or non-targeted screening of mycotoxins lacking reference standards, a tiered identification strategy was implemented utilizing RT prediction and projection models. Afterwards, peak area thresholds, mass accuracy, predicted retention time alignment, isotopic pattern matching, and fragment ions (if available) were confirmed.

2.5. RT prediction

Robust QSRR and projection models were developed to predict RTs of mycotoxins in this study. RT datasets covering 343 compounds (Zhang et al., 2024) and 1820 compounds (Aalizadeh et al., 2021) were integrated. RTs across chromatographic methods (CM) were compared. CM for mycotoxin in this study was marked as CM_M; CM_03 from Zhang et al.'s study was introduced; CM from Aalizadeh et al.'s study was marked as CM_L. RTs in CM_03 were projected to CM_L using the 154 overlapped compounds as calibration, resulting in 1951 RTs in sum. A random selection of 251 compounds including 8 mycotoxins were selected as the test set, and the remaining 1708 compounds were used as

Table 1
Information of 41 mycotoxins.

	Compound	Abbreviation	Molecular formula	Monoisotopic mass	Adduct ions
1	(−)-Citreoviridin	CTV	$C_{23}H_{30}O_6$	402.2043	$[M+H]^+$
2	(−)-Citrinin	CIT	$C_{13}H_{14}O_5$	250.0841	$[M+H]^+$
3	15-acetyl-deoxynivalenol	15-ADON	$C_{17}H_{22}O_7$	338.1366	$[M+H]^+$
4	3-acetyl-deoxynivalenol	3-ADON	$C_{17}H_{22}O_7$	338.1366	$[M+H]^+$
5	Aflatoxin B_1	AFB_1	$C_{17}H_{12}O_6$	312.0634	$[M+H]^+$
6	Aflatoxin B_2	AFB_2	$C_{17}H_{14}O_6$	314.0790	$[M+H]^+$
7	Aflatoxin G_1	AFG_1	$C_{17}H_{12}O_7$	328.0583	$[M+H]^+$
8	Aflatoxin G_2	AFG_2	$C_{17}H_{14}O_7$	330.0739	$[M+H]^+$
9	Alternariol	AOH	$C_{14}H_{10}O_5$	258.0528	$[M-H]^-$
10	Altertoxin I	ATX I	$C_{20}H_{16}O_6$	352.0947	$[M-H]^-$
11	Brevianamide F	Bre F	$C_{16}H_{17}N_3O_2$	283.1321	$[M+H]^+$
12	Cytochalasin E	Cyto E	$C_{28}H_{33}NO_7$	495.2257	$[M+NH4]^+$
13	Deoxynivalenol	DON	$C_{15}H_{20}O_6$	296.1260	$[M+H]^+$
14	Destruxin B	DESB	$C_{30}H_{51}N_5O_7$	593.3788	$[M+H]^+$
15	Diacetoxyscirpenol	DAS	$C_{19}H_{26}O_7$	366.1678	$[M+NH4]^+$
16	fumagillin	FUM	$C_{26}H_{34}O_7$	458.2304	$[M+H]^+$
17	Fumigacin	FUG	$C_{33}H_{44}O_8$	568.3036	$[M+NH4]^+$
18	Fumonisin B_1	FB_1	$C_{34}H_{59}NO_{15}$	721.3885	$[M+H]^+$
19	Fumonisin B_2	FB_2	$C_{34}H_{59}NO_{14}$	705.3936	$[M+H]^+$
20	Fumonisin B_3	FB_3	$C_{34}H_{59}NO_{14}$	705.3936	$[M+H]^+$
21	Fusarenon-X	Fus-X	$C_{17}H_{22}O_8$	354.1315	$[M+H]^+$
22	Gliotoxin	GT	$C_{13}H_{14}N_2O_4S_2$	326.0395	$[M+H]^+$
23	HT-2 Toxin	HT-2	$C_{22}H_{32}O_8$	424.2097	$[M+NH4]^+$
24	Neosolaniol	NEO	$C_{19}H_{26}O_8$	382.1628	$[M+NH4]^+$
25	Nivalenol	NIV	$C_{15}H_{20}O_7$	312.1209	$[M+H]^+$
26	Ochratoxin A	OTA	$C_{20}H_{18}ClNO_6$	403.0823	$[M+H]^+$
27	Ochratoxin B	OTB	$C_{20}H_{19}NO_6$	369.1212	$[M+H]^+$
28	Ochratoxin C	OTC	$C_{22}H_{22}ClNO_6$	431.1136	$[M+H]^+$
29	Paxilline	PAX	$C_{27}H_{33}NO_4$	435.2410	$[M+H]^+$
30	PENICILLIC ACID	PA	$C_8H_{10}O_4$	170.0579	$[M+H]^+$
31	Roquefortine C	Roq-C	$C_{22}H_{23}N_5O_2$	389.1852	$[M+H]^+$
32	Rugulosin	RUG	$C_{30}H_{22}O_{10}$	542.1213	$[M+H]^+$
33	Sterigmatocystin	STC	$C_{18}H_{12}O_6$	324.0634	$[M+H]^+$
34	T-2 toxin	T-2	$C_{24}H_{34}O_9$	466.2203	$[M+NH4]^+$
35	Verruculogen	VER	$C_{27}H_{33}N_3O_7$	511.2318	$[M+H]^+$
36	Zearalanone	ZAN	$C_{18}H_{24}O_5$	320.1624	$[M-H]^-$
37	Zearalenone	ZEN	$C_{18}H_{22}O_5$	318.1467	$[M-H]^-$
38	α-Zearalanol	α-ZAL	$C_{18}H_{26}O_5$	322.1780	$[M-H]^-$
39	α-Zearalenol	α-ZEL	$C_{18}H_{24}O_5$	320.1624	$[M-H]^-$
40	β-Zearalanol	β-ZAL	$C_{18}H_{26}O_5$	322.1780	$[M-H]^-$
41	β-Zearalenol	β-ZEL	$C_{18}H_{24}O_5$	320.1624	$[M-H]^-$

