ASMS 2015

WP 052

Strategies for the Screening and Identification of Mycotoxins in Food by UHPLC/Q-TOF/MS

Elisabeth Varga[1], Emma Rennie[2], Thomas Glauner[3], Michael Sulyok[1], Maria Vandamme[2], Rudolf Krska[1], Franz Berthiller[1]

[1]University of Natural Resources and Life Sciences, Vienna (BOKU), Tulln, Austria; [2]Agilent Technologies Inc., Santa Clara, CA; [3]Agilent Technologies Sales & Services GmbH, Waldbronn, Germany

Introduction

Mycotoxins are toxic secondary metabolites of fungi which can occur in a variety of food and feed. They can cause hepatotoxic, mutagenic, carcinogenic, estrogenic or immunosuppressive effects in humans and animals. The formation of mycotoxins during grain production is a severe problem which can cause major financial harm. To ensure food safety the analysis of mycotoxins in food and feed is essential. There is a growing interest in multi-mycotoxin methods to harmonize the analysis of different commodities, to identify mycotoxins in unlikely matrices and to extend the knowledge of emerging mycotoxins from *Aspergillus*, *Penicillium*, *Fusarium* or *Alternaria* species. Modern high-resolution accurate mass LC/MS instruments allow the analysis of a virtually unlimited number of contaminants below the maximum limits stipulated by legislation.

In this poster we show the application of UHPLC/Q-TOF/MS/MS combined with an accurate mass library for mycotoxins and related metabolites for the screening and identification of contaminants in 3 representative matrices (maize, hazelnut, and wine).

Full details of this work are available as Agilent application note (5991-5667EN).

Experimental

Sample preparation and LC/MS method

Maize and nut samples were extracted with acidified aqueous acetonitrile. Wine samples and extracts of maize and hazelnut were spiked with a neat standard solution of 45 characteristic mycotoxins, diluted, and injected into the UHPLC/Q-TOF/MS system. An Agilent 1290 Infinity UHPLC system was coupled to an Agilent G6550 quadrupole time-of-flight (Q-TOF) LC/MS system equipped with a Dual Spray Agilent Jet Stream Ionization source. Separation was achieved using a Poroshell 120 EC-C18 column (100 x 2.1 mm, 2.7 µm) using a gradient between water and methanol, both containing 5 mM ammonium formate and 0.1% formic acid. The Q-TOF was operated either in target MS/MS mode or in All Ions MS/MS mode using 2 distinct collision energies.

For data evaluation the new Agilent accurate mass Personal Compound Database and Library (PCDL) for Mycotoxins and Related Metabolites (G5883CA) has been used.

Results and discussion

PCDL for Mycotoxins and Related Metabolites

The new Agilent accurate mass Personal Compound Database and Library (PCDL) for Mycotoxins and Related Metabolites (G5883CA) contains entries for more than 450 mycotoxins, fungal metabolites or bacterial metabolites relevant for molds in food (or the environment). For more than 300 compounds accurate mass MS/MS library spectra are included. The spectra were acquired by injecting single-analyte solutions in target MS/MS mode for all relevant molecular ions including $[M+H]^+$, $[M-H]^-$, $[M+NH_4]^+$, and $[M+HCOO]^-$ for three collision energies. This is shown for T-2 toxin in figure 1. Ions from MS/MS spectra were compared to theoretical fragment formulas and corrected to their theoretical masses. Moreover, a spectral noise filter and minimum base peak threshold were applied to ensure good ion statistics for all fragment ions.

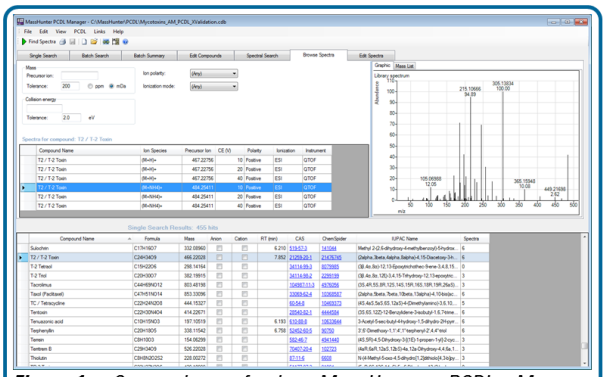

Figure 1. *Screenshot of the MassHunter PCDL Manager software showing the accurate mass spectrum for the $[M+NH_4]^+$ species of T-2 toxin at a collision energy of 10 eV.*

All Ions MS/MS screening and verification

The All Ions MS/MS workflow is illustrated in figure 2. Accurate mass data is collected for the precursor ions in a low-energy channel and for fragments in high-energy channels, using two different collision energies without precursor selection. For aspergillimide the 9 most abundant fragments were automatically extracted and 7 of them showed perfect co-elution with the precursor ion. This is obvious from the overlay of chromatograms (2B) but also from the co-elution plot (2C) and the co-elution scores in the compound table (2D). In summary, mass deviations for the precursor masses of the 45 spiked compounds were generally below 1 ppm and most of the compounds could be verified with at least one fragment ion in either positive or negative mode. A minimum co-elution score of 80 (out of 100) has been specified as the criterion for compound verification.

