

Simultaneous determination of 16 mycotoxins in cereals using an Agilent Triple Quadrupole LC/MS system and e-Method

Application Note

Food Testing and Agriculture

Abstract

A method for the simultaneous determination of 16 mycotoxins in major cereal species has been established using an Agilent Triple Quadrupole LC/MS system. Crushed samples of wheat and maize were extracted with an acetonitrile-water-acetic acid solution (70:29:1, v/v/v). The resulting sample solutions were then separated using an Agilent Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm), which was monitored by an Agilent Triple Quadrupole LC/MS system equipped with an Agilent Jet Stream electrospray ionization source. Fourteen ¹³C-labeled mycotoxins were employed as stable isotope-labeled internal standards to improve method accuracy. The resulting method is rapid, simple, accurate, and cost effective, and exhibited good linear correlation, with regression coefficients (R^2) greater than 0.996. The recoveries of 16 mycotoxins in wheat and maize matrices at 3 levels were basically within 85-120%, and RSDs of the replicate sample assay fell within 0.7-8.2%. An FAPAS-positive sample was detected using this method, and the measured values agreed well with the specified values of FAPAS, demonstrating the accuracy of this method. Furthermore, a UHPLC-MS/MS e-Method for the simultaneous determination of 16 mycotoxins was established, greatly simplifying the process for method establishment and optimization. Inter-laboratory validation using the e-Method on different models of Agilent LC/MS systems in a number of laboratories worldwide verified the accuracy of the method and the utility of the e-Method.



Authors

Ye Jin, Wu Yu, Wang Songxue Academy of State Administration of Grain, China

Guo Qilei, Lu Meiling, Wu Cuiling, Chen Yuhong Agilent Technologies

Introduction

Mycotoxins are a range of toxic or harmful substances emerging from the fungal growth process. Currently, more than 400 types of mycotoxins have been discovered in nature^{1,2} that have carcinogenic, teratogenic, and mutagenic effects on humans and animals.^{3,4} A variety of mycotoxins can be detected in grain; commonly found mycotoxins include aflatoxins (AFT), ochratoxins (OTA), zearalenone (ZEN), deoxynivalenols (DON), fumonisins (FB), and T-2 toxin (T-2). In order to provide safe grain for humans and animals, timely and rapid monitoring, detection, and control of mycotoxin contaminants in grain has become an important topic to countries all over the world.

At present, the primary assay methods for mycotoxins include thin-layer chromatography, fluorospectrometry, ELISA, liquid chromatography (LC), and LC/MS. For multi-residue and multi-contaminant analysis, liquid chromatography-tandem mass spectrometry has been proved to be a robust tool for enabling simultaneous detection of multiple mycotoxins in different cereal species with high sensitivity and selectivity.⁵ A reliable quantitative method for the rapid and simultaneous determination of 16 mycotoxins commonly found in major cereal species has been established using an Agilent Triple Quadrupole LC/MS system. This method can meet the requirements for high-throughput and rapid monitoring of multiple mycotoxins commonly found in cereals.

Experimental

Reagents and Samples

All reagents and solvents were chromatographic or analytical grade. Methanol and acetonitrile were purchased from Merck, Germany. Formic acid, acetic acid, and ammonium acetate were purchased from Sigma-Aldrich, Inc. The following 16 mycotoxins and 14 isotope-labeled internal standards were purchased from Romer Labs in Austria: nivalenol (NIV), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3G), 3-acetyldeoxynivalenol

Table 1. Concentrations of constituents in the mixed standard working solution for 16 mycotoxins.

| Name | Concentration (µg/mL) | Name | Concentration (µg/mL) |
|------------------|--------------------------|-----------------|--------------------------|
| AFB ₁ | 0.1 | NIV | 20 |
| AFB ₂ | 0.03 | DON | 15 |
| AFG ₁ | 0.1 | DON-3G | 2.5 |
| AFG ₂ | 0.03 | 3-AcDON | 4 |
| 15-AcDON | 2 | HT-2 | 1 |
| ZEN | 2 | ST | 0.1 |
| OTA | 0.2 | FB ₁ | 2 |
| T-2 | 0.2 | FB ₂ | 1 |

