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- › Acrylamide
- › Melamine
- › Phenolic Pollutants
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Application Note: 319

Quantitation of Acrylamide in Food Samples on the TSQ Quantum Discovery by LC/APCI-MS/MS

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Key Words

- TSQ Quantum Discovery™
- Hypercarb™
- Acrylamide
- Quantitation
- Triple Quadrupole MS

Introduction

Acrylamide has been identified as a potential human carcinogen. This is important not only because acrylamide is a common industrial chemical, but acrylamide has been shown to be present at significant levels in food samples,[1] particularly cooked foods high in carbohydrates. This has led many government health agencies around the world to assess the risk of short- and long-term exposure to acrylamide in humans.

This has led to the development of LC-MS/MS methodology for the quantitative analysis of acrylamide in foodstuffs.[1-5] While a GC/MS protocol for the analysis of acrylamide exists, this method requires extensive sample cleanup and chemical derivatization.[6] The advantage of LC-MS/MS is that chemical derivatization is not necessary prior to acrylamide analysis.

To date, most LC-MS/MS methods for the assay of acrylamide have utilized an electrospray ionization (ESI) source for the production of acrylamide ions.[1-4] Yet it is well-known that ESI-MS is problematic when highly aqueous solutions, such as those required for the reversed-phase LC separation of acrylamide, are used.[7] On the other hand, water does not pose a problem for the formation of a stable corona discharge used in APCI. One published report has demonstrated that APCI is a viable ion source for the production of acrylamide ions for LC-MS/MS detection.[5] Furthermore, a study comparing ESI and APCI ion sources for the LC-MS/MS analysis of acrylamide showed that under the same chromatographic conditions, APCI-MS/MS yielded an improved detection limit.[8]

This report presents data acquired on the Thermo Scientific TSQ Quantum Discovery for the analysis of acrylamide. A simple LC-MS/MS method using the APCI source is used to measure acrylamide, via selective reaction monitoring (SRM), over a wide concentration range. A small selection of food samples was analyzed for acrylamide content following extraction with water. To preclude the need for a time-consuming solid-phase extraction procedure, a column-switching method was employed to selectively "fractionate" acrylamide from polar matrix interferences prior to LC-MS/MS detection.

Goals

1. **Development**–A sensitive and rugged LC/APCI-MS/MS assay for the analysis of acrylamide
2. **Application**–An on-line column-switching technique to aqueous food extracts as an alternative to solid-phase extraction (SPE) cleanup
3. **Measurement**–Acrylamide content in selected food samples

Experimental

Chemicals and Reagents: Acrylamide (>99.0%) was purchased from Fluka (Buchs SG, Switzerland). 2,3,3-d3-acrylamide (98%) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). HPLC grade water was acquired from Burdick and Jackson (Muskegon, MI, USA). All chemicals were used as received without further purification.

Sample Preparation: Standards were prepared by dilution of a stock solution of 1.0 mg/mL acrylamide or 1.0 mg/mL d3-acrylamide in water. The stock solutions were stored at 4 °C for a period of no longer than two weeks.

Two brands of potato chips and two brands of breakfast cereals were purchased and stored at room temperature until processed. After homogenizing approximately 50 grams of a food sample, two grams were weighed into a 35 mL polypropylene centrifuge tube. Aqueous extraction of acrylamide was initiated by the addition of 20 mL water containing 1000 ng d3-acrylamide as the internal standard (final concentration = 50 ng/mL). The sample was vortexed for 30 s then subsequently centrifuged at 18,000 g for 15 minutes. Ten milliliters of the supernatant was decanted into a clean 35 mL centrifuge tube and centrifuged at 18,000 g for 10 minutes. Prior to analysis, 0.49 mL of the aqueous extract was filtered through a 0.45 µm centrifuge filter (Millipore Corp., Bedford, MA, USA) at 9,000 g for 5 minutes.

Sample Analysis: LC experiments were conducted with the Thermo Scientific Surveyor™ HPLC system. A Thermo Scientific Hypercarb 2.1 × 50 mm column was utilized as the analytical LC column. Separations of acrylamide were achieved under isocratic conditions using 100% water as the mobile phase at a flow rate of 0.4 mL/min. The injection volume for all LC experiments was 10 µL.

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To eliminate the need for solid phase extraction (SPE) purification prior to the analysis of the food sample extracts, a column-switching LC method was employed. Briefly, the sample extract was loaded onto a 2.1 × 50 mm Thermo Scientific Aquasil™ C18 column, which was positioned before a 6-port switching valve. The eluent from the C18 column was diverted to waste except for the period when acrylamide eluted from the C18 column, whereby the valve was switched to the Hypercarb column for MS/MS detection. This column-switching method required a second Thermo Scientific Surveyor MS pump, which also delivered 100% water at 0.4 mL/min. Both Surveyor MS pumps and the 6-port switching valve were controlled using Xcalibur™ version 1.3 software.

The experimental conditions for the TSQ Quantum Discovery were as follows:

Source: APCI
Ion polarity: Positive
Vaporizer Temperature: 375 °C
Discharge Current: 5 µA
Ion Transfer Capillary Temperature: 250 °C
Source CID Offset: 6 V
Scan Mode: Selective Reaction Monitoring
Q2 Pressure: 1.0 mTorr argon
SRM Transitions: *m/z* 72 → 55 for acrylamide;
m/z 75 → 58 for d3-acrylamide
Collision Energy: 13 eV
Scan Width: 1.0 u
Scan Time: 0.3 s (each SRM transition)
Q1, Q3 Resolution: Unit (0.7 u FWHM)

Results and Discussion

Prior to the acquisition of acrylamide standards, it was important to determine if there was any detectable native acrylamide contribution originating from the deuterated internal standard. As shown in Figure 1, there is no acrylamide signal observed for the *m/z* 72 → 55 SRM transition at the same retention time as the 50 ng/mL d3-acrylamide standard.

The limit of quantitation (LOQ) for acrylamide on the TSQ Quantum Discovery was 0.25 ng/mL acrylamide or 2.5 pg on column (Figure 2). This compares favorably to LOQs previously reported by other research groups, including an 8-fold improvement over the mass LOQ by LC/ESI-MS/MS (20 pg)[1] and a 40-fold improvement over the concentration LOQ on the TSQ 7000 (10 ng/mL),[5] which used an LC/APCI-MS/MS method.

The calibration curve for acrylamide from 0.25 ng/mL to 2500 ng/mL is displayed in Figure 3. This calibration curve was generated using the column-switching LC method just prior to the acquisition of the food extracts data. A linear regression fit to these data using 1/x weighting yielded the following equation: $y = 5.5997 \times 10^{-4} + 0.0206125x$. The correlation coefficient for this curve was $r^2 = 0.9999$, indicating excellent linearity across the four orders of magnitude dynamic range. Table 1 summarizes the statistical results for the acrylamide calibration curve. At the LOQ,

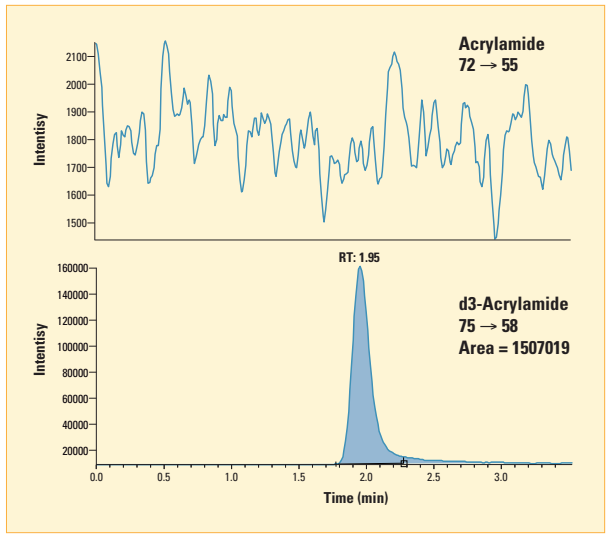

Figure 1: SRM chromatograms for 50 ng/mL d3-acrylamide

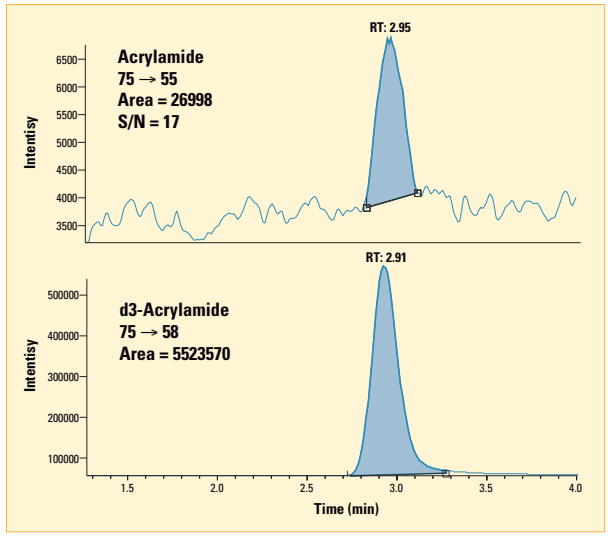

Figure 2: SRM chromatograms for 0.25 ng/mL acrylamide (LOQ) with 50 ng/mL d3-acrylamide

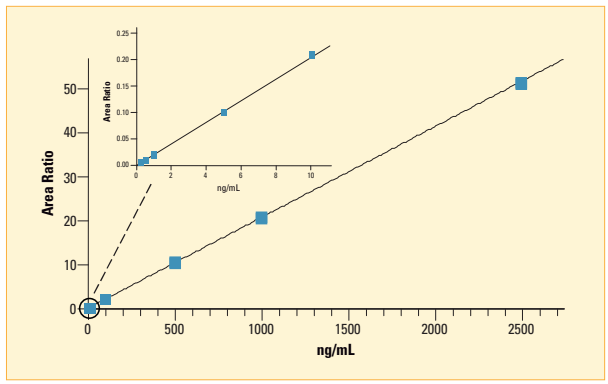

Figure 3: Calibration curve for acrylamide using column-switching LC method with APCI-MS/MS detection

Nominal (ng/mL)	Mean Conc. (ng/mL)	% Rel. Error	% CV	Number of Replicates
0.250	0.253	1.1	12.1	5
0.500	0.485	-2.9	6.7	5
1.00	1.00(4)	0.4	4.6	5
5.00	4.86	-2.7	0.9	5
10.0	10.2	2.1	0.7	5
100	101	0.7	0.5	5
500	512	2.4	0.8	3
1000	1006	0.6	0.6	3
2500	2481	-0.8	0.6	3

Table 1: Statistical data for the calibration curve of acrylamide

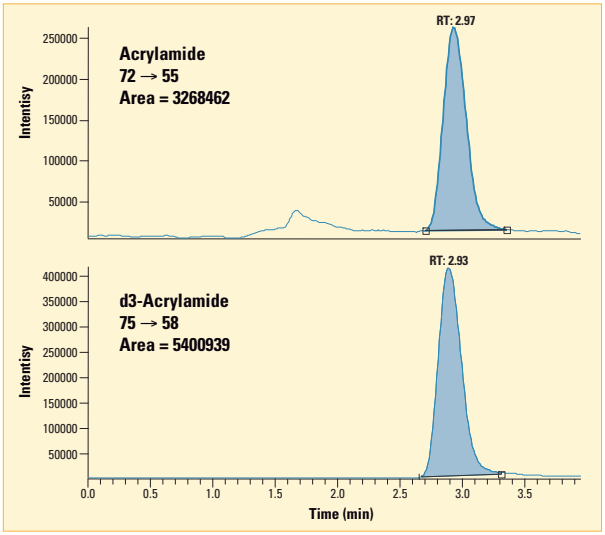

Figure 4: SRM chromatograms of the Potato Chip 2 sample aqueous extract

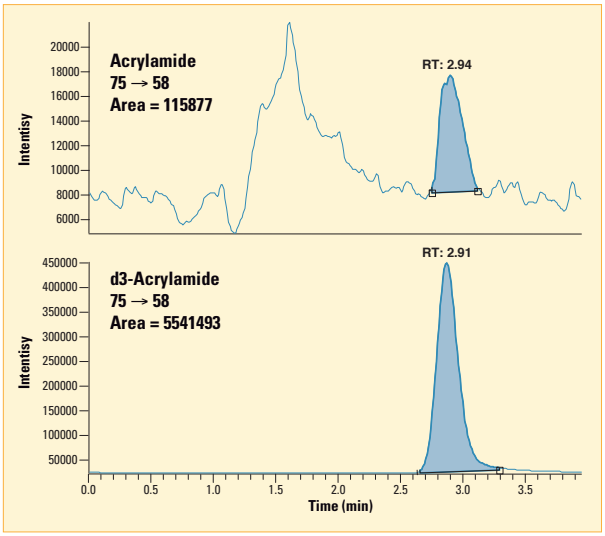

Figure 5: 1 ng/mL acrylamide standard analyzed directly after duplicate injections of the aqueous extract of the Potato Chip 2 sample

the accuracy, as a percent relative error, is 1.1% and the precision, as a percent coefficient of variance (%CV), is 12.1% for five replicate injections. Above the LOQ, the relative error varied from -2.9 to +2.4% and the %CV ranged from 0.5 to 6.7%.

Results obtained from the aqueous extract of Potato Chip 2 are presented in Figure 4. By utilizing a C18 column positioned before a switching valve to selectively elute acrylamide onto the Hypercarb column, background interferences are reduced. Unlike most of the other acrylamide reports where SPE cleanup was used following extraction of the sample with water,[1-4] the column-switching LC method employed here provides an on-line means of acrylamide fractionation. This has the advantage of minimizing sample losses during SPE and greatly reduces sample preparation time.

To monitor the consistency and reproducibility of the column-switching LC-MS/MS method, a 1 ng/mL acrylamide standard was analyzed immediately following each food sample. An example of this quality control standard analyzed after the Potato Chip 2 sample is shown in Figure 5. Although the baseline for the *m/z* 72 → 55 SRM transition is somewhat elevated near the retention time for acrylamide, the calculated concentration for this standard is 0.99 ng/mL, equating to a relative error of -1.0%.

Table 2 reports the results for four different food samples that were assayed for acrylamide using the column-switching LC method and MS/MS detection. The acrylamide concentrations in each food sample were calculated by multiplying the measured solution concentration from duplicate injections by the extraction volume and dividing by the food sample mass that was extracted. The determined acrylamide concentrations correlated well to those reported elsewhere for these classes of food.[1-5]

	Cereal 1	Cereal 2	Potato Chip 1	Potato Chip 2
Injection 1	17.17 ng/mL	55.93 ng/mL	57.11 ng/mL	29.18 ng/mL
Injection 2	17.00 ng/mL	56.18 ng/mL	56.52 ng/mL	29.14 ng/mL
Mean	17.09 ng/mL	56.06 ng/mL	56.82 ng/mL	29.16 ng/mL
Extraction Vol.	20.0 mL	20.0 mL	20.0 mL	20.0 mL
Mass Sample	2.003 g	2.007 g	2.021	1.995
Acrylamide Conc.	171 ng/g	559 ng/g	562 ng/g	292 ng/g

Table 2: Results of acrylamide assay from food samples

Conclusions

An LC-MS/MS method has been developed for the measurement of acrylamide on the TSQ Quantum Discovery. Using APCI for the analysis of acrylamide from 100% water, an LOQ of 0.25 ng/mL acrylamide or 2.5 pg on column was achieved. Incorporation of a column-switching LC method prior to MS/MS detection of acrylamide eliminated the need to purify food sample extracts by SPE. The method was successfully demonstrated for the analysis of four brands of food samples using TSQ Quantum Discovery in conjunction with a column-switching LC method.

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Application Note: 424

Analysis of Melamine and Cyanuric Acid in Food Matrices by LC-MS/MS

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Key Words

- TSQ Quantum Ultra™
- Cyanuric Acid
- Food Safety
- LC-MS/MS

Introduction

In March 2007, several North American manufacturers of pet food voluntarily issued nationwide recall notices for some of their products that were reportedly associated with renal failure in pets[1]. The raw material wheat gluten, used to manufacture the pet food, was imported from China and was identified as the source of contamination[2].

Although initial reports suggested that contamination was confined to pet food, further investigations revealed that melamine-tainted fodder may have been used to feed animals intended for human consumption.[3,4,5] In particular, it was discovered that melamine-contaminated ingredients had been used to prepare feed for chickens, swine, and catfish.[3,4] Consequently, the U.S. Food and Drug Administration (FDA)[3] and the U.S. Department of Agriculture (USDA)[4] have developed methods for the analysis of melamine residues in animal tissue. Both methods use tandem mass spectrometric detection and employ disposable strong cation exchange solid phase extraction (SPE) cartridges to prepare samples for liquid chromatographic analysis.

Experimental

Chemicals and reagents

Unless stated otherwise, all organic solvents used in this work were HPLC grade quality and were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Melamine, cyanuric acid, and 30% (w/w) aqueous ammonia were purchased from Sigma (St. Louis, MO, USA). The internal standards $^{13}C_3$-melamine and -cyanuric acid were prepared using $^{13}C_3$-cyanuric chloride, which was also obtained from Sigma. 18 MΩ water was obtained from a Milli-Q™ (Millipore Corporation, Billerica, MA, US) purification system.

Calibration Standards

Individual solutions (1000 µg/mL) of cyanuric acid and melamine were prepared by dissolving the crystalline compounds in 50% (v/v) aqueous methanol. Aliquots (1 mL) of these solutions were combined and then diluted with 1:3 water-acetonitrile, respectively, to obtain a 10 µg/mL stock solution, from which eight working standards in the range of 1-1000 ng/mL were prepared by serial dilutions with acetonitrile. Calibration standards were prepared by adding 50 µL of the stock solution of the internal standards to 1 mL of each of the eight working standards.

Sample Preparation

Solid samples were homogenized using an Ultra-Turrax® (IKA®-Werke GmbH & Co. KG, Staufen, Germany) homogenizer. After extraction into aqueous 1:1 Water:MeOH, and addition of the internal standards, the samples were prepared by offline ion exchange chromatography using SPE cartridges.

Liquid Chromatography

Aliquots (10-25 µL) of the above extracts were chromatographed on a BioBasic™AX (Thermo Fisher Scientific, Bellefonte, PA) analytical column (2.1×150 mm, 5 µm), which was kept at 30 °C in an oven. The initial mobile phase was composed of acetonitrile-isopropanol-50 mM aqueous ammonium acetate in the ratio of 85:10:5, respectively, and was pumped through the column at a flow of 400 µL per minute.

After 5 min, the mobile phase composition and flow were immediately changed to 9:1 water-acetonitrile, and 500 µL per minute, respectively. These conditions were maintained for 5 min before returning the mobile phase to the initial composition. After 5 min of equilibration, the flow through the column was returned to 400 µL per minute. The column effluent was diverted to waste for the first 1.5 minutes and then switched to the detector for the remaining run time.

MS Conditions – Melamine

MS: Thermo Scientific TSQ Quantum Ultra
Source: Heated Electrospray (H-ESI)
Ionization: Positive ESI
Sheath Gas: 65 units
Auxiliary Gas: 35 units at 250 °C
Ion Transfer Tube Temp: 350 °C
Scan Time: 200 ms/transition
Q1/Q3 Resolution: 0.7 FWHM

SRM Transitions:

Melamine:	*Melamine $^{13}C_3$ (Internal Standard):*
m/z 127→68 @ 32 eV	*m/z* 130→70 @ 32 eV
m/z 127→85 @ 18 eV	*m/z* 130→87 @ 18 eV

QED-MS/MS Conditions:
Collision Energy: 30 eV
Reverse Energy Ramp (RER): 50 eV

MS Conditions – Cyanuric Acid

MS: TSQ Quantum Ultra
Source: Heated Electrospray (H-ESI)
Ionization: Negative ESI
Sheath Gas: 75 units
Auxiliary Gas: 10 units at 250 °C
Ion Transfer Tube Temp: 350 °C
Scan Time: 200 ms/transition
Q1/Q3 Resolution: 0.7 FWHM

SRM Transitions:

Cyanuric Acid:	*Cyanuric Acid $^{13}C_3$ (Internal Standard):*
m/z 128→42 @ 17 eV	*m/z* 131→43 @ 17 eV
m/z 128→85 @ 11 eV	*m/z* 131→87 @ 11 eV

Results

A chromatogram showing a standard mixture of both melamine and cyanuric acid, along with their associated internal standards, is shown in Figure 1. Calibration curves ranging from 1-1000 ppb are shown in Figure 2 and Figure 3 for melamine and cyanuric acid, respectively. The calibrations are linear over the entire range, and a close-up of the lower portion of the calibration curve (1-100 ppb) is shown in the same figure.

Melamine and cyanuric acid were spiked into a matrix of catfish and processed as described in the method section above. A chromatogram of this sample, spiked at 10 ppb for melamine and 50 ppb for cyanuric acid, is shown in Figure 4. Very low noise is observed, emphasizing the effectiveness of the cleanup procedure for a complicated matrix.

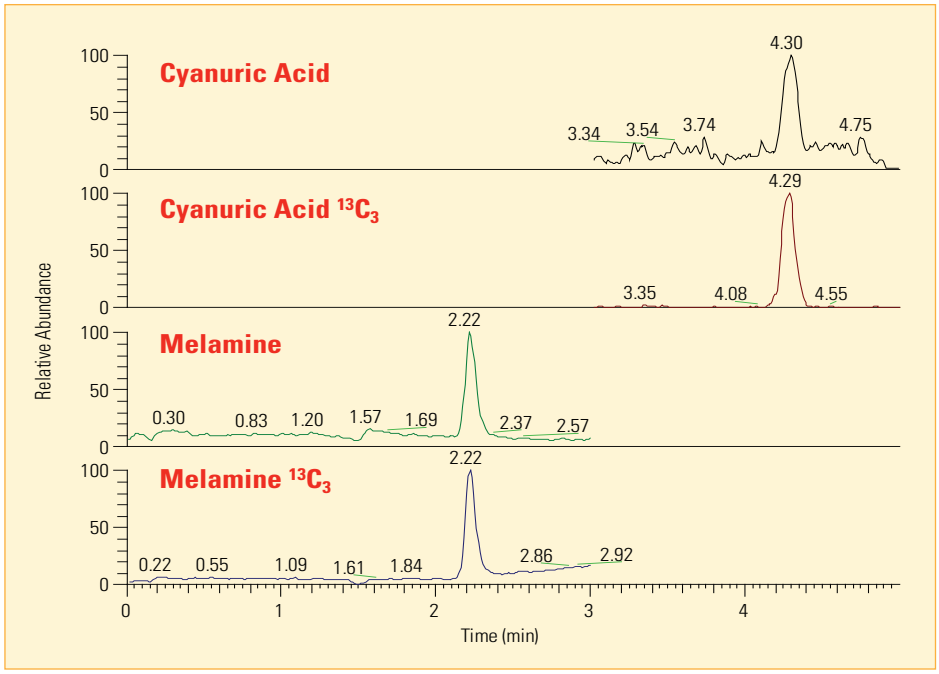

Figure 1. Melamine, cyanuric acid, and their internal standards at a concentration of 1 ppb. From top to bottom, cyanuric acid, cyanuric acid $^{13}C_3$, melamine, and melamine $^{13}C_3$.

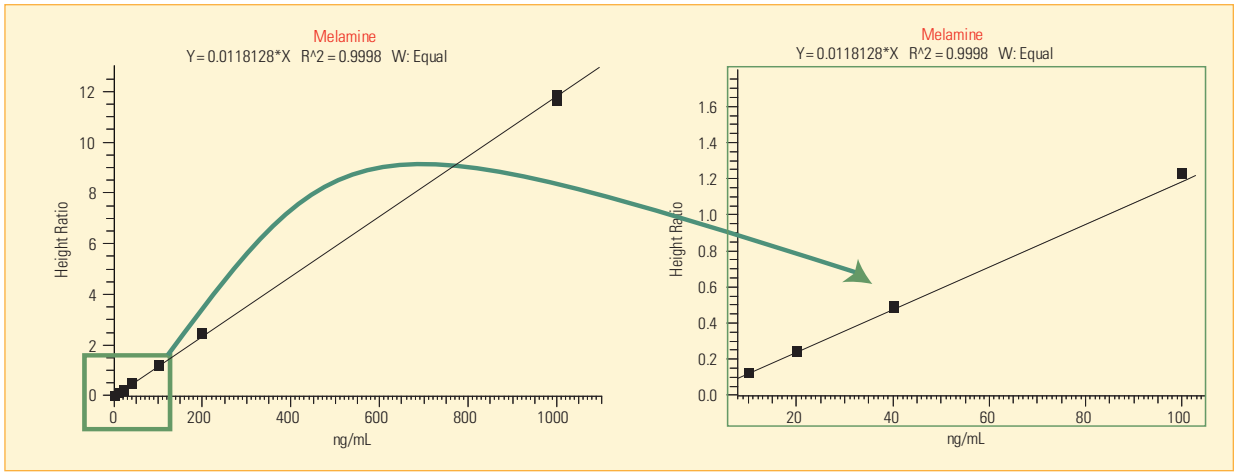

Figure 2. Calibration curve for melamine from 1-1000 ng/mL. The left figure shows the entire calibration range, while the right figure shows the expanded range from 10-100 ng/mL.

Additionally, full spectra data was collected using the standard Quantitation-Enhanced Data-Dependent MS/MS (QED-MS/MS) scan function. QED-MS/MS works by monitoring SRM data, and when the response of a particular SRM reaches a threshold level, the full scan MS/MS is activated. The resulting full scan spectra for melamine at 100 ppb and its internal standard are shown in Figure 5. The full scan data allows for further confirmation of results by eliminating "false positives" and also provides the opportunity to perform a library search. When a full scan QED-MS/MS spectra collected from a catfish sample spiked at 10 ppb was searched against the library, the library search returned melamine as the most likely hit. The results of the library search are shown in Figure 6. The spectrum of the sample and the spectrum that is stored in the library are visible in the lower left quadrant of the figure. The top spectrum is the catfish sample, while the lower spectrum is the reference spectrum. There is good agreement between the two spectra, even though the reference spectrum was generated using standards without matrix.

