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Normalization of LC-MS mycotoxin analysis using the *N*-alkylpyridinium-3-sulfonates (NAPS) retention index system

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ABSTRACT: A major challenge for LC-MS analysis is the ability to compare data between laboratories and across instrument platforms. Normalization of mycotoxin analysis has two major challenges: the difficulty of acquiring authentic standards for many mycotoxins and the wide range of analytical methodologies being used. Currently, identification relies on dereplication strategies based on physicochemical properties such as the *m/z* of the precursor and product ions. Unlike these intrinsic properties, retention time (RT) is an extrinsic property impacted by LC conditions, including mobile phases and column chemistry, making exchange of data between groups difficult. To address this, we propose using an electrospray compatible, retention index (RI) system based on a series of *N*-alkylpyridinium-3-sulfonates (NAPS). These standards of differing alkyl chain length span RI units from 100 to 2000, are UV active, and have fixed positive and negative charges for electrospray ionization in either mode. Using a Thermo Q-Exactive LC-MS, the RIs of 96 mycotoxins and fungal secondary metabolites were normalized with the NAPS RI system under multiple pH, column and gradient chromatographic conditions. Additionally, we developed software that allows users to convert published RIs to a predicted RT value for their specific analysis conditions. As proof of concept, we were able to substantially decrease the number of false positives from a crude extract of *Penicillium roqueforti* by using an RI filter as well as a secondary correction for increased accuracy. Implementation of the NAPS RI will greatly enhance the sharing of fungal metabolite and mycotoxin data between laboratories and improve individual laboratory dereplication of unknown natural products.

Fungi are capable of producing an immense diversity of natural products. Some fungal metabolites, including aflatoxins and fumonisins, contaminate agricultural crops and foodstuffs, posing significant risks to human and animal health, resulting in large economic losses.[1] Fungi are also responsible for the production of beneficial metabolites such as penicillin and cephalosporin that are the basis for the most widely used antibiotics.[2] One of the major challenges for mycotoxin analysis is the difficulty in comparing data across laboratories, which employ different analytical methodologies and instrument platforms. The identification of mycotoxins and fungal metabolites is particularly challenging as the number of commercially available standards is limited or are restrictive due to their costs. To this end, great effort has been made to develop tools for the identification of fungal metabolites based on their unique physicochemical properties, without direct comparison to standards.[3] Liquid chromatography (LC) is an extensively used chemical screening technique, enabling compounds to be distinguished based on their partitioning between a solid phase material and a liquid mobile phase. Identifying compounds using only retention times (RT) is problematic; RTs are extrinsic properties that are influenced by column chemistry, mobile phase compositions, the gradient program, and temperature. Similar to approaches in gas chromatography,[4] an analyte's RT can be converted into a more universal retention index (RI) by comparison to the RTs of a homologous series of standards, each with a defined RI value.[5-7] Frisvad (1987), reported the UV spectra of 182 fungal metabolites and used a series of alkylphenones to calculate their RIs.[8] This was the first major attempt to normalize fungal metabolite analysis and provided a tool for dereplication of unknowns. Since that time, major technological innovations, notably electrospray ionization (ESI)[9] and high resolution mass spectrometry (HRMS) have dramatically advanced fungal metabolite analysis. HRMS allows the high-throughput measurement of intrinsic physicochemical properties, specifically accurate *m/z* and isotopic pattern for chemical formula determination.[10] The development of spectral libraries such as MassBank[11,12] and METLIN,[13] which house tens of thousands of product ion spectra have also greatly assisted in the confident identification of unknowns based on their specific MS/MS dissociation patterns.

