Screening and Verifying Mycotoxins in Food with Q-TOF LC/MS and an Accurate Mass Library

Application Note

Food and Agriculture

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Abstract

This application note describes the creation of an accurate mass library for mycotoxins and related metabolites and its application for the screening of mycotoxins in food. An Agilent 1290 Infinity LC coupled to an Agilent 6550 iFunnel Q-TOF LC/MS was operated in positive and negative electrospray with dual-spray Agilent Jet Stream Technology. Accurate mass spectra were acquired for a large collection of mycotoxins and related metabolites in one or both ionization modes and for all relevant ion species.

Three different matrices were extracted and spiked with 44 indicator compounds. Samples were analyzed using target MS/MS and All Ions MS/MS acquisition. This work demonstrates the value of both acquisition modes, combined with an efficient data analysis workflow and the Mycotoxins and Related Metabolites Personal Compound Database and Library (PCDL), for the screening and verification of mycotoxins in complex matrices.

Agilent Technologies

Introduction

Mycotoxins are toxic secondary metabolites of fungi, which can occur in various food and feed products including cereals, nuts, fruits, spices, wine, and coffee [1]. They can cause hepatotoxic, mutagenic, carcinogenic, estrogenic, or immunosuppressive effects in humans and animals. The several hundreds of mycotoxins and secondary fungal metabolites belong to different chemical classes with very different physicochemical properties. Currently, only about a dozen compounds are considered as major health risks and are, therefore, regulated in food and feed. In Europe, Commission Regulation (EC) 1881/2006 and its amendments specify maximum levels for aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, patulin, and zearalenone in food [2]. In addition, there are indicative levels for T-2 and HT-2 toxin specified in Commission Recommendation 2013/165/EU [3].

Comprehensive data on the occurrence of mycotoxins apart from the regulated compounds is limited, especially in food matrices other than raw cereals [4]. This is one reason why, in recent years, single mycotoxin methods have been increasingly replaced by LC/MS-based multitarget methods [4,5]. The harmonization of methods for different commodities, the identification of mycotoxins in unlikely matrices, and the increase in knowledge of emerging mycotoxins from *Aspergillus*, *Penicillium*, *Fusarium*, or *Alternaria* species are just a few reasons for this trend. These developments were aided by the increase in performance of modern LC/MS instruments over the last few years, and the development of software tools that enhance productivity. Modern high-resolution, accurate-mass LC/Q-TOF instruments can analyze a virtually unlimited number of contaminants [6]. They also allow retrospective data analysis to find contaminants that were not considered at the time of the measurement [7].

While most multitarget methods were developed for screening contaminants, they also allow acquisition of quantitative information for regulated mycotoxins. The challenges are the efficient extraction of analytes with largely different physicochemical properties from many food products, and the huge differences in naturally occurring toxin concentrations.

This application note describes the creation and use of an accurate mass LC/MS/MS database and library. The library contains spectra for more than 300 LC/MS-amenable mycotoxins and fungal or bacterial metabolites, to screen and identify these compounds in food samples. The sample preparation method comprises a single extraction with an acidified acetonitrile-water mixture. Two different screening strategies are used. In the conventional approach, the Q-TOF LC/MS system is first operated in TOF mode and a database search is conducted. Using a second injection, a targeted MS/MS method of the list of suspects was used and the obtained spectra were compared to the MS/MS library. In a second approach, the Q-TOF operates in the All Ions MS/MS mode with two collision energies. The All Ions technique features easy setup of the acquisition method and verification of the mycotoxins using the MS/MS spectral library. This method produces chromatographic coelution of the precursor and product ions. We show method performance parameters for 44 representative indicator compounds in maize, hazelnuts, and wine.

Experimental

Reagents and standards

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile, methanol, and formic acid were purchased from VWR International (Vienna, Austria). Ammonium formate solution (p/n G1946-85021) came from Agilent. Ultrapure water was produced using a Purelab Ultra system (ELGA LabWater, Celie, Germany). Analytical standards of the fungal and bacterial metabolites were purchased from Enzo Life Sciences (Lausen, Switzerland), Bioviotica Naturstoffe GmbH (Dransfeld, Germany), Bioaustralis (distributed by Tebu-Bio, Germany), Iris Biotech GmbH (Marktredwitz, Germany), Romer Labs (Tulln, Austria), or Sigma-Aldrich Corp. (Vienna, Austria). Other standards were provided as isolates from research groups around the world.

Stock standard solutions were prepared by dissolving the reference compounds in acetonitrile, methanol, water, or mixtures of these, depending on the physicochemical properties of the substance. The individual standard solutions were combined to a multi-analyte working solution that was used for calibration and for spiking blank samples. Stock standard solutions and the multi-analyte working solution were stored until use at –20 °C. Calibration samples were prepared by diluting the working solution with a mixture of acetonitrile:water:formic acid (79:20.9:0.1, v:v:v). The extraction solvent had the same composition.

Sample preparation

Blank maize and hazelnut samples for spiking experiments were purchased and were checked for the absence of any compounds of interest with an LC/MS/MS method. The samples were ground and homogenized using an electric blender. A 5 g (± 0.01 g) portion of the samples was weighed in a 50 mL polypropylene tube and 20 mL extraction solvent was added. The samples were extracted at room temperature for 90 minutes on a rotary shaker. After settling of the solid residue, an aliquot of the extract was transferred to an HPLC vial. The raw extracts were spiked with the multianalyte working solution at three different concentration levels.