Results and Discussion

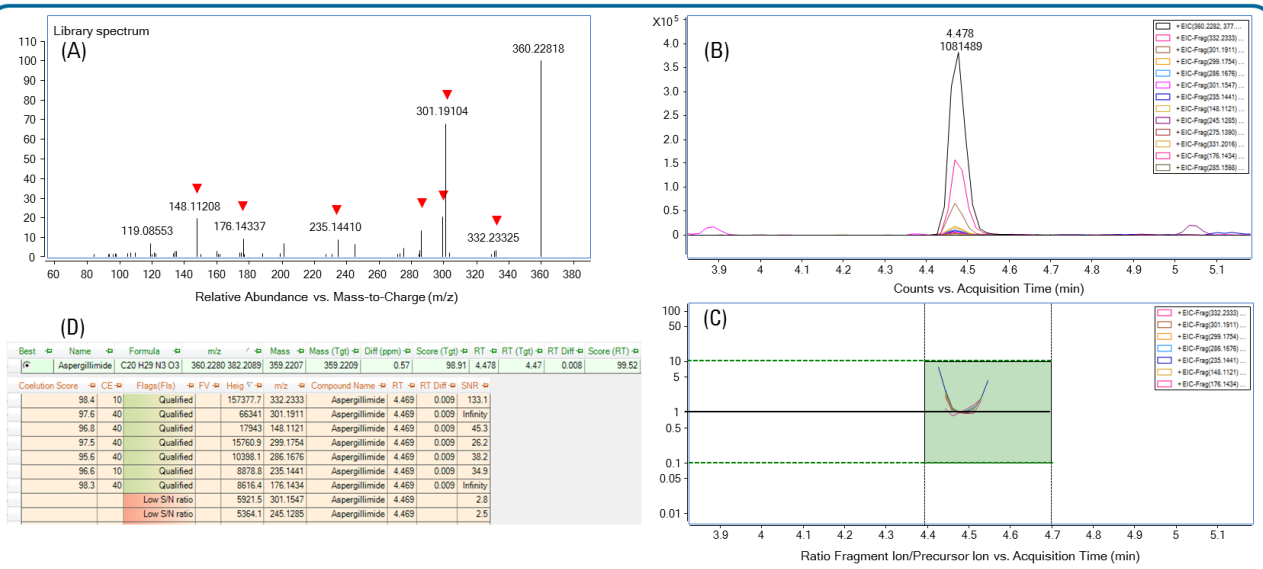

Figure 2. *Results for All Ions MS/MS screening and verification for aspergillimide in a spiked maize sample. (A) Library spectrum from the Agilent Mycotoxins PCDL. Red triangles indicate the automatically extracted fragment ions which are overlaid in the chromatogram (B). Coelution plot (C) visualizes the congruency of the chromatographic peaks. Combined results and scores are shown in the Compound table (D).*

TOF screening and MS/MS library confirmation

The Agilent Mycotoxins PCDL was used with the Find-by-Formula data mining algorithm to find compounds. Chromatograms, MS and MS/MS spectra were extracted automatically. The workflow is illustrated in figure 3.

The comparison of the library spectrum of the $[M+NH_4]^+$ species of T-2 toxin (figure 3C) resulted in a high library score of 92 (out of 100). A minimum library match score of 70 (out of 100) has been specified as criterion for compound verification.