Despite the benefits of using accurate mass and MS/MS to identify compounds, the lack of a normalized convention for de-

scribing retention times has provided some analytical challenges, particularly for isobaric compounds. For example, *Aspergillus niger* was reported to produce the mycotoxin fumonisin B_1, a potent and regulated sphinganine analogue synthesized by *Fusarium verticillioides*,[14] when in fact it makes the isobaric compound, fumonisin B_6.[15-17] Another important example is the analysis of 3-acetyldeoxynivalenol (3ADON) and 15-acetyldeoxynivalenol (15ADON), which are isomers produced by different populations of *F. graminearum*.[18,19] Distinguishing these compounds is especially important since it reveals which population is the source of the contamination and the compounds themselves interact differentially with the host plant's defence mechanisms.[20] Although the retention indices of 474 fungal metabolites calculated using alkylphenone standards have been reported,[3] the hyphenation of LC and MS has presented certain impediments in continuing the implementation of alkylphenone RIs moving forward. Alkylphenones have strong absorbing chromophores that are easily detected by UV, however, lack the acidity or basicity to be readily ionized by ESI thus have low MS sensitivity. To address this problem, a 20 member series of *N*-alkylpyrinium-3-sulfonates (NAPS) that is fully compatible with LC-ESI-MS has recently been introduced (Figure 1).[21] By design, these compounds possess a strong UV chromophore in addition to fixed positive and negative charges, which greatly improves ionization efficiency in both ESI ionization modes.

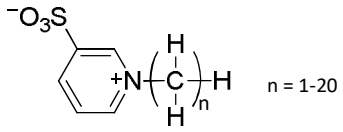

Figure 1. Chemical structure of the *N*-alkylpyridinium-3-sulfonate (NAPS) series for n = 1-20. Defined RI values are 100 × carbon length of alkyl chain (n). In positive ionization mode, the $[M+H]^+$ is the major ion type; in negative ionization mode, the major ion type is as an adduct with formic acid, $[M+HCOO]^-$ when formic acid is present in the mobile phases.

To facilitate modern dereplication efforts, we have performed LC-MS/MS analysis on 96 fungal metabolites and contributed their product ion spectra and NAPS RI values to the NORMAN MassBank library.[12] Furthermore, we have measured these RI values under several chromatographic conditions and produced a python software module allowing analysts to convert the RIs of metabolites found in databases to the predicted RTs of their specific method.

EXPERIMENTAL SECTION

A total of 96 mycotoxins and fungal secondary metabolites were obtained from either commercial sources or were purified from fungal cultures (ESM Table S1). Unless otherwise listed, the standards were prepared and analyzed as 10 µg mL^{-1} solutions in 80% Methanol.

LC-MS/MS analysis

NAPS standards, mixtures of mycotoxins and fungal extracts were analyzed using a Thermo Q-Exactive Orbitrap® mass spectrometer coupled to an Agilent 1290 HPLC system. The following conditions were used for heated electrospray ionization (HESI): capillary voltage 3.9 kV; capillary temperature, 400 °C; sheath gas, 17 arbitrary units; auxiliary gas, 8 units; probe heater temperature, 450 °C and S-Lens RF level, 45%. Full MS analysis was performed at a resolution of 35 000, automatic gain control (AGC) target of 1×10^6 and maximum injection time of 64 ms. MS/MS spectra of each compound were acquired at 5 different collision energies, at a resolution of 17 500, isolation window of 1.2 *m/z*, automatic gain control (AGC) target of 1×10^6 and maximum injection time of 64 ms. MassBank input files were generated for each spectrum and are deposited in the NORMAN MassBank (*https://massbank.eu/MassBank/*) under contributor 'AAFC'.

Retention Index Calculations

The NAPS standards were developed by the National Research Council (NRC) of Canada and produced at a stock concentration of 100 µM in methanol.[21] They are available as a reference material (RM-RILC) from NRC (https://nrc.canada.ca/en/certifications-evaluations-standards/certified-reference-materials/list). Solutions of fungal metabolites were spiked with the NAPS stock solution for a final concentration of 1 µM. These mixtures were first resolved using LC method A: Zorbax Eclipse Plus RRHD C18 (2.1 × 50 mm, 1.8 µm; Agilent Technologies, CA, USA) column, maintained at 35°C and mobile phases were of water with 0.1% formic acid (A), and acetonitrile with 0.1% formic acid (B) (Optima grade, Fisher Scientific, NJ, USA). The gradient, developed for rapid, multi-mycotoxin analysis,[20] consisted of 0% B for 0.5 min before increasing to 100% over 3 min, held at 100% for 3 min and reduced to 0% over 0.5 min. The retention times of the fungal metabolites as well as the 20 NAPS standards were recorded following 5 µL injections and detection in positive ionization mode. The defined RI values and measured RTs of the NAPS standards (200-2000) were fit using the Akima cubic spline function in the SciPy Python module,[22,23] and used to calculate the RIs of the 96 fungal metabolites. A stand-alone RI converter was developed https://github.com/sumarah-lab/Retention-Time-Converter. NAPS-100 was excluded due to co-elution with NAP-200 in the void volume. To understand the effects of varying chromatographic conditions, RI values were calculated in an identical manner as above (ESM Table S2) after changing the C18 column, mobile phases or gradient conditions (Table 1).