LC/MS/MS analyses

Separation was carried out using an Agilent 1290 Infinity UHPLC, consisting of:

- Agilent 1290 Infinity Binary Pump (G4220A),
- Agilent 1290 Infinity High Performance Autosampler (G4226A), and
- Agilent1290 Infinity Thermostatted Column Compartment (G1316C)

The UHPLC system was coupled to an Agilent 6550 iFunnel Quadrupole Time-of-Flight LC/MS equipped with a dual-spray Agilent Jet Stream electrospray ionization source. Reference mass ions were delivered using an Agilent 1260 Infinity Isocratic Pump (G1310B) operated at 1.0 mL/min and using a 1 in 100 flow splitter (p/n G1607-60000). The final flow rate to the reference sprayer was 10 µL/min. The Q-TOF LC/MS instrument was operated with Agilent MassHunter Data Acquisition Software, rev. B.05.01, in 2 GHz extended dynamic range mode with positive or negative ionization with two different methods. In target MS/MS acquisition, a data rate of three scans/s in MS and MS/MS mode was used. All Ions MS/MS acquisition used three scans/s with two discrete collision energies. The use of two collision energies resulted in alternating spectra with a low-energy channel containing the precursor ion and two high-energy channels containing the precursor and product ions.

Chromatographic conditions

Conditions for chromatography

Column:	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)
Mobile phase:	A) 5 mM NH_4 formate + 0.1% formic acid B) 5 mM NH_4 formate + 0.1% formic acid in methanol
Gradient:	Time (min) % B 0.0 10 0.5 10 10.0 98 15.0 98 15.1 10 17.0 10
Stop time:	17.0 min
Temperature:	30 °C
Flow rate:	0.40 mL/min
Injection volume:	2 µL

Conditions for Dual AJS

Gas temperature:	130 °C
Gas flow:	16 L/min
Nebulizer:	30 psig
Sheath gas temperature:	300 °C
Sheath gas flow:	11 L/min
Capillary voltage:	+ve 4,000 V; –ve 4,000 V
Nozzle voltage:	+ve 500 V; –ve 500 V
Reference mass correction:	+ve 121.05087 and 922.00980; –ve 112.98559 and 966.00073

All-Ions MS/MS

Mass range:	40 to 1,300 amu
Scan rate:	3 spectra/s
Collision energies:	0 – 10 – 40 V

Target MS/MS

MS mass range:	80 to 1,300 amu
MS/MS mass range:	40 to 1,300 amu
Scan rate:	3 spectra/s for MS and MS/MS
Collision energy:	20 V
Target masses:	45 (positive and negative), Delta RT 0.5 min

Data were evaluated using MassHunter Qualitative Analysis Software B.07.00. Positive identifications of mycotoxins were reported if the compound was detected by the Find by Formula data-mining algorithm with a mass error below 5 ppm and with a sufficient score (including isotope abundance and isotope spacing). A retention time window of ± 1 minute was specified for peak detection to compensate for retention time shifts due to system-to-system variability.

Creation of the mycotoxins and related metabolites PCDL

Accurate mass spectra were acquired with flow injection or through a short column of single-analyte solutions in target MS/MS mode with collision energies of 10, 20, and 40 eV. All relevant compound species including $[M+H]^+$, $[M-H]^-$, $[M+NH_4]^+$, and $[M+HCOO]^-$ were used as target masses. If precursor ion stability required higher collision energies, extra spectra were acquired in a second run. In either positive or negative ionization mode, meaningful MS/MS spectra were acquired for more than 300 mycotoxins and other fungal or bacterial metabolites. For several compounds, MS/MS library spectra were captured in both ionization modes and for more than one precursor ion species. Solid standards or stock solutions were collected over more than a decade. Most compounds were purchased from different suppliers, the others were either isolated at IFA-Tulln, BOKU or were provided by other research groups. To eliminate mass assignment errors, fragment masses in the acquired spectra were compared to the theoretical fragment formulas and corrected to their theoretical masses. All MS/MS spectra were curated for spectral noise. A minimum base peak threshold was applied to ensure good ion statistics for all fragment ions. The corrected spectra were included in the Agilent Personal Compound Database and Library for Mycotoxins and Related Metabolites (p/n G5883CA), which was used for the screening and verification of mycotoxins in food samples. For the 44 indicator compounds, retention time information was added to the library by analyzing a comprehensive mycotoxin standard with the given UHPLC method.

Figure 1 shows a screen capture of the MassHunter PCDL Manager Software, along with the accurate mass spectrum of the $[M+NH_4]^+$ species of T-2 toxin acquired with a collision energy of 10 eV.