the training set to construct the QSRR model. Molecular structure data were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). 1444 2D molecular descriptors were obtained using the PaDEL-descriptor tool. An artificial neural network (ANN) based on a multilayer perceptron was employed to develop the QSRR model. The Bayesian regularization backpropagation method was employed to train the network, ensuring optimal performance. ANN analysis was conducted on both the training and test sets, utilizing an iterative minimization program to optimize the network parameters and prevent from overfitting. Afterwards, a projection model was constructed using the retention times of 15 mycotoxins in both chromatographic methods. The RTs in the CM_L were projected to CM_M of this study. Detailed procedures were illustrated in the supplementary materials.

2.6. Statistical analysis

Statistical analysis was performed by Excel (Microsoft 365). Figures were completed by chiplot (https://www.chiplot.online/). Pearson correlation was conducted, and a value of $p < 0.05$ was considered as significant.

3. Results and discussion

3.1. Optimization of instrument condition

The structure of different mycotoxins varies a lot with logP ranged from −0.86 to 6.31. A compatible HSS T3 column was selected for the separation. The mobile phases were water and methanol with the addition of formic acid and ammonium acetate, based on its demonstrated dual-mode compatibility, enabling effective ionization for both ESI+ and ESI- detection. Initial percentages of methanol were compared for 5 %, 10 %, 20 %, and 30 %. It was revealed that better peak shapes was found at initial methanol percentage of 5 % for polar compounds such as NIV, PA, AFG_1 and CIT (Fig. S2). 5 % methanol was selected as initial mobile phase, and the gradient elution procedure was shown in Table S2. This optimized approach achieved high-throughput detection with reduced half of the analytical time compared to individual ESI+/ESI- methods. However, dedicated mobile phase optimization based on ionization polarity remains imperative to achieve optimal analytical performance when analyses specific mycotoxin classes or require enhanced sensitivity.

Instrument parameters including sheath gas flow rate, auxiliary gas flow rate, purge gas flow rate, spray voltage, capillary temperature, auxiliary gas temperature, and S lens RF level were optimized for better sensitivity and repeatability. Response intensities, their relative standard deviations (RSDs) were compared and analyzed. Among these parameters, capillary temperature and RF lens were critical influence factors. The results were shown in Fig. 1. Response intensities of mycotoxins increased with increasing capillary temperature generally. However, the stability decreased. For RF lens, the general responses were best at 80, and the responses were stable. 350 °C and 80 were applied because of the relatively high responses and low RSDs. Spray voltage also influenced the sensitivity of mycotoxins, particularly in ESI- mode. Spray voltages of 2 kV for ESI+ and -2 kV for ESI- were applied

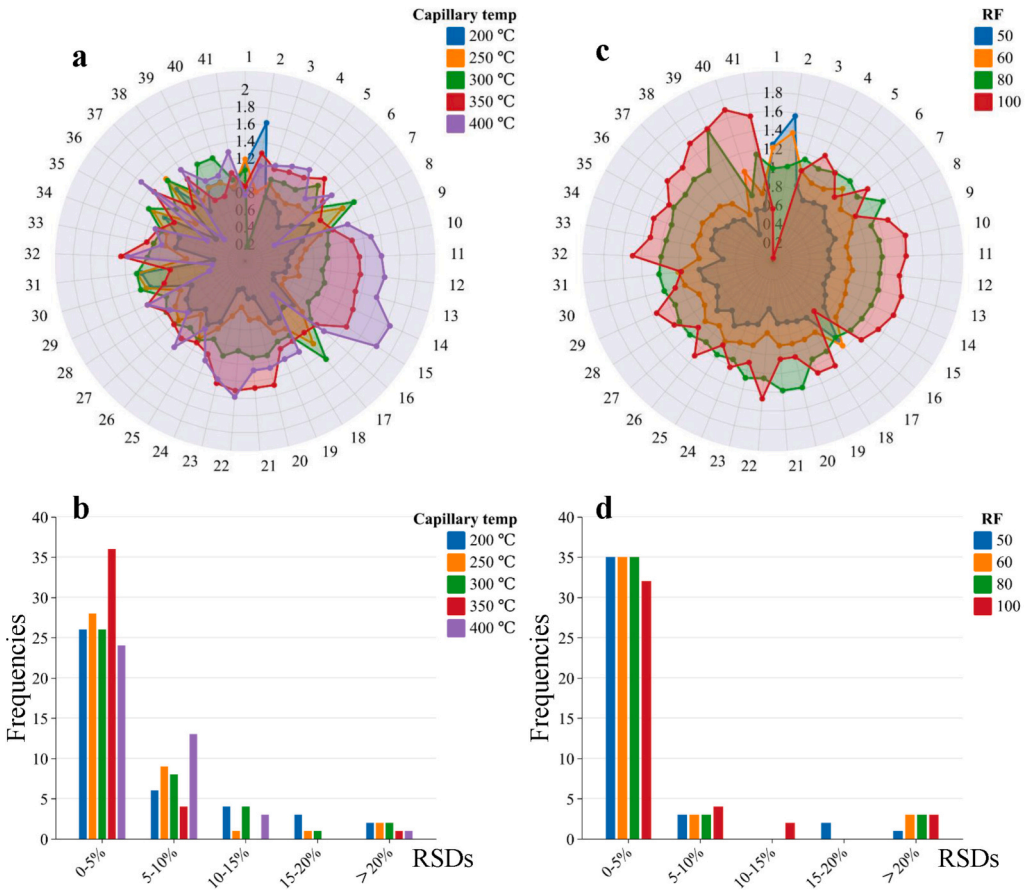


Fig. 1. Optimization of Capillary temperature and RF: normalized intensities of 41 mycotoxins by different Capillary temperatures (a) and RFs (c); Frequencies of intensity RSDs by different Capillary temperatures (b) and RFs (d).