Retention Index Correction Factor

A secondary correction factor to further improve accuracy of RT prediction across various LC methods was explored. Method A was defined as the primary method to which all methods were corrected to. A number of structurally representative compounds, were used as 'anchor' compounds in a multi-point correction. The five anchor compounds used in this study and their MassBank accession codes are: rubrofusarin (AC000705-AC000708), ergocryptinine (AC000303-AC000312), viridicatin (AC000273-AC000277), roridin A (AC000555-AC000563) and macrosporin (AC000185- AC000188)) were selected based on their ability to correct the RIs of the majority of standards. The difference between an anchor compound's RI

in each method and the RI in method A was used to correct for each specific group of compounds. (ESM Table S3). The anchor compounds used here were selected based on their structural similarity with a target analyte, and therefore, analysts could potentially select any analyte to act as the anchor.

Table 1 LC-MS chromatography methods tested.
[a] - Zorbax Eclipse Plus RRHD C18 (2.1mm, 1.8µm particle size)
I -%B: 0 min,0%; 0.5 min,0%; 3.5 min,100%; 5.5 min,100%; 6.0 min, 0%
II -%B: 0 min,0%; 0.5 min,0%; 6.5 min/],100%; 8.5 min,100%; 9.0 min, 0%
III-%B: 0 min,0%; 0.5 min,0%; 15.5 min,100%; 17.5 min,100%; 18.5 min, 0%
IV-%B: 0 min,0%; 0.5 min,0%; 30.5 min,100%; 32.5 min,100%; 33.0 min, 0%

Method	Column[a]	Gradient	H_2O +	ACN +
A	50 mm	I	0.1% FA	0.1% FA
B	100 mm	I	0.1% FA	0.1% FA
C	50 mm	II	0.1% FA	0.1% FA
D	100 mm	II	0.1% FA	0.1% FA
E	50 mm	III	0.1% FA	0.1% FA
F	100 mm	III	0.1% FA	0.1% FA
G	50 mm	IV	0.1% FA	0.1% FA
H	100 mm	IV	0.1% FA	0.1% FA
I	50 mm	I	0.05% FA	0.05% FA
J	50 mm	I	0.15% FA	0.15% FA
K	50 mm	I	0.2% FA	0.2% FA
L	50 mm	I	0.3% FA	0.3% FA
M	100 mm	I	0.3% FA	0.3% FA

Preparation of crude fungal extracts

Penicillium roqueforti isolated from barley was used as proof of concept.[23] Briefly, the strain was inoculated onto 9 cm polystyrene Petri dishes with YES agar media and incubated for 7 d at 21 ºC. A total of six agar plugs were removed using a sterilized 7 mm cork borer as previously described.[24] The plugs were transferred to a polypropylene tube to which 2 mL of ethyl acetate was added. The mixture for vortexed for 30s, followed by sonication at 35 ºC for 15 min. The supernatants were transferred to clean glass vials, dried under a gentle stream of nitrogen. The residue was reconstituted in 1 mL of 8:2 methanol:water and filtered using a 0.45µm PVDF syringe filter into a 2mL amber glass HPLC vial.

RESULTS AND DISCUSSION

Generation of MS/MS library

High resolution tandem mass spectrometry is routinely acquired by time-of-flight or Orbitrap mass analyzers. The MS/MS fragmentation of an analyte is highly dependent on the molecular structure and bond strengths of the precursor ion. Although major advances for *in silico* prediction of product ion spectra have been achieved,[24] accurate and platform specific prediction is not yet a reality. Experimentally observed product ions and their relative ratios are the result of the time frame of the dissociation reaction as well as the relative energetics of multiple competing unimolecular reactions.[25] For this reason, searchable libraries of product ion spectra have been key in allowing researchers to identify unknown analytes by MS/MS. In this study, MS/MS spectra were acquired at five collision energies for each fungal metabolite and uploaded to the searchable NORMAN MassBank database.