 $\begin{array}{l} (3\text{-}AcDON), \ 15\text{-}acetyldeoxynivalenol (15\text{-}AcDON), \ aflatoxin \ B_1 \\ (AFB_1), \ aflatoxin \ B_2 \ (AFB_2), \ aflatoxin \ G_1 \ (AFG_1), \ aflatoxin \ G_2 \ (AFG_2), \\ ochratoxin \ A \ (OTA), \ T\text{-}2 \ toxin \ (T\text{-}2), \ HT\text{-}2 \ toxin \ (HT\text{-}2), \ zearalenone \\ (ZEN), \ fumonisin \ B_1 \ (FB_1), \ fumonisin \ B_2 \ (FB_2), \ sterigmatocystin \\ (ST), \ [^{13}C_{15}]\text{-}NIV, \ [^{13}C_{15}]\text{-}DON, \ [^{13}C_{17}]\text{-}3\text{-}AcDON, \ [^{13}C_{17}]\text{-}AFB_1, \\ [^{13}C_{17}]\text{-}AFB_2, \ [^{13}C_{17}]\text{-}AFG_1, \ [^{13}C_{17}]\text{-}AFG_2, \ [^{13}C_{20}]\text{-}OTA, \ [^{13}C_{24}]\text{-}T\text{-}2, \\ [^{13}C_{22}]\text{-}HT\text{-}2, \ [^{13}C_{18}]\text{-}ZEN, \ [^{13}C_{34}]\text{-}FB_1, \ (1^{3}C_{34}]\text{-}FB_2, \ and \ [^{13}C_{18}]\text{-}ST. \end{array}$

Apparatus and Equipment

Separation was carried out using an Agilent 1290 Infinity LC system equipped with Agilent components in two configurations as follows:

- · G4220A binary pump, G4226A sampler, and G1316C TCC
- G7120A high-speed pump, G7167B Multi-sampler, and G7116B MCT

Agilent 6460/6470/6490/6495 Triple Quadrupole LC/MS systems with AJS electrospray ionization source were used for the analysis. Agilent MassHunter workstation software was used for data acquisition and qualitative and quantitative analysis.

Standards preparation

A single-standard stock solution for 16 mycotoxins was prepared. AFB₁, AFB₂, AFG₁, and AFG₂ were dissolved in methanol; FB₁ and FB₂ were dissolved in acetonitrile:water (50:50, v/v); the remaining compounds were dissolved in acetonitrile. All stock solutions were kept at -20°C. A portion of this mycotoxin singlestandard stock solution was pipetted into a 10 mL volumetric flask and diluted with water with the total volume reaching to 10 mL to make a mixed mycotoxin standard working solution, which was kept at -20°C. The concentrations are shown in Table 1. A portion of the mycotoxin stable isotope-labeled single-standard solution was pipetted into a 10 mL volumetric flask and diluted with water with the total volume reaching to 10 mL to make a mixed stable isotope-labeled internal standard working solution, which was kept at -20 °C in the dark. The concentrations are shown in Table 2.

Table 2. Concentrations of constituents in the mixed standard working solution for 14 stable isotope-labeled internal standards.

| Name | Concentration (µg/mL) | Name | Concentration (µg/mL) |
|--|--------------------------|---|--------------------------|
| [¹³ C ₁₇]-AFB ₁ | 0.01 | [¹³ C ₁₅]-NIV | 2.5 |
| [¹³ C ₁₇]-AFB ₂ | 0.01 | [¹³ C ₁₅]-DON | 2 |
| [¹³ C ₁₇]-AFG ₁ | 0.01 | [¹³ C ₁₇]-3-AcDON | 1 |
| [¹³ C ₁₇]-AFG ₂ | 0.01 | [¹³ C ₁₈]-ZEN | 0.2 |
| [¹³ C ₂₀]-OTA | 0.04 | $[^{13}C_{34}]$ -FB ₁ | 0.5 |
| [¹³ C ₂₄]-T-2 | 0.05 | $[^{13}C_{34}]$ -FB ₂ | 0.3 |
| [¹³ C ₂₂]-HT-2 | 0.125 | | |
| [¹³ C ₁₈]-ST | 0.025 | | |