(Fig. S3). After optimization, the parameters were shown in Table S3.

Full mass, data dependent acquisition (DDA) and data independent acquisition (DIA) mode were the most common acquisition modes for screening analysis (Jia et al., 2019; Liu et al., 2024; Sun et al., 2021; Zhang et al., 2021). For the full mass mode, the structural information is limited without fragment ions, which was not applicable for the qualitative analysis. DIA mode analysis could obtain both parent and product ions, but the data processing is complicated. DDA is an acquisition mode where MS2 fragmentation scan occurred based on intensity in the MS1 scan. Compared to Full Mass mode, DDA is particularly suitable for qualitative analysis of compounds in complex samples with detailed corresponding MS1 and MS2 information. It provides fragment information by focusing on high-intensity precursor ions, enhancing sensitivity and specificity for the discovery of new or unknown compounds. DDA data processing is more streamlined with established workflows for automated data extraction and compound identification than DIA. In this study, full scan-DDA mode was selected for a more comprehensive qualitative analysis. To improve the efficiency, positive+negative-switching scanning mode was adopted in this study. While it is true that the number of data points acquired in positive+negative-switching mode is inherently reduced compared to separate positive/negative mode acquisitions, our systematic validation confirmed that the acquired data remain sufficient for qualitative analysis. As shown in Fig. S4, more than 7 scans could be obtained, which could meet the analytical requirements. This approach significantly enhances the analytical efficiency and throughput, balancing efficiency and sensitivity based on our specific research objective. For studies requiring ultimate sensitivity, replicate injections with separate positive/negative mode acquisitions could be implemented as an alternative workflow. In addition, it was revealed that the inclusion list can improve the accuracy of target and suspect screening results, therefore, the inclusion list of mycotoxins was established and applied.

3.2. Screening ions

In this study, the most common adduct ions were $[M + H]^+$ in ESI+ mode. $[M + NH_4]^+$, $[M + K]^+$, $[M + Na]^+$ also formed because of the addition of ammonium acetate in the mobile phase and the presence of sodium and potassium ions in the instrument system. For example, $[M + H]^+$, $[M + NH_4]^+$ and $[M + Na]^+$ were found for DAS and 3-ADON. The response intensities ranked as follows, $[M + Na]^+ > [M + NH_4]^+ > [M + H]^+$ for DAS and $[M + Na]^+ > [M + H]^+ > [M + NH_4]^+$ for 3-ADON (Fig. S5). However, $[M + Na]^+$ was difficult to break up to obtain abundant fragment ions. $[M + NH_4]^+$ and $[M + H]^+$ were further investigated for fragment ions. The most common ions in ESI- mode was $[M\text{-}H]^-$, and $[M + CH_3COO]^-$ may also be present. $[M\text{-}H]^-$ was the most abundant ion in ESI- mode for mycotoxins in this study. Some mycotoxins could be detected in both ESI+ and ESI- mode, such as ZEN and its metabolites, and their intensities were higher in ESI- mode in this study.

For the mycotoxins in this study, the predominant adduct ions were $[M + H]^+$ for 27 compounds, $[M + NH_4]^+$ for 6 compounds and $[M\text{-}H]^-$ for 8 compounds (Table 1). For suspect and nontargeted screening with no corresponding standards, the different adduct ions should be fully considered for molecular formula assignment.

To build the inhouse database, collision energy was set at 5 different values. The fragment ions were diverse at different CEs. For example, the fragment ions of AFB_1 was shown at Fig. S6. The fragment ions at different CEs should be all took into consideration for screening analysis. Based on preliminary collision-induced dissociation (CID) profiling studies, a stepped energy acquisition protocol (15/35/55 eV) was

strategically implemented to maximize fragmentation coverage across mycotoxin classes. At 15 eV, predominant precursor ions such as $[M + H]^+$, $[M + H\text{-}CO]^+$ or $[M + H\text{-}H_2O]^+$ were preserved for molecular identification, such as *m/z* 313.0707, 285.0758 and 295.0601 for AFB_1. 35 eV induced optimal mid-range fragmentation generating class-specific diagnostic fragment ions, such as m/z 285.0758, 269.0445 and 241.0495 for AFB_1; whereas 55 eV proved essential for mycotoxins with relatively high molecule weight, such as m/z 704.3852 and 473.2831 for FB_1. The integrated spectrum of the three CEs provide sufficient fragment ions for a wide range of mycotoxins in a single injection.

3.3. QSRR retention time prediction model using machine learning

Retention time (RT) was important information for compound identification in HRMS screening (Croley et al., 2012). Correlation between RTs and logP was investigated for compound identification previously (Ng et al., 2020). In this study, correlation between retention time and logP was analyzed and a significantly positive relationship was found (Fig. 2). However, the correlation between RT and logP was not significant when logP was below 0. There were many other factors which may influence the retention behavior.

Retention time predictions from molecule structures in liquid chromatography were increasingly used in MS-based targeted and non-targeted analyses, providing supplementary evidence for molecule annotation (Aalizadeh et al., 2021; Ruan et al., 2023; Song et al., 2024). Previously, we developed a RT database covering 343 molecules under 30 chromatographic methods (Zhang et al., 2024). For the targeted mycotoxins, 37 mycotoxins were included in the RT database. Among the 30 chromatographic methods, CM_03 was similar with the method in this study, and a positive correlation was found for RTs between this study (CM_M) and CM_03 ($R = 0.95$, $p < 0.01$). Therefore, a QSRR model was developed for RT prediction of mycotoxins in this study by using the RTs of this study and CM_03.