Retention Index Calculations

The retention indices of the NAPS standards are defined as the number of carbons in the N-alkyl chain times 100 (Figure 1). NAPS-100, and 200, which contain a methyl and ethyl alkyl chain respectively, both elute in the void volume under all chromatographic conditions tested (Figure 1). Similarly, NAPS-300 was only slightly retained and produced peak splitting. Beginning at NAPS-400, which contains a butyl chain, the series of NAPS compounds were eluted from the column in a predictable manner according to the length of their alkyl moiety (Figure 2).

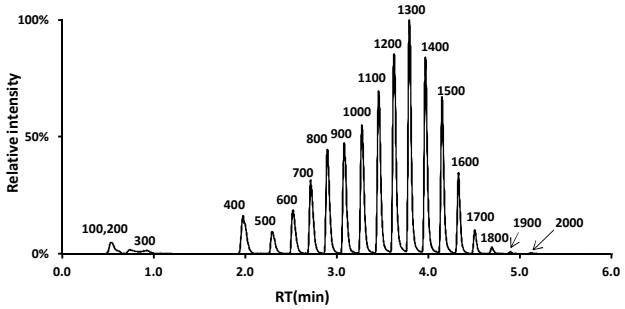

Figure 2. Combined extracted ion chromatograms for NAPS standards (RI 100-2000) using LC method A. NAPS-100 and 200 elute in the void volume while NAPS-300 is not resolved as a single peak.

The NAPS standard mix was co-spiked with the fungal metabolite solutions and the retention times of all analytes were recorded from their extracted ion chromatograms. As shown in Figure 3, the correlation between the NAPS RIs and the RTs can be described using a cubic spline function. In this dataset, only fungal metabolites with calculated RI values ranging from 400-2050 were included.

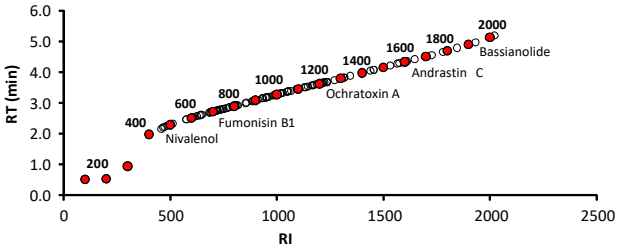

Figure 3. Relationship between RI and RT measured using method A (Table 1). The RT and RI relationship of the 200-2000 NAPS standards (in red) were fitted using an Akima cubic spline function. This function was then used to convert fungal metabolite RTs into RI values (Table 2).

The polar *Fusarium* metabolite, nivalenol, had the smallest RI at 458. The calculated RIs of enniatin A1, and enniatin A occurred above 2100. These metabolites showed high variance between runs as they were outside the NAPS calibration curve and were therefore excluded. Additional NAPS standards with

longer chain lengths will be required for these long-eluting analytes and are being considered for incorporation into a future reference material.

The isobaric trichothecenes 3ADON and 15ADON were not resolved to baseline with this LC method, however, their RTs and therefore RIs were different. The RI of the earlier eluting 15ADON was 700, and 3-ADON was 733, similar to the order of elution observed by Yang and co-workers[26] using a C18 column. Although these isomers have distinguishable retention times in this study, other studies have shown they co-elute.[27] This underlies the fact that LC experimental conditions can drastically affect the resolution of two compounds. Therefore, we measured the RIs of the 96 compounds under several LC conditions (Table 1) to estimate the level of precision possible when reporting RI values. (ESM Table S2).