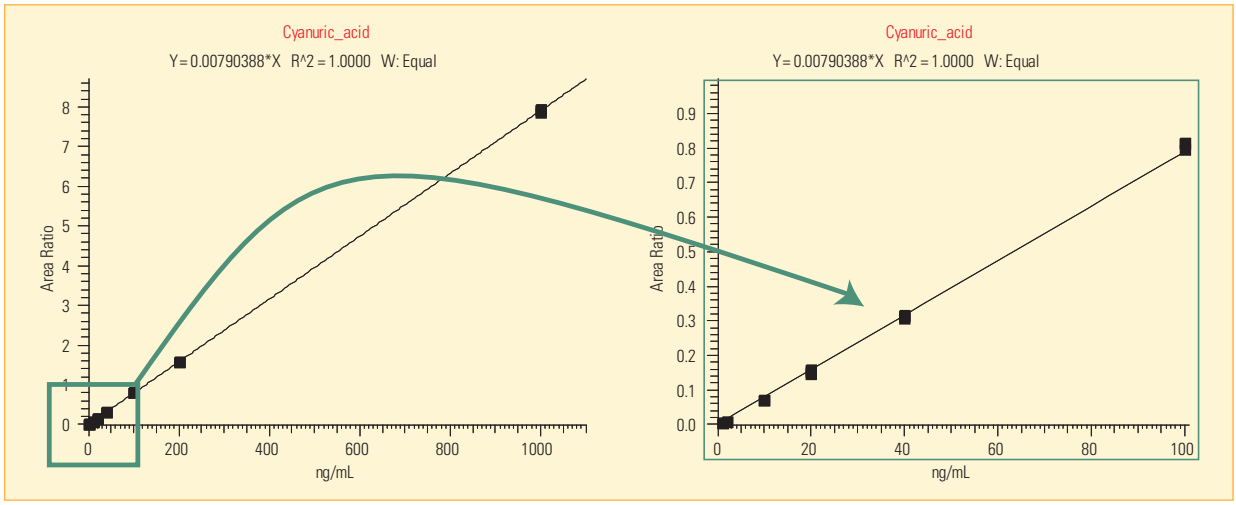

Figure 3: Calibration curve for cyanuric acid from 1-1000 ng/mL. The left figure shows the entire calibration range, while the right figure shows the expanded range from 1-100 ng/mL.

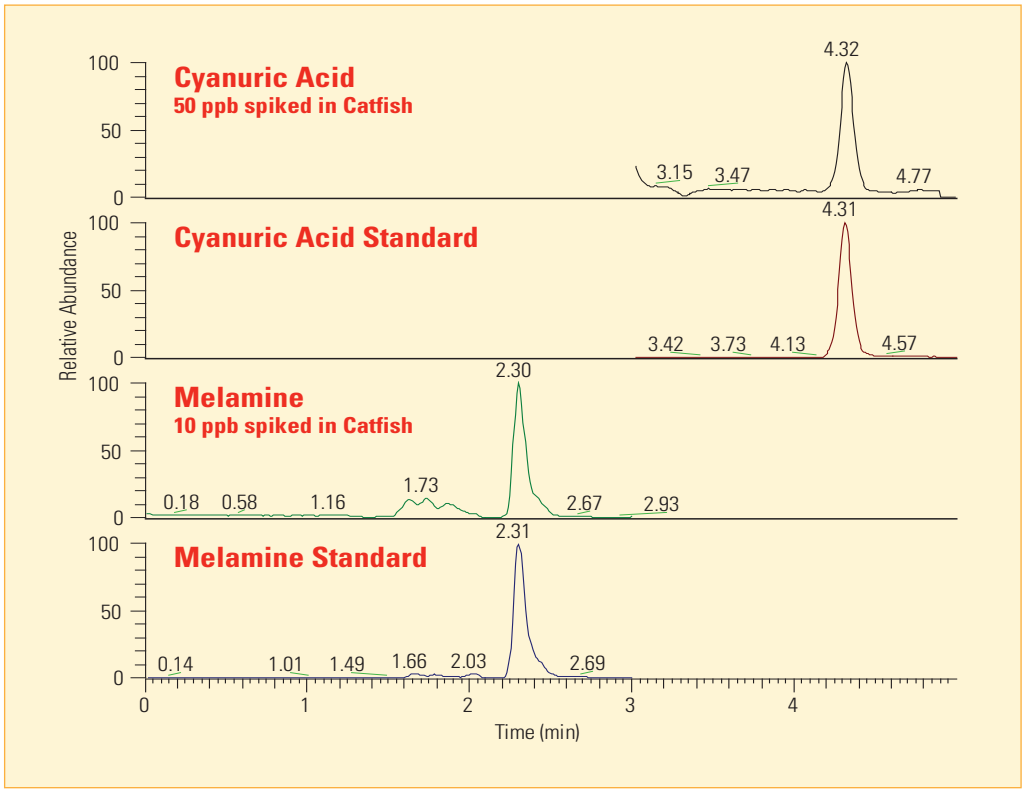

Figure 4: Chromatogram of cyanuric acid and melamine spiked into catfish matrix, at a level of 50 ppb for cyanuric acid, and 10 ppb for melamine

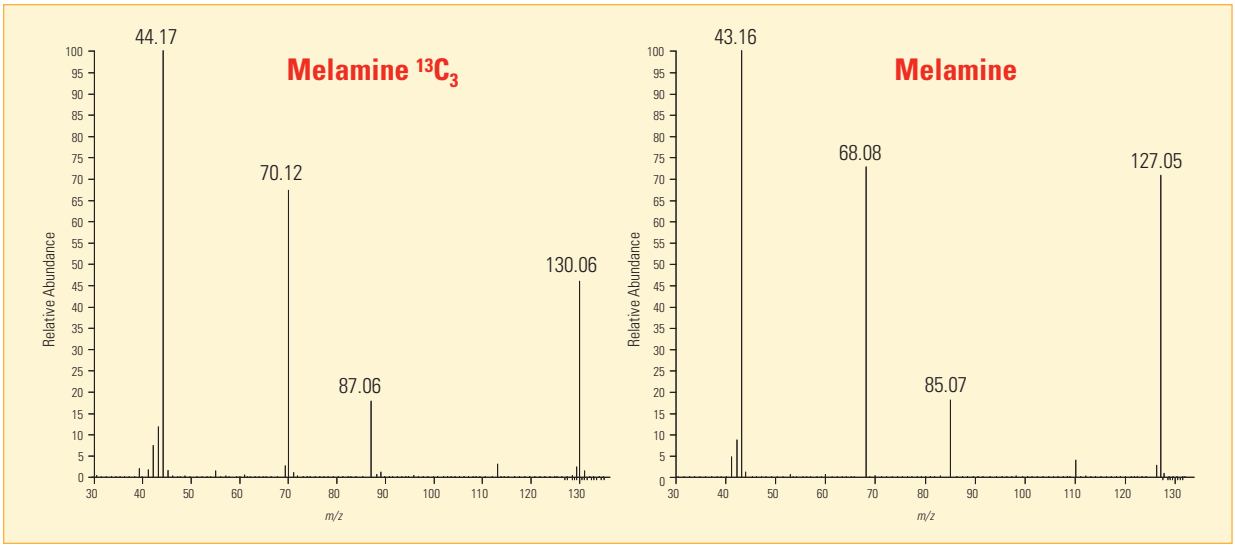

Figure 5: QED-MS/MS spectra for melamine $^{13}C_3$ (left) and melamine (right). Unique, rich, library-searchable spectra are collected in the same chromatographic run, allowing both quantitative and confirmatory full scan data in the same file.

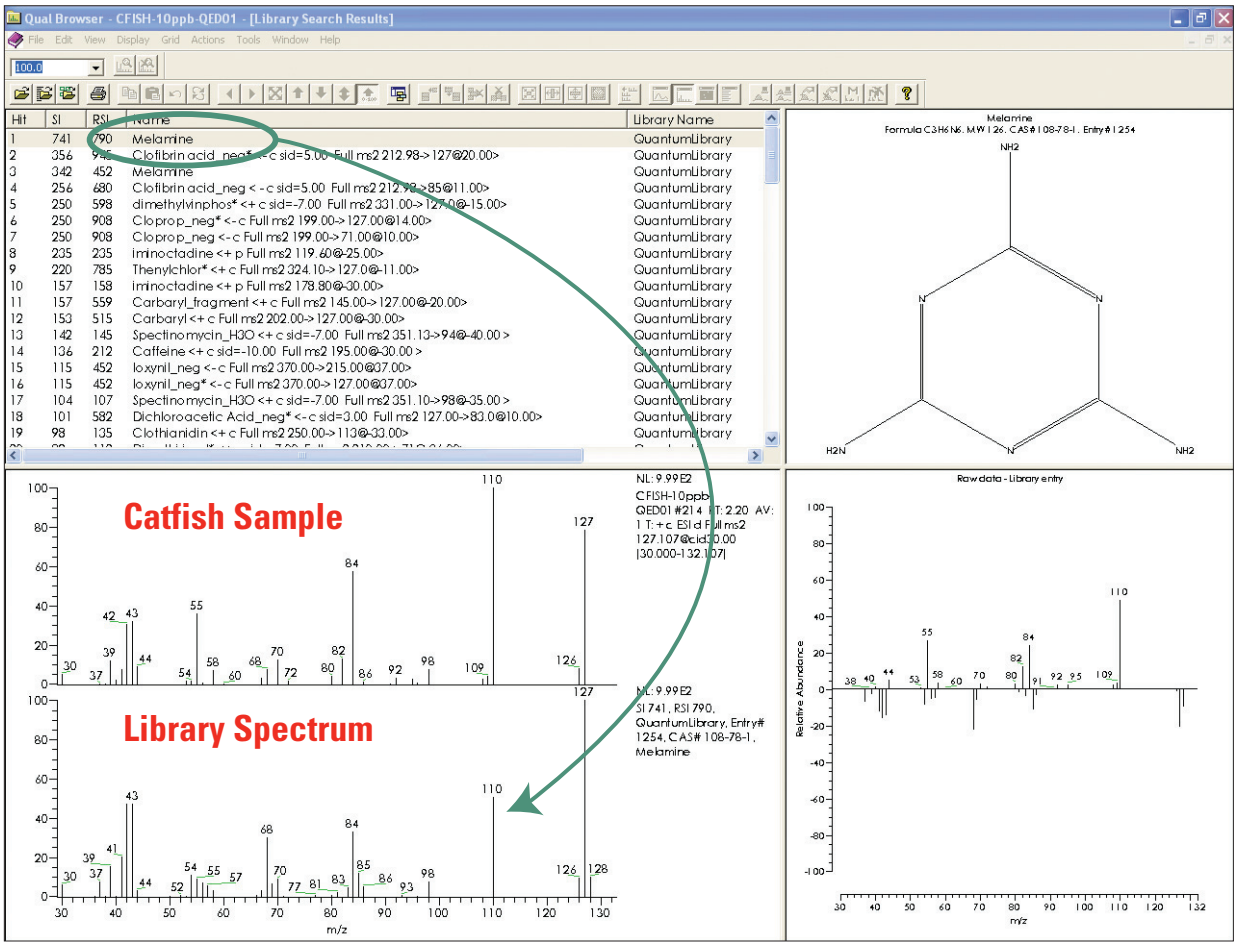

Figure 6: Library search results for melamine spiked at 10 ppb into a catfish matrix. Melamine is the top hit in the search list.

Conclusion

A simple, sensitive, and specific method for the detection and quantitation of melamine and cyanuric acid in food matrices has been demonstrated. The method is robust and allows for the analysis of a large number of samples, without degradation in column performance. Additionally, full scan spectra for Q3 are collected in the same chromatographic run using the QED-MS/MS scan function, permitting a library search of the results to eliminate any false positives.

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High-Throughput Food Safety Control Employing Real Time Ionization (DART) Coupled to Orbitrap High-Resolution Mass Spectrometry

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Key Words
- Exactive
- DART
- Melamine
- Mycotoxins

Introduction

In recent years, substantial developments have taken place in the field of mass spectrometry, enabling the introduction of a number of novel ambient desorption ionization techniques[1] such as direct analysis in real time (DART®),[2] desorption electrospray ionization (DESI),[3] surface desorption atmospheric pressure chemical ionization (DAPCI)[4] and atmospheric solids analysis probe (ASAP™).[5] These novel ion sources are characterized by remarkably high throughput of analyses which can be carried out under ambient conditions without (chromatographic) separation of sample components prior to desorption/ionization or the need for complicated and time demanding sample pre-treatment procedures. The DART technology employed in this study relies upon fundamental principles of atmospheric pressure chemical ionization (APCI). Excited-state helium atoms produce reactive species for analyte ionization.[2] Numerous applications of the DART ion source coupled to various types of mass spectrometers have been reported.[6-16] DART found its use in many areas of analytical chemistry as a tool for rapid qualitative analysis of numerous compounds. Due to the relatively high signal fluctuation of ion intensities obtained by repeated DART measurements, an internal standard usually has to be employed for compensation during quantitative analysis. However, implementation of Vapur® gas ion separator and automatic sampling systems were reported to significantly improve the repeatability for some analytes.[15]

Due to the absence of separation, the whole sample is introduced into a mass spectrometer. This unavoidably leads to a significant number of spectral interferences. In order to correctly determine the masses of relevant compounds and potential unknowns in the case of fingerprinting analysis, it is essential to separate them from the matrix ions. A mass spectrometer based on Orbitrap technology routinely achieves the mass resolving power of up to 100,000 FWHM (full width half maximum) while maintaining excellent mass accuracy of < 5 ppm, without the use of internal mass correction.[17] Those features make it an ideal tool to complement DART ionization for the analysis of complex samples.

This application note shows the possibilities using the DART ion source coupled with the ultra high-resolution Thermo Scientific Exactive mass spectrometer for rapid detection and quantitation of a wide range of food contamin - ants like mycotoxins and food adulterants (melamine).

Experimental

DART-Exactive MS

DART-Exactive MS system used in this study consisted of a new commercial model of DART ion source (DART-SVP) with a 12 Dip-It™ tip scanner autosampler coupled to the Exactive™ benchtop mass spectrometer – see Figure 1. Vapur interface was employed to hyphenate the ion source and the mass spectrometer, low vacuum in the interface chamber

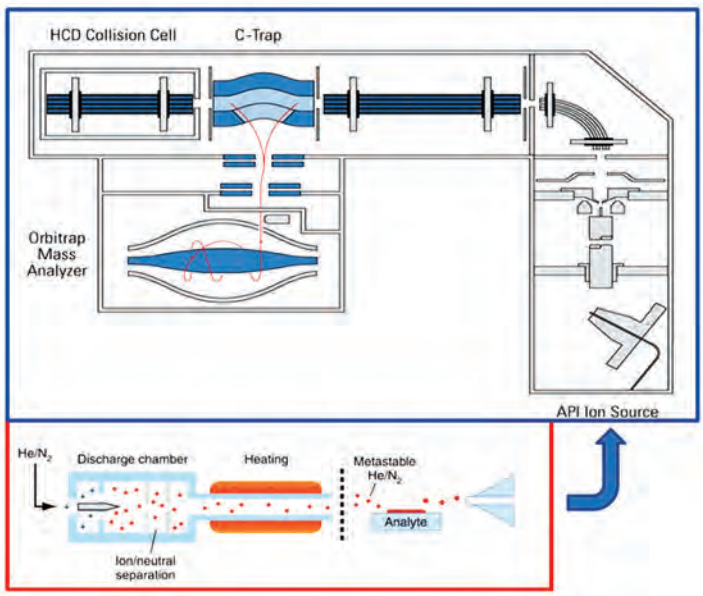

Figure 1: Schematics of a DART-Exactive system (source: *www.ionsense.com*). DART ionization source (bottom), Exactive MS (top).

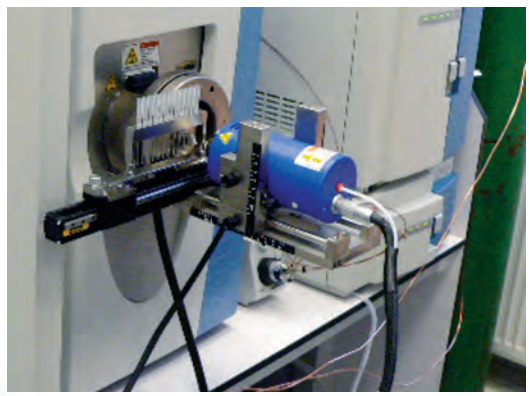

Figure 2: The DART ionization source coupled to Exactive MS

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was maintained by a membrane pump (Vacuubrand, Wertheim, Germany). The use of Vapur gas ion separator during DART ionization was essential in order to maintain stable vacuum within the operating pressure limits of the Exactive instrument. Vapur interface also improved transport efficiency of ions from the sampling area to the atmospheric-pressure interface inlet of the mass spectrometer, thus enhancing both sensitivity and reproducibility of the measurement. The distance between the exit of the DART gun and the ceramic transfer tube of the Vapur was set to 10 mm, the gap between the ceramic tube and the inlet to the heated capillary of the Exactive was 2 mm.

DART-MS instrument was operated either in positive or negative ionization mode; optimized settings of the system parameters were as follows: *(i)* DART positive ionization: helium flow: 2.5 L min^{-1}; gas temperature: 350 °C; discharge needle voltage: -5000 V; grid electrode: +200 V. *(ii)* DART ionization negative ionization: helium flow: 2.5 L min^{-1}; gas temperature: 350 °C; discharge needle voltage: -5000 V; grid electrode: +350 V. *(iii)* Mass spectrometric detection: capillary voltage: ±55 V; tube lens voltage: ±130 V; capillary temperature: 250 °C. Sheath, auxiliary and sweep gases were disabled during DART-MS analysis. The acquisition rate was set according to desired resolving power of the Exactive mass analyzer, and was 10 spectra s^{-1} at 10,000 FWHM (full width at half maximum), 4 spectra s^{-1} at 25,000 FWHM and 2 spectra s^{-1} at 50,000 FWHM. In all cases, the mass resolving power was calculated for *m/z* 200.

Semi-automatic analysis of liquid samples was carried out with the use of 12 Dip-It tip scanner autosampler. Dip-It tips were inserted into a holder and immersed in sample extracts placed in deepwell micro-plate (Life Systems Design, Merenschwand, Switzerland). The holder was mounted on the body of the autosampler. Subsequently, the Dip-It tips automatically moved at a constant speed of 0.5 mm s^{-1} through the helium gas beam in perpendicular direction to the axis leading from DART gun exit to the mass spectrometers inlet. Using the above moving speed, the time of desorption from the surface of each tip was 9 s; total run time of 12 analyses was approx. 4.2 min. To enable and/or enhance ionization of certain analytes, 2 mL autosampler vial containing dopant solution was placed in the distance of 20 mm from the DART gun exit. Aqueous solution of ammonia (25%, *w/w*, Penta, Chrudim, Czech Republic) and neat dichloromethane (Scharlau, Barcelona, Spain) were used as dopants in positive and negative ionization mode, respectively.

Mycotoxin Analysis

Chemicals and standards

Standards of 3-acetyldeoxynivalenol (3-ADON), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-Glc), fusarenon-X (FUS-X), nivalenol (NIV), HT-2 toxin (HT-2), T-2 toxin (T-2), diacetoxyscirpenol (DAS), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin B2 (AFG2), ochratoxin A (OTA), fumonisin B1 (FB1), fumonisin B2 (FB2), sterigmatocystin, zearalenone (ZEA), $^{13}C_{15}$-deoxynivalenol ($^{13}C_{15}$-DON), $^{13}C_{15}$-nivalenol ($^{13}C_{15}$-NIV) and $^{13}C_{18}$-zearalenone ($^{13}C_{18}$-ZEA) were supplied by Biopure (Tulln, Austria). Standards of deepoxy-deoxynivalenol (deepoxy-DON), altenuene, alternariol, alternariolmethylether (alternariol-met), ergocornine, ergocrystine and ergosine were obtained from Sigma-Aldrich (Steinheim, Germany).

Composite standard was prepared in acetonitrile containing each of analytes (isotope-labeled compounds not included) at concentration level of 5000 ng mL^{-1} and further diluted to obtain solvent standards at 500 ng mL^{-1}. Individual solvent solutions of ^{13}C-labeled internal standards were prepared at 5000 µg mL^{-1} in acetonitrile. Matrix-matched standards in the concentration range 10 to 1000 ng mL^{-1} (corresponding to 50 to 5000 µg kg^{-1} in matrix) were obtained by spiking of blank wheat and maize extracts (prepared by procedures described below), additionally, isotopically labelled compounds were added at level 100 ng mL^{-1} (500 µg kg^{-1} in matrix).

Acetonitrile and methanol, both of HPLC-grade, were supplied by Merck (Darmstadt, Germany). Pure water was obtained from Milli-Q® purification system. Anhydrous magnesium sulphate, sodium chloride and ammonium formate (≥ 99% purity), were from Sigma-Aldrich. Primary secondary amine (PSA) sorbent was obtained from Varian (Harbor City, CA, USA), formic acid (≥ 98% purity) was from AppliChem GmbH (Darmstadt, Germany).

Samples and sample preparation

Modified QuEChERS procedure[18] was employed to extract target analytes from the examined matrices (wheat, maize and millet). 2 g of homogenized sample were weighed into a 50 mL polypropylene (PP) centrifuge tube, 7.5 mL of deionized water and 10 mL of acetonitrile were added. Vigorous shaking of the mixture (4 min) was followed by the addition of 4 g $MgSO_4$, 1 g NaCl, further shaking for 3 min and centrifugation (5 min, 10,000 rpm, 20 °C). 4 mL aliquot of the upper organic phase was transferred into a 15 mL PP tube containing 200 mg of PSA and 600 mg $MgSO_4$ and shaken for 3 min to perform solid phase extraction (SPE) clean-up of the extract. After centrifugation (3 min, 10,000 rpm, 20 °C), approx. 600 µL were taken for DART-Exactive MS analysis.

Analysis of Melamine

Chemicals and standards

Solid standard of melamine (MEL, ≥ 99.0%) was supplied by Sigma-Aldrich; isotopically labeled $^{13}C_3$-melamine ($^{13}C_3$-MEL, ≥ 98.0%) was from Witega (Berlin, Germany). Individual stock solutions of MEL and $^{13}C_3$-MEL were prepared at 1000 µg mL^{-1} in water. By further dilution, aqueous solutions at 100 and 10 µg mL^{-1} were obtained and used for preparation of matrix matched standards and spiking experiments. Matrix-matched calibration was prepared by spiking of blank raw milk, standards containing MEL in the range from 25 to 2500 ppb and fixed amount of $^{13}C_3$-MEL at 250 ppb were obtained this way. Water used in this study was purified with the use of Milli-Q purification system.

Samples and sample preparation

Raw milk samples were analyzed without any pre-treatment. Prior to DART-MS analyses, blank milk was spiked with MEL at 100 and 500 ppb and with $^{13}C_3$-MEL at 250 ppb. Additionally real-life samples (n = 2) representing contaminated powdered milk were, according to producers instructions, reconstituted in water (1:10, *w/v*), spiked with $^{13}C_3$-MEL and subjected to instrumental analysis.

Results and Discussion

DART-Exactive Analysis of Mycotoxins

The efficiency and practical applicability of DART technology for ionization of aflatoxins, fusarium toxins, alternaria toxins, ochratoxins, ergot alkaloids, and sterigmatocystin (analytes possessing relatively largely differing physico-chemical properties) was evaluated in this part of the study. For this purpose, solvent standards containing respective mycotoxin at level 500 ng mL^{-1} were analyzed. Various settings (100 – 400 °C) of ionization gas temperature and grid electrode voltage were tested in order to obtain best sensitivity and best efficiency of analyte's thermo-desorption. As shown below, most mycotoxins could be transferred into gaseous phase at temperature 350 °C which was found as an optimal compromise between signal intensity and analytes' thermal desorption speed. While the use of lower grid voltage (200 V) in positive ionization mode enabled approx. 50% intensity increase compared to 350 V setting, 350 V potential was optimal for analytes ionized in negative mode. It was also found that ionization of some mycotoxins is improved by introducing dopant vapours (dichloromethane or ammonia) into the region between the ion source exit and Vapur interface ceramic tube inlet.

The list of ions generated by DART, when analyzing mycotoxin standard solutions, is provided in Table 1. As it can be seen, most of the examined mycotoxins could be effectively ionized in positive or negative ion mode, either as pseudomolecular ions or forming charged adducts supposing dichloromethane or ammonia vapors were present in the ionization region. Relatively poor ionization efficiencies were obtained for aflatoxins where electrospray ionization (ESI)[19, 20] was documented to be option for their control at ultra trace levels which are of regulatory interest.