Sample preparation

Wheat and maize samples were crushed with a pulverizer, passed through a test sieve with a 1 mm pore size, then mixed well. A 5 g sample was put into a 50 mL centrifuge tube, into which 20 mL of acetonitrile-water-acetic acid mixed solution (70:29:1, v/v/v) as extration solvent was added, then the mixture was vortexed for 1 min and placed on a rotary shaker for 30 minutes. The solution was centrifuged at 6,000 r/min for 10 minutes; 0.5 mL of supernatant was pipetted into a 1.5 mL centrifuge tube, into which 0.5 mL water was added and mixed well by vortex mixing. The sample was then centrifuged at 12,000 r/min for 10 minutes at 4°C, and the supernatant was passed through a PTFE filter membrane with a 0.2 µm pore size. 180 µL of filtrate was pipetted into a 400 µL insertion tube, into which 20 µL mixed stable isotope-labeled internal standard working solution was added and then mixed well by vortex mixing for analysis.

LC conditions

| Column: | Agilent Eclipse Plus C18, 2.1 × 100 mm, | | | | | |
|-------------------|--|----------|--|--|--|--|
| Flow rate: | 0.3 mL/min | 3704-302 | | | | |
| Temperature: | 35°C | | | | | |
| Injection volume: | 2 µL | | | | | |
| Mobile phase: | A) Aqueous solution containing 1% acetic acid and 5 mmol/L ammonium acetate | | | | | |
| | B) Methanol | | | | | |
| Gradient elution | Time (min) | B% | | | | |
| procedure: | 0.0 | 10.0 | | | | |
| | 2.0 | 10.0 | | | | |
| | 3.0 | 20.0 | | | | |
| | 7.0 | 24.0 | | | | |
| | 10.5 | 30.0 | | | | |
| | 13.5 | 60.0 | | | | |
| | 15.0 | 70.0 | | | | |
| | 18.0 | 75.0 | | | | |
| | 18.1 | 95.0 | | | | |
| | 21.9 | 95.0 | | | | |
| | 22.0 | 10.0 | | | | |

Mass spectrometric conditions

Agilent Triple Quadrupole LC/MS systems used in this study were equipped with an AJS electrospray ionization source; specific ionization source parameters are shown in Table 3. For Agilent 6490/6495 Triple Quadrupole LC/MS systems utilizing iFunnel ion transmission and focusing technique, the high pressure RF was 150(+)/90(-) and the low pressure RF was 60(+)/60(-).

Based on compound retention times, time segments were set for MS data acquisition (Table 4). Delta EMV was set depending on the model of each instrument in use separately to meet the requirements for sensitivity. To minimize the amount of matrix going into the spray chamber, the HPLC flow was diverted to waste for the first 1.8 minutes of the analysis, then again from 18 minutes until the end of the analysis.

MRM parameters of 16 mycotoxins and 14 isotopelabeled internal standards are shown in Table 5.

Table 3. Ionizaiton source parameters.

| Parameter | Agilent 6460/6470 MS/MS | Agilent 6490/6495 MS/MS |
|------------------------|----------------------------|----------------------------|
| Dry gas temperature | 300°C | 150°C |
| Dry gas flow rate | 7 L/min | 15 L/min |
| Nebulizer pressure | 35 psi | 35 psi |
| Sheath gas temperature | 350°C | 370°C |
| Sheath gas flow rate | 11 L/min | 12 L/min |

Table 4. Time segments.