285 compounds were set as the training set and 58 compounds as the test set to build the QSRR model. Machine learning was employed using Gaussian Process Regression (GPR). For the mycotoxins, 29 compounds were in the training set (TR) while 8 compounds were in the test set (TE). The remaining 3 compounds were in the external test set (ETE). After training, the prediction errors ranged from 0.1 min to 2.1 min for testing set, which may result from the diversity of mycotoxin structures. The prediction result could not meet the requirement for the suspect and non-targeted analysis. To improve the accuracy of the prediction model, a dataset of 1820 compounds in the literature was further introduced to increase the accuracy of the QSRR model (Aalizadeh et al., 2021). The chromatography method from the literature was marked as CM_L. 154 compounds were overlapped between CM_03 and CM_L. RTs in CM_03 were projected to CM_L using the 154 overlapped compounds as calibration, resulting in 1951 RTs in sum. 251 compounds including 8 mycotoxins were selected as the test set, and the remaining 1708 compounds were used as the training set to construct the QSRR model. After the training, the optimal QSRR model was applied. Afterwards, a projection model was constructed using 15 mycotoxins as calibrators. The RTs in the CM_L were projected to CM_M of this study. The predicted RTs were compared with the experimental RTs (Fig. 3). The results indicated that the correlation coefficient between the predicted and experimental RTs was >0.97 (Fig. 3a). And the correlation coefficients were all above 0.94 for TR, TE and ETE (Fig. 3b). The errors were all below 0.5 min. The root mean square error (RMSE) of RTs was 0.2 min, and the relative RMSE was 3.29 %. The result of the model was satisfactory and the model in this study could be applied in RT prediction of mycotoxins without standards, which could improve the confidence level of qualitative screening.

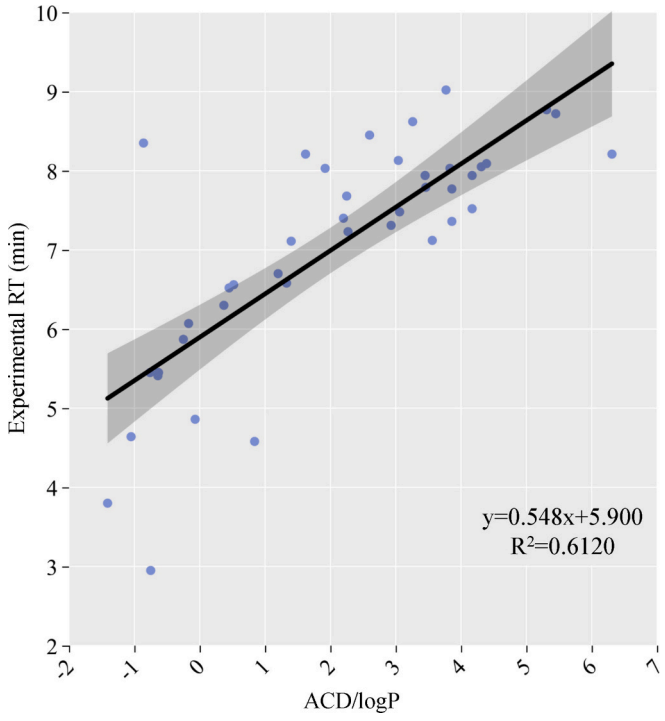


Fig. 2. Correlation between ACD/logP and experimental RT.

3.4. Investigation of screening parameters

Screening parameters including thresholds of peak area, mass accuracy and retention time were optimized using a matrix-matched standard solution (100 μg kg^{-1}). We designed three parameters each with three distinct threshold levels, resulting in a total of 27 screening methods (Fig. 4, Fig. S7), where true positives (TPs), true negatives (TNs), false positives (FPs), and false negatives (FNs) were evaluated. The boundary is 175, the total number of mycotoxins in the targeted and suspect list. The performance evaluation of the developed screening methods incorporated both FPs and FNs as critical metrics. However, divergent performance between FPs and FNs were observed across different methods, necessitating a composite assessment of overall accuracy. The performance metrics including true positive rate (TPR), false negative rate (FNR), true negative rate (TNR), false positive rate (FPR), precision, false discovery rate (FDR), Accuracy, F1 score, and Matthew's correlation coefficient (MCC) were calculated (Table S6) (Fisher et al., 2022), among which Accuracy, F1 score, and MCC emerged as the most robust indicators, demonstrating superior discriminative power in optimizing sensitivity-specificity balance and error minimization. It was revealed that Method 14 and 15 demonstrated the highest accuracy, F1 score and MCC of 0.9773, 0.9500 and 0.9358, respectively. The difference between these 2 methods was the threshold of retention time. For the target screening, a narrower threshold of 0.3 min was suggested, while for the suspect or non-targeted screening, a threshold of 0.5 min with predicted RT was recommended. Notably, practical applications may require differential emphasis on error types depending on analytical objectives. In scenarios prioritizing comprehensive compound detection, minimization of FNs becomes paramount, as potential FPs can be subsequently resolved through confirmatory targeted analysis. For confirmatory analyses demanding high specificity, stringent FPs control should be prioritized. To address such different requirements in error control, differential weighting factors can be systematically assigned to FPs and FNs. This adaptive approach ensures alignment with specific analytical priorities. Isotopic pattern fit threshold is an important parameter for screening analysis (Knolhoff, Callahan and Croley, 2014). Isotopic fit of 41 mycotoxins ranged from 78 to 100. The threshold was set to 70 in this study. The default isotope fit threshold of 90 may result in a certain