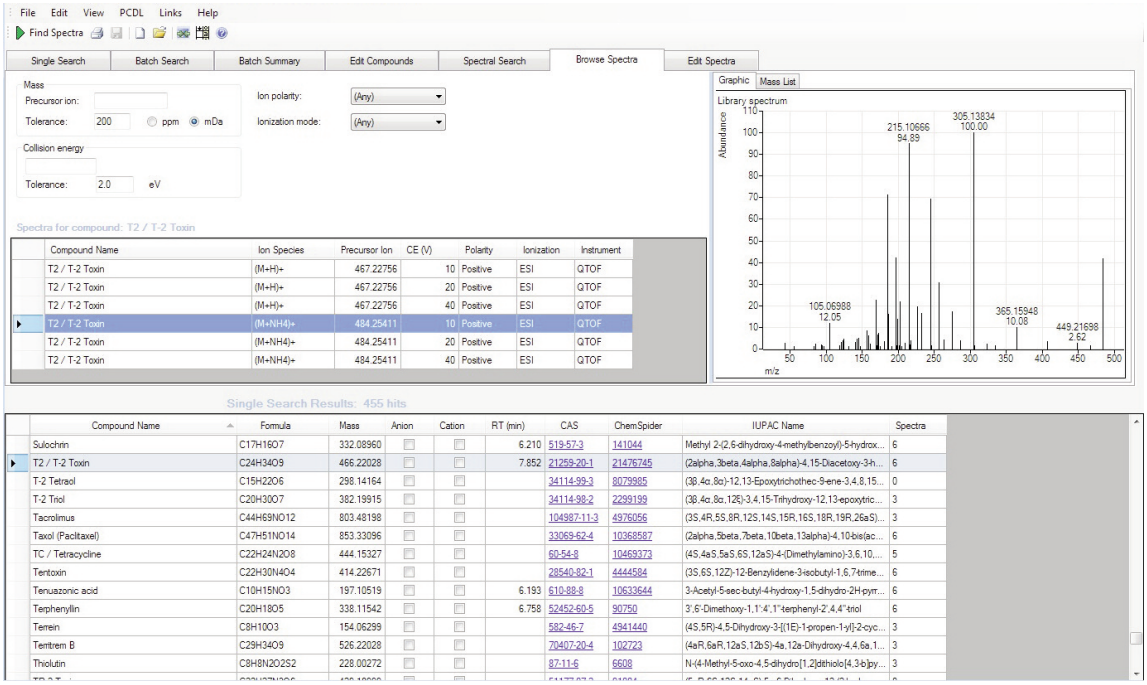

Figure 1. Agilent MassHunter PCDL Manager Software showing the Mycotoxins and Related Metabolites PCDL and the accurate mass spectrum for the $[M+NH_4]^+$ species of T-2 toxin at a collision energy of 10 eV.

Results and Discussion

All Ions MS/MS acquisition for simultaneous screening and verification

Maize and hazelnut extracts, and red wine samples, were spiked with 44 indicator compounds from the group of mycotoxins and fungal metabolites. All regulated compounds, some polar and nonpolar analytes, and poor ionizing compounds were selected. In the All Ions MS/MS workflow, accurate mass data were collected without fragmentation in a low-energy channel. At the same time, compounds were fragmented with two different collision energies without precursor selection. Accurate mass fragment data were recorded in two high-energy channels. When the data are analyzed using the Find by Formula algorithm, precursor information is derived from the Mycotoxins and Related Metabolites PCDL and compound chromatograms are extracted for all specified ion species. For putative identifications, the spectra stored in the PCDL are used, and for a specified number of the most abundant fragments chromatograms are automatically extracted from the high-energy channels. For example, Figure 2A shows the accurate mass library spectrum of aspergillimide from the Mycotoxins and Related Metabolites PCDL compared to the cleaned high-energy spectrum from a spiked maize sample (Figure 2B). The red triangles indicate the fragment ions automatically selected from the library spectrum for evaluation. While the library spectrum is based on a collision energy of 40 eV, the cleaned high-energy spectrum combines information from both high-energy channels acquired with 10 and 40 eV.

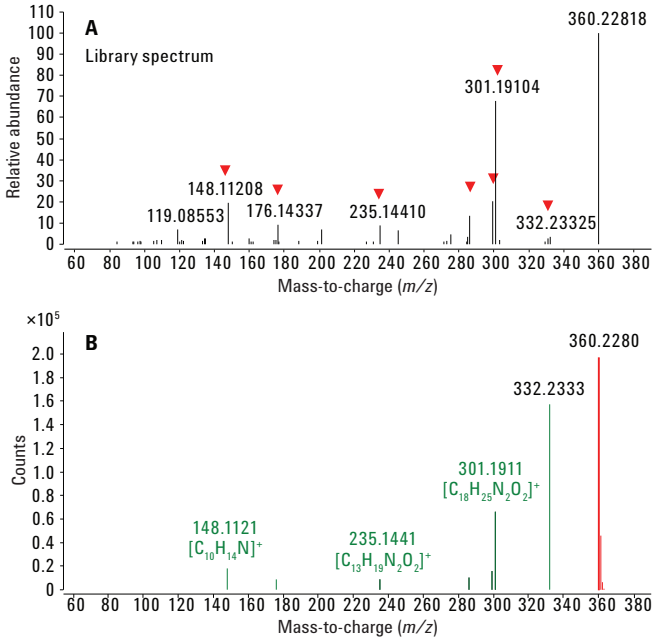

Figure 2. Accurate-mass library spectrum for aspergillimide at a collision energy of 40 eV (A) compared to the acquired high-energy spectrum (B) from a spiked maize sample (cleaned spectrum). Red triangles in the library spectrum indicate ions automatically selected for the All Ions MS/MS evaluation.