| No. | Start time (min) | Valve | Capillary voltage (V) | Nozzle voltage (V) |
|-----|---------------------|----------|--------------------------|-----------------------|
| 1 | 0 | To waste | 3500(+/-) | 0(+)/1000(-) |
| 2 | 1.8 | To MS | 3500(+/-) | 0(+)/1000(-) |
| 3 | 3.6 | To MS | 3500(+/-) | 0(+)/1000(-) |
| 4 | 8.0 | To MS | 3500(+/-) | 0(+)/1000(-) |
| 5 | 12.4 | To MS | 3500(+/-) | 0(+)/1000(-) |
| 6 | 14.9 | To MS | 3500(+/-) | 500(+)/1000(-) |
| 7 | 16.5 | To MS | 2500(+/-) | 500(+)/1000(-) |
| 8 | 18.0 | To waste | 3500(+/-) | 0(+)/1000(-) |

| Time segment | Compound name | Precursor ion (m/z) | Product ion (m/z) | Fragmentor (V) | Collision energy (V) | Polarity |
|--------------|-------------------------------------|---------------------|-------------------|----------------|----------------------|----------|
| 2 | [¹³ C]-NIV | 386 | 295.1 | 90 | 10 | Negative |
| 2 | NIV | 371 | 281.1*/59.1 | 90 | 10/30 | Negative |
| | [¹³ C]-DON | 370.1 | 279.0 | 90 | 10 | Negative |
| 3 | DON | 355.1 | 265.0*/247.0 | 90 | 10/10 | Negative |
| | DON-3G | 517.1 | 427.0*/247.0 | 120 | 20/20 | Negative |
| | 15-AcDON | 356.1 | 261.0/137.0* | 90 | 15/15 | Positive |
| 4 | [¹³ C]-3-AcDON | 356.2 | 245.1 | 110 | 10 | Positive |
| | 3-AcDON | 339.2 | 231.1*/213.1 | 110 | 10/20 | Positive |
| | [¹³ C]-AFB ₁ | 330.1 | 301.1 | 160 | 21 | Positive |
| | AFB1 | 313.2 | 285.1*/241.1 | 160 | 22/38 | Positive |
| r. | [¹³ C]-AFB ₂ | 332.2 | 303.0 | 160 | 21 | Positive |
| | AFB ₂ | 315.2 | 287.1*/259.1 | 160 | 24/30 | Positive |
| 5 | [¹³ C]-AFG ₁ | 346.1 | 257.1 | 150 | 25 | Positive |
| | AFG ₁ | 329.2 | 311.1*/243.1 | 150 | 20/25 | Positive |
| | [¹³ C]-AFG ₂ | 348.1 | 330.1 | 160 | 23 | Positive |
| | AFG ₂ | 331.2 | 313.1*/245.1 | 160 | 23/30 | Positive |
| | [¹³ C]-FB ₁ | 756.5 | 356.4 | 180 | 45 | Positive |
| | FB ₁ | 722.4 | 352.3/334.3* | 180 | 45/45 | Positive |
| 6 | [¹³ C]-T-2 | 508.3 | 322.1 | 110 | 10 | Positive |
| 0 | T-2 | 484.3 | 305.1*/185.1 | 110 | 10/20 | Positive |
| | [¹³ C]-HT-2 | 464.1 | 278.1 | 100 | 10 | Positive |
| | HT-2 | 442.2 | 263.1/215.1* | 100 | 10/10 | Positive |
| | [¹³ C]-FB ₂ | 740.5 | 358.3 | 180 | 45 | Positive |
| | FB ₂ | 706.5 | 336.3*/318.3 | 180 | 45/45 | Positive |
| | [¹³ C]-OTA | 424.2 | 250.1 | 120 | 25 | Positive |
| 7 | OTA | 404.1 | 358.0/239.0* | 120 | 10/25 | Positive |
| 1 | [¹³ C]-ST | 343.1 | 327.1 | 150 | 20 | Positive |
| | ST | 325 | 310.0/281.0* | 150 | 20/36 | Positive |
| | [¹³ C]-ZEN | 335.2 | 185.0 | 190 | 25 | Negative |
| | ZEN | 317.1 | 175.0*/130.8 | 190 | 25/33 | Negative |

Table 5. MRM parameters of 16 mycotoxins and 14 isotope-labeled internal standards.