				Detected Ions		
Compound	**Elemental Formula**	**Exact MW**	**Ionization Mode**	**Ion**	**Elemental Composition**	**Exact Mass**
ADON	$C_{17}H_{22}O_7$	338.1360	Negative	$[M+Cl]^-$	$C_{17}H_{22}O_7Cl$	373.1049
DON	$C_{15}H_{20}O_6$	296,1254	Negative	$[M+Cl]^-$	$C_{15}H_{20}O_6Cl$	331.0943
Deepoxy-DON	$C_{15}H_{20}O_5$	280,1305	Negative	$[M+Cl]^-$	$C_{15}H_{20}O_5Cl$	315.0993
FUS-X	$C_{17}H_{22}O_8$	354,1309	Negative	$[M+Cl]^-$	$C_{17}H_{22}O_8Cl$	389.0998
NIV	$C_{15}H_{20}O_7$	312,1204	Negative	$[M+Cl]^-$	$C_{15}H_{20}O_7Cl$	347.0903
ZEA	$C_{18}H_{22}O_5$	318,1462	Negative	$[M-H]^-$	$C_{18}H_{21}O_5$	317.1394
				$[M+Cl]^-$	$C_{18}H_{22}O_5Cl$	353.1150
HT-2	$C_{22}H_{32}O_8$	424,2092	Positive	$[M+NH_4]^+$	$C_{22}H_{36}NO_8$	442.2435
T-2	$C_{24}H_{34}O_9$	466,2197	Positive	$[M+NH_4]^+$	$C_{24}H_{38}NO_9$	484.2541
DAS	$C_{19}H_{26}O_7$	366,1673	Positive	$[M+H]^+$	$C_{19}H_{27}O_7$	367.1750
				$[M+NH_4]^+$	$C_{19}H_{30}NO_7$	384.2017
Altenuene	$C_{15}H_{16}O_6$	292,0941	Negative	$[M-H]^-$	$C_{15}H_{15}O_6$	291.0874
				$[M+Cl]^-$	$C_{15}H_{16}O_6Cl$	327.0630
Alternariol	$C_{14}H_{10}O_5$	258,0523	Negative	$[M-H]^-$	$C_{14}H_9O_5$	257.0455
				$[M+Cl]^-$	$C_{14}H_{10}O_5Cl$	293.0211
Alternariol-met	$C_{15}H_{12}O_5$	272,0679	Negative	$[M-H]^-$	$C_{15}H_{11}O_5$	271.0612
				$[M+Cl]^-$	$C_{15}H_{12}O_5Cl$	307.0368
AFB1	$C_{17}H_{12}O_6$	312,0628	Positive	$[M+H]^+$	$C_{17}H_{13}O_6$	313.0712
AFB2	$C_{17}H_{14}O_6$	314,0785	Positive	$[M+H]^+$	$C_{17}H15O_6$	315.0868
AFG1	$C_{17}H_{12}O_7$	328,0578	Positive	$[M+H]^+$	$C_{17}H_{13}O_7$	329.0661
AFG2	$C_{17}H_{14}O_7$	330,0734	Positive	$[M+H]^+$	$C_{17}H_{15}O_7$	331.0712
Sterigmatocystin	$C_{18}H_{12}O_6$	324,0628	Positive	$[M+H]^+$	$C_{18}H_{13}O_6$	325.0707
OTA	$C_{20}H_{18}ClNO_6$	403,0817	n.d.	n.d.	–	–
FB1	$C_{34}H_{59}NO_{15}$	721,3879	n.d.	n.d.	–	–
FB2	$C_{34}H_{59}NO_{14}$	705,3930	n.d.	n.d.	–	–
Ergocornine	$C_{31}H_{39}N_5O_5$	561,2946	n.d.	n.d.	–	–
Ergocristine	$C_{39}H_{39}N_5O_5$	657,2946	n.d.	n.d.	–	–
Ergosine	$C_{30}H_{37}N_5O_5$	547,2789	n.d.	n.d.	–	–

n.d. - signal not detected

Table 1: Overview of most intensive mycotoxins ions detected under optimized DART-Exactive MS conditions in solvent standard (500 ng mL^{-1})

Material Description	Analyte (Assigned/Certified Value)	DART-Exactive MS External Calibration/Isotope Dilution	
		Mean (µg kg^{-1})	RSD (%)[a]
CRM, maize flour	DON (474 ± 30 µg kg^{-1})	459/486	9.0/5.9
CRM, wheat flour	DON (2800 ± 200 µg kg^{-1})	2608/2819	6.7/5.4
CRM, ground millet	ZEA (648 ± 140 µg kg^{-1})	583/613	7.5/6.0
CRM, maize flour	ZEA (60 ± 9 µg kg^{-1})	< LCL[b]	– / –

a Relative standard deviation (RSD) calculated from 3 analyses.
b The concentration of analyte was below LCL of the method.

Table 2: Trueness of data obtained by DART-Exactive MS analysis of certified reference materials

No ions were obtained under tested conditions for a few other mycotoxins, such as ochratoxin A, fumonisins or ergocornine, ergocrystine and ergosine. These compounds are rather polar, and especially in case of fumonisins and ergot alkaloids, have relatively high molecular weight (MW). Both of these properties are associated with low volatility that hampers the transfer of such analytes into gaseous phase. To facilitate and/or enhance DART ionization of troublesome mycotoxins, derivatization of polar functional groups, which enables avoiding hydrogen bonding, may represent a conceivable strategy.[21]

Quantitative Analysis

For quantitative purposes, the most abundant ions yielded by respective mycotoxins (see Table 1) were used and narrow isolation window of 4 ppm was employed to extract ion records (chronograms) of target analytes with high selectivity. The quantitative parameters of the method for DON and ZEA, demonstrated by analysis of available certified reference materials containing incurred *Fusarium* toxins, are presented in Table 2. For evaluation of repeatability, peak areas were preferred since they were shown to give better results compared to calculations based on peak heights. Typical RSDs for cereals spiked by mycotoxins at 500 µg mL^{-1} level ranged from 8.1 to 14.3%. Further decrease of RSDs (4.7–8.7%) and improved linearity of calibration plots compared to external calibration, was obtained when isotopically labelled internal standards were employed for compensation of absolute signal fluctuation. In case of regulated mycotoxins (DON, ZEA) DART-MS method lowest calibration levels allowed a reliable control of maximum limits established for tested matrices.[22] The recoveries of all target mycotoxins at both tested spiking levels 150 and 500 µg kg^{-1} were in the range 82–120% when external calibration based on matrix matched standards was employed for quantification. Regarding the requirements for performance characteristics in analysis of regulated analytes,[23] these were reliably met for both target toxins.

Melamine Analysis

Under experimental conditions, both MEL and $^{13}C_3$-MEL were detected as $[M+H]^+$ ions in positive DART ionization mode. The efficiency of ionization was comparable for both compounds. Very good mass accuracy, with mass error less than 3 ppm was achievable with Exactive mass analyzer (operated under mass resolving power setting 50,000 FWHM) within all measurements in this study; analyte confirmation based on elemental composition estimation could be performed. As shown in Figure 3, abundant spectral interference observed at *m/z* 127.04 was detected both in blank and spiked samples (in contrast to solvent standards). At mass resolving power ~3,500 FWHM obtained by time-of-flight mass analyzer it was not possible to resolve signals of analyte and interference. Especially at low concentration levels of MEL, the signal of analyte was completely overlapped by the interference making its detection impossible. On the other hand, employing high mass resolving power of DART-Exactive MS, reliable detection of MEL in milk, was feasible even at low concentration levels. Using

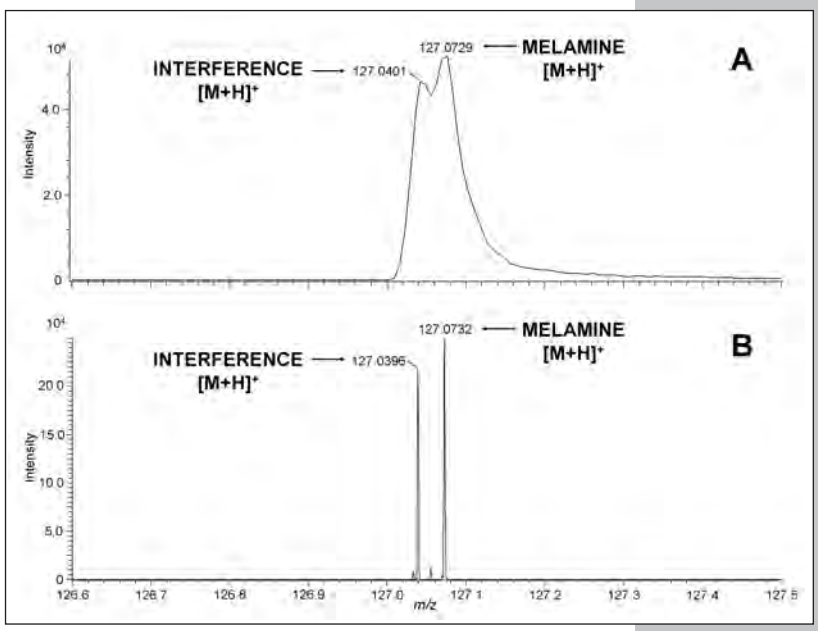

Figure 3: The improvement of mass separation by applying high mass resolution during analysis of melamine in milk sample at 2.5 mg/kg.
(A) DART-TOFMS (mass resolving power 3,500 FWHM);
(B) DART-Exactive MS (mass resolving mass power 50,000 FWHM).

Sample	DART-Exactive MS		LC-MS/MS	
	Mean (ppb)	RSD (%)	Mean (ppb)	RSD (%)
Milk powder 1	501	8.2	530	5.1
Milk powder 1	2496	6.4	2612	2.1

Table 3: Concentration of MEL in real-life samples as measured by DART-Exactive MS and LC-MS/MS

accurate mass of interference ion, elemental composition $C_6H_7O_3$ was estimated. This value corresponds to protonated molecule of hydroxymethyl furfural (HMF) which is typically formed during thermal processing of sugars containing foods.

The detectability of the method was characterized as lowest calibration level (LCL). Generally used limit of detection could not be calculated due to absence of noise in obtained records. LCL for melamine in milk was 25 ppb. For quantification purpose, isotope dilution technique was used. Figure 4 shows record of calibration standards analysis of which can be completed within 4.2 min (duplicate of each standard), Figure 5 documents acceptable linearity obtained for calibration curve in the range 25 to 2500 ppb ($R^2 \geq 0.99$). The LCL for melamine in milk was determined at 25 ppb and recoveries calculated at 100 and 500 ppb were in the range 98–119% and 101–109%. Repeatability at 100 ppb (n = 5) was 7.2%.

The results of real life samples analyses were compared to those obtained by validated LC–MS/MS method. Good agreement between respective values was observed (see Table 3).

Conclusions

The results presented in this application note demonstrate the feasibility of DART ionization source in combination with Exactive mass spectrometer for the rapid detection and quantification of various food contaminants, including set of priority mycotoxins and melamine selected as an example. Comparable trueness of generated results was achieved by applying isotope dilution-based quantification and matrix-matched calibration to compensate for signal suppression and other matrix effects that unavoidably occur during direct analysis of real matrix samples.

The major advantages of the combination of ambient ionization technique with Exactive mass spectrometry are the simplicity of operation, day-to-day robustness and broad application range. In addition, ultra high-resolution provided by Exactive mass analyzer helps to solve some of the problems caused by isobaric interferences from matrix components. The choice of ultra high-resolution mass spectrometer such as Exactive is one of the key requirements when considering the application of DART ionization as a reliable tool in the food laboratory.

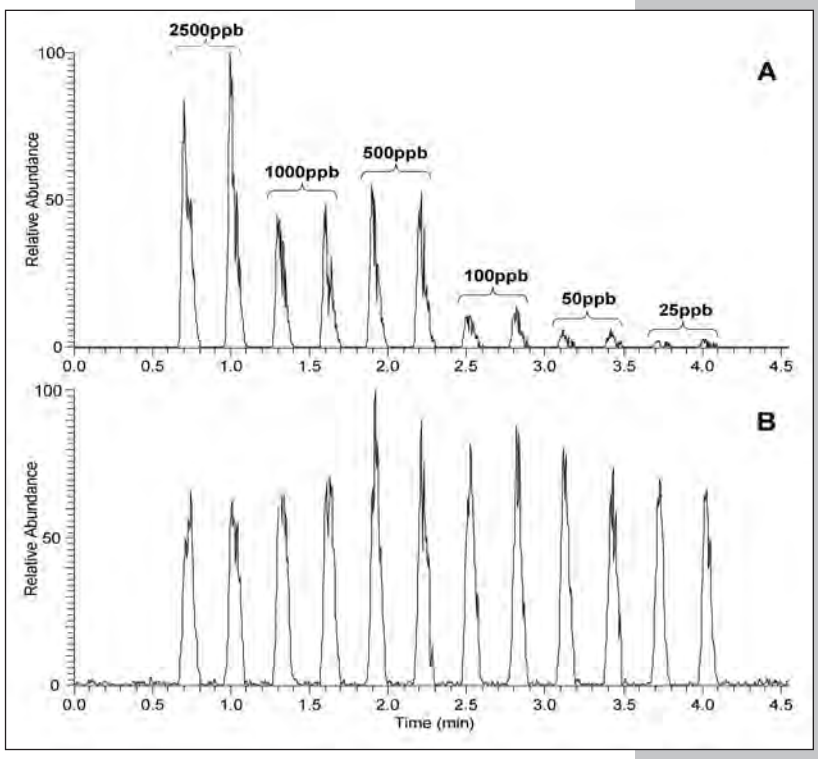

Figure 4: DART-Exactive MS record of milk spiked with MEL in the range 25 to 2500 ppb. (A) MEL (m/z 127.0726 ± 3 ppm); (B) $^{13}C_3$-MEL (m/z 130.0827 ± 3 ppm).

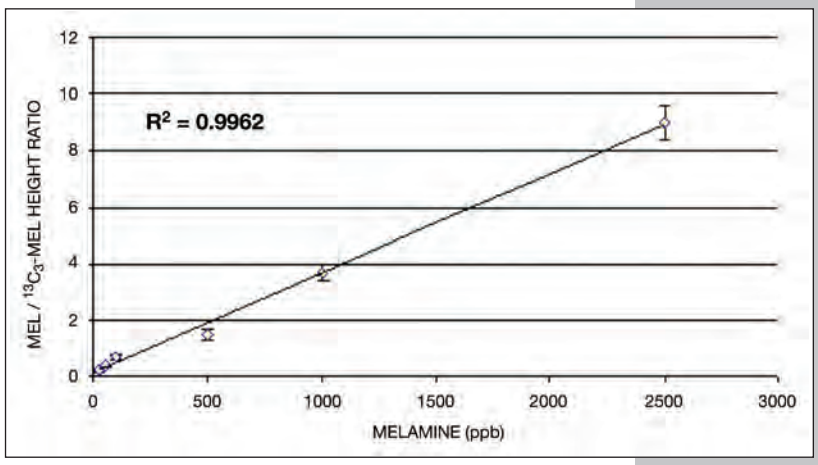

Figure 5: Calibration curve of melamine obtained by DART-Exactive MS analysis of matrix-matched standards constructed by plotting analyte-to-internal standard peak height ratio. Error bars are standard deviation (n = 3).

Acknowledgements

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Application Note: 502

Simple and Rapid Screening of Melamine in Milk Products with High Resolution Accurate Mass Benchtop Orbitrap LC-MS

Kefei Wang, Chunang (Christine) Gu
Thermo Fisher Scientific, San Jose, CA, USA

Key Words

- Exactive
- HRAM High Resolution Accurate Mass
- High throughput screening
- Milk products

Introduction

Generally used for industrial manufacturing, melamine, a nitrogen-rich white crystal, has been found as an adulterant used to falsify the protein levels in many milk products. In the 2008 Chinese milk scandal, thousands of young children who consumed melamine-contaminated milk products were reported to have developed sickness related to kidney stones and renal failure. More recently in January 2010, China reported another recall of melamine-tainted condensed and powdered milk products. Contaminated milk products were also found during the 2008 scandal in many other countries and regions, causing widespread concern and demand for monitoring melamine in various milk products.

Different countries vary in setting the Maximum Residue Limit (MRL) for melamine, but generally follow the United States Food and Drug Administration (US FDA) MRL of 1 ppm for infant formula and 2.5 ppm for other milk products.[1] Most advanced food testing labs employ mass spectrometry-based methods, particularly liquid chromatography tandem mass spectrometry (LC-MS/MS), for detecting sub-ppm to low ppb levels of melamine.

Milk is a complex matrix containing soluble proteins, sugars and lipids, with additional enriched nutrients such as vitamins and minerals added to infant formula. Sample cleanup is critical and two approaches are generally used. First is the dilute-and-shoot approach in which the milk products are dissolved in diluted acid, followed by protein precipitation with acetonitrile. US FDA uses such a method for reporting a limit of quantitation (LOQ) of 250 ppb on LC-MS/MS.[1] In the second approach, more time-consuming and labor-intensive solid phase extraction (SPE) is used to remove the interferences and enrich the sample melamine for more sensitive quantitation at low ppb level by LC-MS/MS.

Another complication in analyzing melamine with LC/MS is that melamine, being a strong polar small molecule, cannot be retained in conventional reverse-phase HPLC. Ion paring or HILIC (Hydrophilic Interaction Chromatography) mode is used.

In this study, we evaluate a simple and rapid LC/MS method to screen trace levels of melamine in milk products by utilizing a benchtop high resolution, accurate mass Orbitrap mass spectrometer. The sample preparation uses dilute-and-shoot. Analysis is fast and requires only a 1-minute LC separation.

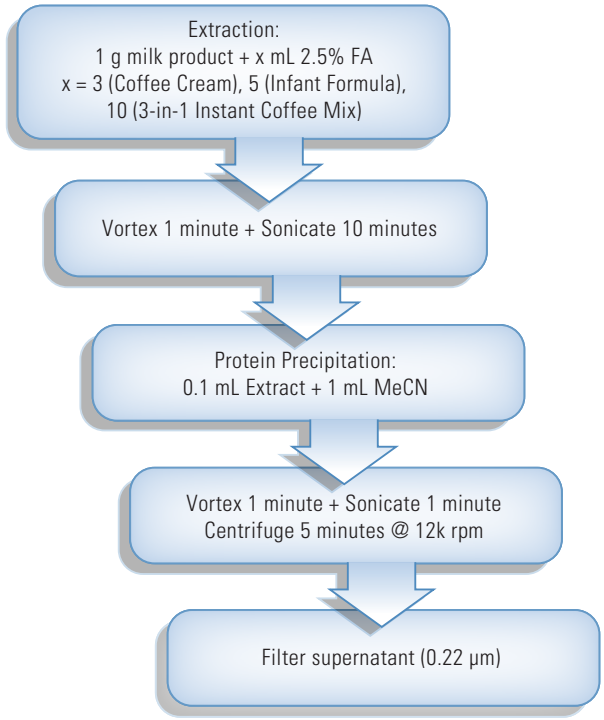

Figure 1: Sample preparation flowchart

Methods

Samples: Concentrated infant formula, instant coffee mix (3-in-1 with coffee, creamer and sugar) and liquid coffee creamer (unflavored) were purchased from local supermarkets.

Sample Preparation: Milk samples were extracted with 2.5% formic acid followed by protein removal with acetonitrile following the US FDA published procedures with modification as shown in Figure 1. The total dilution factors as a result of sample preparation are given in Table 1.

Sample	Total Dilution Factor
Infant Formula	65
Coffee Cream	44
3-in-1 Coffee Mix	110

Table 1. Total dilution factor

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Figure 2: Exactive mass spectrometer and Accela liquid chromatography system

LC Conditions:

LC:	Thermo Scientific Accela liquid chromatography system
Column:	Thermo Scientific BioBasic AX 50 mm x 3 mm, 5 µm
Eluent:	95:5 MeCN (0.1% FA) / Water (0.1% FA), isocratic at 500 µL/min
Injection:	5 µL (with loop)
Run time:	1 minute

Mass Spectrometer Conditions:

Mass spectrometer:	Thermo Scientific Exactive benchtop high resolution, accurate mass system (Figure 2)
MS parameter settings:	See Figure 3
Resolution:	High (R=50,000 FWHM at *m/z* 200)
Lock mass:	*m/z* 195.0877 (Caffeine)
Ion source:	HESI-II, +3.5 KV
Vaporizer temp:	300 °C
Tube lens:	94 V
Sheath/Aux gas:	30/10 units with N_2
Capillary temp:	270 °C

Results and Discussion

The goal of this study was to explore high resolution benchtop mass spectrometry for a simple and rapid method to test melamine in milk products with a detection limit lower than 250 ppb, the reporting LOQ set by the US FDA method for infant formula on a triple quadrupole mass spectrometer. The sample preparation followed the dilute-and-shoot approach without the use of a laborious and time-consuming SPE procedure.

Other than the conventional ion source tuning, the Exactive™ mass spectrometer setup required only one parameter to be changed: resolution was set to High (R=50,000 FWHM at *m/z* 200). The *m/z* 195.0877 of caffeine was used as a lock mass because caffeine was conveniently present in the tuning solution; after each tuning, the residue caffeine peak can be used as a lock mass in subsequent sample analysis. The additional caffeine peak can be found in coffee samples. Under these conditions, melamine (*m/z* 127.0727) can be unambiguously identified with mass accuracy better than 2 ppm.

The Exactive mass spectrometer sensitivity and linear response range were evaluated with the melamine standards. Figure 4 shows the chromatogram and accurate mass spectra of a representative 0.1 ppb solution, and Figure 5 displays a representative calibration curve demonstrating a linear response from 0.1 to 100 ppb.

Milk samples were found to have a strong matrix effect that resulted in severe ion suppression. Preliminary experiments with loop injection without any LC separation failed to detect 1 ppb melamine spiked in any of the three matrices even with a further 5x dilution. Thus it was decided that a simple LC separation is still required.

The LC separation employed a 1-minute run on a BioBasic™ AX weak anion exchange column with a strong organic mobile phase (95% v/v MeCN), creating a HILIC condition[2] that separated the melamine (R.T. ~0.54-0.6 min) from the major interference species eluting either in the void volume (0.35-0.4 min) or after the melamine. An isocratic run was chosen to eliminate the column equilibration time between each injection, thus increasing throughput.

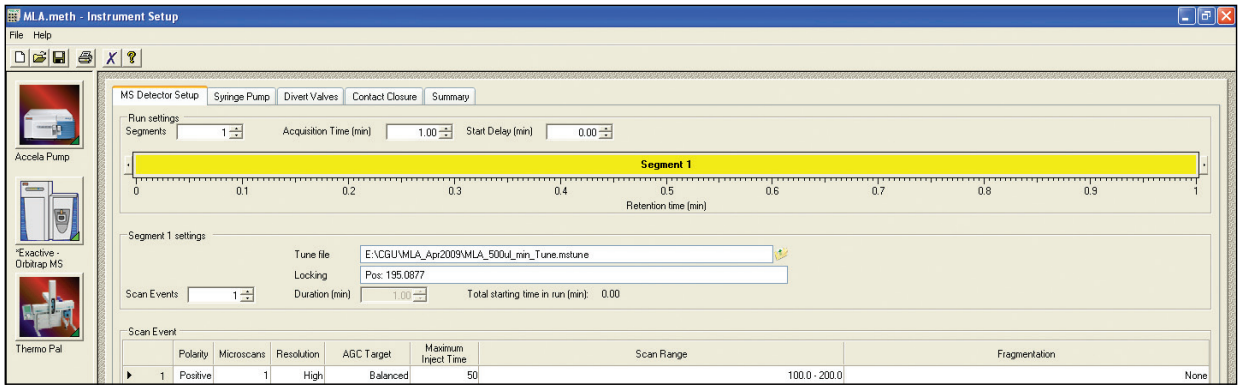

Figure 3. MS parameter settings.

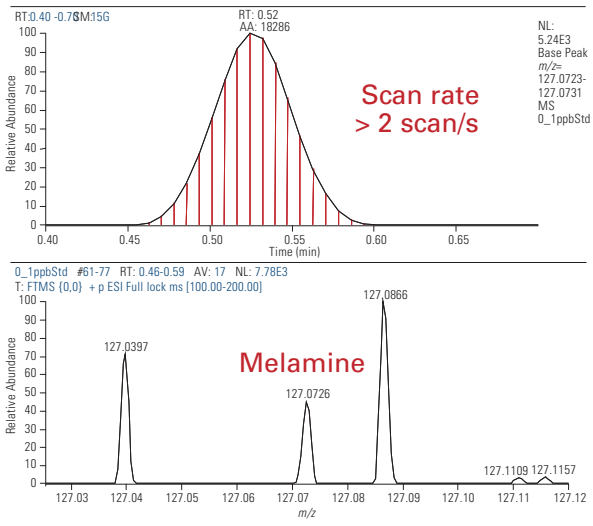

Figure 4: Chromatogram and spectra of 0.1 ppb (0.5 pg on column) melamine standard

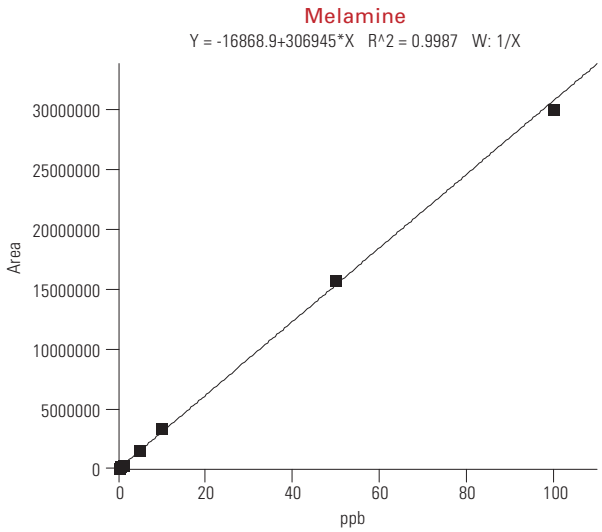

Figure 5: Calibration curve of melamine standard solution (0.1 to 100 ppb). Four orders of linear dynamic range were observed.