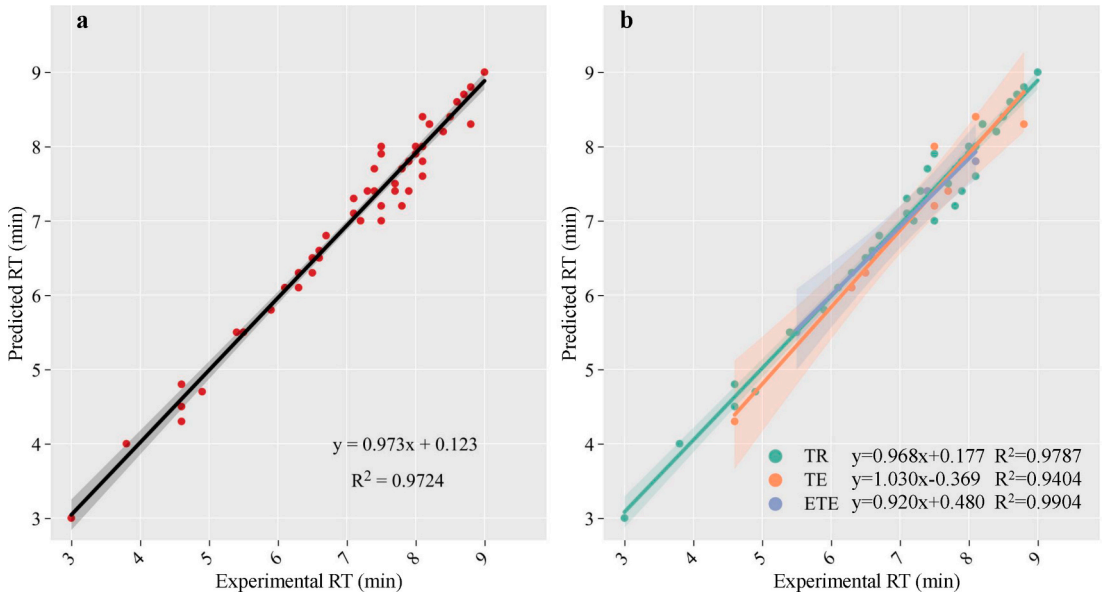
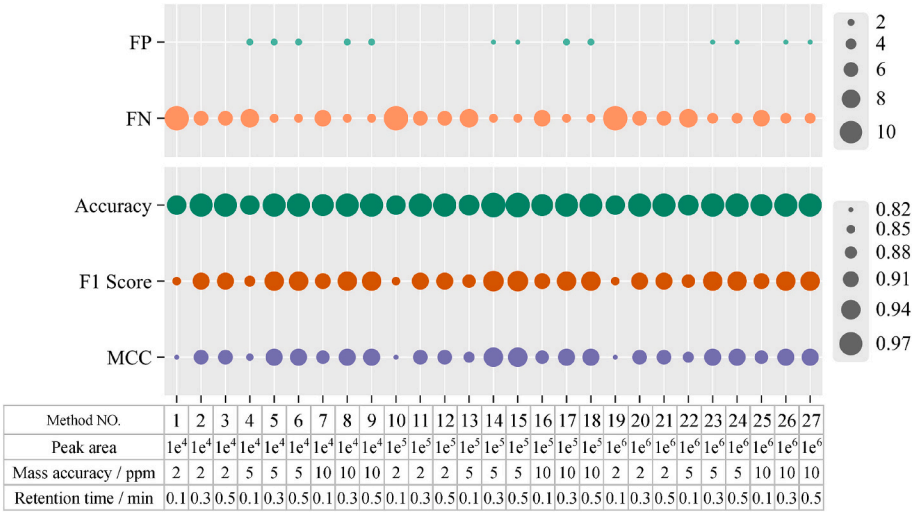


Fig. 3. Correlation analysis between experimental RTs and predicted RTs using: a. all data; b. individual groups of training set (TR), test set (TE) and external test set (ETE).



Method NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Peak area	$1e^4$	$1e^4$	$1e^4$	$1e^4$	$1e^4$	$1e^4$	$1e^4$	$1e^4$	$1e^4$	$1e^5$	$1e^5$	$1e^5$	$1e^5$	$1e^5$	$1e^5$	$1e^5$	$1e^5$	$1e^5$	$1e^6$	$1e^6$	$1e^6$	$1e^6$	$1e^6$	$1e^6$	$1e^6$	$1e^6$	$1e^6$
Mass accuracy / ppm	2	2	2	5	5	5	10	10	10	2	2	2	5	5	5	10	10	10	2	2	2	5	5	5	10	10	10
Retention time / min	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5

Fig. 4. FP, FN, accuracy, F1 score and MCC across 27 methods with different screening parameters.

probability of false negatives. Moreover, a minimum of 2 fragment ions were also set to improve the confidence level.

3.5. Method validation and implication

Currently, there are no standards for the method validation of mycotoxins screening in food matrix. In this study, the method validation was conducted referring to the EU SANTE/12682/2019 and previous studies (European Commission, 2019; Huang et al., 2022; Regueiro et al., 2017)(Bai et al., 2022). When the screening method is only intended to be used as a qualitative method, there are no requirements with regard to recovery of the analytes. The screening detection limit (SDL) of the qualitative screening method is the lowest level at which an analyte has been detected (not necessarily meeting the MS-identification criteria) in at least 95 % of the samples.