By overlaying chromatograms for both precursor and fragment ions and the calculation of a coelution score, the identity of the fungal metabolite aspergillimide was confirmed. The coelution score accounts for factors such as abundance, peak shape (symmetry), peak width, and retention time. The scores were plotted and made available for inspection in a coelution plot. Figure 3A shows the overlay of the precursor chromatogram with the fragment chromatograms from the high-energy channels. From the automatically extracted fragments, six fragment chromatograms showed coelution with the precursor ion, as revealed by the coelution plot in Figure 3B. Figure 3C shows the detailed identification results in the compound table.

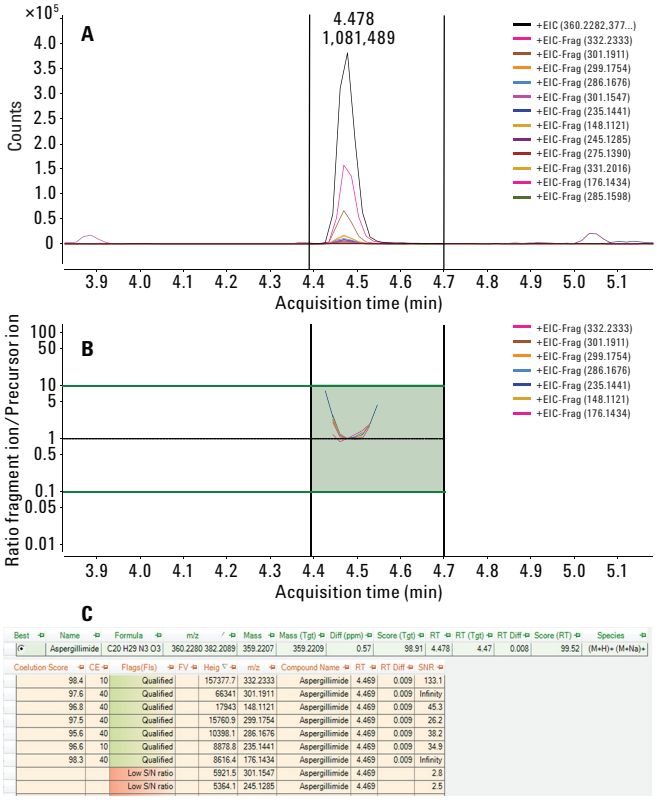

Figure 3. Overlay of precursor and fragment ion traces for aspergillimide in a spiked maize sample (A), coelution plot (B), and compound identification results including the coelution score (C).

Table 1 lists all 44 mycotoxins and fungal metabolites spiked into a maize extract at 30 ng/mL. Compounds were measured with positive or negative ionization and the predominant species for each analyte are given. Several compounds were detected and qualified in both polarities. In these cases, results for the more sensitive ionization mode are shown. At this concentration, most compounds were found by automatically searching using the Find by Formula algorithm. The mass deviations of the measured masses compared to the theoretical masses were generally below 1 ppm. We observed a mass deviation between 2 and 5 ppm for only 11 compounds. Therefore, target scores, including retention time, mass accuracy, isotope abundance, and isotope spacing, were typically above 90 (out of 100). Most of the compounds were verified with at least one extra fragment ion in either positive or negative ion mode. A minimum coelution score of 80 (out of 100) has been specified as the criterion for compound verification.

Screening and verification of mycotoxins in food by target MS/MS acquisition

The same samples were also analyzed using the same chromatographic method, but with target MS/MS acquisition. The Mycotoxins and Related Metabolites PCDL was used with the Find by Formula data-mining algorithm to find the compounds. Chromatograms for the expected ion species, MS and MS/MS spectra, were extracted automatically for the identified compounds. The results were scored based on the agreement of the accurate monoisotopic mass, the isotope ratio, the isotope spacing, and the retention time.

Figure 4 shows the compound chromatogram and peak spectrum of T-2 toxin spiked into a maize extract. The predominant ion species for T-2 toxin were $[M+NH_4]^+$ and $[M+Na]^+$ ions. The measured *m/z* signals for these species (blue) were in good agreement with the expected isotope ratio (red boxes). In total, the software assigned 10 ions to the $[M+H]^+$, $[M+NH_4]^+$, and $[M+Na]^+$ species of T-2 toxin, including their isotope signals. The good mass accuracy and isotope pattern matching was reflected in a good target score of 98.5 (out of 100). Target scores for the other compounds (not shown) were comparable to the values in Table 1 for the All Ions MS/MS workflow.

The red diamond indicates that an MS/MS spectrum was acquired for that *m/z*. MS/MS spectra were extracted automatically over the peak range and matched against the library spectra contained in the PCDL.