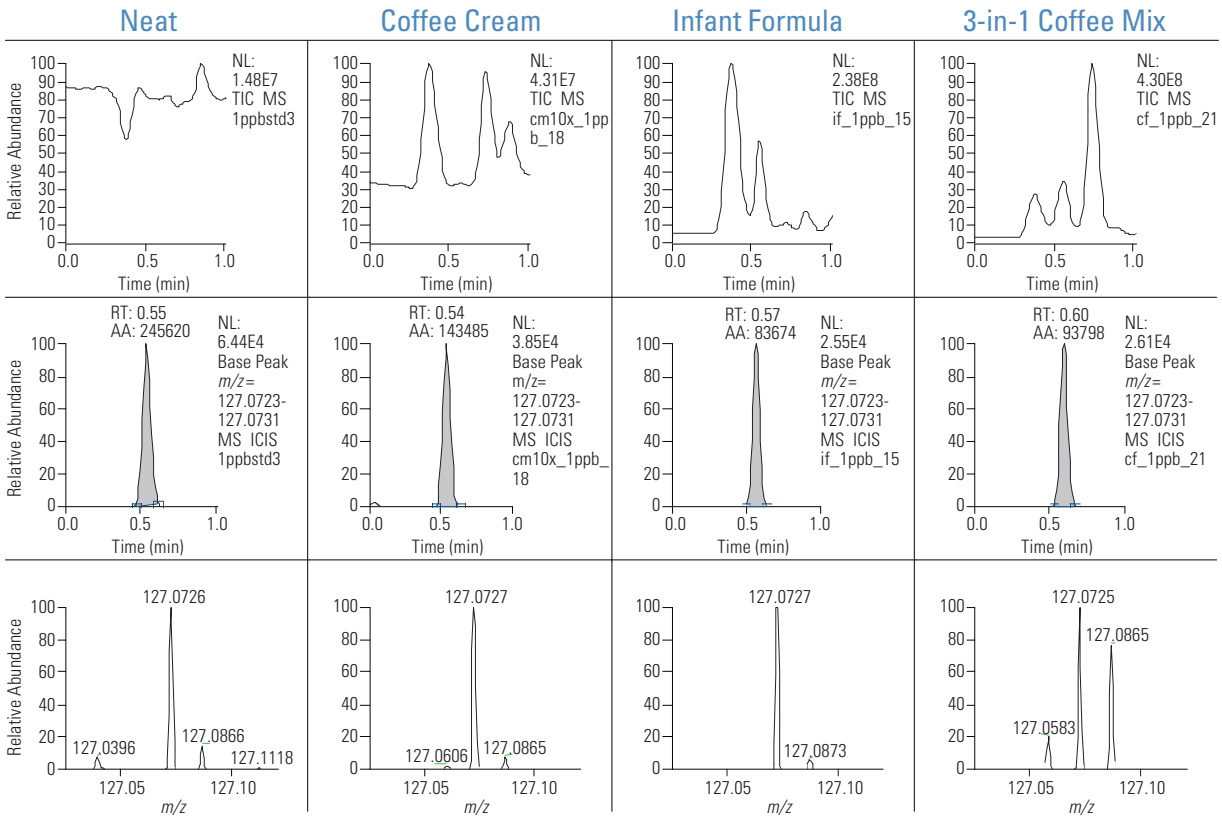

Figure 6: Comparison of 1 ppb melamine in standard (neat) and spiked in extract sample matrix (top: TIC; middle: chromatographic peak, bottom: mass spectra).

Figure 6 shows the comparison of 1 ppb melamine spiked in a mobile phase (neat) and in three extracted sample matrices. As shown, 1 ppb spikes can be detected. Based on the dilution factor from sample preparation in Table 1, the detection of 1 ppb spike corresponds to 65, 44, and 110 ppb in infant formula, coffee cream and 3-in-1 instant coffee mix, respectively.

The responses of 1 ppb melamine in matrices are only 30%-50% of that in the neat solution, but responses were found to be consistent in each sample extract in the spiked 1-10 ppb range evaluated. The average response factor (RF) values from spiking 1, 5, and 10 ppb in each of three sample matrices are given in Table 2. A constant response factor in each extract matrix makes it possible to use the standard addition method for melamine quantitation.

The overall recovery was also evaluated by spiking a 300 ppb level of melamine in three milk products prior to the extraction. The recovery values were found to be 75%-91%.

Sample	RF	RSD%
Coffee Cream	0.50	8.0%
Infant Formula	0.31	7.2%
3-in-1 Coffee Mix	0.34	3.4%

Table 2. Melamine response factor (RF) in sample matrix compared to neat standard (RF=1) and RSD% (n=3)

Conclusions

The high resolution, accurate mass Exactive mass spectrometer was shown to be sensitive in detecting <0.1 ppb melamine (0.5 pg on column) in neat standard, and response is linear from 0.1 to 100 ppb. The error for mass accuracy is <2 ppm with lock mass.

Milk samples prepared by dilute-and-shoot showed severe ion suppression that was reduced with a simple 1-minute isocratic HILIC LC separation, after which a consistent response factor of 0.3-0.5 for each sample matrix was obtained for quantitation.

Quantitation limits were less than 44, 65, and 110 ppb for coffee cream, infant formula and 3-in-1 instant coffee mix, respectively, exceeding the requirement of 250 ppb LOQ as stated by US FDA for infant formula.

References

(1) http://www.cfsan.fda.gov/~frf/lib4421.html, Determination of Melamine and Cyanuric Acid Residues in Infant Formula using LC-MS/MS, Laboratory Information Bulletin No. 4421, October 2008, US FDA.

(2) Varelis, P., Beck, J., Wang, K., and Ghosh, D., Analysis of Melamine and Cyanuric Acid in Food Matrices by LC-MS/MS, Thermo Fisher Scientific App. Note 424.

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Application Note: 377

Analysis of Mycotoxins in Various Cattle Forages and Food Matrices with the TSQ Quantum Discovery MAX

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Key Words

- TSQ Quantum Discovery MAX™
- Food and Environmental
- H-SRM
- Quantitation

Introduction

Mycotoxins are toxic metabolites produced by certain species of fungi that can infect and colonize on various agricultural crops in the field and during storage. Environmental factors such as temperature and humidity influence the occurrence of these toxins on grains, nuts and other commodities susceptible to mold infestation. In addition, any crop that is stored for more than a few days is a target for mold growth and mycotoxin formation.

Most mycotoxins are relatively stable compounds that are not destroyed by food processing or cooking. Although the generating organisms might not survive processing, the toxin can still be present. Mycotoxins pose a potential threat to human and animal health through the ingestion of contaminated food products. Mycotoxins can have both chronic and acute effects on human and animal health. They can be teratogenic, mutagenic, or carcinogenic in susceptible animal species. They are linked to various diseases in domestic animals, livestock, and humans in many parts of the world. Most mycotoxins are toxic in very low concentrations and therefore require sensitive and reliable methods for their detection.

This application note describes an LC-MS/MS method for the determination of mycotoxins in various cattle forages. Using this method it is possible to simultaneously measure the following 12 mycotoxins within 12 minutes: Nivalenol (NIV), Deoxynivalenol (DON), Aflatoxin G1, Aflatoxin G2, Aflatoxin B1, Aflatoxin B2, Fumonisin B1, Fumonisin B2, Diacetoxyscripenol (DAS), T2-Toxine, Ochratoxin A, and Zearalenon (ZEN). See Figure 1.

The TSQ Quantum Discovery MAX triple quadrupole system has been evaluated for round-the-clock analysis of different mycotoxins. Multiple samples with different matrices (cattle forages, food matrices) have been analyzed.

Goal

To demonstrate that the TSQ Quantum Discovery MAX, with its H-SRM capabilities and H-ESI source, is ideally suited for the rigorous demands of high-throughput analyses of mycotoxins in various matrices.

Experimental Conditions

Sample Preparation

The samples analyzed were various extracts of cattle forages and food products. The following sample extraction procedure, adapted from TLR International Laboratories, was used. To begin, 25 g of grounded sample was dissolved in 100 mL of acetonitrile:water (80:20 v/v). The extract was then mixed for two hours. Afterwards, the extracts were filtered and diluted four times with water. The resulting solution was acetonitrile:water 20:80 v/v.

HPLC

HPLC analysis was performed using the Surveyor HPLC System (Thermo Scientific, San Jose, CA). Each 20 µL sample was injected directly onto a Hypersil GOLD™ 100 × 2.1 mm, 5 µm analytical column (Thermo Scientific, Bellefonte, PA). A gradient LC method used mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.3 mL/min. The gradient is described in Figure 2.

Mass Spectrometry

MS analysis was carried out on a TSQ Quantum Discovery MAX triple stage quadrupole mass spectrometer with a heated electrospray ionization (H-ESI) probe (Thermo Scientific, San Jose, CA). The MS conditions were as follows:
Ion source polarity: Positive ion mode
Spray voltage: 4000 V
Vaporizer temperature: 300°C
Sheath gas pressure (N2): 30 units
Auxiliary gas pressure (N2): 30 units
Ion transfer tube temperature: 350°C
Scan Type: SRM

The MS conditions and the H-SRM transitions were obtained by automatic optimization with the auto-tune software. Figures 3 and 4 show two examples of the collision energy optimization. Figure 5 summarizes all of the H-SRM transitions that were used.

Two product ions were measured for all compounds; one was used as the quantifier ion and the other was used as the qualifier ion. In this way, the ion ratio confirmation was done as an identity confirmation. See Figure 5 for further details.

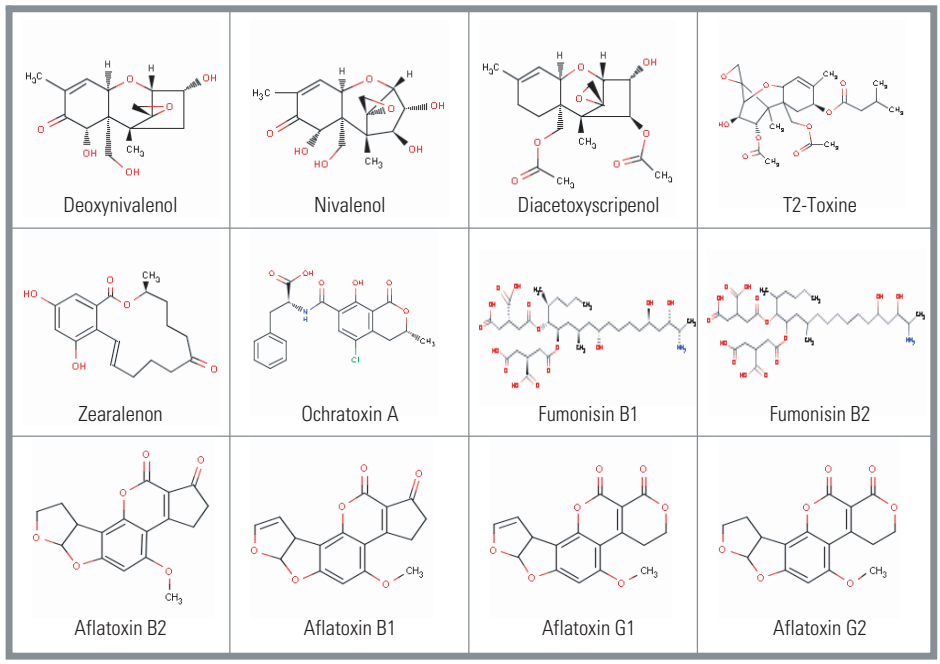
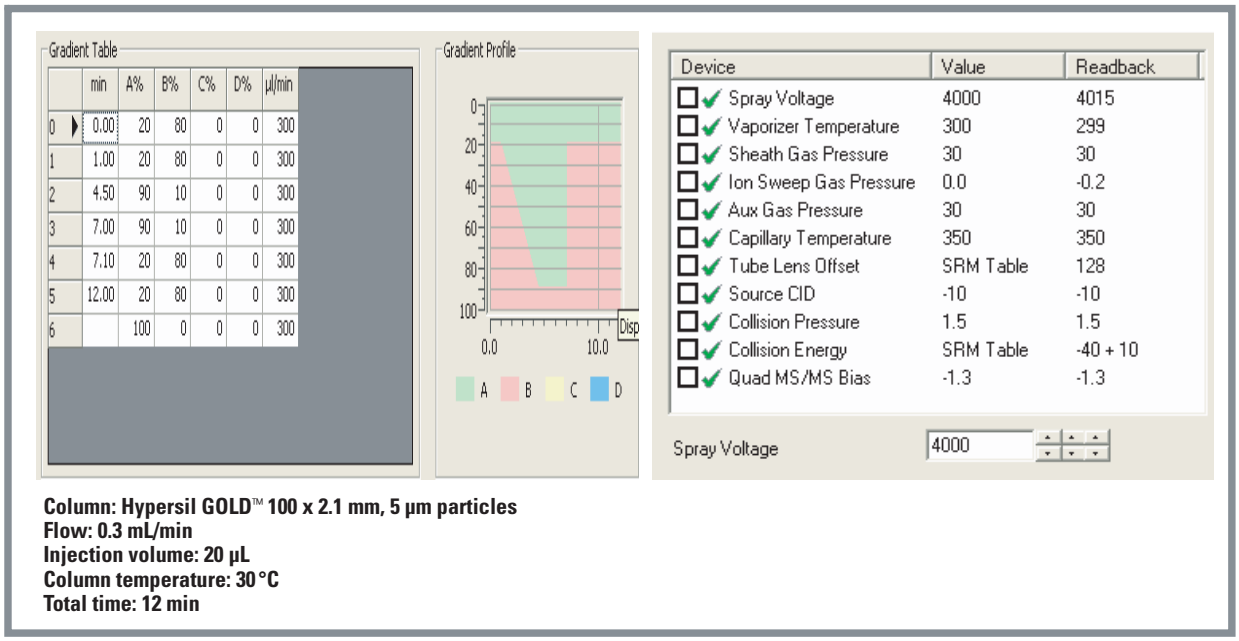


Figure 1: Structures of 12 mycotoxins



Column: Hypersil GOLD™ 100 x 2.1 mm, 5 µm particles
Flow: 0.3 mL/min
Injection volume: 20 µL
Column temperature: 30 °C
Total time: 12 min

Figure 2: LC/MS conditions

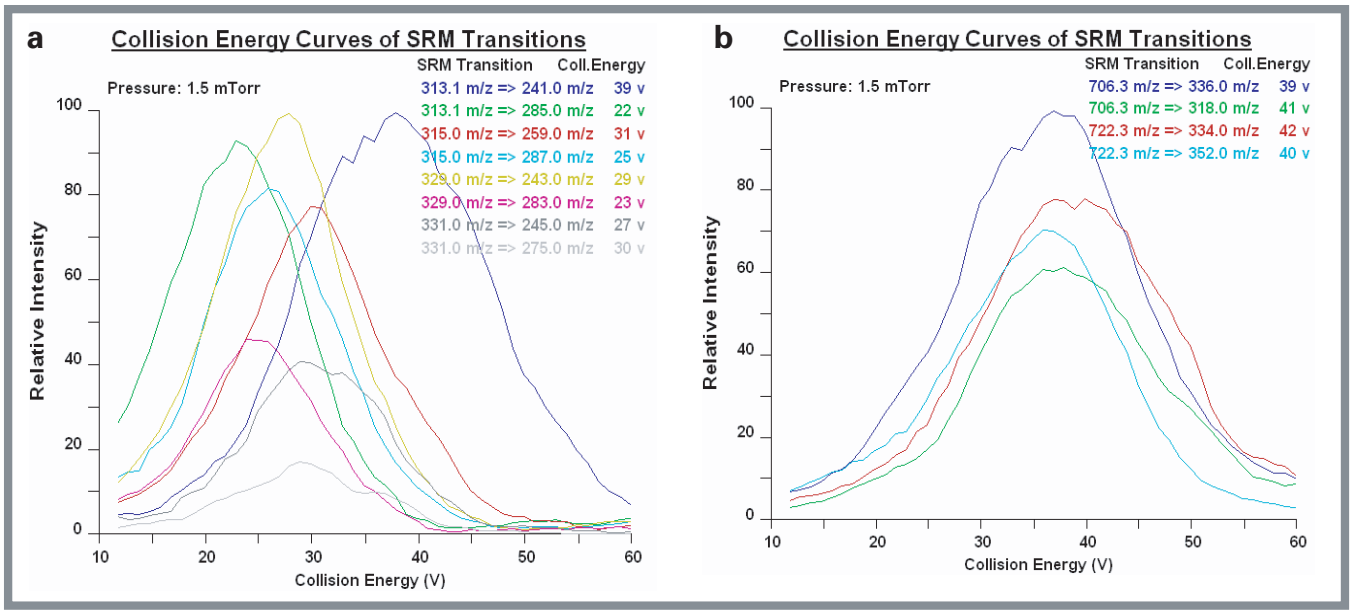


Figure 3: Optimization of collision energies of Aflatoxin A1/B1/G2

Figure 4: Optimization of collision energies of Fumonsin B1/B2

Compound	Parent	Daughter (Quan)	Daughter (Qual)	Rt	Conc Range (ppb)
Aflatoxin B1	313	241	285	4.8	0.1–100
Aflatoxin B2	315	259	287	4.5	0.1–100
Aflatoxin G1	329	243	283	5.0	0.1–100
Aflatoxin G2	331	245	275	4.8	0.1–100
Fumonisin B1	722	334	352	4.4	0.1–1000
Fumonisin B2	706	336	318	4.8	0.1–1000
Ochratoxin	404	239	221	5.6	0.1–1000
Zearalenon	319	187	185	5.7	0.1–100
Deoxynivanlenol	297	249	231	1.2	0.1–100
Diacetoxyscripenol	367	307	289	4.4	0.1–100

Figure 5: H-SRM transitions

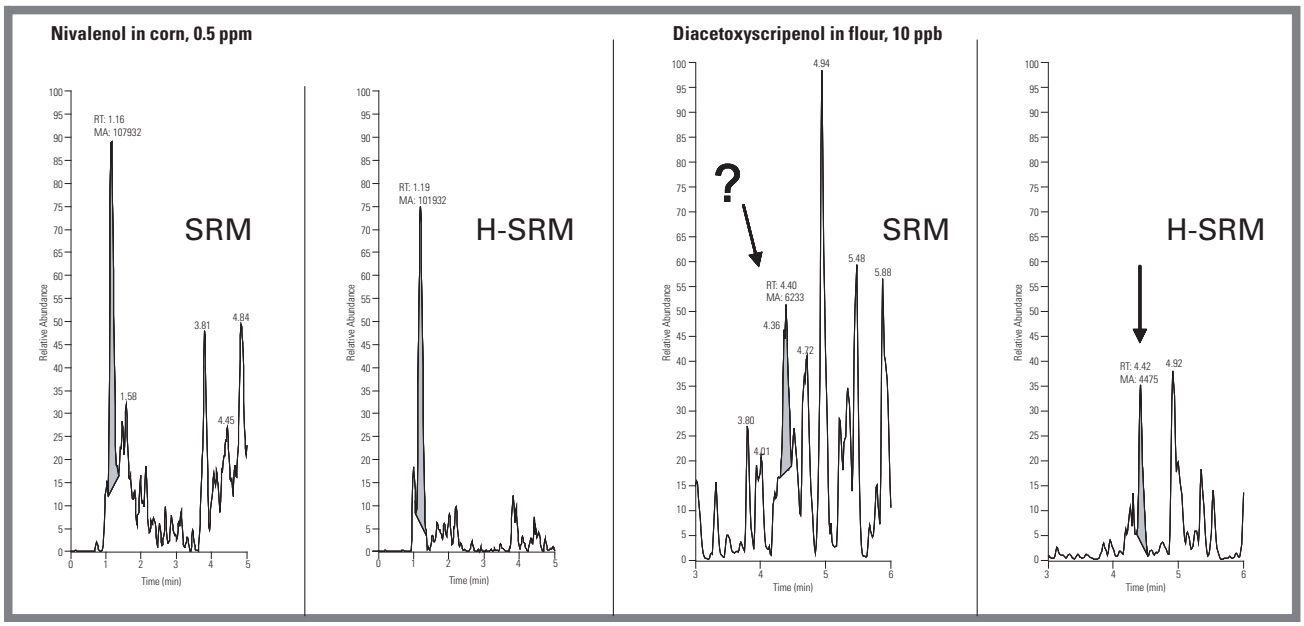


Figure 6: Comparison of SRM and H-SRM data for two samples

Results and Discussion

The TSQ Quantum Discovery MAX offers the unique capability of highly-selective reaction monitoring (H-SRM). Setting the resolution of Q1 at 0.1 FWHM helps to decrease the background noise and eliminate isobaric interferences. This improves the signal-to-noise ratio and results into a lower limit of quantification. Figure 6 compares SRM and H-SRM data for two samples.

The calibration curves were generated by dilutions in acetonitrile:water 20:80 v/v. Figure 7 presents the linear fit calibration curves for five mycotoxins using H-SRM.

The calibration curves have R^2 values that are greater than 0.998, which indicate excellent linear fits over the dynamic range.

The mycotoxin levels found in the various matrices were in the expected range. For example, in a QC-sample used as an internal control, Aflatoxin B1 was expected on a level of 5 ppb (in extract). The detected amounts (ppb in solution) are presented in Table 1. This level for Aflatoxin B1, is subjected to EU legislation as the low limit of quantification.

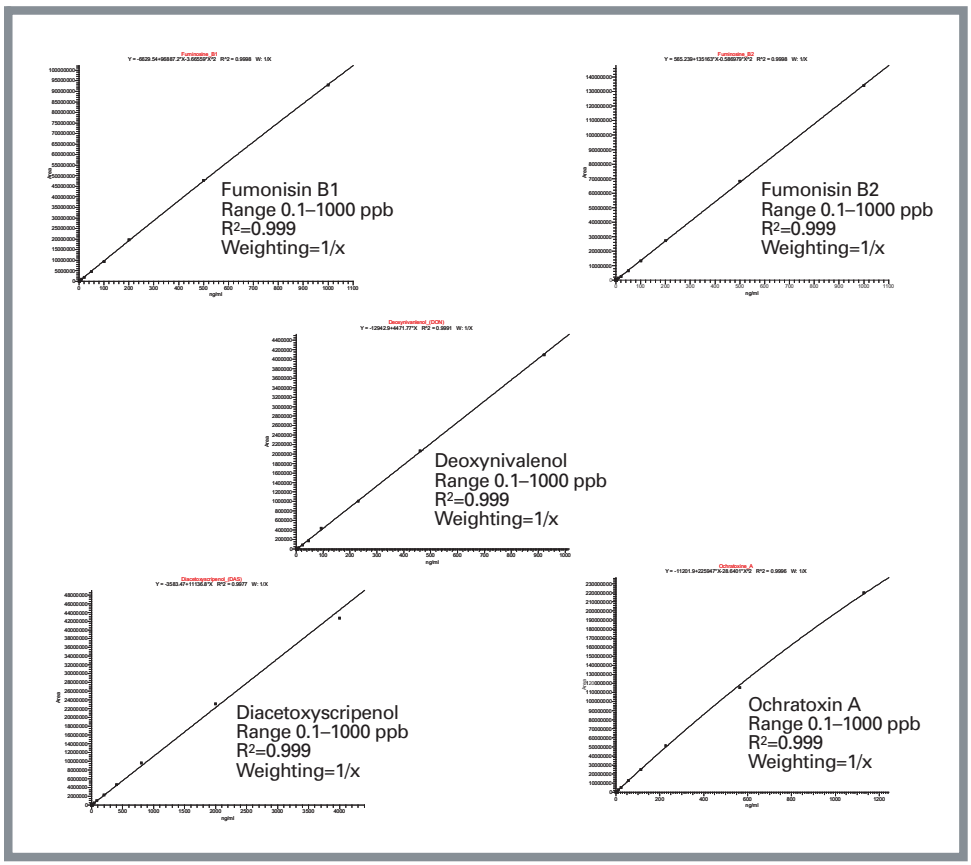


Figure 7: Calibration curves for five mycotoxins

Sample	Detected Amount (ppb)
Sample-01	1.19
Sample-02	1.28
Sample-03	1.43
Sample-04	1.25
Sample-05	1.15
Sample-06	1.37
Average	1.28
RSD	0.1
RSD%	8.3%
Average in Extract	**5.11**

Table 1. Detected Amounts of Aflatoxin B1 in Solution

For the analysis of mycotoxins in various matrices, the heated electrospray ionization (H-ESI) probe provides significant advantages. The dual desolvation zone design increases the ionization efficiency and helps to get rid of the clustering solvents. (See Figure 8.) This leads to higher signals with better %RSDs. The H-ESI probe also handles higher LC flows (up to 1 mL/min) without losing ionization efficiency. This helps to speed up the method without the need to split the LC flow. Figures 9 and 10 describe the increased sensitivity of the H-ESI probe with two samples of mycotoxins.