Results and Discussion

Optimization of UHPLC-MS/MS conditions

Due to differences in the sensitivity of each compound, a singlestandard solution with a concentration of 0.5-2.0 μ g/mL was chosen to optimize MS parameters. Mycotoxin compounds are likely to form adduct ions such as $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, [M-H]⁻, [M+HCOO]⁻ and [M+CH₃COO]⁻, and the response intensity of each adduct ion depends on the nature of the compound and the composition of the mobile phase used. Both [M+H]⁺ and [M-H]⁻ for NIV showed weak response, and in the mobile phase containing acetic acid, the response of [M+CH₃COO]⁻ increased significantly, DON-3G showed the same trend. Consequently, NIV, DON, and DON-3G were chosen for analysis in negative mode using [M+CH₃COO]⁻ as the precursor ion. ZEN was also detected in negative mode, with [M-H]⁻ as the precursor ion. Among mycotoxins detected in positive mode, [M+NH₄]⁺ was selected as the precursor ion for 15-AcDON, T-2, and HT-2, while [M+H]⁺ was chosen as the precursor ion for the remaining mycotoxins. For most compounds, the highest sensitivity was achieved when the nozzle voltage was set to 0 V under positive mode. However, for FB₁ and FB₂, setting an appropriate nozzle voltage led to a higher response. In this method, the nozzle voltage was set to 500 V during the time segment when FB₁ and FB₂ eluted. Although the sensitivity was lost to some extent for the other compounds during the same acquisition time segment, the response of FB1 and FB₂ increased by over 50% compared to that obtained with the nozzle voltage of zero, enhancing the overall performance of this method.

To ensure formation of different adduct ions such as [M+H]⁺, [M+NH₄]⁺, [M-H]⁻ and [M+CH₃COO]⁻, sufficient concentrations of acetic acid and ammonium acetate were required in the mobile phase. Since 3-AcDON and 15-AcDON are isomers, the possibility of ion pair interference still remained, even though different ion pairs had been targeted. To minimize misjudgment in the case of a complex matrix, it was essential to optimize chromatographic conditions to separate both isomers by baseline. With the Eclipse Plus C18 column, an optimized gradient elution profile was applied to realize the baseline separation for the isomer pair, i.e., 3-AcDON and 15-AcDON. Consequently, 16 mycotoxins were well separated within 18 minutes (Figure 1). Among the 16 mycotoxins, 12 were detected under positive mode and 4 were detected under negative mode. According to the retention time, time segments were set for data acquisition. Since ZEN and OTA were coeluted in the same time segment but detected under different polarity, rapid polarity switching mode was selected in the corresponding time segment.



Figure 1. Overlaid MRM chromatograms of the mixed standard working solution for 16 mycotoxins.

Calibration curve

Appropriate volumes of the mixed mycotoxin standard working solution were accurately pipetted and serially diluted with acetonitrile-water-acetic acid solution (35:64.5:0.5, v/v/v) to make a series of mixed standard working solutions with different concentrations. Thereafter, a 20 µL mixed standard working solution for 14 stable isotope-labeled internal standards was added to a 400 µL insertion tube, into which 180 µL of each prepared serial standard working solution was pipetted. After vortex mixing, the mixture was loaded for the generation of internal standard calibration curves (Figure 2). All 16 mycotoxins showed good linearity, with correlation coefficients (R²) greater than 0.996. Since neither DON-3G nor 15-AcDON have corresponding commercialized stable isotope-labeled internal standards, [¹³C₁₅]-DON and [¹³C₁₇]-3-AcDON, which are analogous to them in structure, were used as their internal standards, respectively.



Figure 2. Calibration curves for 16 mycotoxins.

Recovery and positive sample analysis

Mixed mycotoxin standard working solutions with high, medium, and low concentrations were added to the blank samples (wheat and maize), followed by sample preparation and instrumental analysis based on the above optimized method. The spiked concentrations, recovery rates, and relative standard deviations (RSDs) are shown in Table 6. For 16 mycotoxins at three levels in two kinds of cereal matrices, the majority of the spiked recoveries were within 85–120% and the RSDs of the replicate duplicate sample assays were within 0.7–8.2%. Figure 3 shows the overlaid TIC chromatograms of 6 consecutive injections for the spiking recovery test in the maize matrix, demonstrating the method is highly stable.

Analysis of the FAPAS test sample showed good agreement between the measured and specified values (Table 7), demonstrating the reliability of this method. The sample preparation procedure in this method is simple, with no need for a cleanup step, and enabled accurate quantitation of 16 mycotoxins in cereals.