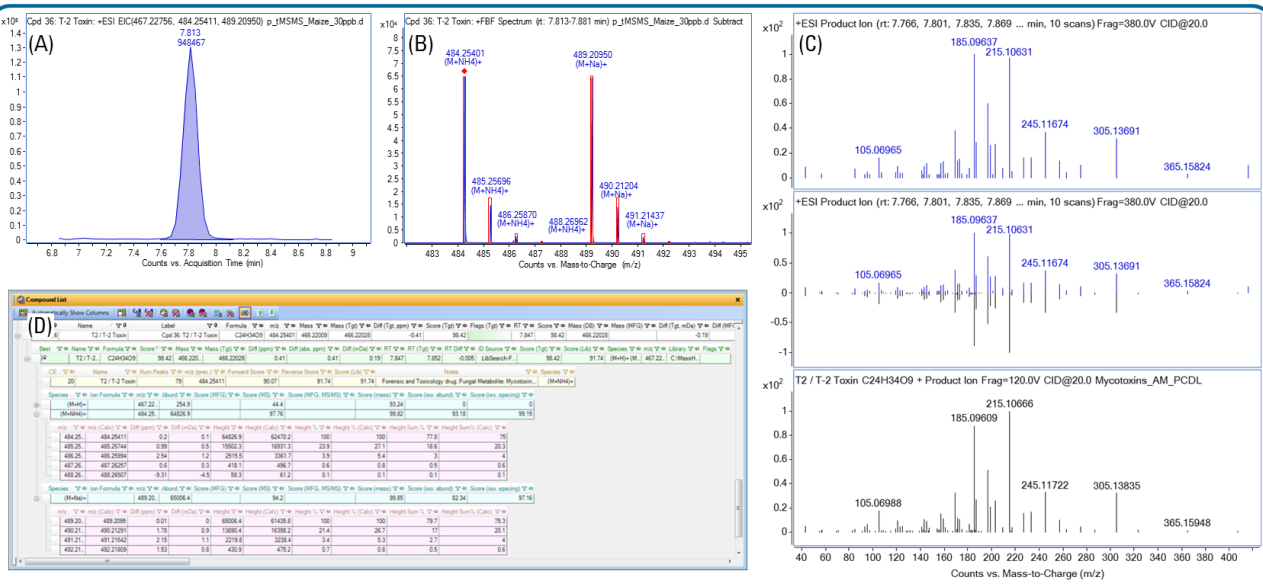

Figure 3. *Results for TOF screening and identification by library comparison of a spiked maize sample. (A) Chromatogram and (B) spectrum from Find-by-Formula data mining. Library comparison (C) of the measured spectrum of T-2 toxin with the reference spectrum from the Agilent Mycotoxins PCDL. Combined results and scores are shown in the Compound table (D).*

Results and Discussion

Cross validation of both workflows

Accurate-mass screening for mycotoxins and fungal metabolites, combined with verification using 2 different workflows, was applied to spiked food extracts. Table 2 shows the comparison of results for 3 matrices spiked to 30 ng/mL. Red wine showed strongest suppression, resulting in lower detection rates. Target MS/MS acquisition combined with library matching resulted in similar verification rates as the All Ions MS/MS acquisition with fragment co-elution. Precursor isolation improved identification in heavier matrices. Due to fast acquisition with All Ions MS/MS, differentiation of closely eluting isomers such as ergosine and ergosinine becomes possible.

Table 2. *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.*

Target MS/MS			Compound name	All Ions MS/MS		
Red wine	Maize	Hazelnut		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 co-elution of fragments

Compound found by FBF, not verified

Compound not found by FBF

Analysis of monitoring samples

Several nut samples were extracted according to the method and were injected into the Q-TOF LC/MS system using All Ions MS/MS in positive and negative mode. Figure 8 shows the chromatograms of six fungal metabolites which were found in a single hazelnut sample. For all compounds, the mass deviation of precursor and fragment ions was below 5 ppm, which is required for identification of the compound.

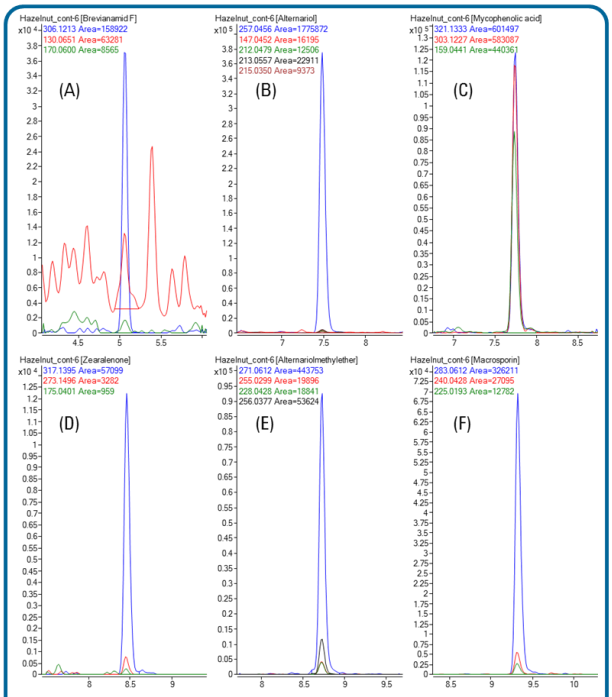

Figure 8. *Chromatograms of mycotoxins detected in a naturally contaminated hazelnut sample. (A) brevianamid F, (B) alternariol, (C) mycophenolic acid, (D) zearalenone, (E) alternariolmethylether, (F) macrosporin.*

Conclusions

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 45 fungal metabolites. Both acquisition modes, in combination with the Mass Hunter Software were effective in verifying the presence of mycotoxins in the sample. The method presented here is an appropriate supplement to single analyte or analyte-group detection methods, to increase knowledge of the occurrence of mycotoxins in various food commodities.