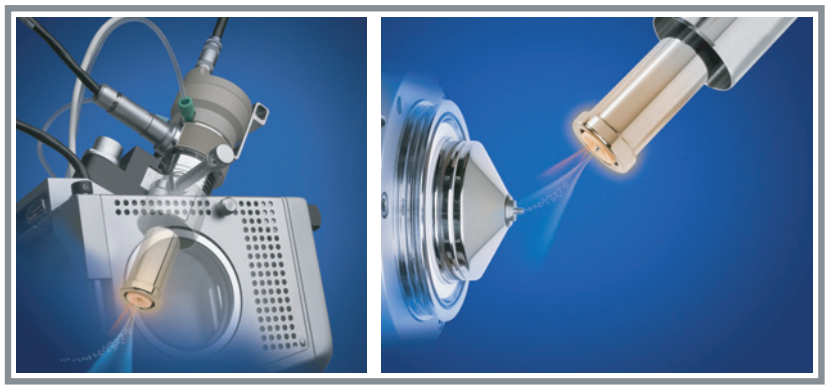


Figure 8: H-ESI – Heated Electrospray Ionization probe

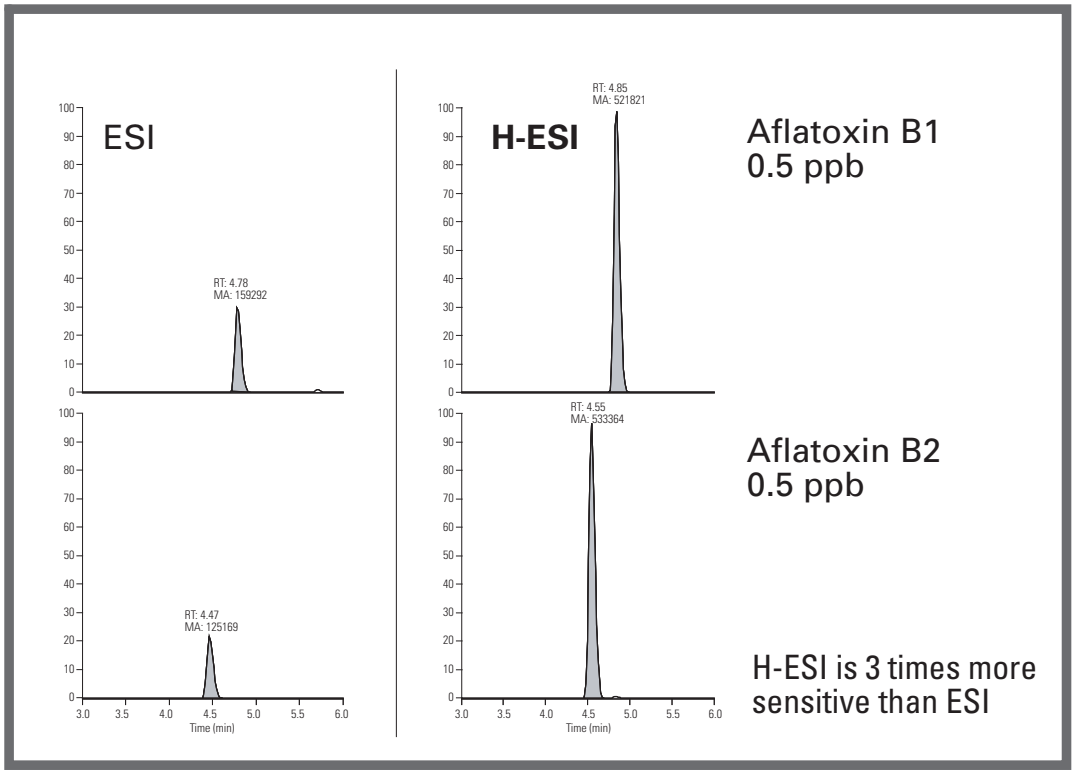


Figure 9: Increased sensitivity with the H-ESI probe in Aflatoxin data

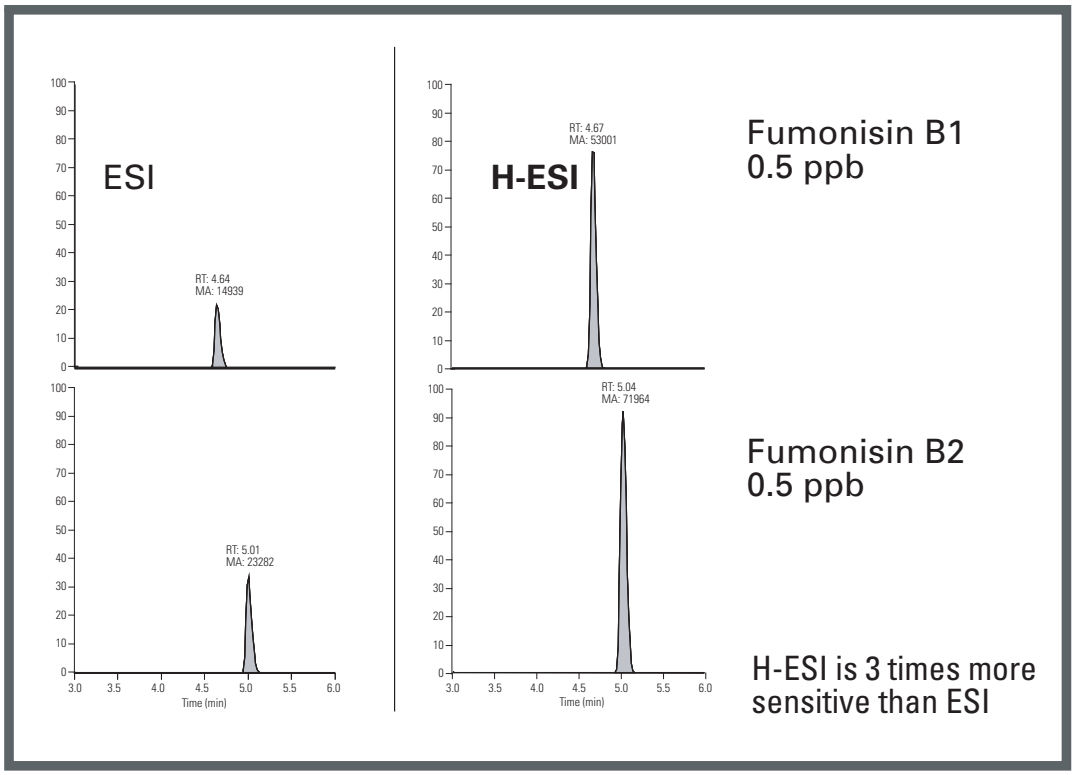


Figure 10: Increased sensitivity with the H-ESI probe in Fumonisin data

Conclusion

LC-MS/MS is a major technique for all kinds of environmental safety and food control laboratories. The TSQ Quantum Discovery MAX is the workhorse of the Quantum series for round the clock productivity. Matrix effects are always an issue with LC-MS/MS methods. However this application note shows that the TSQ Quantum Discovery MAX can help overcome these effects with its unique features of H-SRM and H-ESI. The results presented here were obtained without extensive preparation. A wide range of matrices were analyzed and excellent results were obtained.

Acknowledgements

Drs. Ing. Harm Janssens and BSc Gerard Franken of TLR International Laboratories, www.tlr.nl, are acknowledged for supplying the basis for this method, which they developed for feed.

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Application Note: 52146

High-Throughput Food Safety Control Employing Real Time Ionization (DART) Coupled to Orbitrap High-Resolution Mass Spectrometry

Lukas Vaclavik, Jana Hajslova, Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Czech Republic
Michal Godula, Thermo Fisher Scientific, Food Safety Group, Czech Republic

Key Words
- Exactive
- DART
- Melamine
- Mycotoxins

Introduction

In recent years, substantial developments have taken place in the field of mass spectrometry, enabling the introduction of a number of novel ambient desorption ionization techniques[1] such as direct analysis in real time (DART®),[2] desorption electrospray ionization (DESI),[3] surface desorption atmospheric pressure chemical ionization (DAPCI)[4] and atmospheric solids analysis probe (ASAP™).[5] These novel ion sources are characterized by remarkably high throughput of analyses which can be carried out under ambient conditions without (chromatographic) separation of sample components prior to desorption/ionization or the need for complicated and time demanding sample pre-treatment procedures. The DART technology employed in this study relies upon fundamental principles of atmospheric pressure chemical ionization (APCI). Excited-state helium atoms produce reactive species for analyte ionization.[2] Numerous applications of the DART ion source coupled to various types of mass spectrometers have been reported.[6-16] DART found its use in many areas of analytical chemistry as a tool for rapid qualitative analysis of numerous compounds. Due to the relatively high signal fluctuation of ion intensities obtained by repeated DART measurements, an internal standard usually has to be employed for compensation during quantitative analysis. However, implementation of Vapur® gas ion separator and automatic sampling systems were reported to significantly improve the repeatability for some analytes.[15]

Due to the absence of separation, the whole sample is introduced into a mass spectrometer. This unavoidably leads to a significant number of spectral interferences. In order to correctly determine the masses of relevant compounds and potential unknowns in the case of fingerprinting analysis, it is essential to separate them from the matrix ions. A mass spectrometer based on Orbitrap technology routinely achieves the mass resolving power of up to 100,000 FWHM (full width half maximum) while maintaining excellent mass accuracy of < 5 ppm, without the use of internal mass correction.[17] Those features make it an ideal tool to complement DART ionization for the analysis of complex samples.

This application note shows the possibilities using the DART ion source coupled with the ultra high-resolution Thermo Scientific Exactive mass spectrometer for rapid detection and quantitation of a wide range of food contamin - ants like mycotoxins and food adulterants (melamine).

Experimental

DART-Exactive MS

DART-Exactive MS system used in this study consisted of a new commercial model of DART ion source (DART-SVP) with a 12 Dip-It™ tip scanner autosampler coupled to the Exactive™ benchtop mass spectrometer – see Figure 1. Vapur interface was employed to hyphenate the ion source and the mass spectrometer, low vacuum in the interface chamber

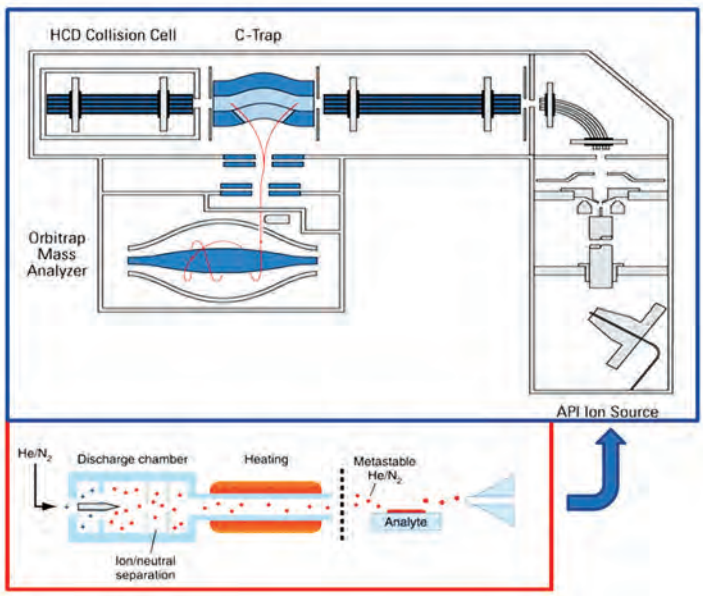

Figure 1: Schematics of a DART-Exactive system (source: *www.ionsense.com*). DART ionization source (bottom), Exactive MS (top).

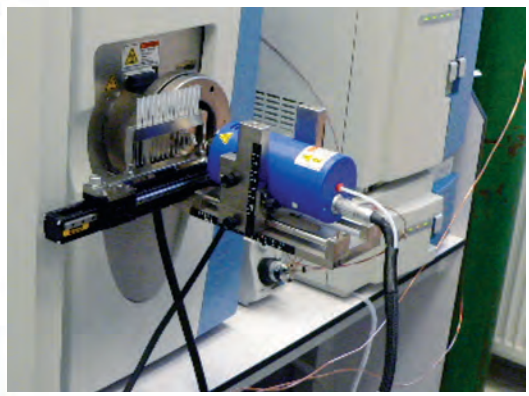

Figure 2: The DART ionization source coupled to Exactive MS

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was maintained by a membrane pump (Vacuubrand, Wertheim, Germany). The use of Vapur gas ion separator during DART ionization was essential in order to maintain stable vacuum within the operating pressure limits of the Exactive instrument. Vapur interface also improved transport efficiency of ions from the sampling area to the atmospheric-pressure interface inlet of the mass spectrometer, thus enhancing both sensitivity and reproducibility of the measurement. The distance between the exit of the DART gun and the ceramic transfer tube of the Vapur was set to 10 mm, the gap between the ceramic tube and the inlet to the heated capillary of the Exactive was 2 mm.

DART-MS instrument was operated either in positive or negative ionization mode; optimized settings of the system parameters were as follows: *(i)* DART positive ionization: helium flow: 2.5 L min^{-1}; gas temperature: 350 °C; discharge needle voltage: -5000 V; grid electrode: +200 V. *(ii)* DART ionization negative ionization: helium flow: 2.5 L min^{-1}; gas temperature: 350 °C; discharge needle voltage: -5000 V; grid electrode: +350 V. *(iii)* Mass spectrometric detection: capillary voltage: ±55 V; tube lens voltage: ±130 V; capillary temperature: 250 °C. Sheath, auxiliary and sweep gases were disabled during DART-MS analysis. The acquisition rate was set according to desired resolving power of the Exactive mass analyzer, and was 10 spectra s^{-1} at 10,000 FWHM (full width at half maximum), 4 spectra s^{-1} at 25,000 FWHM and 2 spectra s^{-1} at 50,000 FWHM. In all cases, the mass resolving power was calculated for *m/z* 200.

Semi-automatic analysis of liquid samples was carried out with the use of 12 Dip-It tip scanner autosampler. Dip-It tips were inserted into a holder and immersed in sample extracts placed in deepwell micro-plate (Life Systems Design, Merenschwand, Switzerland). The holder was mounted on the body of the autosampler. Subsequently, the Dip-It tips automatically moved at a constant speed of 0.5 mm s^{-1} through the helium gas beam in perpendicular direction to the axis leading from DART gun exit to the mass spectrometers inlet. Using the above moving speed, the time of desorption from the surface of each tip was 9 s; total run time of 12 analyses was approx. 4.2 min. To enable and/or enhance ionization of certain analytes, 2 mL autosampler vial containing dopant solution was placed in the distance of 20 mm from the DART gun exit. Aqueous solution of ammonia (25%, *w/w*, Penta, Chrudim, Czech Republic) and neat dichloromethane (Scharlau, Barcelona, Spain) were used as dopants in positive and negative ionization mode, respectively.

Mycotoxin Analysis

Chemicals and standards

Standards of 3-acetyldeoxynivalenol (3-ADON), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-Glc), fusarenon-X (FUS-X), nivalenol (NIV), HT-2 toxin (HT-2), T-2 toxin (T-2), diacetoxyscirpenol (DAS), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin B2 (AFG2), ochratoxin A (OTA), fumonisin B1 (FB1), fumonisin B2 (FB2), sterigmatocystin, zearalenone (ZEA), $^{13}C_{15}$-deoxynivalenol ($^{13}C_{15}$-DON), $^{13}C_{15}$-nivalenol ($^{13}C_{15}$-NIV) and $^{13}C_{18}$-zearalenone ($^{13}C_{18}$-ZEA) were supplied by Biopure (Tulln, Austria). Standards of deepoxy-deoxynivalenol (deepoxy-DON), altenuene, alternariol, alternariolmethylether (alternariol-met), ergocornine, ergocrystine and ergosine were obtained from Sigma-Aldrich (Steinheim, Germany).

Composite standard was prepared in acetonitrile containing each of analytes (isotope-labeled compounds not included) at concentration level of 5000 ng mL^{-1} and further diluted to obtain solvent standards at 500 ng mL^{-1}. Individual solvent solutions of ^{13}C-labeled internal standards were prepared at 5000 µg mL^{-1} in acetonitrile. Matrix-matched standards in the concentration range 10 to 1000 ng mL^{-1} (corresponding to 50 to 5000 µg kg^{-1} in matrix) were obtained by spiking of blank wheat and maize extracts (prepared by procedures described below), additionally, isotopically labelled compounds were added at level 100 ng mL^{-1} (500 µg kg^{-1} in matrix).

Acetonitrile and methanol, both of HPLC-grade, were supplied by Merck (Darmstadt, Germany). Pure water was obtained from Milli-Q® purification system. Anhydrous magnesium sulphate, sodium chloride and ammonium formate (≥ 99% purity), were from Sigma-Aldrich. Primary secondary amine (PSA) sorbent was obtained from Varian (Harbor City, CA, USA), formic acid (≥ 98% purity) was from AppliChem GmbH (Darmstadt, Germany).

Samples and sample preparation

Modified QuEChERS procedure[18] was employed to extract target analytes from the examined matrices (wheat, maize and millet). 2 g of homogenized sample were weighed into a 50 mL polypropylene (PP) centrifuge tube, 7.5 mL of deionized water and 10 mL of acetonitrile were added. Vigorous shaking of the mixture (4 min) was followed by the addition of 4 g $MgSO_4$, 1 g NaCl, further shaking for 3 min and centrifugation (5 min, 10,000 rpm, 20 °C). 4 mL aliquot of the upper organic phase was transferred into a 15 mL PP tube containing 200 mg of PSA and 600 mg $MgSO_4$ and shaken for 3 min to perform solid phase extraction (SPE) clean-up of the extract. After centrifugation (3 min, 10,000 rpm, 20 °C), approx. 600 µL were taken for DART-Exactive MS analysis.

Analysis of Melamine

Chemicals and standards

Solid standard of melamine (MEL, ≥ 99.0%) was supplied by Sigma-Aldrich; isotopically labeled $^{13}C_3$-melamine ($^{13}C_3$-MEL, ≥ 98.0%) was from Witega (Berlin, Germany). Individual stock solutions of MEL and $^{13}C_3$-MEL were prepared at 1000 µg mL^{-1} in water. By further dilution, aqueous solutions at 100 and 10 µg mL^{-1} were obtained and used for preparation of matrix matched standards and spiking experiments. Matrix-matched calibration was prepared by spiking of blank raw milk, standards containing MEL in the range from 25 to 2500 ppb and fixed amount of $^{13}C_3$-MEL at 250 ppb were obtained this way. Water used in this study was purified with the use of Milli-Q purification system.

Samples and sample preparation

Raw milk samples were analyzed without any pre-treatment. Prior to DART-MS analyses, blank milk was spiked with MEL at 100 and 500 ppb and with $^{13}C_3$-MEL at 250 ppb. Additionally real-life samples (n = 2) representing contaminated powdered milk were, according to producers instructions, reconstituted in water (1:10, *w/v*), spiked with $^{13}C_3$-MEL and subjected to instrumental analysis.

Results and Discussion

DART-Exactive Analysis of Mycotoxins

The efficiency and practical applicability of DART technology for ionization of aflatoxins, fusarium toxins, alternaria toxins, ochratoxins, ergot alkaloids, and sterigmatocystin (analytes possessing relatively largely differing physico-chemical properties) was evaluated in this part of the study. For this purpose, solvent standards containing respective mycotoxin at level 500 ng mL^{-1} were analyzed. Various settings (100 – 400 °C) of ionization gas temperature and grid electrode voltage were tested in order to obtain best sensitivity and best efficiency of analyte's thermo-desorption. As shown below, most mycotoxins could be transferred into gaseous phase at temperature 350 °C which was found as an optimal compromise between signal intensity and analytes' thermal desorption speed. While the use of lower grid voltage (200 V) in positive ionization mode enabled approx. 50% intensity increase compared to 350 V setting, 350 V potential was optimal for analytes ionized in negative mode. It was also found that ionization of some mycotoxins is improved by introducing dopant vapours (dichloromethane or ammonia) into the region between the ion source exit and Vapur interface ceramic tube inlet.

The list of ions generated by DART, when analyzing mycotoxin standard solutions, is provided in Table 1. As it can be seen, most of the examined mycotoxins could be effectively ionized in positive or negative ion mode, either as pseudomolecular ions or forming charged adducts supposing dichloromethane or ammonia vapors were present in the ionization region. Relatively poor ionization efficiencies were obtained for aflatoxins where electrospray ionization (ESI)[19, 20] was documented to be option for their control at ultra trace levels which are of regulatory interest.

				Detected Ions		
Compound	**Elemental Formula**	**Exact MW**	**Ionization Mode**	**Ion**	**Elemental Composition**	**Exact Mass**
ADON	$C_{17}H_{22}O_7$	338.1360	Negative	$[M+Cl]^-$	$C_{17}H_{22}O_7Cl$	373.1049
DON	$C_{15}H_{20}O_6$	296,1254	Negative	$[M+Cl]^-$	$C_{15}H_{20}O_6Cl$	331.0943
Deepoxy-DON	$C_{15}H_{20}O_5$	280,1305	Negative	$[M+Cl]^-$	$C_{15}H_{20}O_5Cl$	315.0993
FUS-X	$C_{17}H_{22}O_8$	354,1309	Negative	$[M+Cl]^-$	$C_{17}H_{22}O_8Cl$	389.0998
NIV	$C_{15}H_{20}O_7$	312,1204	Negative	$[M+Cl]^-$	$C_{15}H_{20}O_7Cl$	347.0903
ZEA	$C_{18}H_{22}O_5$	318,1462	Negative	$[M-H]^-$	$C_{18}H_{21}O_5$	317.1394
				$[M+Cl]^-$	$C_{18}H_{22}O_5Cl$	353.1150
HT-2	$C_{22}H_{32}O_8$	424,2092	Positive	$[M+NH_4]^+$	$C_{22}H_{36}NO_8$	442.2435
T-2	$C_{24}H_{34}O_9$	466,2197	Positive	$[M+NH_4]^+$	$C_{24}H_{38}NO_9$	484.2541
DAS	$C_{19}H_{26}O_7$	366,1673	Positive	$[M+H]^+$	$C_{19}H_{27}O_7$	367.1750
				$[M+NH_4]^+$	$C_{19}H_{30}NO_7$	384.2017
Altenuene	$C_{15}H_{16}O_6$	292,0941	Negative	$[M-H]^-$	$C_{15}H_{15}O_6$	291.0874
				$[M+Cl]^-$	$C_{15}H_{16}O_6Cl$	327.0630
Alternariol	$C_{14}H_{10}O_5$	258,0523	Negative	$[M-H]^-$	$C_{14}H_9O_5$	257.0455
				$[M+Cl]^-$	$C_{14}H_{10}O_5Cl$	293.0211
Alternariol-met	$C_{15}H_{12}O_5$	272,0679	Negative	$[M-H]^-$	$C_{15}H_{11}O_5$	271.0612
				$[M+Cl]^-$	$C_{15}H_{12}O_5Cl$	307.0368
AFB1	$C_{17}H_{12}O_6$	312,0628	Positive	$[M+H]^+$	$C_{17}H_{13}O_6$	313.0712
AFB2	$C_{17}H_{14}O_6$	314,0785	Positive	$[M+H]^+$	$C_{17}H15O_6$	315.0868
AFG1	$C_{17}H_{12}O_7$	328,0578	Positive	$[M+H]^+$	$C_{17}H_{13}O_7$	329.0661
AFG2	$C_{17}H_{14}O_7$	330,0734	Positive	$[M+H]^+$	$C_{17}H_{15}O_7$	331.0712
Sterigmatocystin	$C_{18}H_{12}O_6$	324,0628	Positive	$[M+H]^+$	$C_{18}H_{13}O_6$	325.0707
OTA	$C_{20}H_{18}ClNO_6$	403,0817	n.d.	n.d.	–	–
FB1	$C_{34}H_{59}NO_{15}$	721,3879	n.d.	n.d.	–	–
FB2	$C_{34}H_{59}NO_{14}$	705,3930	n.d.	n.d.	–	–
Ergocornine	$C_{31}H_{39}N_5O_5$	561,2946	n.d.	n.d.	–	–
Ergocristine	$C_{39}H_{39}N_5O_5$	657,2946	n.d.	n.d.	–	–
Ergosine	$C_{30}H_{37}N_5O_5$	547,2789	n.d.	n.d.	–	–

n.d. - signal not detected

Table 1: Overview of most intensive mycotoxins ions detected under optimized DART-Exactive MS conditions in solvent standard (500 ng mL^{-1})

Material Description	Analyte (Assigned/Certified Value)	DART-Exactive MS External Calibration/Isotope Dilution	
		Mean (µg kg^{-1})	RSD (%)[a]
CRM, maize flour	DON (474 ± 30 µg kg^{-1})	459/486	9.0/5.9
CRM, wheat flour	DON (2800 ± 200 µg kg^{-1})	2608/2819	6.7/5.4
CRM, ground millet	ZEA (648 ± 140 µg kg^{-1})	583/613	7.5/6.0
CRM, maize flour	ZEA (60 ± 9 µg kg^{-1})	< LCL[b]	– / –

a Relative standard deviation (RSD) calculated from 3 analyses.
b The concentration of analyte was below LCL of the method.

Table 2: Trueness of data obtained by DART-Exactive MS analysis of certified reference materials

No ions were obtained under tested conditions for a few other mycotoxins, such as ochratoxin A, fumonisins or ergocornine, ergocrystine and ergosine. These compounds are rather polar, and especially in case of fumonisins and ergot alkaloids, have relatively high molecular weight (MW). Both of these properties are associated with low volatility that hampers the transfer of such analytes into gaseous phase. To facilitate and/or enhance DART ionization of troublesome mycotoxins, derivatization of polar functional groups, which enables avoiding hydrogen bonding, may represent a conceivable strategy.[21]

Quantitative Analysis

For quantitative purposes, the most abundant ions yielded by respective mycotoxins (see Table 1) were used and narrow isolation window of 4 ppm was employed to extract ion records (chronograms) of target analytes with high selectivity. The quantitative parameters of the method for DON and ZEA, demonstrated by analysis of available certified reference materials containing incurred *Fusarium* toxins, are presented in Table 2. For evaluation of repeatability, peak areas were preferred since they were shown to give better results compared to calculations based on peak heights. Typical RSDs for cereals spiked by mycotoxins at 500 µg mL^{-1} level ranged from 8.1 to 14.3%. Further decrease of RSDs (4.7–8.7%) and improved linearity of calibration plots compared to external calibration, was obtained when isotopically labelled internal standards were employed for compensation of absolute signal fluctuation. In case of regulated mycotoxins (DON, ZEA) DART-MS method lowest calibration levels allowed a reliable control of maximum limits established for tested matrices.[22] The recoveries of all target mycotoxins at both tested spiking levels 150 and 500 µg kg^{-1} were in the range 82–120% when external calibration based on matrix matched standards was employed for quantification. Regarding the requirements for performance characteristics in analysis of regulated analytes,[23] these were reliably met for both target toxins.

Melamine Analysis

Under experimental conditions, both MEL and $^{13}C_3$-MEL were detected as $[M+H]^+$ ions in positive DART ionization mode. The efficiency of ionization was comparable for both compounds. Very good mass accuracy, with mass error less than 3 ppm was achievable with Exactive mass analyzer (operated under mass resolving power setting 50,000 FWHM) within all measurements in this study; analyte confirmation based on elemental composition estimation could be performed. As shown in Figure 3, abundant spectral interference observed at *m/z* 127.04 was detected both in blank and spiked samples (in contrast to solvent standards). At mass resolving power ~3,500 FWHM obtained by time-of-flight mass analyzer it was not possible to resolve signals of analyte and interference. Especially at low concentration levels of MEL, the signal of analyte was completely overlapped by the interference making its detection impossible. On the other hand, employing high mass resolving power of DART-Exactive MS, reliable detection of MEL in milk, was feasible even at low concentration levels. Using

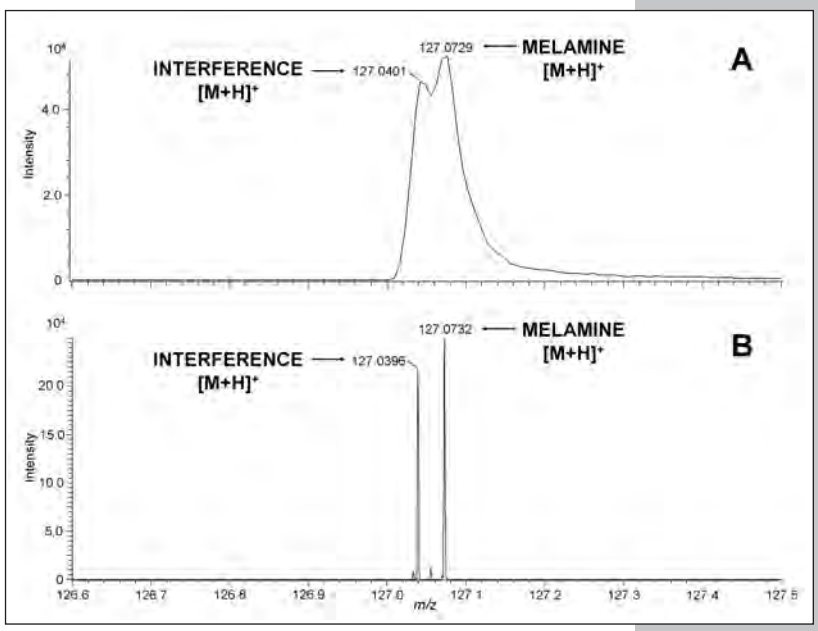

Figure 3: The improvement of mass separation by applying high mass resolution during analysis of melamine in milk sample at 2.5 mg/kg.
(A) DART-TOFMS (mass resolving power 3,500 FWHM);
(B) DART-Exactive MS (mass resolving mass power 50,000 FWHM).