In this study, the lowest concentration at which the compound could be detected in all matrix spiked samples was determined as SDL. A blank maize sample was selected for matrix spiked experiment. The SDLs of mycotoxins in maize were shown in Table 2. SDLs of 12 mycotoxins were $\leq$ 1 μg kg^{-1}, 12 mycotoxins were between 1 and 10 μg kg^{-1}, 9 mycotoxins were between 10 and 50 μg kg^{-1}, and 2 mycotoxins were between 50 and 100 μg kg^{-1}. The SDLs of FB_2 and FB_3 were 500 and 2000 μg kg^{-1} respectively. It was revealed that covalent or non-covalent binding to the matrix such as starch or proteins occurred for FBs in cereals (Knutsen et al., 2018). Temperature, water proportion and other extraction conditions may affect the extraction efficiency of FBs. The pretreatment conditions should be further optimized and validated for the detection of FBs. Considering the maximum limits for mycotoxins, SDLs of 39 mycotoxins were lower than 100 μg kg^{-1}, which can meet the requirements of mycotoxins in cereals and derived products in China, EU, USA and some other countries and organizations. The method can be used for high-throughput qualitative screening of mycotoxins in cereals and derived products at confidence level 1 (Schymanski et al., 2014).

Moreover, the method was applied in the analysis of 4 polluted maize samples. 11 mycotoxins were screened including ZEN, CTV, FB_1, FB_2, FB_3, AFB_1, OTA, Bre F, Roq-C, RUG and AOH. No false positive was found in the maize sample. The results of the screening method

Table 2
SDLs of 41 mycotoxins in maize sample.

Compound	SDL (μg·kg^{-1})	Compound	SDL (μg·kg^{-1})
CTV	10	GT	50
CIT	50	HT-2	10
15-ADON	1	NEO	10
3-ADON	1	NIV	50
AFB_1	1	OTA	50
AFB_2	1	OTB	10
AFG_1	1	OTC	1
AFG_2	1	PAX	10
AOH	1	PA	10
ATX I	10	Roq-C	10
Bre F	50	RUG	50
Cyto E	10	STC	1
DON	100	T-2	10
DESB	1	VER	50
DAS	10	ZAN	1
FUM	50	ZEN	1
FUG	50	α-ZAL	10
FB_1	100	α-ZEL	10
FB_2	2000	β-ZAL	10
FB_3	500	β-ZEL	10
Fus-X	10		

established in this study were also compared with the default screening method (Table S7). False negative and false positive detections were found by the default method, resulting in low Accuracy, F1 score and MCC. The results further demonstrated the importance of screening parameters optimization.

4. Conclusion

This study presents an innovative strategy to improve the confidence level of qualitative screening using HRMS via systematic optimization of mycotoxin detection in maize. By integrating Full mass-DDA in ESI positive+negative-switching mode with machine learning based RT prediction and projection models, we established a robust approach that significantly improves reliability while minimizing false identifications. Critical parameters including mass accuracy deviation threshold, peak area threshold, retention time deviation and isotopic fit scoring were comprehensively evaluated through diverse performance metrics. Accuracy, F1 score, and MCC emerged as the most robust indicators, demonstrating superior discriminative power in optimizing sensitivity-specificity balance and error minimization. The strategy allows adaptation to diverse compound classes beyond mycotoxins, offering potential for food safety monitoring and environmental contaminant screening.

CRediT authorship contribution statement

Yan Gao: Writing – original draft, Data curation. **Mengyu Feng:** Formal analysis, Data curation. **Xiuqin Li:** Writing – review & editing, Conceptualization. **Yan Zhang:** Validation, Investigation. **Jinglei Hu:** Investigation. **Kangcong Li:** Investigation. **Jianhua Duan:** Investigation. **Qinghe Zhang:** Funding acquisition, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102467.

Data availability

Data will be made available on request.

References

Aalizadeh, R., Alygizakis, N. A., Schymanski, E. L., Krauss, M., Schulze, T., Ibáñez, M., … Thomaidis, N. S. (2021). Development and application of liquid chromatographic retention time indices in HRMS-based suspect and nontarget screening. *Analytical Chemistry, 93*(33), 11601–11611. https://doi.org/10.1021/acs.analchem.1c02348

Angeles, L. F., Singh, R. R., Vikesland, P. J., & Aga, D. S. (2021). Increased coverage and high confidence in suspect screening of emerging contaminants in global environmental samples. *Journal of Hazardous Materials, 414*, Article 125369. https://doi.org/10.1016/j.jhazmat.2021.125369

Ates, E., Godula, M., Stroka, J., & Senyuva, H. (2014). Screening of plant and fungal metabolites in wheat, maize and animal feed using automated on-line clean-up coupled to high resolution mass spectrometry. *Food Chemistry, 142*, 276–284. https://doi.org/10.1016/j.foodchem.2013.07.054

Bai, M., Tang, R., Li, G., She, W., Chen, G., Shen, H., Zhu, S., Zhang, H., & Wu, H. (2022). High-throughput screening of 756 chemical contaminants in aquaculture products using liquid chromatography/quadrupole time-of-flight mass spectrometry. *Food Chemistry: X, 15*, Article 100380. https://doi.org/10.1016/j.fochx.2022.100380

Beccari, G., Caproni, L., Tini, F., Uhlig, S., & Covarelli, L. (2016). Presence of Fusarium species and other toxigenic fungi in malting barley and multi-mycotoxin analysis by liquid chromatography–high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry, 64*(21), 4390–4399. https://doi.org/10.1021/acs.jafc.6b00702

Charbonnet, J. A., McDonough, C. A., Xiao, F., Schwichtenberg, T., Cao, D., Kaserzon, S., … Higgins, C. P. (2022). Communicating confidence of per- and polyfluoroalkyl substance identification via high-resolution mass spectrometry. *Environmental Science & Technology Letters, 9*(6), 473–481. https://doi.org/10.1021/acs.estlett.2c00206