Table 2 Measured RI values of Fungal Secondary Metabolites in Method A

Compound	RI	Compound	RI	Compound	RI
AAL toxin TB	689	DON-3-glucoside	465	Mycophenolic acid	1049
Aflatoxin B1	910	3-ADON	733	Neosolaniol	772
Aflatoxin B2	859	15-ADON	700	Nivalenol	458
Aflatoxin M1	750	3,15-DiacetylDON	963	NX Toxin	722
Alantrypinone	716	Diacetoxyscirpenol	884	Ochratoxin A	1188
α-Cyclopiazonic acid	1345	Emodin	1317	Penicillic Acid	649
Altenuene	794	Enniatin B	1846	Penitrem A	1533
Alternaric acid	1135	Enniatin B1	2021	Phacidin	1439
Alternariol	947	Ergocornine	689	Roquefortine A	576
Alternariol monomethyl	1212	Ergocorninine	716	Roquefortine C	733
Altersolanol A	605	Ergocristine	738	Roridin A	1106
Andrastin C	1577	Ergocristinine	778	Rubrofusarin	605
Atranone B	1647	Ergocryptine	722	Sambucinol	811
Aurantiamide	1100	Ergocryptinine	750	Satratoxin G	952
Aurofusarin	1236	Frequentin	1236	Satratoxin H	989
Averufin	1566	Fumiquinazoline F	1212	Stachybocin B	1456
Bassianolide	1933	Fumiquinazoline H	1027	Stachybocin C	1456
4',5-Bisdeoxy-dothistromin	1055	Fumonisin B1	700	Sterigmatocystin	1271
Beauvericin	1780	Fumonisin B2	794	Stipitatic acid	500
Bostrycin	639	Fumonisin B3	761	T-2 Toxin	1164
Brevianamide A	816	Fusarenone-X	576	HT-2 Toxin	931
7,8-Dihydroxycalonectrin	805	Herqueichrysin	1612	T-2 Tetraol	488
Cercosporin	1206	Isomarticin	952	Tentoxin	936
Chaetoglobosin A	1236	Iso-Roridine E	1294	Traversianal	1728
Chaetoglobosin C	1317	Koninginin G	1022	Trichodermamide C	644
Chetomin	1224	Koninginin A	1129	Trichoverrol A	1016
Citrinin	513	Koninginin E	1170	Verrucarin J	1400
Culmorin	1147	Kotanin	1176	Verrucarol	605
5-Hydroxyculmorin	624	Macrosporin	1300	Viridicatin	1066
Cyclopenin	890	Marticin	1005	Viridicatol	853
Deoxynivalenol (DON)	504	Meleagrin	684	Walleminone	1094
DON-3-glucuronide	473	Mellein	1094	Zearalenone	1218

Effects of experimental conditions on measured RI

Utilization of the NAPS RI system for mycotoxin and fungal secondary metabolite LC-MS analysis in addition to the sharing of MS/MS spectra through spectral databases will greatly enhance the dereplication of known compounds between laboratories. However, the precision, and effects of different experimental conditions on the measured RI values needs to be understood. In this work, a total of 13 LC methods and resulting RIs were compared. For 7 of the tested LC conditions, the average absolute error in RIs compared to method A was less than 20 (Figure 4). Methods I-M showed that small differences in the concentration of formic acid (0.05%-0.3%) had limited effects on the RI value. In contrast, four methods had average RI errors above 30. The main difference with these LC methods from method A, is the length of the gradient, which was either 15 or 30 min compared to the 3 min gradient of method A (Table 1).

In order to improve the accuracy of a measured RI between methods, an optional secondary correction strategy is proposed. Altering the LC conditions led to a decrease in measured RI values of some compounds, while others increased. Compounds with similar structures were generally affected by changes to LC conditions in a similar manner (ESM Table S2). Accurate secondary corrections could be achieved by assigning 'anchor' compounds for other similar compounds. For example, the ergot alkaloid, ergocryptinine has a RI of 750 in method A, however, in the longer method G, the value is 851 (Δ+101 RI). Using this compound to apply a 101 RI unit correction on other alkaloids such as ergocorninine and ergocristinine, reduced their errors from 103 and 97 RI units to only 2 and 4, respectively. Within this dataset, we determined that as little as five anchor compounds were structurally similar to approximately half of the 96 compounds and their use could improve RI determination (Figure 4). The correction factor significantly improved the within method variation of 48 compounds (Figure 4, Wilcoxon Singed-ranks test; $p < 0.01$). Additionally, the retention index variation between the compounds was significantly reduced (Figure 5, Wilcoxon Signed-ranks test; $p < 0.05$).