Sample	DART-Exactive MS		LC-MS/MS	
	Mean (ppb)	RSD (%)	Mean (ppb)	RSD (%)
Milk powder 1	501	8.2	530	5.1
Milk powder 1	2496	6.4	2612	2.1

Table 3: Concentration of MEL in real-life samples as measured by DART-Exactive MS and LC-MS/MS

accurate mass of interference ion, elemental composition $C_6H_7O_3$ was estimated. This value corresponds to protonated molecule of hydroxymethyl furfural (HMF) which is typically formed during thermal processing of sugars containing foods.

The detectability of the method was characterized as lowest calibration level (LCL). Generally used limit of detection could not be calculated due to absence of noise in obtained records. LCL for melamine in milk was 25 ppb. For quantification purpose, isotope dilution technique was used. Figure 4 shows record of calibration standards analysis of which can be completed within 4.2 min (duplicate of each standard), Figure 5 documents acceptable linearity obtained for calibration curve in the range 25 to 2500 ppb ($R^2 \geq 0.99$). The LCL for melamine in milk was determined at 25 ppb and recoveries calculated at 100 and 500 ppb were in the range 98–119% and 101–109%. Repeatability at 100 ppb (n = 5) was 7.2%.

The results of real life samples analyses were compared to those obtained by validated LC–MS/MS method. Good agreement between respective values was observed (see Table 3).

Conclusions

The results presented in this application note demonstrate the feasibility of DART ionization source in combination with Exactive mass spectrometer for the rapid detection and quantification of various food contaminants, including set of priority mycotoxins and melamine selected as an example. Comparable trueness of generated results was achieved by applying isotope dilution-based quantification and matrix-matched calibration to compensate for signal suppression and other matrix effects that unavoidably occur during direct analysis of real matrix samples.

The major advantages of the combination of ambient ionization technique with Exactive mass spectrometry are the simplicity of operation, day-to-day robustness and broad application range. In addition, ultra high-resolution provided by Exactive mass analyzer helps to solve some of the problems caused by isobaric interferences from matrix components. The choice of ultra high-resolution mass spectrometer such as Exactive is one of the key requirements when considering the application of DART ionization as a reliable tool in the food laboratory.

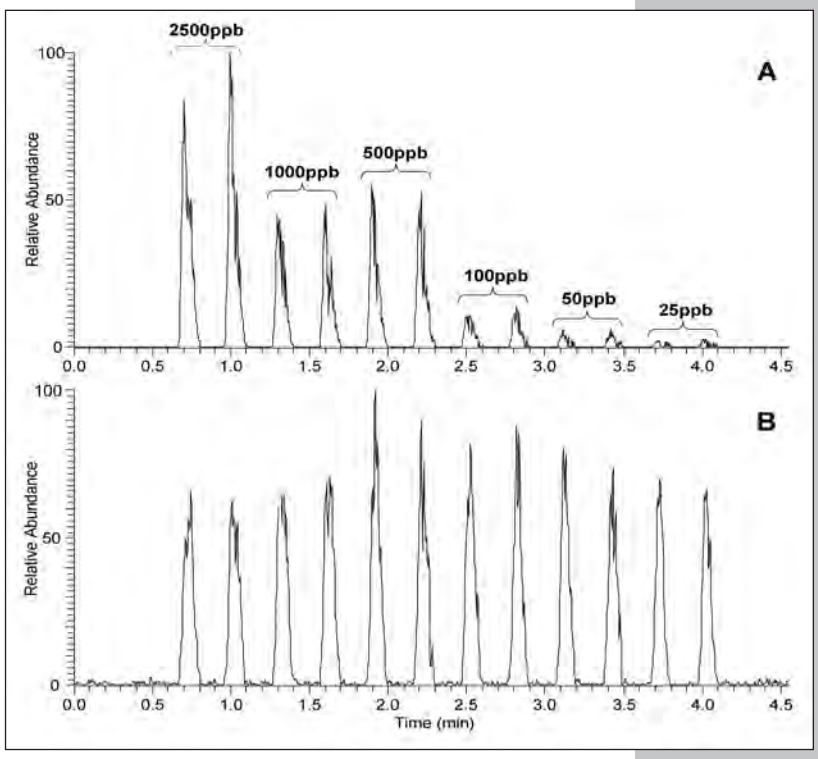

Figure 4: DART-Exactive MS record of milk spiked with MEL in the range 25 to 2500 ppb. (A) MEL (m/z 127.0726 ± 3 ppm); (B) $^{13}C_3$-MEL (m/z 130.0827 ± 3 ppm).

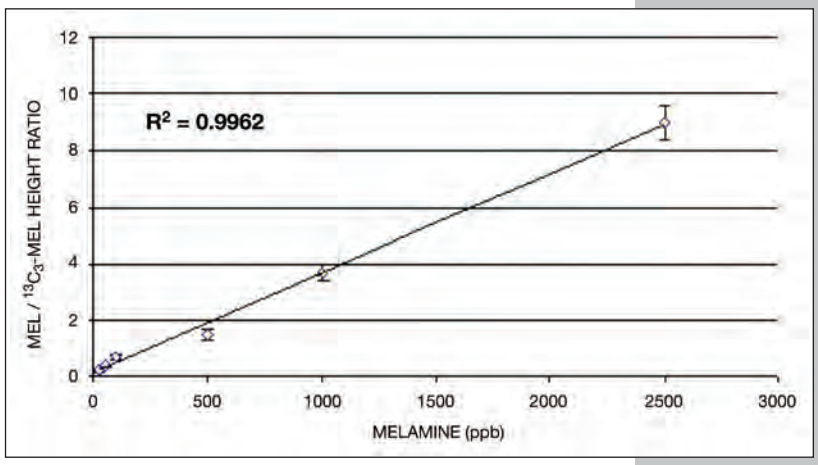

Figure 5: Calibration curve of melamine obtained by DART-Exactive MS analysis of matrix-matched standards constructed by plotting analyte-to-internal standard peak height ratio. Error bars are standard deviation (n = 3).

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Application Note: 411

Analyzing Phenolic Pollutants in Water Using U-HPLC

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Key Words

- Hypersil GOLD™ Columns
- Accela™ High Speed U-HPLC
- Surveyor Plus™ HPLC
- Phenols
- US EPA and EU Standards
- Water Pollutants

Overview

This study demonstrates analysis optimization by variation of column chemistry, and the viability of reducing stationary phase particle size to significantly increase analysis speed, while maintaining separation parameters and increasing sensitivity.

Introduction

Phenolic compounds are of particular environmental importance due to their relatively high toxicity at low levels and their presence in environmental waters and organic matter, following degradation of a range of industrial products such as pesticides and herbicides, as well as naturally occurring humic substances and tannins.

Previous studies[1,2] have shown that reversed-phase liquid chromatography (RP-LC) coupled to atmospheric pressure chemical ionization mass spectrometry (APCI-MS) can effectively separate and detect a range of phenolic compounds at low ppb levels, following various extraction methods. Such methods provide a realistic alternative to traditional analysis approaches using gas chromatography (GC), which involve lengthy sample preparation/analysis times and difficulty in derivatization of certain phenols.

In this study, the effect on the separation and analysis speed of a number of priority phenols cited within the U.S. Environmental Protection Agency (EPA) and European Union (EU) lists of priority pollutants[3] have been assessed by changing the chemistry and reducing the particle size of the stationary phase.

Materials and Methods

HPLC Columns

The effect of particle/column size variation on analysis speed and separation efficiency was studied using the following Hypersil GOLD columns (Thermo Fisher Scientific, Bellefonte, PA) and experimental conditions:

150 × 2.1 mm (5 µm particle size)
100 × 2.1 mm (3 µm particle size)
100 × 2.1 mm (1.9 µm particle size).
Mobile Phase: A) 0.1% Acetic Acid in Water
B) 0.1% Acetic Acid in Methanol.
Temperature: 60°C
Detection: UV Diode array (270-320 nm),
Gradients, flow rates and injection volumes are listed in Table 1.
Phenols were prepared at a concentration of 5 ppm in Water:Methanol (95:5).

Stationary phase chemistry

The effect of stationary phase chemistry on the separation of five phenols (2-Chlorophenol, 4-Chlorophenol, 2-Nitrophenol, 4-Nitrophenol and 2,4-Dinitrophenol), using 1.9 µm particles, was studied using three column types (all 100 × 2.1 mm):

Hypersil GOLD
Hypersil GOLD aQ™ (polar endcapped C18)
Hypersil GOLD PFP (perfluorinated phenyl).
Analysis conditions were equivalent to those described within U-HPLC Method 1 (Table 1).

Instrumentation

A Thermo Scientfic Surveyor Plus HPLC system was used for 5 and 3 µm particle analyses, and a Thermo Scientific Accela U-HPLC system was used for 1.9 µm analyses.

Results

Effect of particle/column size on analysis speed and quality

The analysis times of eleven priority phenolic pollutants were significantly improved by reducing column dimensions from 150 to 100 mm and particle size from 5 µm to 3 µm. Further improvements were achieved by changing to 1.9 µm particles, using the Accela High Speed LC System.

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Typical chromatograms demonstrating improvements in analysis speed are provided in Figures 1 to 3.

Analysis time was further reduced by increasing the flow rate of the U-HPLC analysis to 1000 µL/min, without any adverse effects on resolution (Figure 3). This is illustrated in the Table inset in Figure 3, which indicates peak width and resolution values for all separations.

Stationary phase chemistry

The Hypersil GOLD 1.9 µm phase produced the optimal overall separation of the chloro- and nitrophenols under the standard conditions used.

The Hypersil GOLD PFP (perfluorinated phenyl) phase showed superior selectivity between chlorophenol components, likely due to the unique selectivity enabled by the presence of fluorine in the stationary phase. However, the separation performance between the chloro- and nitrophenols was slightly reduced.

Chromatograms illustrating the effect on the separation of using different stationary phases are given in Figure 4, along with resolution values between 4- and 2-chlorophenol (R_S 6,7) and between 2-Nitro and 4-Chlorophenol (R_S 4,6).

Method A (Column 150x2.1 mm, 5 µm). Flow = 600 µL/min. Injection Volume = 5 µL.		**Method B** (Column 100x2.1 mm, 3 µm). Flow = 600 µL/min. Injection Volume = 1 µL.		**UHPLC Method 1** (Column 100x2.1 mm, 1.9 µm). Flow = 600 µL/min. Injection Volume = 1 µL.		**UHPLC Method 2** (Column 100x2.1 mm, 1.9 µm). Flow = 1000 µL/min. Injection Volume = 1 µL.	
Time (min)	**Eluent B (%)**	**Time (min)**	**Eluent B (%)**	**Time (min)**	**Eluent B (%)**	**Time (min)**	**Eluent B (%)**
0.0	5	0.0	5	0.0	5	0.0	5
1.5	5	1.0	5	1.0	5	0.6	5
19.5	95	13.0	95	13.0	95	7.8	95
21	95	14.0	95	14.0	95	8.4	95
21.01	5	14.01	5	14.01	5	8.41	5
22.5	5	15.0	5	15.0	5	9.0	5

Table 1: HPLC and U-HPLC gradients, flow rates, and injection volumes.

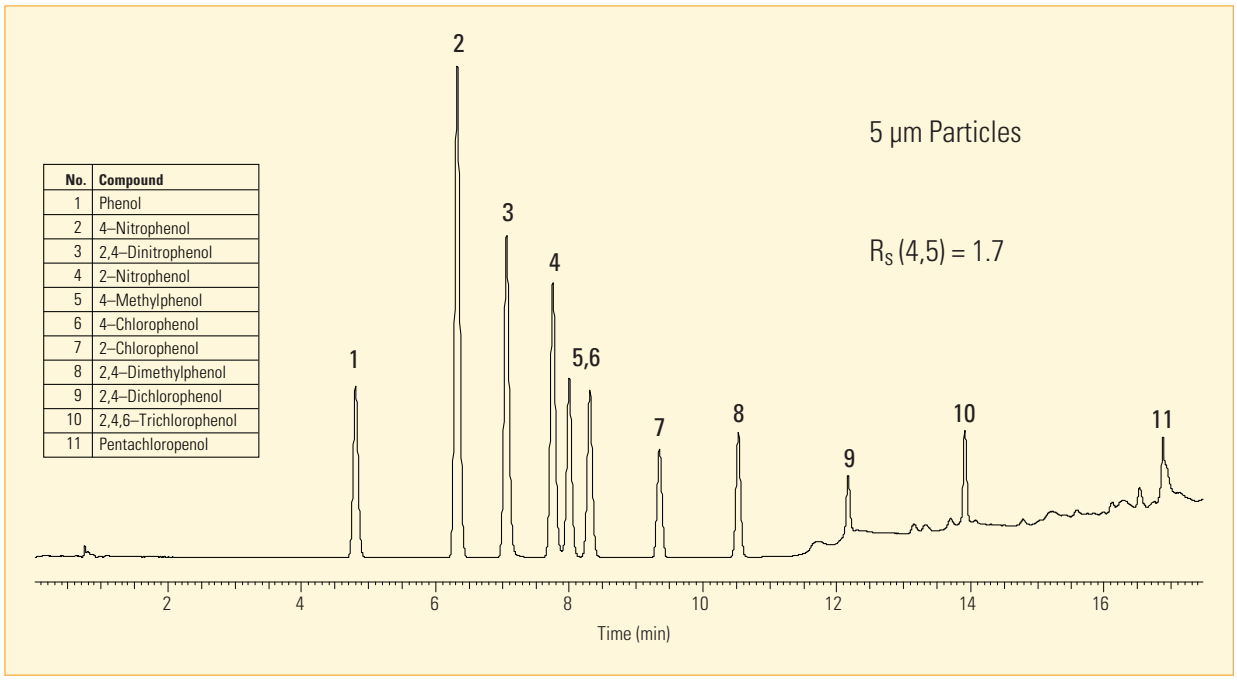


Figure 1: Separation of priority phenolic pollutants. using a 5 µm particle packed column.

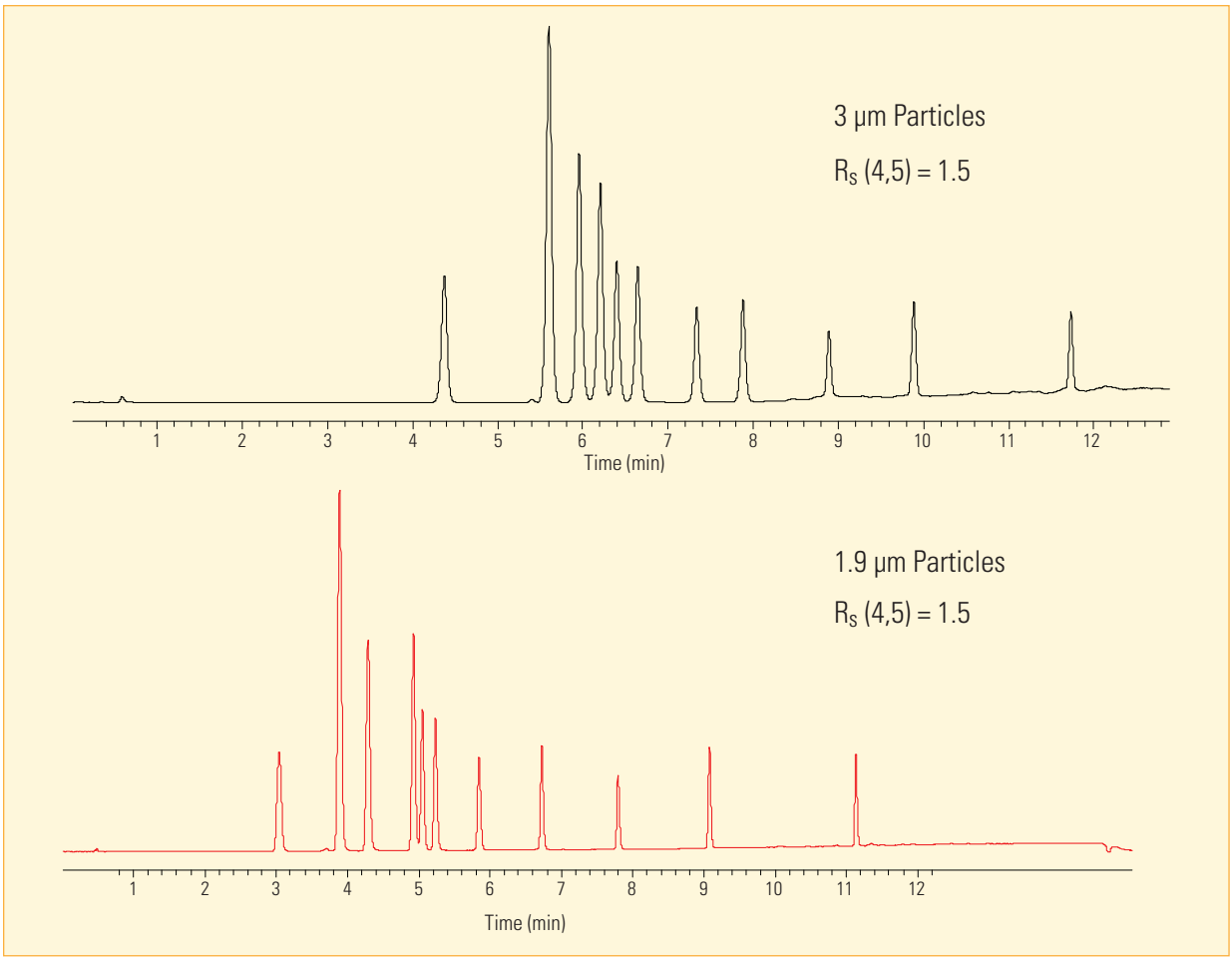
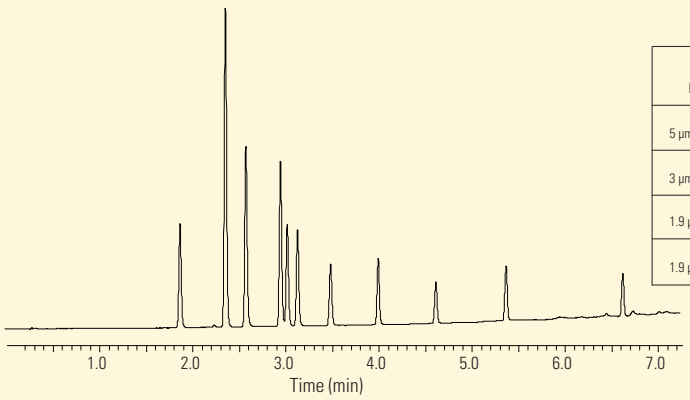


Figure 2: Chromatographic effect of variation in column dimensions (3 and 1.9 µm, 100 x 2.1 mm).



Column Dimensions	Method	Average Peak Width (mins)	Resolution (peaks 4,5)
5 µm. 150 x 2.1 mm	A	0.14	1.7
3 µm. 100 x 2.1 mm	B	0.13	1.5
1.9 µm. 100 x 2.1 mm	U-HPLC 1	0.09	1.5
1.9 µm. 100 x 2.1 mm	U-HPLC 2	0.06	1.3

Figure 3: Increased throughput using U-HPLC and 1.9 µm particles. Comparison of peak width (at 10% height) and resolution.

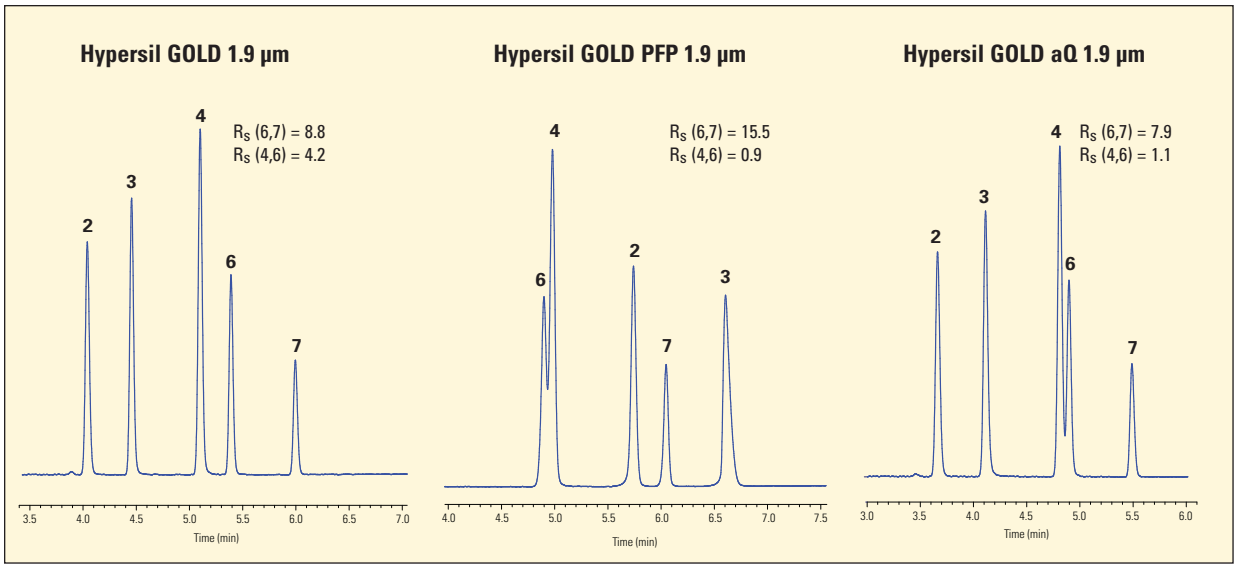


Figure 4: Comparison of 1.9 µm stationary phase chemistries for the separation of chloro- and nitrophenols.

Conclusions

A number of priority phenols can be successfully separated in shorter analysis times by transferring to U-HPLC methods using Hypersil GOLD 1.9 µm particle columns, without losing any significant resolution.

The increased peak efficiency observed for 1.9 µm particle packed columns indicates that low level phenol analyses in environmental matrices described in previous studies,[1,2] would be further enhanced with increased sensitivity.

Different column chemistries create important differences in selectivity for method development purposes, which may aid studies involving, for example, the separation of halophenols using a Hypersil GOLD PFP phase.

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Application Note: 52007

Quantitative Analysis of Carbonyl-DNPH Derivatives by UHPLC/UV

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Key Words

- Accela
- Hypersil GOLD
- Carbonyls
- Environmental Analysis
- UHPLC

Goal

Develop a fast, accurate and robust method for the separation and quantitation of parts per billion (ppb) concentrations of low molecular weight carbonyls using UHPLC/UV.

Introduction

Carbonyl compounds from motor vehicle and industrial emissions are precursors to ground-level ozone, a major component of smog and strongly associated with respiratory and pulmonary problems. Moreover, several volatile aldehydes and ketones have direct adverse effects on human health and are defined as hazardous air pollutants under the Clean Air Act Amendments of 1990.[1,2] Formaldehyde, the most abundant airborne carbonyl, is classified as a probable human carcinogen by the United States Environmental Protection Agency (EPA) and designated carcinogenic to humans by the International Agency for Cancer Research (IARC).[3,4] Acetaldehyde is another abundant carbonyl pollutant that is categorized as a suspected or known carcinogen by regulatory agencies.[4,5] In accordance with the Clean Air Act, the EPA enforces ambient monitoring of three carbonyl pollutants – formaldehyde, acetaldehyde and acetone – and recommends surveillance of several others in areas with persistently high ozone levels.[6] The California Air Resources Board (CARB) requires monitoring of formaldehyde, acetaldehyde, acrolein and methyl ethyl ketone in major urban areas.[7]

Carbonyls are also sources of pollution in indoor living and working environments. Formaldehyde, a ubiquitous indoor pollutant, is released from multiple diverse sources including plywood, particle board, furniture, paper products, resins, glues, tobacco smoke, fuel-burning appliances, textiles and cosmetics. Indeed, in a recent study of over two hundred homes of non-smoking families in different U.S. cities, the median concentrations of nine carbonyls were found to be significantly higher indoors than outdoors; for formaldehyde, the median indoor and outdoor concentrations were 20.1 µg/m^3 (16 ppb) and 6.42 µg/m^3 (5 ppb), respectively.[8] Airborne formaldehyde levels of 0.1 ppm can cause irritation of the upper respiratory tract in sensitive individuals.[9] The U.S. Occupational Safety and Health Agency (OSHA) has set the legal permissible exposure limit for formaldehyde in the workplace at 0.75 ppm measured as an 8-hour time-weighted average, and established a 15-minute short-term exposure limit at 2 ppm.[9] There are currently no federal government regulations or guidelines for formaldehyde levels in residential settings, but the

California Office of Environmental Health Hazard Assessment (OEHHA) has established acute (1 hour) exposure levels of 55 µg/m^3 (44 ppb) and set both the eight-hour and chronic reference exposure levels at 9 µg/m^3 (7 ppb).[10] Carbonyls are also encountered in food and drinking water.[11] Alcohol represents a major source of acetaldehyde exposure and has been associated with increased cancer risk in individuals with aldehyde dehydrogenase deficiency.[12] Determination of exposure pathways, health outcomes and effective pollution control strategies requires sensitive and accurate methods for trace-level analysis of carbonyl compounds in a range of matrices.