Croley, T. R., White, K. D., Callahan, J. H., & Musser, S. M. (2012). The chromatographic role in high resolution mass spectrometry for non-targeted analysis. *Journal of the American Society for Mass Spectrometry, 23*(9), 1569–1578. https://doi.org/10.1007/s13361-012-0392-0

Deng, H., Xu, Z., Luo, L., Gao, Y., Zhou, L., Chen, X., … Yin, Q. (2023). High-throughput detection and dietary exposure risk assessment of 44 mycotoxins in mango, litchi, longan, and their products in South China. *Food Chemistry: X, 20*, Article 101002. https://doi.org/10.1016/j.fochx.2023.101002

European Commission. (2019). *Analytical quality control and method validation procedures for pesticide residues analysis in food and feed*. SANTE/12682/2019.

von Eyken, A., & Bayen, S. (2019). Optimization of the data treatment steps of a non-targeted LC-MS-based workflow for the identification of trace chemical residues in honey. *Journal of the American Society for Mass Spectrometry, 30*(5), 765–777. https://doi.org/10.1007/s13361-019-02157-y

Fisher, C. M., Peter, K. T., Newton, S. R., Schaub, A. J., & Sobus, J. R. (2022). Approaches for assessing performance of high-resolution mass spectrometry–based non-targeted analysis methods. *Analytical and Bioanalytical Chemistry, 414*(22), 6455–6471. https://doi.org/10.1007/s00216-022-04203-3

Huang, Q., Guo, W., Zhao, X., Cao, H., Fan, K., Meng, J., Nie, D., Wu, Y., & Han, Z. (2022). Universal screening of 200 mycotoxins and their variations in stored cereals in Shanghai, China by UHPLC-Q-TOF MS. *Food Chemistry, 387*, 132869. https://doi.org/10.1016/j.foodchem.2022.132869

Jia, W., Shi, L., Zhang, F., Fan, C., Chang, J., & Chu, X. (2019). Multiplexing data independent untargeted workflows for mycotoxins screening on a quadrupole-Orbitrap high resolution mass spectrometry platform. *Food Chemistry, 278*, 67–76. https://doi.org/10.1016/j.foodchem.2018.11.056

Kern, S., Fenner, K., Singer, H. P., Schwarzenbach, R. P., & Hollender, J. (2009). Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. *Environmental Science & Technology, 43*(18), 7039–7046. https://doi.org/10.1021/es901979h

Kiefer, K., Müller, A., Singer, H., & Hollender, J. (2019). New relevant pesticide transformation products in groundwater detected using target and suspect screening for agricultural and urban micropollutants with LC-HRMS. *Water Research, 165*, Article 114972. https://doi.org/10.1016/j.watres.2019.114972

Klitgaard, A., Iversen, A., Andersen, M. R., Larsen, T. O., Frisvad, J. C., & Nielsen, K. F. (2014). Aggressive dereplication using UHPLC–DAD–QTOF: Screening extracts for up to 3000 fungal secondary metabolites. *Analytical and Bioanalytical Chemistry, 406*(7), 1933–1943. https://doi.org/10.1007/s00216-013-7582-x

Knolhoff, A. M., Callahan, J. H., & Croley, T. R. (2014). Mass accuracy and isotopic abundance measurements for HR-MS instrumentation: Capabilities for non-targeted analyses. *Journal of the American Society for Mass Spectrometry, 25*(7), 1285–1294. https://doi.org/10.1007/s13361-014-0880-5

Knutsen, H., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., … Dall'Asta, C. (2018). Appropriateness to set a group health-based guidance value for

fumonisins and their modified forms. *EFSA Journal, 16*(2), 5172. https://doi.org/10.2903/j.efsa.2018.5172

Li, S., Zhang, S., Li, X., Zhou, S., Ma, J., Zhao, X., Zhang, Q., & Yin, X. (2023). Determination of multi-mycotoxins in vegetable oil via liquid chromatography-high resolution mass spectrometry assisted by a complementary liquid–liquid extraction. *Food Chemistry: X, 20*, Article 100887. https://doi.org/10.1016/j.fochx.2023.100887

Liu, H., Wang, R., Zhao, B., & Xie, D. (2024). Assessment for the data processing performance of non-target screening analysis based on high-resolution mass spectrometry. *Science of the Total Environment, 908*, 167967. https://doi.org/10.1016/j.scitotenv.2023.167967

Liu, S., Feng, M., Li, X., Tang, G., Zhang, Q., & Li, X. (2021). High accuracy liquid chromatography-isotopic dilution mass spectrometry method for determination of multi-mycotoxins in maize. *Chinese Journal of Analytical Chemistry, 49*, 546–553. https://doi.org/10.19756/j.issn.0253-3820.201650

Man, Y., Wang, W., Mao, L., Zhu, L., Zhang, Y., Zhang, L., … Liu, X. (2022). Degradation of kresoxim-methyl in different soils: Kinetics, identification of transformation products, and pathways using high-resolution-mass-spectrometry-based suspect and non-target screening approaches. *Journal of Agricultural and Food Chemistry, 70*(51), 16146–16155. https://doi.org/10.1021/acs.jafc.2c07488

Mao, J., Zheng, N., Wen, F., Guo, L., Fu, C., Ouyang, H., Zhong, L., Wang, J., & Lei, S. (2018). Multi-mycotoxins analysis in raw milk by ultra high performance liquid chromatography coupled to quadrupole orbitrap mass spectrometry. *Food Control, 84*, 305–311. https://doi.org/10.1016/j.foodcont.2017.08.009