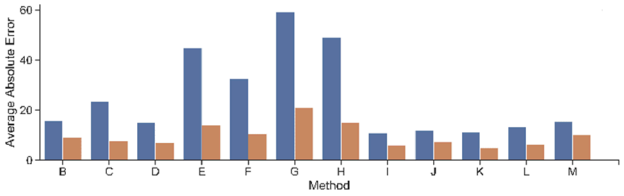

Figure 4. Average sum of the absolute errors of the corrected compounds for each method as compared to method A. Blue bars are uncorrected values and orange bars are corrected values.

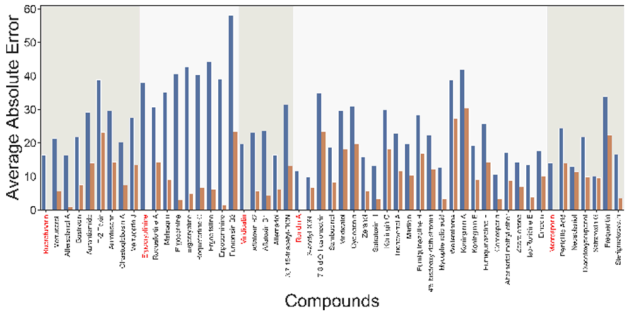

Figure 5. Average absolute errors for corrected compounds. Blue bars are uncorrected values and orange bars are corrected values. Red highlighted values are the anchor compounds used to correct all compounds in each shaded section.

Application: Analysis of Fungal Culture Extract

To demonstrate the benefits of using RI values for dereplication of a complex fungal extract, a sample derived from *P. roqueforti* was analyzed by LC-MS/MS on a 50mm C18 column using a 16.5-min method. Using the retention times of the NAPS standards within these samples, the RIs of the deposited 96 mycotoxins were then converted to predicted RTs for this method using our software.

Table 3: Comparison of LC-MS analysis at 50ppm and 5ppm without using RT and at 5ppm with use of RI and 5ppm with RI and a five point correction (RI'). This eliminated 7 false positives leaving two known *P. roqueforti* metabolites roquefortine A and C.

	50ppm no RT	5ppm no RT	5ppm RI ± 1 min	5ppm RI' ± 0.15 min
Roquefortine A	+	+	+	+
Roquefortine C	+	+	+	+
Iso-Roridine E	+	+	+	-
Mellein	+	+	-	-
Roridin A	+	+	-	-
Chaetoglobosin A	+	-	-	-
Trichoverrol A	+	-	-	-
NX-Toxin	+	-	-	-
Trichodermamide C	+	-	-	-

The fungal extract was initially screened for the 96 mycotoxins based only on accurate *m/z* (± 50 ppm) without any RT filtering (Table 3). In this approach, 9 putative analytes of the 96 were detected. Applying a ± 5 ppm accurate mass cutoff lowered the number of putative detections to five. When analytes were also scrutinized by their predicted retention times using RI (± 1 min), roquefortine A, and C, which are known metabolites of *Penicillium* were detected, along with iso-roridine E. The addition of a secondary correction using ergocryptinine as the anchor compound for Roquefortine A and C reduced the RI error between predicted and measured values. Conversely, when Iso-roridin E was corrected with its anchor compound roridin A (Figure 5), its RT error was exacerbated, reflecting that it was a false positive. A comparison to the published chemotaxonomy of *P. roqueforti* is consistent with these data, which document that roquefortine A and C are produced by *P. roqueforti* [28] but not iso-roridin E.

Conclusions

Application of the NAPS RI to mycotoxin analysis will allow for more accurate dereplication strategies leading to more identification of unknowns from food and feed samples. It will also facilitate ease of data exchange between laboratories and will reduce the number of misidentified compounds reported in the literature.