Highly volatile and reactive, low molecular weight carbonyls are typically converted to stable derivatives prior to analysis. The most commonly used derivatizing agent is 2,4-dinitrophenylhydrazine, which reacts readily with carbonyls in acidic conditions to form 2,4-dinitrophenylhydrazones (DNPH) derivatives. While GC-based methods have been developed to detect these compounds, HPLC coupled with UV detection is the most widely recognized technique for the analysis of carbonyl-DNPH derivatives. EPA methods for the determination of carbonyls in ambient air (EPA TO-11), ambient indoor air (EPA 8315A Procedure 2), drinking water (EPA 554) and aqueous, soil, waste and stack samples (EPA 8315A Procedure 1) utilize DNPH derivatization and HPLC/UV analysis.[13-15] Likewise, CARB Method 1004 specifies HPLC analysis of carbonyl-DNPH derivatives with UV detection for the monitoring of aldehydes and ketones in automotive engine exhaust.[16] However, long run times, poor resolution and low separation efficiencies can limit the utility of conventional HPLC in this application. Ultra high performance liquid chromatography (UHPLC) enables faster separations and higher resolution through the use of sub-2 µm diameter particles.

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The Thermo Scientific Accela UHPLC system offers the flexibility of performing both HPLC and UHPLC separations on a single platform. The Accela™ 1250 Pump delivers precise flows and accurate gradients at an expansive range of flow rates (up to 2 mL/min) and pressures (up to 1250 bar), and accelerates method development and maximizes method flexibility through quaternary gradient capabilities. The Accela UHPLC system together with Thermo Scientific 1.9 µm Hypersil GOLD columns enables fast chromatographic separations with high efficiency and resolution. In this application note, we demonstrate fast, accurate and robust separation, detection and quantitation of ppb levels of carbonyl-DNPH derivatives using the Accela UHPLC system and high performance columns.

Materials and Methods

Sample Preparation

DNPH-derivatized carbonyl standards (100 µg/mL) were purchased from AccuStandard® (New Haven, CT, USA). Stock solutions were prepared by diluting five-fold with 60:40 acetonitrile:water (v/v). Calibration solutions, with concentrations of 98-50000 ng/mL, were prepared by serial dilution of the stock solutions in 60:40 (v/v) acetonitrile:water.

LC/UV Analysis

Instrumentation

LC separations were performed on an Accela 1250 UHPLC system with an Accela autosampler (Thermo Fisher Scientific, San Jose, CA, USA). UV absorbance was monitored at 360 nm using an 80 Hz Accela PDA detector (Thermo Fisher Scientific, San Jose, CA, USA).

LC Parameters

Separation of Carbonyls Listed in EPA Method 8315A Procedure 1

Column:	Thermo Scientific Hypersil GOLD column (2.1 × 100 mm, 1.9 µm particle size)			
Mobile Phase:	A: Water B: Acetonitrile			
Column Temperature:	40 °C			
Sample Injection Volume:	2 µL			
Gradient:	Time (min)	A %	B %	µL/min
	0.00	68.0	32.0	800
	7.00	25.0	75.0	800
	7.10	20.0	80.0	800
	9.00	18.0	82.0	800
	9.1	68.0	32.0	800

Separation of Carbonyls Listed in EPA Method 8315A Procedure 2

(a) Column:	HALO Phenyl-Hexyl (2.1 × 100 mm, 2.7 µm particle size)					
Mobile Phase:	A: Water B: Acetonitrile C: 50:50 THF:water D: Methanol					
Column Temperature:	40 °C					
Sample Injection Volume:	2 µL					
Gradient:	Time (min)	A%	B%	C%	D%	µL/min
	0.00	51.0	38.0	8.0	3.0	450
	2.00	51.0	38.0	8.0	3.0	450
	2.10	51.0	38.0	8.0	3.0	400
	4.00	43.0	40.0	14.0	3.0	400
	4.10	43.0	40.0	14.0	3.0	450
	5.00	38.0	40.0	16.0	6.0	450
	5.10	38.0	40.0	16.0	6.0	380
	7.50	30.0	40.0	16.0	14.0	380
	7.60	30.0	40.0	16.0	14.0	450
	9.00	23.0	45.0	16.0	16.0	450
	10.00	51.0	38.0	8.0	3.0	450
(b) Column:	Waters ACQUITY UPLC® BEH Phenyl column (2.1 × 100 mm, 1.7 µm particle size), Waters ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm particle size), and Thermo Scientific Hypersil GOLD column (2.1 × 100 mm, 1.9 µm particle size)					
Mobile Phase:	A: Water B: Acetonitrile C: 50:50 THF:water D: Methanol					
Column Temperature:	40 °C					
Sample Injection Volume:	2 µL					
Gradient:	Time (min)	A%	B%	C%	D%	µL/min
	0.00	57.0	32.0	8.0	3.0	700
	2.00	57.0	32.0	8.0	3.0	700
	2.10	57.0	32.0	8.0	3.0	600
	3.00	55.0	32.0	10.0	3.0	600
	6.00	43.0	38.0	16.0	3.0	500
	8.00	40.0	38.0	16.0	16.0	500
	10.00	30.0	29.0	16.0	3.0	550

Quantitative Analysis

Column:	Thermo Scientific Hypersil GOLD (2.1 × 100 mm, 1.9 µm particle size)					
Mobile Phase:	A: Water B: Acetonitrile C: 50:50 THF:water D: Methanol					
Column Temperature:	40 °C					
Sample Injection Volume:	2 µL					
Gradient:	Time (min)	A%	B%	C%	D%	µL/min
	0.00	67.0	22.0	8.0	3.0	620
	5.00	61.0	28.0	8.0	3.0	620
	9.00	37.0	31.0	14.0	18.0	620
	13.00	35.0	31.0	14.0	20.0	620
	13.10	67.0	22.0	8.0	3.0	620

Results and Discussion

Separation of Carbonyl-DNPH Standards

The most well-established approach for the analysis of carbonyls in environmental samples relies on derivatization with 2,4 dinitrophenylhydrazine followed by separation and detection of the carbonyl-DNPH derivatives using HPLC and UV absorption. HPLC methods using conventional C18 columns packed with 3 and 5 µm particles typically require long analysis times of up to an hour and have limited resolving power. The use of sub-2 µm particle columns facilitates rapid analysis of challenging samples by improving chromatographic resolution, speed and sensitivity. Using the Accela 1250 UHPLC system, a single Hypersil GOLD™ column (1.9 µm, 2.1 × 100 mm) and a simple acetonitrile/water gradient, a mixture of the DNPH standards of 12 carbonyls targeted by EPA Method 8315A Procedure 1 was successfully separated and detected under 8 minutes (Figure 1). All the DNPH derivatives were baseline resolved and eluted in order of increasing hydrophobicity: formaldehyde, acetaldehyde, propanal, crotonaldehyde, butanal, cyclohexanone, pentanal, hexanal, heptanal, octanal, nonanal and decanal. This analysis was performed using a flow rate of 800 µL/minute, which generated back pressures up to over 1000 bar. The Accela 1250 pump is the only commercially available LC platform that is capable of handling such high operational pressures due to its very low internal back pressures.

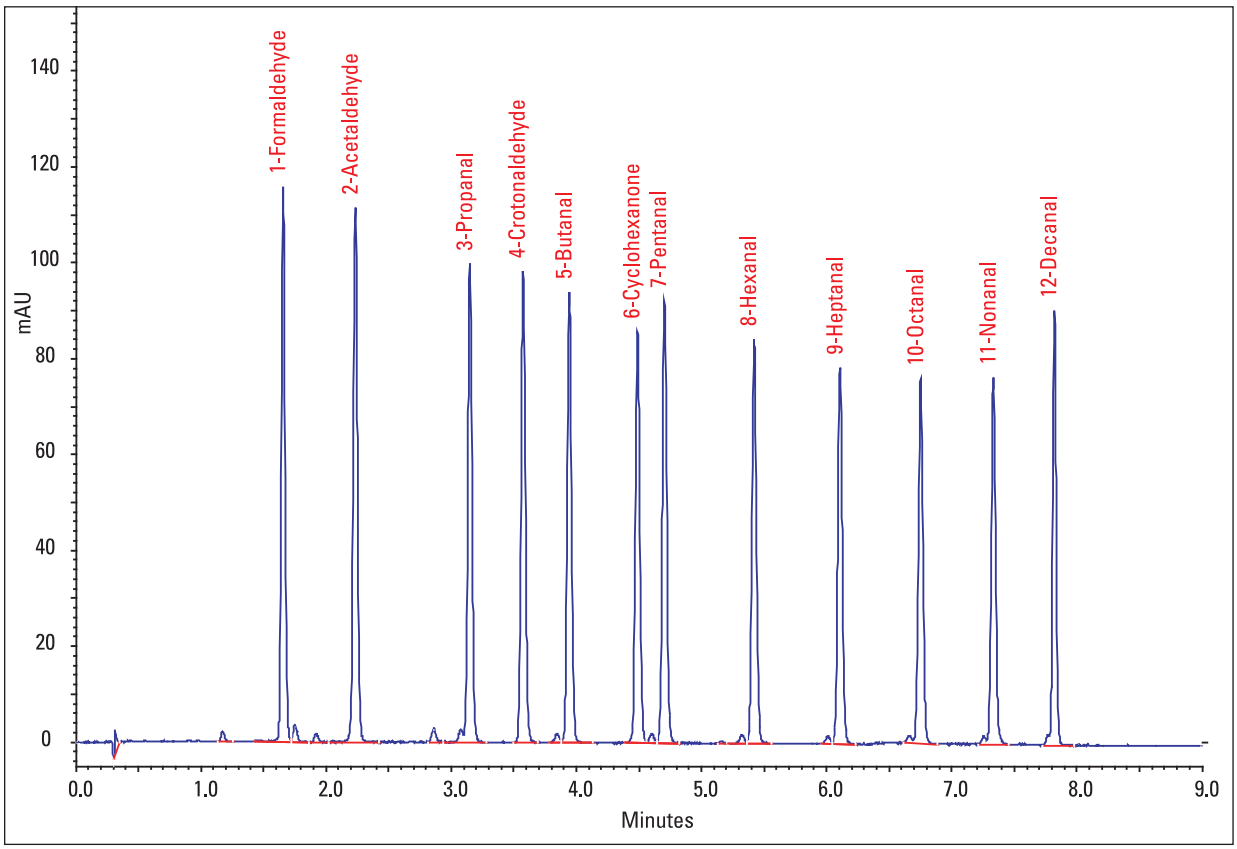

Figure 1: UHPLC separation of 12 carbonyl-DNPH derivatives at 20 µg/mL concentrations

The group of 15 carbonyls targeted by EPA Method 8315A Procedure 2 is difficult to separate and resolve by LC using conventional C18 columns and simple binary gradient systems. Higher resolution may be achieved with more complex gradients, and columns with phenyl functionalities may also help to enhance retention and improve resolution when separating difficult or complex mixtures of aromatic compounds. Both conventional and sub-2 µm columns were evaluated for the separation of the carbonyl derivatives. Figure 2a shows the gradient separation of a standard mixture of 15 carbonyl-DNPH derivatives using a HALO Phenyl-Hexyl column (2.7 µm, 2.1 × 100 mm) at 40 °C. Two organic mobile phases and THF were used, and flow rates were in the range of 380-450 µL/min. The carbonyl derivatives separated in about 8 minutes, with an elution order of formaldehyde, acetaldehyde, acetone, acrolein, propanal, crotonaldehyde, butanal, benzaldehyde, isovaleraldehyde, pentanal, o-tolualdehyde, p-tolualdehyde, m-tolualdehyde, hexanal, and 2,5-dimethylbenzaldehyde. Benzaldehyde and isovaleraldehyde were not baseline separated and the tolualdehyde isomers were not well resolved with this column. Figure 2b shows the separation of these compounds using a Waters ACQUITY BEH Phenyl column (1.7 µm, 2.1 × 100 mm) and a gradient with flow rates adjusted for UHPLC (Figure 2b). Comparable separation power was observed with the HALO Phenyl-Hexyl column and the Waters BEH Phenyl column.

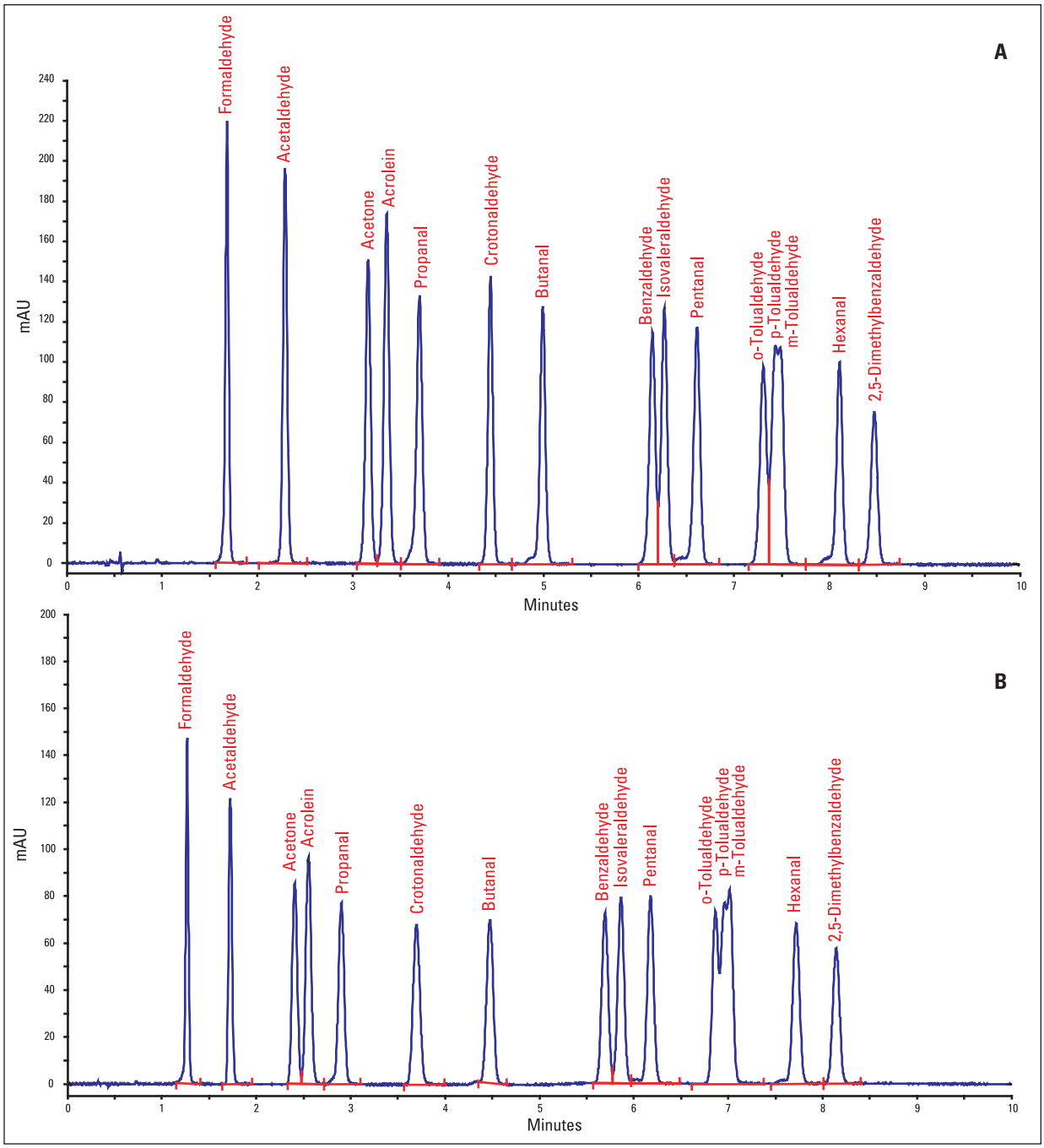

Figure 2: Separation of 15 carbonyl-DNPH derivatives at 20 µg/mL concentrations using a (A) Phenyl-Hexyl column and a (B) BEH Phenyl column

The Hypersil GOLD (1.9 µm, 2.1 × 100 mm) and Waters BEH C18 (1.7 µm, 2.1 × 100 mm) columns were also evaluated. The Hypersil GOLD column exhibited resolving power and efficiencies that were comparable to the phenyl-functionalized columns (Figure 3a), while the Waters BEH C18 column (1.7 µm, 2.1 × 100 mm) was less efficient in separating the more hydrophobic analytes (Figure 3b). The Hypersil GOLD column was selected for quantitative carbonyl analysis.

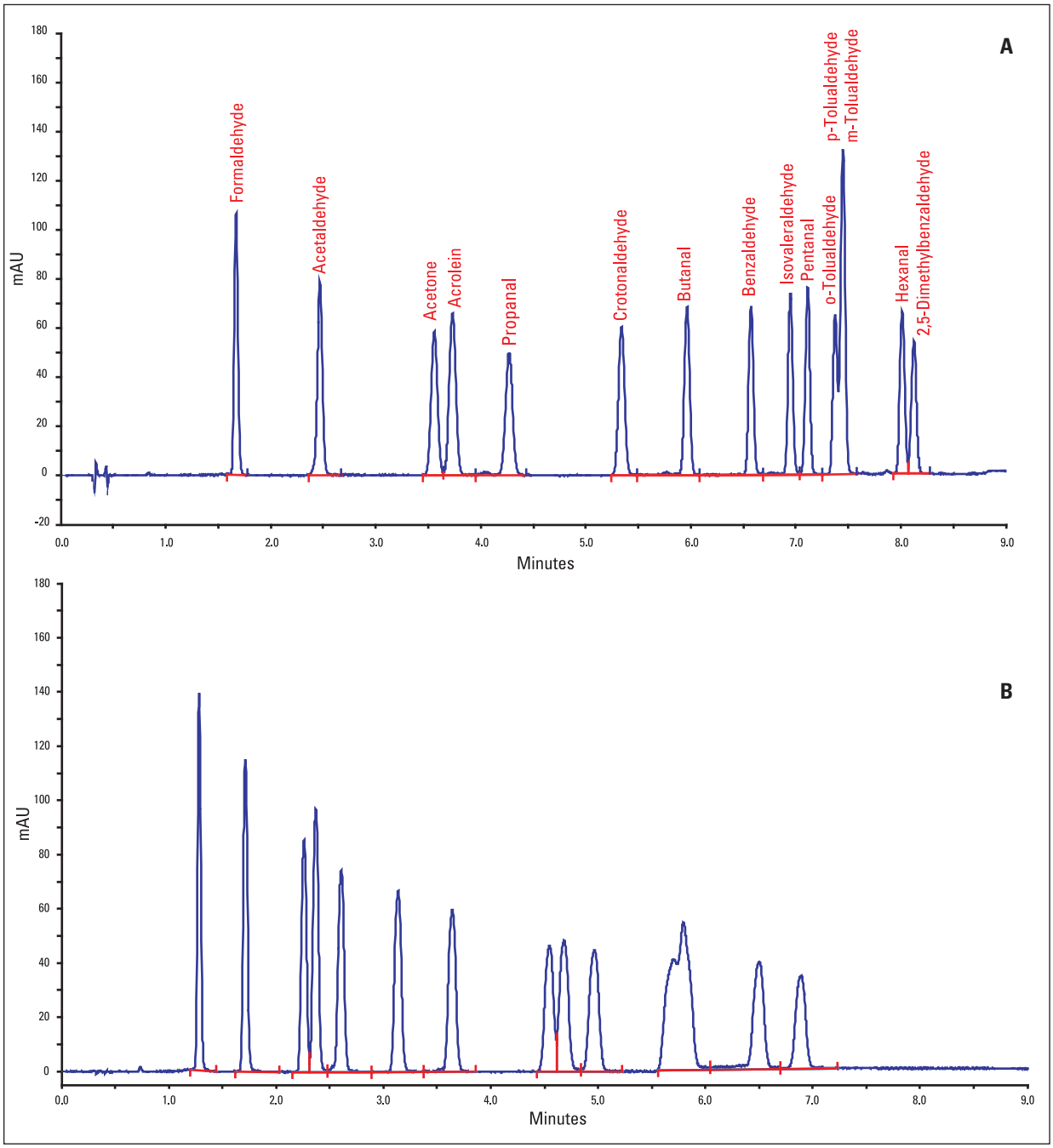

Figure 3: Separation of 15 carbonyl-DNPH derivatives at 20 µg/mL concentrations using a (A) Hypersil GOLD column and a (B) Waters ACQUITY BEH C18 column

Quantitative Analysis

Linearity and Sensitivity

Figure 4 demonstrates UHPLC separation of the 15 carbonyl-DNPH derivatives using the Hypersil GOLD column and a 13-minute gradient. All compounds but the tolualdehyde isomers were baseline resolved. While m-tolualdehyde and p-tolualdehyde co-elute, partial resolution of the o-tolualdehyde peak was achieved under these chromatographic conditions. The 13-minute gradient enabled better separation of the acetone and acrolein peaks as well as the hexanal and 2,5-dimethylbenzaldehyde peaks compared to the 8-minute gradient. This method was used for quantitative analysis of the carbonyl-DNPH standards.

The m- and p-tolualdehyde-DNPH derivatives were quantified together since they co-elute. Excellent linearity in detector response was observed over the range of 98-50000 ng/mL (ppb) (196-100000 ng/mL (ppb) for m- and p-tolualdehyde combined), with correlation coefficients greater than 0.999 for all analytes. Representative calibration curves are shown in Figure 5.

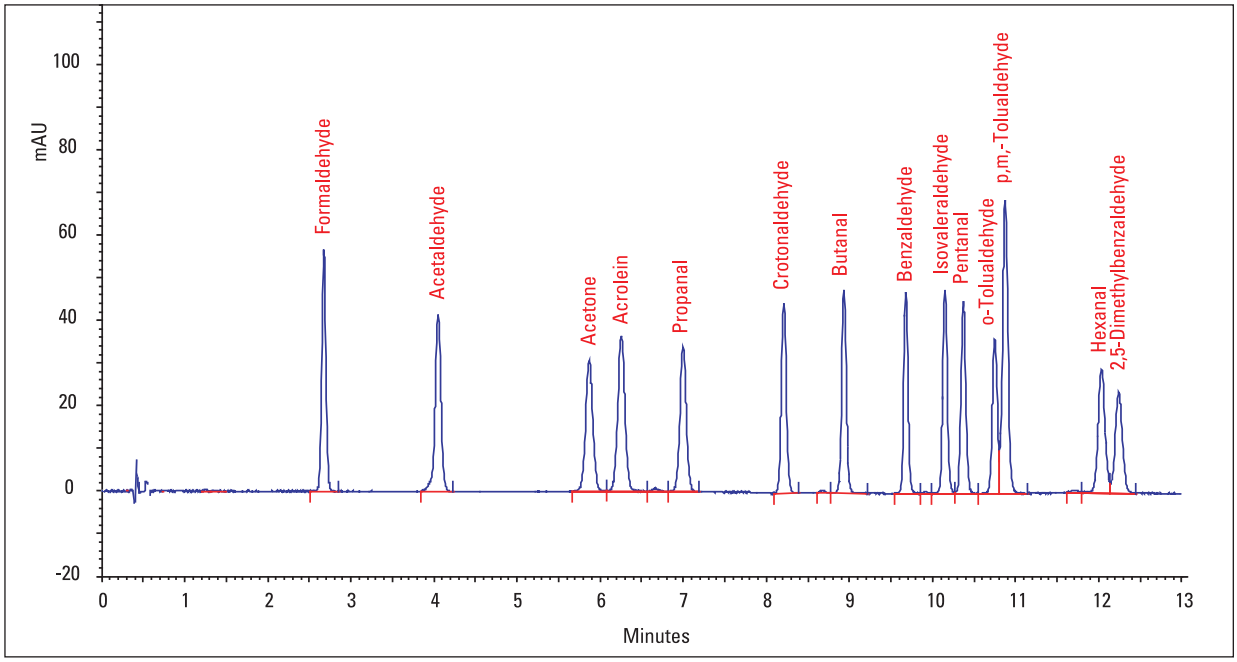

Figure 4: UHPLC separation of 15 carbonyl-DNPH derivatives at 20 µg/mL concentrations using a Hypersil GOLD column and 13-minute gradient

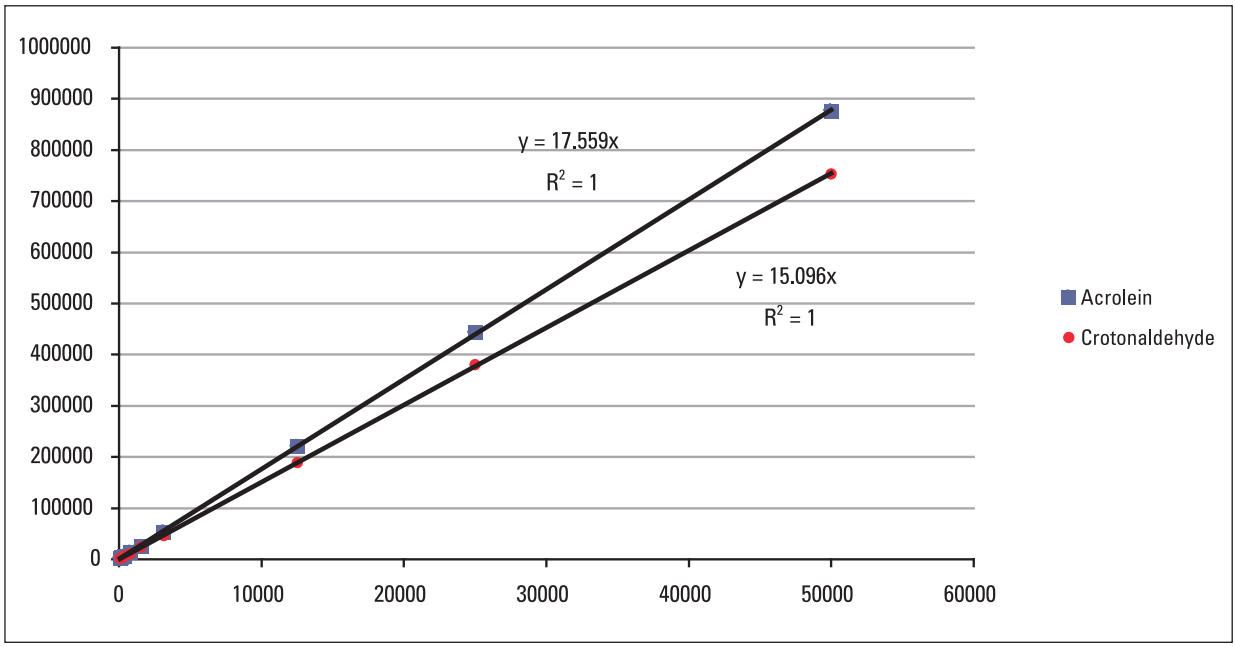

Figure 5: Representative calibration curves of acrolein-DNPH and crotonaldehyde-DNPH standards

Limits of detection (LODs) and limits of quantitation (LODs), defined as S/N ratio of 3 and 10, respectively, are shown in Table 1. LODs ranged from 33.9 to 104.5 ng/mL (ppb), and LOQs ranged from 181.2 to 396.8 ng/mL (ppb). These LODs would be sufficient for the detection of carbonyls in complex real-world samples since they are typically enriched 200-fold prior to analysis.