Figure 3. Overlaid TIC spectra of 6 consecutive injections of spiked samples in maize matrix.

Table 6. Spike recovery and precision values of 16 mycotoxins in wheat and maize matrices.

| | Spiked | Wh | eat | Ма | ize | | Spiked | Wheat | | Maize | |
|------------------|--------------------------|-----------------|---------|-----------------|---------|------------------|--------------------------|-----------------|---------|-----------------|---------|
| Compound | concentration (µg/kg) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Compound | concentration (µg/kg) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| NIV | 100 | 97.2 | 1.6 | 103.1 | 1.8 | AFB ₁ | 0.5 | 113.3 | 2.2 | 123.6 | 3.1 |
| | 200 | 88.0 | 2.3 | 97.5 | 4.1 | - | 1 | 100.4 | 3.6 | 108.1 | 7.3 |
| | 500 | 92.3 | 0.9 | 87.3 | 1.3 | - | 2.5 | 102.4 | 2.3 | 91.1 | 4.9 |
| DON | 75 | 109.3 | 1.8 | 109.7 | 2.7 | HT-2 | 5 | 113.8 | 2.6 | 113.0 | 3.8 |
| | 150 | 101.5 | 1.9 | 105.0 | 4.1 | - | 10 | 107.2 | 3.2 | 111.7 | 6.1 |
| | 375 | 100.7 | 1.8 | 92.9 | 1.8 | - | 25 | 101.9 | 3.8 | 101.0 | 4.5 |
| DON-3G | 12.5 | 105.6 | 4.8 | 106.1 | 2.5 | FB ₁ | 10 | 87.7 | 5.5 | 94.3 | 8.2 |
| | 25 | 88.8 | 2.1 | 97.2 | 4.2 | | 20 | 70.9 | 6.0 | 86.0 | 8.1 |
| | 62.5 | 93.5 | 6.2 | 85.7 | 1.6 | - | 50 | 85.5 | 5.7 | 91.4 | 5.4 |
| 3-AcDON | 20 | 110.8 | 1.9 | 113.1 | 2.0 | 0TA | 1 | 114.4 | 4.0 | 113.8 | 7.3 |
| | 40 | 92.8 | 1.0 | 105.4 | 3.4 | - | 2 | 95.2 | 1.1 | 102.8 | 4.0 |
| | 100 | 100.0 | 0.9 | 93.5 | 1.2 | - | 5 | 95.1 | 4.0 | 86.4 | 2.8 |
| 15-AcDON | 10 | 115.9 | 2.7 | 115.9 | 3.1 | T-2 | 1 | 119.5 | 2.4 | 114.9 | 2.3 |
| | 20 | 102.1 | 1.4 | 106.5 | 3.2 | - | 2 | 108.8 | 4.0 | 112.1 | 4.2 |
| | 50 | 103.4 | 1.1 | 97.2 | 1.9 | - | 5 | 99.6 | 3.5 | 97.4 | 3.3 |
| AFG ₂ | 0.15 | 102.8 | 5.3 | 127.3 | 4.0 | ZEN | 10 | 116.7 | 1.5 | 107.6 | 2.8 |
| | 0.3 | 105.5 | 6.1 | 106.2 | 3.0 | - | 20 | 106.9 | 1.7 | 105.3 | 6.0 |
| | 0.75 | 96.1 | 4.0 | 93.5 | 2.8 | - | 50 | 106.0 | 1.9 | 95.2 | 2.0 |
| AFG ₁ | 0.5 | 118.7 | 3.1 | 128.2 | 2.8 | ST | 0.5 | 111.3 | 1.5 | 113.2 | 2.1 |
| | 1 | 102.1 | 4.1 | 109.5 | 4.9 | - | 1 | 96.4 | 4.4 | 102.4 | 4.2 |
| | 2.5 | 96.1 | 4.0 | 94.5 | 3.4 | - | 2.5 | 100.6 | 3.0 | 92.7 | 2.7 |
| AFB ₂ | 0.15 | 129.4 | 5.5 | 104.9 | 4.8 | FB ₂ | 5 | 107.5 | 3.8 | 109.8 | 4.7 |
| | 0.3 | 105.6 | 1.4 | 105.0 | 3.4 | - | 10 | 85.1 | 2.4 | 93.1 | 0.7 |
| | 0.75 | 99.9 | 1.9 | 94.4 | 6.7 | - | 25 | 94.1 | 5.7 | 89.9 | 4.0 |