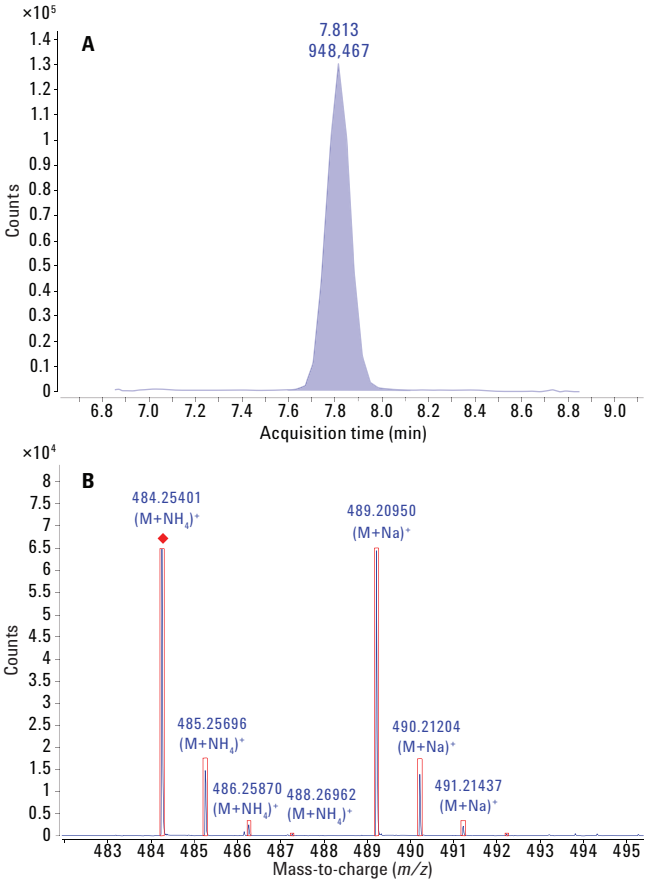

Figure 4. Compound chromatogram (A) and peak spectrum (B) obtained by the Find by Formula algorithm for T-2 toxin spiked in a maize sample at 30 ng/mL.

Table 1. Analysis of 44 mycotoxins and fungal metabolites spiked into maize extract at 30 ng/mL, and measured with positive or negative All Ions MS/MS acquisition.

Compound	Retention time (min)	Formula	Ion species	Mass	Mass deviation (ppm)	Target score	Coelution score
15-Monoacetoxyscirpenol	5.61	$C_{17}H_{24}O_6$	$[M+NH_4]^+$	324.1573	-2.52	96.8	96.3
16-Keto-Aspergillimide	5.85	$C_{20}H_{27}N_3O_4$	$[M+H]^+$	373.2002	-1.61	94.9	94.9
3-Acetyldeoxynivalenol	4.88	$C_{17}H_{22}O_7$	$[M+HCOO]^-$	338.1366	-2.73	98.0	
Aflatoxin B_1	6.36	$C_{17}H_{12}O_6$	$[M+H]^+$	312.0634	-0.59	98.2	90.2
Aflatoxin B_2	6.12	$C_{17}H_{12}O_6$	$[M+H]^+$	314.0790	0.86	98.5	96.9
Aflatoxin G_1	5.83	$C_{17}H_{12}O_7$	$[M+H]^+$	328.0583	-0.65	99.6	97.0
Aflatoxin G_2	5.56	$C_{17}H_{14}O_7$	$[M+H]^+$	330.0740	1.06	98.3	96.5
Aflatoxin M_1	5.62	$C_{17}H_{12}O_7$	$[M+H]^+$	328.0583	-0.14	97.7	95.3
Agroclavine	4.77	$C_{16}H_{18}N_2$	$[M+H]^+$	238.1470	-0.41	99.2	96.5
Alternariol	7.48	$C_{14}H_{10}O_5$	$[M-H]^-$	258.0528	0.27	97.5	96.6
Alternariolmethylether	8.72	$C_{15}H_{12}O_5$	$[M-H]^-$	272.0685	0.10	98.6	97.0
Aspergillimide	4.48	$C_{20}H_{29}N_3O_3$	$[M+H]^+$	359.2209	-0.57	99.1	97.2
Beauvericin	10.09	$C_{45}H_{57}N_3O_9$	$[M+NH_4]^+$	783.4095	0.24	99.2	95.5
Brevianamid F	4.99	$C_{16}H_{17}N_3O_2$	$[M+H]^+$	283.1321	-0.63	96.8	98.7
Curvularin	7.10	$C_{16}H_{20}O_5$	[M+H]+	292.1311	-0.86	99.1	97.8
Cyclopiazonic acid	8.86	$C_{20}H_{20}N_2O_3$	$[M+H]^+$	336.1474	-3.01	97.3	96.7
Cyclosporin A	10.45	$C_{62}H_{111}N_{11}O_{12}$	$[M+HCOO]^-$	1201.8414	-4.30	85.5	96.4
Diacetoxyscirpenol	6.34	$C_{19}H_{26}O_7$	$[M+NH_4]^+$	366.1679	-1.48	96.9	92.3
Deoxynivalenol	2.99	$C_{15}H_{20}O_6$	$[M+Na]^+$	296.1260	-1.80	97.6	
Emodin	7.39	$C_{15}H_{10}O_5$	$[M-H]^-$	270.0528	-2.46	91.1	
Enniatin B	9.90	$C_{33}H_{57}N_3O_9$	$[M+NH_4]^+$	639.4095	-2.96	95.2	92.5
Ergosine	6.29	$C_{30}H_{37}N_5O_5$	$[M+H]^+$	547.2795	-3.30	94.2	82.2
Ergosinine	6.19	$C_{30}H_{37}N_5O_5$	$[M+H]^+$	547.2795	-1.18	95.9	93.5
Ergotaminine/ergotamine	6.46	$C_{33}H_{35}N_5O_5$	$[M+H]^+$	581.2638	-1.29	97.5	86.3
Fumonisin B_1	7.78	$C_{34}H_{59}NO_{15}$	$[M+H]^+$	721.3885	-3.12	87.2	
Fumonisin B_2	8.69	$C_{34}H_{59}NO_{14}$	$[M+H]^+$	705.3936	-2.42	97.3	96.6
Fusarenon-X	3.91	$C_{17}H_{22}O_8$	$[M+Na]^+$	354.1315	-4.80	89.0	
HT-2 toxin	7.30	$C_{22}H_{32}O_8$	$[M+NH_4]^+$	424.2097	-2.05	97.8	97.8
Macrosporin	9.36	$C_{16}H_{12}O_5$	$[M-H]^-$	284.0685	0.33	97.6	96.6
Moniliformin	0.72	$C_4H_2O_3$	$[M-H]^-$	98.0004	-3.01	91.2	
Mycophenolic acid	7.75	$C_{17}H_{20}O_6$	$[M+H]^+$	320.1260	-0.09	97.7	97.3
Nivalenol	2.15	$C_{15}H_{20}O_7$	$[M+HCOO]^-$	312.1209	-3.22	87.4	
Ochratoxin A	8.38	$C_{20}H_{18}ClNO_6$	$[M+H]^+$	403.0823	-1.81	91.7	97.7
Paraherquamide A	6.26	$C_{28}H_{35}N_3O_5$	$[M+H]^+$	493.2577	-0.85	96.7	96.8
Patulin	2.38	$C_7H_6O_4$	$[M-H]^-$	154.0266	-1.07	99.7	92.0
Roquefortine C	7.35	$C_{22}H_{23}N_5O_2$	$[M+H]^+$	389.1852	-0.36	98.9	97.3
Skyrin	10.23	$C_{30}H_{18}O_{10}$	$[M-H]^-$	538.0900	-1.19	98.0	99.1
Stachybotrylactam	9.22	$C_{23}H_{31}NO_4$	$[M+H]^+$	385.2253	-1.10	99.5	97.0
Sulochrin	6.21	$C_{17}H_{16}O_7$	$[M-H]^-$	332.0896	-0.71	98.1	97.8
T-2 toxin	7.83	$C_{24}H_{34}O_9$	$[M+NH_4]^+$	466.2203	-0.18	97.4	97.7
Tenuazonic acid	6.19	$C_{10}H_{15}NO_3$	$[M+H]^+$	197.1052	0.96	99.1	
Terphenyllin	6.75	$C_{20}H_{18}O_5$	$[M+H]^+$	338.1154	-1.79	98.3	95.4
Viridicatin	7.97	$C_{15}H_{11}NO_2$	$[M+H]^+$	237.0790	1.26	99.0	96.2
Zearalenone	8.36	$C_{18}H_{22}O_5$	$[M-H]^-$	318.1467	0.10	99.3	96.0