Ng, B., Quinete, N., & Gardinali, P. R. (2020). Assessing accuracy, precision and selectivity using quality controls for non-targeted analysis. *Science of the Total Environment, 713*, Article 136568. https://doi.org/10.1016/j.scitotenv.2020.136568

Pereira, K. L., Ward, M. W., Wilkinson, J. L., Sallach, J. B., Bryant, D. J., Dixon, W. J., … Lewis, A. C. (2021). An automated methodology for non-targeted compositional analysis of small molecules in high complexity environmental matrices using coupled ultra performance liquid chromatography Orbitrap mass spectrometry. *Environmental Science & Technology, 55*(11), 7365–7375. https://doi.org/10.1021/acs.est.0c08208

Regueiro, J., Negreira, N., Hannisdal, R., & Berntssen, M. H. G. (2017). Targeted approach for qualitative screening of pesticides in salmon feed by liquid chromatography coupled to traveling-wave ion mobility/quadrupole time-of-flight mass spectrometry. *Food Control, 78*, 116–125. https://doi.org/10.1016/j.foodcont.2017.02.053

Ruan, T., Li, P., Wang, H., Li, T., & Jiang, G. (2023). Identification and prioritization of environmental organic pollutants: From an analytical and toxicological perspective. *Chemical Reviews, 123*(17), 10584–10640. https://doi.org/10.1021/acs.chemrev.3c00056

Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H. P., & Hollender, J. (2014). Identifying small molecules via high resolution mass spectrometry: Communicating confidence. *Environmental Science & Technology, 48*(4), 2097–2098. https://doi.org/10.1021/es5002105

Song, D., Tang, T., Wang, R., Liu, H., Xie, D., Zhao, B., Dang, Z., & Lu, G. (2024). Enhancing compound confidence in suspect and non-target screening through machine learning-based retention time prediction. *Environmental Pollution, 347*, 123763. https://doi.org/10.1016/j.envpol.2024.123763

Su, Q., Vera, P., & Nerín, C. (2023). Combination of structure databases, in silico fragmentation, and MS/MS libraries for untargeted screening of non-volatile migrants from recycled high-density polyethylene milk bottles. *Analytical Chemistry, 95*(23), 8780–8788. https://doi.org/10.1021/acs.analchem.2c05389

Sun, F., Tan, H., Li, Y., De Boevre, M., Zhang, H., Zhou, J., Li, Y., & Yang, S. (2021). An integrated data-dependent and data-independent acquisition method for hazardous compounds screening in foods using a single UHPLC-Q-Orbitrap run. *Journal of Hazardous Materials, 401*, 123266. https://doi.org/10.1016/j.jhazmat.2020.123266

Turnipseed, S. B., Lohne, J. J., Storey, J. M., Andersen, W. C., Young, S. L., Carr, J. R., & Madson, M. R. (2014). Challenges in implementing a screening method for veterinary drugs in milk using liquid chromatography quadrupole time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry, 62*(17), 3660–3674. https://doi.org/10.1021/jf405321w

Vergeynst, L., Van Langenhove, H., & Demeestere, K. (2015). Balancing the false negative and positive rates in suspect screening with high-resolution Orbitrap mass spectrometry using multivariate statistics. *Analytical Chemistry, 87*(4), 2170–2177. https://doi.org/10.1021/ac503426k

Wang, L., Xiao, Q., Yuan, M., & Lu, S. (2022). Discovery of 18 organophosphate esters and 3 organophosphite antioxidants in food contact materials using suspect and nontarget screening: Implications for human exposure. *Environmental Science & Technology, 56*(24), 17870–17879. https://doi.org/10.1021/acs.est.2c05888

Wu, W., Huang, X., Liang, R., Guo, T., Xiao, Q., Xia, B., Wan, Y., & Zhou, Y. (2023). Determination of 63 mycotoxins in grain products by ultrahigh-performance liquid chromatography coupled with quadrupole-Orbitrap mass spectrometry. *Food Control, 150*, 109772. https://doi.org/10.1016/j.foodcont.2023.109772

Yang, G., Zhang, J., Tang, Y., Kong, C., Li, S., Wang, S., … Chi, H. (2024). Development and validation of rapid screening of 192 veterinary drug residues in aquatic products using HPLC-HRMS coupled with QuEChERS. *Food Chemistry: X, 22*, Article 101504. https://doi.org/10.1016/j.fochx.2024.101504

Yang, Y., Yin, Y., Wang, S., & Dong, Y. (2021). Simultaneous determination of zearalenone and ochratoxin A based on microscale thermophoresis assay with a bifunctional aptamer. *Analytica Chimica Acta, 1155*, Article 338345. https://doi.org/10.1016/j.aca.2021.338345

Zhang, X., Qu, J., Dai, Z., Lin, Y., Lu, G., Yang, S., You, Y., Liu, H., Wu, Y., Jiang, G., & Li, Y. (2021). Data-dependent acquisition based high-resolution mass spectrum for trace Alternaria mycotoxin analysis and sulfated metabolites identification. *Food Chemistry, 364*, 130450. https://doi.org/10.1016/j.foodchem.2021.130450

Zhang, Y., Liu, F., Li, X., Gao, Y., Li, K., & Zhang, Q. (2024). Generic and accurate prediction of retention times in liquid chromatography by post–projection calibration. *Communications Chemistry, 7*(1), 54. https://doi.org/10.1038/s42004-024-01135-0

Zheng, M., Liu, H., Ye, J., Ni, B., Xie, Y., & Wang, S. (2022). Target-responsive aptamer-cross-linked hydrogel sensors for the visual quantitative detection of aflatoxin B1 using exonuclease I-triggered target cyclic amplification. *Food Chemistry: X, 15*, Article 100395. https://doi.org/10.1016/j.fochx.2022.100395