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References

(1) Wild, C. P.; Miller, J. D.; Groopman, J. D. *Mycotoxin control in low-and middle-income countries*; International Agency for Research on Cancer Lyon, France, 2015.
(2) Demain, A. L. *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 185-201.
(3) Nielsen, K. F.; Smedsgaard, J. *J. Chromatogr. A* **2003**, *1002*, 111-136.
(4) Kovats, v. E. *Helv. Chim. Acta* **1958**, *41*, 1915-1932.
(5) Smith, R. M. *J. Chromatogr. A* **1982**, *236*, 313-320.
(6) Smith, R. M. In *Journal of Chromatography Library*; Elsevier, 1995, pp 93-144.
(7) Hill, D. W.; Kelley, T. R.; Langner, K. J.; Miller, K. W. *Anal. Chem.* **1984**, *56*, 2576-2579.
(8) Frisvad, J. C.; Thrane, U. *J. Chromatogr. A* **1987**, *404*, 195-214.
(9) Yamashita, M.; Fenn, J. B. *J. Phys. Chem.* **1984**, *88*, 4451-4459.
(10) Kind, T.; Fiehn, O. *BMC Bioinf.* **2007**, *8*, 105.
(11) Horai, H.; Arita, M.; Kanaya, S.; Nihei, Y.; Ikeda, T.; Suwa, K.; Ojima, Y.; Tanaka, K.; Tanaka, S.; Aoshima, K. *J. Mass Spectrom.* **2010**, *45*, 703-714.
(12) Schulze, T.; Schymanski, E.; Stravs, M.; Neumann, S.; Krauss, M.; Singer, H.; Hug, C.; Gallampois, C.; Hollender, J.; Slobodnik, J. *NORMAN Network Bulletin* **2012**, *9*.
(13) Smith, C. A.; O'Maille, G.; Want, E. J.; Qin, C.; Trauger, S. A.; Brandon, T. R.; Custodio, D. E.; Abagyan, R.; Siuzdak, G. *Ther. Drug Monit.* **2005**, *27*, 747-751.
(14) Palencia, E. R.; Mitchell, T. R.; Snook, M. E.; Glenn, A. E.; Gold, S.; Hinton, D. M.; Riley, R. T.; Bacon, C. W. *J. Food Prot.* **2014**, *77*, 805-813.
(15) Mansson, M.; Klejnstrup, M. L.; Phipps, R. K.; Nielsen, K. F.; Frisvad, J. C.; Gotfredsen, C. H.; Larsen, T. O. *J. Agric. Food. Chem.* **2009**, *58*, 949-953.
(16) Qi, T. F.; Renaud, J. B.; McDowell, T.; Seifert, K. A.; Yeung, K. K.-C.; Sumarah, M. W. *J. Agric. Food. Chem.* **2016**, *64*, 1583-1589.
(17) Renaud, J. B.; Kelman, M. J.; Qi, T. F.; Seifert, K. A.; Sumarah, M. W. *Rapid Commun. Mass Spectrom.* **2015**, *29*, 2131-2139.
(18) Miller, J.; Taylor, A.; Greenhalgh, R. *Can. J. Microbiol.* **1983**, *29*, 1171-1178.
(19) Miller, J. D.; Greenhalgh, R.; Wang, Y.; Lu, M. *Mycologia* **1991**, 121-130.
(20) Renaud, J. B.; Sumarah, M. W. *Anal. Bioanal.Chem.* **2016**, *408*, 3083-3091.
(21) Quilliam, M. A.; Patents WO2017/053706A1, **2017**, and US10,228,356B2, **2019**.
(22) Jones, E.; Oliphant, T.; Peterson, P. **2014**.
(23) Kelman, M. J. **2016**.
(24) Allen, F.; Pon, A.; Wilson, M.; Greiner, R.; Wishart, D. *Nucleic Acids Res.* **2014**, *42*, W94-W99.
(25) Baer, T.; Mayer, P. M. *J. Am. Soc. Mass. Spectrom.* **1997**, *8*, 103-115.
(26) Yang, D.; Geng, Z.; Yao, J.; Zhang, X.; Zhang, P.; Ma, H. *World Mycotoxin J.* **2013**, *6*, 117-125.
(27) Blesa, J.; Moltó, J.-C.; El Akhdari, S.; Mañes, J.; Zinedine, A. *Food Control* **2014**, *46*, 1-5.
(28) Nielsen, K. F.; Sumarah, M. W.; Frisvad, J. C.; Miller, J. D. *J. Agric. Food Chem.* **2006**, *54*, 3756-3763.