Figure 5 shows the MS/MS spectrum for T-2 toxin acquired in the maize extract spiked at 30 ng/mL (top panel) in comparison to the library spectrum from the PCDL (bottom panel). The middle panel is a mirror representation of the difference spectrum. All major fragment ions in the library spectrum of T-2 toxin were found in the measured spectrum within a narrow mass extraction window and in a similar ratio as in the reference spectrum, for a collision energy of 20 eV. Thus, the reverse search against the accurate mass library resulted in a score of 91.7 out of 100 and verified the presence of T-2 toxin in the sample. The detailed compound identification results are shown in the compound table in Figure 6. MS/MS scores for all individual compounds are not shown, but had to be above 60 to achieve verification (see Figure 7).

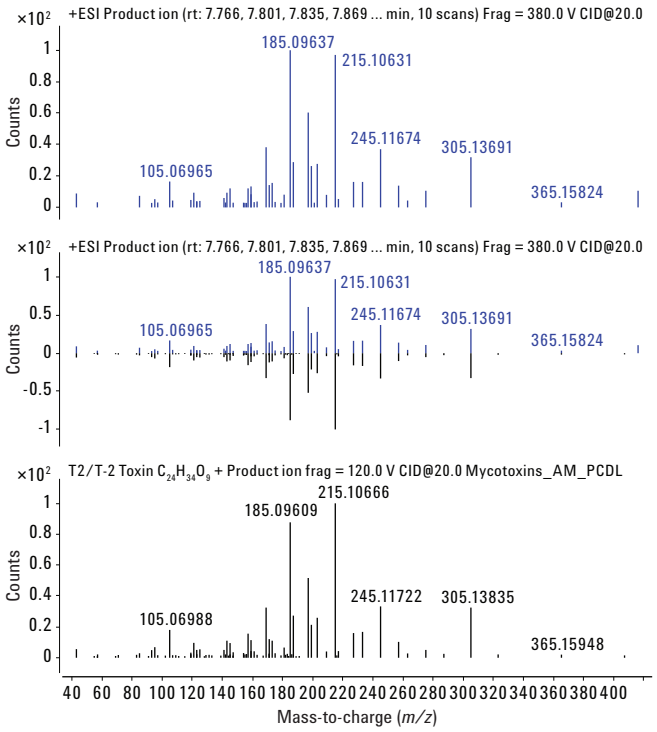

Figure 5. Comparison of the measured spectrum of T-2 toxin in the spiked maize extract with the reference spectrum from the Agilent Mycotoxins and Related Metabolites PCDL.