Table 7. Specified and measured values of the FAPAS sample.

| Specified value (µg⁄kg) | Measured value (µg∕kg) |
|----------------------------|--|
| 1247±193 | 1239.4 |
| 5.42±1.19 | 5.2 |
| 797±202 | 760.3 |
| 360±91.5 | 330.3 |
| 160±33.7 | 145.7 |
| 105±23.1 | 108.2 |
| 286±55.2 | 303.4 |
| 2.4±0.53 | 2.3 |
| | Specified value (µg/kg) 1247±193 5.42±1.19 797±202 360±91.5 160±33.7 105±23.1 286±55.2 2.4±0.53 |

Use of the UHPLC-MS/MS e-Method

Introduction of the e-Method

As many parameters are involved in an LC/MS multi-residue assay, a lot of instrumental parameters have to be optimized when creating a new LC-MS/MS method, requiring much time and effort. To enhance efficiency, we took advantage of state-ofthe-art performance of the Agilent LC/MS system in combination with unique features of its MS software to establish an e-Method with "plug-and-play" functionality. Using such an approach, data acquisition and quantitative analysis can be quickly carried out after verifying the instrumental configuration, selecting the corresponding e-Method, and simple testing. Thereby, the developing time can be greatly shortened and the application difficulty can be reduced. Application of an e-Method allows standardization of the analysis process within a laboratory and easy promotion of the method among laboratories.

The e-Method consists of three parts:

- *.m file A method file that can be directly invoked by MassHunter acquisition software. The method file in this format contains LC parameters, ionization source parameters for the triple quadrupole LC/MS system, and acquisition parameters of an MRM mode for 16 mycotoxins and 14 stable isotope-labeled internal standards.
- *.quantmethod.xml file A target compound quantitative method file that can be directly used by MassHunter quantitative analysis software. 16 mycotoxins are quantitated by an internal standard method, and a concentration series of isotope-labeled internal standard calibration curves is set in accordance with the industrial standard of grain and oils inspection (Inspection of Grain and Oils–Determination of 16 Mycotoxins in Cereals).
- 3. *.pdf file Contains user instructions and remarks for the e-Method.

Invoking the e-Method acquisition method

After verifying instrumental configuration and opening acquisition software, the *.m acquisition method file corresponding to the instrument model can be directly invoked. After checking the parameter settings for the LC and MS modules sequentially, sample analysis can be conducted.

The standard solution at the lowest point of the concentration series for calibration curves should be examined first, and the retention time of each compound checked to ensure that the time segment setting in the method under the specific laboratory conditions is appropriate. The starting time point of a time segment can be adjusted according to the elution time window for the compounds when necessary. The peak area of each compound should be checked to see if the Δ EMV setting in the method is appropriate, and the Δ EMV setting in corresponding time segment can be modified according to the actual peak area when necessary.

Invoking and updating the e-Method quantitative method

To perform quantitative analysis, open MassHunter Quantitative Analysis software, select "Create batch" and import data to be quantitatively analyzed into the batch. Select data for a higher concentration standard, invoke quantitative method *.quantmethod.xml in the e-Method as shown in Figure 4, and enter the editing interface of quantitative method.

| 1 | Agilent Mas | sHunter Quar | ntitative A | nalysis (fo | r QQC | 2) - Method - [C:\MassHunter\Methods\Mycot | oxins] |
|----|-------------|--------------|-------------|-------------|--------|--|---------|
| Fi | ile Edit Vi | ew Analyze | Method | Update | Librar | y Report Tools Help | |
| | New | | |) | In | curred Blank Offset 🛛 Layout: 🔝 😰 😰 | |
| | Open | | |) | • | Open Method from Existing File | |
| | Append | | |) | · | Open Method from Existing Batch | els: 1(|
| ľ | Edit | | | F10 | | Open and Apply from Existing File | |
| | Validate | | | | | Open and Apply from Existing Batch | 1 |

Figure 4. Invoking the quantitative method.