Comparison of the two workflows

Accurate-mass screening for mycotoxins and fungal metabolites, combined with verification of the identified contaminants by MS/MS library searching or All Ions MS/MS acquisition, was applied to spiked food extracts. Figure 7 shows the comparison of both acquisition modes for the three different matrices at 30 ng/mL. In general, red wine showed strong suppression effects, resulting in lower detection and verification rates compared to the other matrices. Target MS/MS acquisition with a single collision energy combined with library matching resulted in similar verification rates as the All Ions MS/MS acquisition with fragment coelution. In particular, the precursor isolation improved identification compared to the All Ions MS/MS fragmentation in the heavier matrix and for low mass contaminants. Conversely, All Ions MS/MS acquisition was very fast and allowed the differentiation of closely eluting isomers such as ergosine and ergosinine.

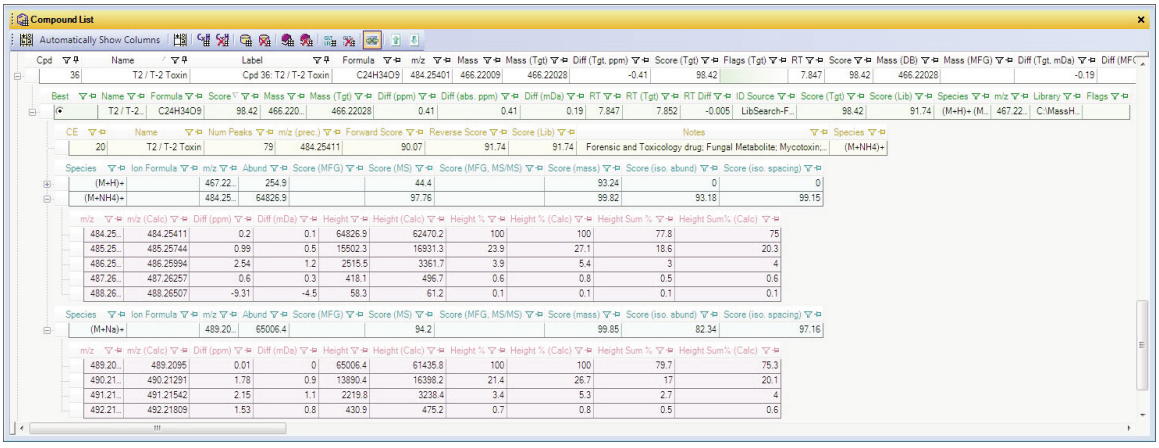

Figure 6. Compound identification results for T-2 toxin in a spiked maize sample including mass accuracy and isotope information from MS spectra, and MS/MS spectrum comparison.

Target MS/MS				All Ions MS/MS		
Red wine	Maize	Hazelnut	Compound name	Red wine	Maize	Hazelnut
			15-Monoacetoxyscirpenol			
			16-Keto-Aspergillimide			
			3-Acetyldeoxynivalenol			
			Aflatoxin B_1			
			Aflatoxin B_2			
			Aflatoxin G_1			
			Aflatoxin G_2			
			Aflatoxin M_1			
			Agroclavine			
			Alternariol			
			Alternariolmethylether			
			Aspergillimide			
			Beauvericin			
			Brevianamid F			
			Curvularin			
			Cyclopiazonic acid			
			Cyclosporin A			
			Diacetoxyscirpenol			
			Deoxynivalenol			
			Emodin			
			Enniatin B			
			Ergosine			
			Ergosinine			
			Ergotaminine/Ergotamine			
			Fumonisin B_1			
			Fumonisin B_2			
			Fusarenon-X			
			HT-2 toxin			
			Macrosporin			
			Moniliformin			
			Mycophenolic acid			
			Nivalenol			
			Ochratoxin A			
			Paraherquamide A			
			Patulin			
			Roquefortine C			
			Skyrin			
			Stachybotrylactam			
			Sulochrin			
			T-2 toxin			
			Tenuazonic acid			
			Terphenyllin			
			Viridicatin			
			Zearalenone			

Compound found by FBF and verified by library matching or coelution of fragments

Compound found by FBF, not verified

Compound not found by FBF

Figure 7. Results of screening and verification of mycotoxins and fungal metabolites in three different matrices at 30 ng/mL, using target MS/MS acquisition and library searching or All Ions MS/MS acquisition. Green: compound automatically found and presence verified by MS/MS library matching or fragment coelution; yellow: compound automatically found but no qualified MS/MS spectrum acquired.

Analysis of a real sample

In addition to the spiked matrices, a contaminated hazelnut sample was extracted according to the method and was injected into the Q-TOF LC/MS system using All Ions MS/MS in positive and negative modes. The precursor and fragment information from the All Ions MS/MS evaluation of a calibration sample was exported to MassHunter Quantitative Analysis for the rapid creation of a quantitative data processing method. After data processing, results were reviewed by sample and compound in the Compounds-at-a Glance module. Figure 8 shows the chromatograms of six fungal metabolites found as contaminants in the hazelnut sample. Alternariol, alternariolmethylether, zearalenone, and macrosporin were analyzed in negative mode. Brevianamid F and mycophenolic acid were analyzed in positive mode. For all compounds, the mass deviation of the precursor *m/z* and at least one fragment ion was below 5 ppm, which is required for the identification of the compound.

Conclusions

The method presented here comprises fast, easy, and cheap solvent extraction and the subsequent injection of the diluted raw extract into the UHPLC/Q-TOF/MS system. It takes full advantage of the low delay volumes of the Agilent 1290 Infinity LC and its ability to handle high backpressures in UHPLC separations to increase chromatographic resolution. The method benefits from the sensitivity of the Agilent 6550 iFunnel Q-TOF, and from the versatile ionization capabilities of the Agilent Jet Stream ionization source.