In the editing interface of the quantitative method, choose "Update Retention Times..." as shown in Figure 5, select the compound(s) for which the retention time needs to be updated, and click "Select All"; the software will update the retention time automatically in the quantitative method according to the acquired data. Following a similar procedure, you can update ratios of qualitative ions to quantitative ions by clicking "Update Qualifier Ratios...".

| 📅 A | gilent MassHunter Quantitative Analysis (f | or QQQ) - Method - | [C:\MassHunter\M | ethods\Mycotoxins] |
|-----|--|--------------------|------------------|--------------------|
| Fil | e Edit View Analyze Method Update | Library Report To | ools Help | |
| | Update Retention Times | Incurred Blank C | Offset Layout: | |
| | Update Retention Times from ISTD | od Table | | |
| | Average Retention Times | vel Name Prefix: | # of | Levels: 10 |
| | Shift Retention Time | ample | | |
| 75 | Update Qualifier Batios | Name | Data File | Туре |
| - | | P5 | P5_5_01.d | Sample |
| | Average Qualifier Ratios | Quantifier | | |
| | Update Mass Assignments | Name | TS | Transition |



Figure 5. Updating retention times and qualifier ratios.

In the *.quantmethod.xml file, the concentration series for calibration curves of 16 mycotoxins are set in accordance with the industrial standard of grain and oils inspection (Inspection of Grain and Oils–Determination of 16 Mycotoxins in Cereals). Researchers may also modify the concentration series for calibration curves under the "Concentration Setup" section of the editing interface, as needed.

Inter-laboratory validation using the e-Method

Using the e-Method established in this application note, interlaboratory validation was carried out among a number of laboratories worldwide, involving instruments such as Agilent 6460/6470/6490/6495 Triple Quadrupole LC/MS systems. All results met the required validation standards, demonstrating the accuracy of the method and the feasibility of applying the e-Method among laboratories. The e-Method makes it easier to promote and standardize the LC-MS/MS determination method among laboratories, greatly simplifying method establishment and optimization and making instrument operation easier and faster, thus shortening the analysis cycle and improving efficiency.

Conclusion

In this application note, an analytical method capable of rapid and accurate determination of mycotoxins in major cereal species has been established and successfully applied to the analysis of 16 mycotoxins in wheat and maize. An FAPAS-positive sample was analyzed using the method established in this application note, and the measured values agreed well with the specified values from FAPAS. This method allows simple preparation, requires less time, and yields good linear correlation, with sensitivity, recovery, and precision meeting the requirement for analysis of mycotoxins in cereals. It is therefore promising for application to routine monitoring of mycotoxins in cereals. The establishment and application of the e-Method simplifies its development and enables "plug-and-play" functionality, benefitting both the promotion and standardization of the LC-MS/MS method among laboratories.

The e-Method described in this application note is available at www.agilent.com/zh-cn/promotions/020617-mycotoxin-grain-cn

References

- Huertas-Perez J F, Arroyo-Manzanares N, Gamiz-Gracia L, et al. Simple and efficient methodology to determine mycotoxins in cereal syrups. Food Chemistry, 2015, 177(6): 274-279.
- Jinap S, Yibadatihan S, Mahyudin N A. Simultaneous determination of multi-mycotoxins in palm kernel cake (PKC) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Food Additives & Contaminants. Part A, 2014, 31(14): 2071-2079.
- Hussein H S and Brasel J M. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicol., 2001, 167: 101-134.
- Bennett J W and Klich M. Mycotoxins. Clin. Microbiol. Rev. 2003, 16: 497-516.
- Song W D, Su Z, Hui X D et al. Advances in research of LC-MS technique applied in assay of mycotoxins in foods. Sci. Tech. Food Ind., 2016, 37(17): 395–399.

www.agilent.com/chem

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc., 2017 Published in the USA, Feb. 16, 2017 5991-7862EN