An accurate mass PCDL for Mycotoxins and Related Metabolites was created and applied for screening and verification of mycotoxins in food samples. Target MS/MS and All Ions MS/MS acquisition were evaluated by analyzing food samples containing 44 fungal metabolites. Both acquisition modes, with Agilent MassHunter Software and the unique built-in identification criteria, effectively verified the presence of mycotoxins in the sample. While the target MS/MS information gave more confidence for low molecular weight compounds, the All Ions MS/MS data can be reinterrogated later for compounds that were not in the scope of the analysis during initial measurement. For efficient data review, the quantitative analysis software was used. This allowed visualization of quantifier and qualifier ions, including quality criteria such as mass accuracy, qualifier ratios, library match scores, and retention time matching.

The method is an appropriate supplement to single analyte or analyte-group detection methods, to increase knowledge of the occurrence of mycotoxins in various food commodities.

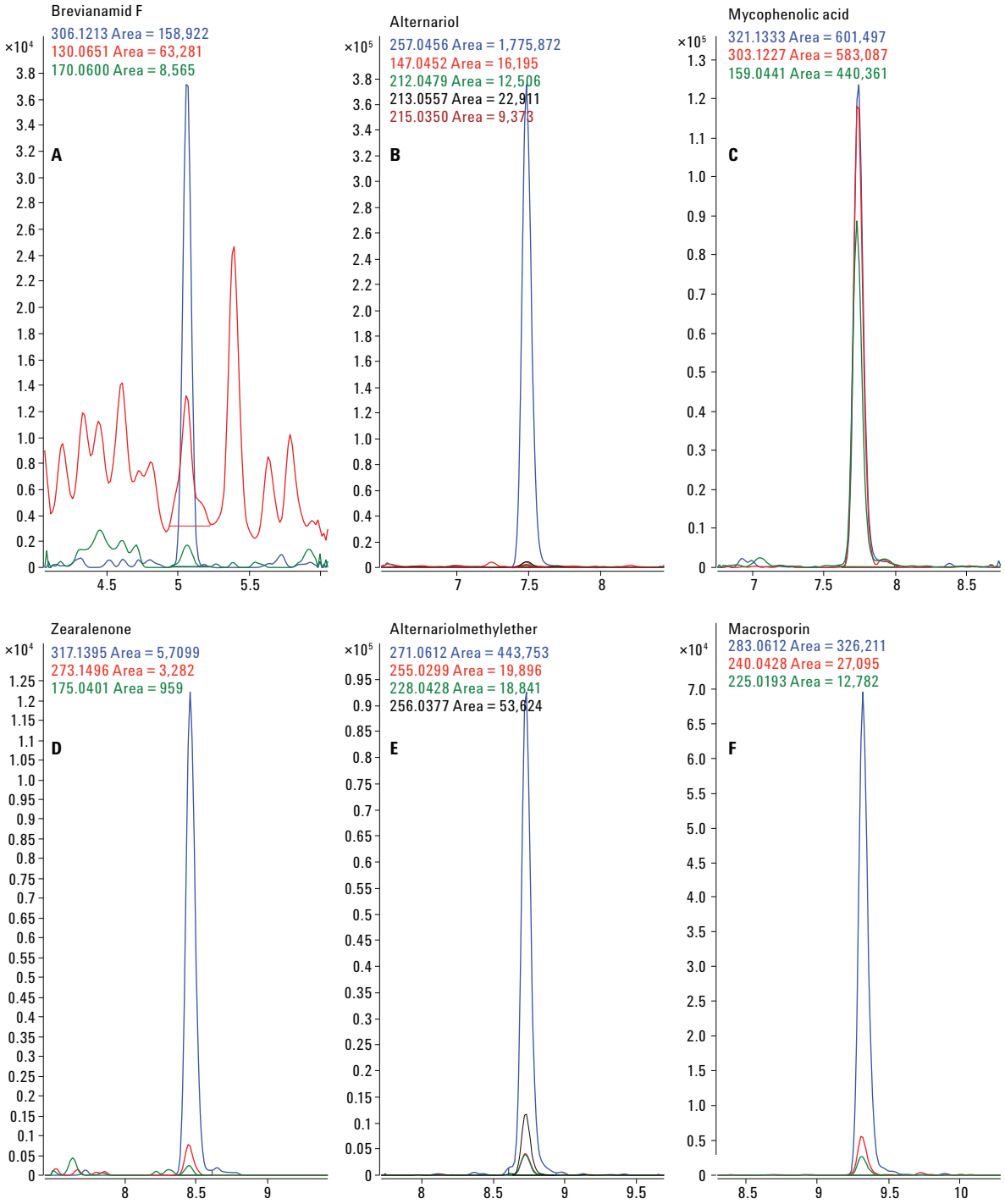

Figure 8. Chromatograms of mycotoxins detected in a naturally contaminated hazelnut sample. (A) brevianamid F (<LLOQ), (B) alternariol (310 µg/kg), (C) mycophenolic acid (6,100 µg/kg), (D) zearalenone (21 µg/kg), (E) alternariolmethylether (220 µg/kg), (F) macrosporin (520 µg/kg).

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