Reproducibility and Accuracy

Reproducibility was investigated by analyzing five replicate injections of each analyte. With four channel mixings of the solvents at various viscosities, retention time RSDs ranged from 0.52-2.22% while peak area RSDs ranged from 0.46-4.91% (Table 1), indicating excellent method reproducibility, particularly of the LC pump.

Quantitative accuracy for all carbonyl-DNPH derivatives were evaluated at two levels of concentrations, 400 ppb and 2000 ppb, using external calibration method. The accuracy of two representative analytes, benzaldehyde-DNPH and o-tolualdehyde-DNPH, were given in Table 2. The values of 96.3% and 103.6% at 400 ppb, respectively, and 99.8% and 99.9% at 2000 ppb, respectively were achieved with the UHPLC method.

Compound	LOD	LOQ	RT %RSD	Area %RSD	Linear Dynamic Range ng/mL
Formaldehyde	34.9	181.2	0.59	0.60	98-50000
Acetaldehyde	56.8	236.4	0.99	0.50	98-50000
Acetone	101.1	319.4	0.77	0.76	98-50000
Acrolein	82.1	266.7	1.53	0.64	98-50000
Propanal	85.2	281.2	2.22	0.59	98-50000
Crotonaldehyde	53.3	218.5	1.37	0.46	98-50000
Butanal	45.5	200.1	0.71	1.24	98-50000
Benzaldehyde	66.3	219.9	0.54	4.91	98-50000
Isovaleraldehyde	44.1	196.4	0.52	2.87	98-50000
Pentanal	33.9	198.2	0.60	2.65	98-50000
o-Tolualdehyde	104.5	321.1	0.75	1.99	98-50000
p,m,-Tolualdehyde	54.6	271.6	0.77	1.33	196-100000
Hexanal	62.9	318.9	0.84	0.66	98-50000
2,5-Dimethylbenzaldehyde	84.7	396.8	1.26	1.29	98-50000

Table 1: Quantitation data for 15 carbonyl-DNPH standards

Compound	400 ppb	% Accuracy	20000 ppb	% Accuracy
Benzaldehyde	385.3	96.3	1996	99.8
o-Tolualdehyde	414.4	103.6	1997	99.9

Table 2: Accuracy data for two carbonyl-DNPH standards

Conclusion

- The Accela 1250 UHPLC system offers the flexibility of performing both HPLC and UHPLC separations. With its very low internal back pressure, this system is capable of operational pressures up to 1250 bar, which is significantly higher compared to other commercial UHPLC systems.
- The Accela 1250 UHPLC system coupled with sub-2 µm Hypersil GOLD columns enabled highly efficient and reproducible separations of carbonyl-DNPH derivatives.
- Fast, accurate and robust quantitative analysis of low molecular weight carbonyls at ppb levels was achieved using Accela 1250 UHPLC followed by UV detection.
- UHPLC significantly improves resolution and speed of analysis and provides a powerful alternative to the HPLC-based procedures currently recommended by regulatory agencies for environmental monitoring of carbonyls.

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Application Note: 493

Quantitative Analysis of Environmental Air Contaminants Using APCI-MS/MS in Mobile Laboratories

Germain Tremblay, Lise Blanchard, Dominic Lortie – Ministère du Développement durable, de l'Environnement et des Parcs, Laval, Québec, Canada; Calin G. Znamirovschi – Thermo Fisher Scientific, West Palm Beach, Florida, USA

Key Words

- Environmental Monitoring
- TSQ Series Triple Quadrupole MS

Introduction

There are many potential hazards in our environment. Chemical emissions, accidental chemical spills and fires are of particular concern. A real-time analytical atmospheric pressure chemical ionization-tandem mass spectrometry (APCI-MS/MS) method for the quantitative analysis of air contaminants has been developed using a customized, direct-sampling APCI device coupled with a Thermo Scientific TSQ series triple stage quadrupole mass spectrometer. This method is critical for both environmental monitoring in areas of steady or long-term exposure and also for accidental or emergency instances. In such situations, timely and accurate qualitative and quantitative information on the types and levels of various toxic chemical contaminants is required to evaluate the hazard and prevent public exposure. Methods have been developed for chemicals related to the ambient air quality criteria, governed by the Ministère du Développement durable, de l'Environnement et des Parcs (MDDEP) of Québec, Canada. Criteria are illustrated in Table 1, for a limited selection of contaminants. A TSQ Series triple stage quadrupole mass spectrometer, with a customized APCI device for direct sampling, has been used (Figure 1).

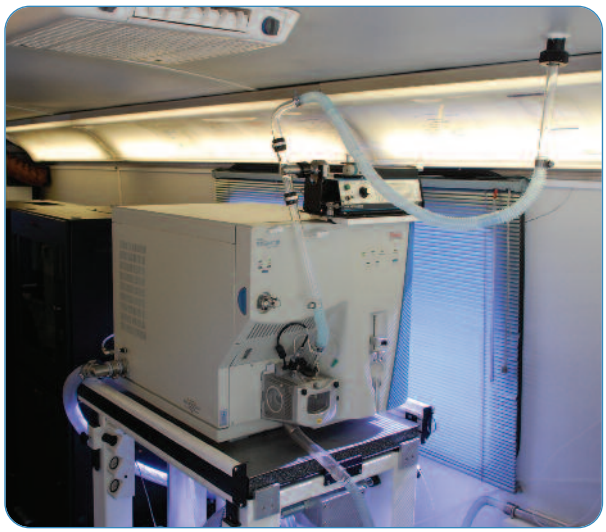

Figure 1: TSQ Series triple stage quadrupole with the ion source customized for direct air sampling.

Table 1. Ambient air quality criteria for common contaminants (limits of acceptance)

Compound	Limit Concentration (maximum mean/4 minutes) (µg/m³)	Limit of Detection (MS/MS) (µg/m³)
Acetone	8600	4
Acrylic acid	270	0.1
Ethyl-3-ethoxypropionate	300	0.02
Ethylacetate	20	16
Hydrogen chloride	1150	8
Methyl-ethyl ketone	740	6
Naphthalene	200	2
Phenol	160	0.4
Propylene glycol monomethyl ether (PGME)	ND	1
Sulfur dioxide	1050	0.3
Triethylamine	22	5

Goal

1) To develop a rapid, on-site, real-time air analysis method to identify and quantitate several common air contaminants.
2) To demonstrate the advantages of using the Thermo Scientific Ion Max source and tandem mass spectrometry (MS/MS) for the detection and determination of a selected range of atmospheric pollutants.
3) To establish and validate methods for air quality control programs, emission inventory and reporting, compliance and enforcement.

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Experimental

Preparation of Standards

Standards were prepared by infusing saturated vapor of standard-grade samples of phenol, propylene glycol monomethyl ether (PGME), methyl-ethyl ketone (MEK), and ethylacetate, respectively into a flow of ambient air using a gastight syringe pumping system connected to the Ion Max™ source of the mass spectrometer (Figure 2). The concentrations of standards were calculated as a function of the infusion rate of saturated vapor of the respective standards into a non-contaminated, continuous flow of atmospheric air, drawn under normal conditions of temperature and pressure. See Tables 2 and 3.

$$Conc(ppb) = \frac{Ps}{Pa} \times \frac{I}{F} \times 1000$$

Ps = *Vapor pressure of the compound (mm Hg at 21 °C)*

Pa = *Atmospheric pressure (mm Hg at 21 °C)*

I = *Infusion rate (µL/min)*

F = *Sampling pump flow (L/min)*

$$Conc(\mu g/m^3) = Conc(ppb) \times \frac{W}{V}$$

W = *Molecular weight of analyzed compound*

V = *Volume (24 liters at 21 °C)*

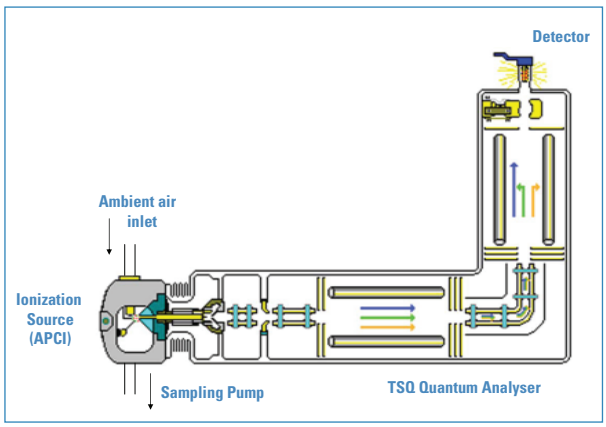

Figure 2: Block diagram of the TSQ Series triple stage quadrupole mass spectrometer custom source.

Table 3. Sample calculation of concentrations of compounds of interest.

	Phenol	Ethylacetate	MEK	PGME
Vapor pressure (Ps)	0.62	75.1	75.6	12
Syringe capacity (mL)	5	1	1	1
Speed setting	9	7	2	7
Infusion rate (µL/min)	1250	100	15	100
Sampling pump flow (L/min)	57	57	55	57
Molecular weight	94	88	72	90
Concentration (ppb)	18	173	27	28
Concentration (µg/m³)	70	636	81	104

Sample Analysis

Air samples were drawn directly from open atmosphere into the Ion Max source housing through the built-in probe aperture. The set-up consisted of an infusion pump regenerative blower, with the drain tube of the source chamber serving as the outlet. Following APCI, the resulting ions entered the mass spectrometer through the ion transfer tube interface.

MS Conditions

Mass spectrometer:	Thermo Scientific TSQ Quantum Discovery MAX
APCI corona voltage:	4 kV (- 4 kV in negative ion mode)
Ion transfer tube temperature:	180 °C
Skimmer offset:	5 V
CID gas pressure:	1.5 mTorr
Resolution:	Unit Resolution (0.7 FWHM)
Analytical scan type:	Selective reaction monitoring (SRM)
SRM conditions:	Scan time: 50 ms Scan width: 1.000 Da

The MS/MS experimental conditions for SRM are shown in Table 4.

Table 2. Calibration of the infusion pump (Correlation between syringe speed and infusion rate).

Syringe capacity	Syringe speed								
	1	2	3	4	5	6	7	8	9
	Flow rate (µL/min)								
10 µL	0.1	0.15	0.20	0.35	0.50	0.75	1.0	1.5	2.5
100 µL	1.0	1.5	2.0	3.5	5.0	7.5	10	15	25
1 mL	10	15	20	35	50	75	100	150	250
2.5 mL	25	38	50	88	125	188	250	375	625
5 mL	50	75	100	175	250	375	500	750	1250
10 mL	100	150	200	350	500	750	1000	1500	2500
50 mL	350	560	720	1230	1800	2560			

Table 4. MS/MS experimental conditions for SRM.

Compound	Precursor Ion *(m/z)*	Product Ion *(m/z)*	Tube Lens Voltage (V)	Collision Energy (V)
$^{13}C_2$-acetic acid	94	61	56	11
d_6-acetone	65	33	82	18
Ethylacetate	89	61	45	8
MEK	73	43	108	13
PGME	91	31	54	21
PGME	91	73	54	5
Phenol	126	93	35	13

Results and Discussion

In negative ion mode, $^{13}C_2$-acetic acid was used as an internal standard. Acetic acid produced a deprotonated molecule (*m/z* 94) $[^{13}CH_3{}^{13}COOH{\cdot}O_2]^-$ which, under CID conditions, produces CH_3COO^- (*m/z* 61). Phenol forms an analog adduct $[C_6H_5OH{\cdot}O_2]^-$ (*m/z* 126), which yields a product ion at *m/z* 93, $C_6H_5O^-$.

In positive ion mode, acetone-d_6 (*m/z* 65 to *m/z* 33) was used as an internal standard. Two precursor ion – product ion transitions were monitored, *m/z* 91 to *m/z* 31 and *m/z* 91 to *m/z* 73, respectively, in multiple reaction monitoring (MRM) mode for the analysis of PGME.

The limit of detection (LOD) is the concentration equivalent of 3x standard deviation of the response at the background level (i.e., ambient air, in the absence of the subject compound).

The calibration data for ethylacetate, MEK, PGME, and phenol are shown in Figures 3 through 10. The quantitative results are listed in Tables 5 through 8.

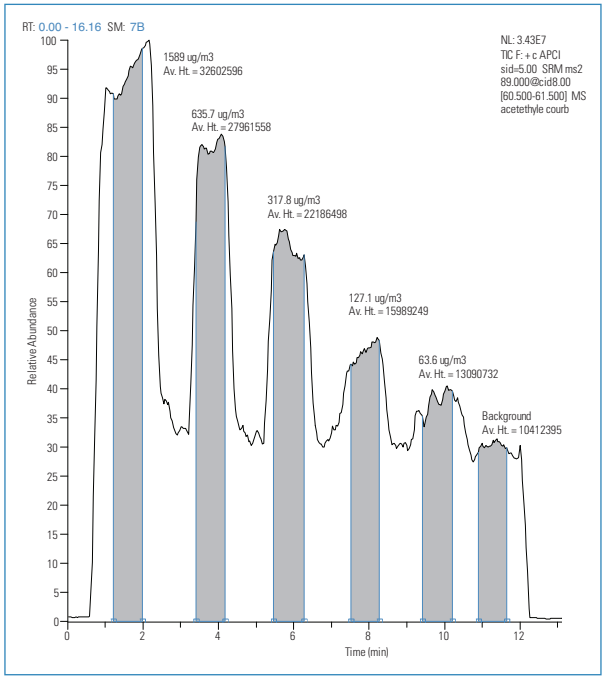

Figure 3: Reconstructed ion trace for ethylacetate to produce the calibration curve.

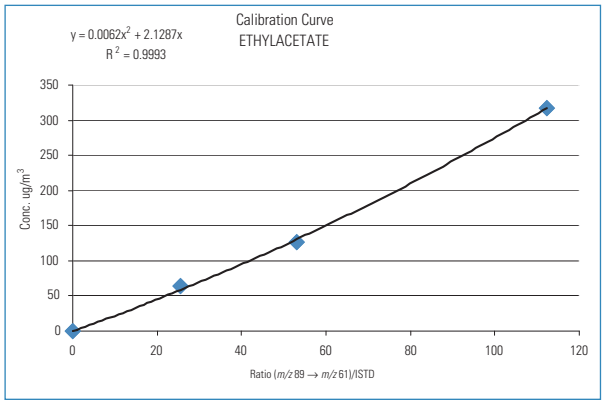

Figure 4: Calibration curve for ethylacetate.

Table 5. Quantitative results for ethylacetate and LOD determination.

ETHYLACETATE

ISTD: Acetone d_6, syringe 1 mL, Speed 2					
SRM (*m/z* 65→*m/z* 33)					
RT (min.)	Syringe Speed	Average Height	Background Subtracted		
10.5	OFF	26000			
15.55	2	130873	104873		

Ethylacetate						
SRM (*m/z* 89→*m/z* 61)						
RT (min)	Syringe Speed	Average Height	Background Subtracted	Response/ISTD Ratio	Concentration ($\mu g/m^3$)	
1.2 - 2.0	9	32602596	22190201			
3.4 - 4.2	7	27961558	17549163			
5.5 - 6.3	5	22186498	11774103	112.3	317.8	
7.5 - 8.3	3	15989249	5576854	53.2	127.1	
9.4 - 10.2	1	13090732	2678337	25.5	63.6	
	OFF	10412395	0	0.0	0	Std. Dev. = 218065
		(3 x Std. Dev.) =	654194	6.24	16.2	**LOD**

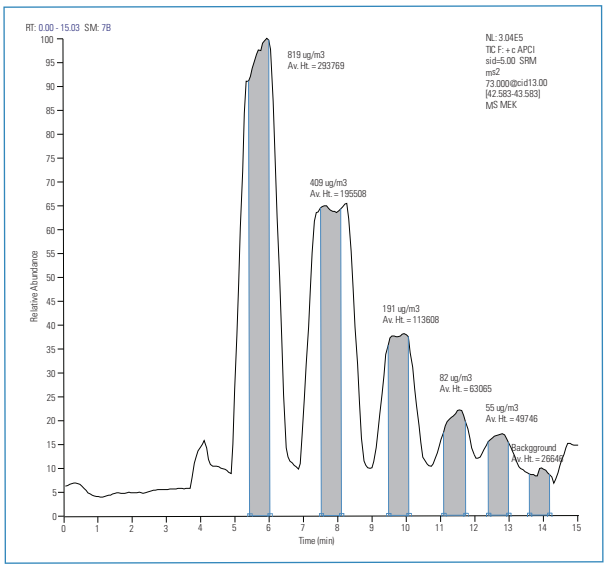

Figure 5: Reconstructed ion trace for MEK to produce the calibration curve.

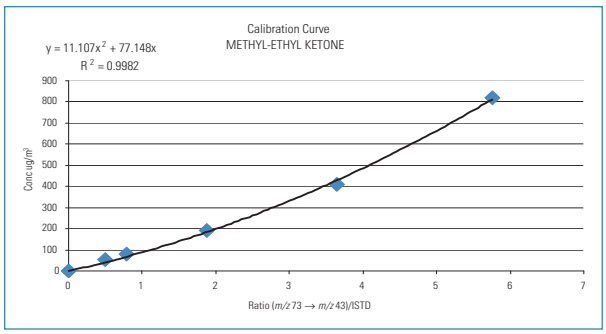

Figure 6: Calibration curve for MEK.

Table 6. Quantitative results for MEK, and LOD determination.

METHYL-ETHYL KETONE (MEK)

ISTD: Acetone d_6, syringe 1 mL, Speed 2					
SRM (*m/z* 65→*m/z* 33)					
RT	Syringe Speed	Aver Height	Background Subtracted		
3.03	OFF	45240			
2.38		91628	46388		

Methyl-ethyl ketone						
SRM (*m/z* 65→*m/z* 33)						
RT (min)	Syringe Speed	Average Height	Background Subtracted	Response/ISTD Ratio	Concentration ($\mu g/m^3$)	
5.4 - 6.0	9	293769	267123	5.8	819	
7.5 -8.1	7	195508	168862	3.6	409	
9.5 - 10.1	5	113608	86962	1.9	191	
11.1 - 10.7	3	63065	36419	0.8	82	
12.4 - 13.0	1	49746	23100	0.5	55	
13.6 - 14.2	OFF	26646	0	0.0	0	Std. Dev. = 1100
		(3 x Std. Dev.) =	3300	0.1	6	**LOD**

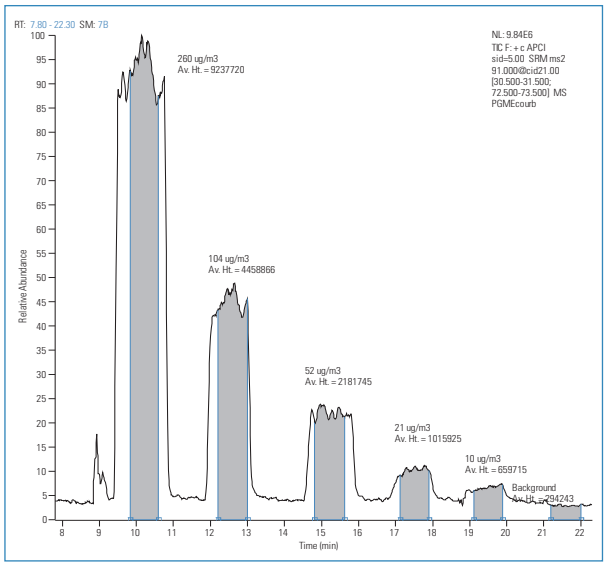

Figure 7: Reconstructed ion trace for PGME to produce the calibration curve.

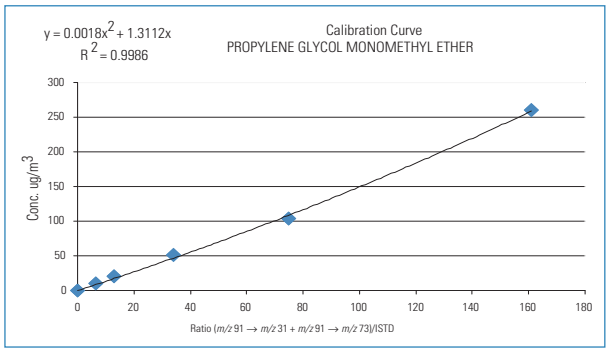

Figure 8: Calibration curve for PGME.

Table 7. Quantitative results for PGME, and LOD determination.

PROPYLENE GLYCOL MONOMETHYL ETHER (PGME)

ISTD: Acetone d_6, syringe 1 mL, Speed 2					
SRM (*m/z* 65→*m/z* 33)					
RT	Syringe Speed	Aver Height	Background Subtracted		
11.00	OFF	12500			
7.30		68039	55539		

Propylene Glycol Monomethyl Ether (PGME)						
MRM (*m/z* 91→*m/z* 33 + *m/z* 91→*m/z* 73)						
RT (min)	Syringe Speed	Average Height	Background Subtracted	Response/ISTD Ratio	Concentration (µg/m^3)	
9.8 - 10.6	9	9237720	8943477	161.0	260	
12.2 -13.0	7	4458866	4164623	75.0	104	
14.8 - 15.6	5	2181745	1887502	34.0	52	
17.1 - 17.9	3	1015925	721682	13.0	21	
19.1 - 19.9	1	659715.5	365473	6.6	10	
21.2 - 22.0	OFF	294243	0	0.0	0	Std. Dev. = 15262
		(3 x Std. Dev.) =	45785	0.8	1	**LOD**

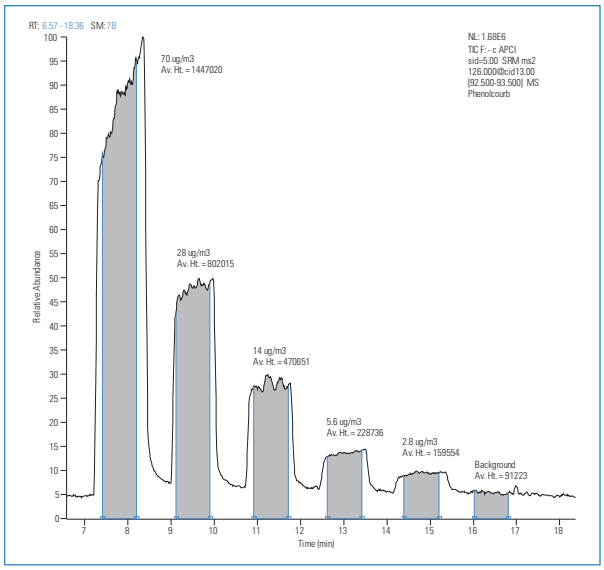

Figure 9: Reconstructed ion trace for phenol to produce the calibration curve.

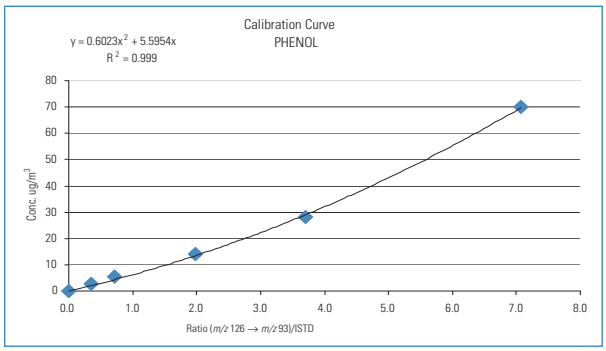

Figure 10: Calibration curve for phenol.

Table 8. Quantitative results for phenol, and LOD determination.

PHENOL

ISTD: Acetic acid $^{13}D_2$, syringe 1 mL, Speed 2					
SRM (*m/z* 94→*m/z* 61)					
RT	Syringe Speed	Aver Height	Background Subtracted		
6.0	OFF	57639			
4.6		249205	191566		

Phenol						
SRM (*m/z* 126→*m/z* 93)						
RT (min)	Syringe Speed	Average Height	Background Subtracted	Response/ISTD Ratio	Concentration (µg/m³)	
7.4 - 8.2	9	1447020	1355797	7.1	70	
9.1 - 9.9	7	802015	710792	3.7	28	
10.9 - 11.7	5	470651	379428	2.0	14	
12.6 - 13.4	3	228736	137513	0.7	5.6	
14.4 - 15.2	1	159554	68331	0.4	2.8	
16.0 - 16.8	OFF	91223	0	0.0	0	Std. Dev. = 3871
		(3 x Std. Dev.) =	11613	0.06	0.4	**LOD**

Conclusion

The custom TSQ Series triple stage quadrupole mass spectrometer system allows the detection and quantitative analysis of a series of chemical pollutants in ambient air. Concentration of these pollutants can be determined in a real-time fashion for immediate action in case of chemical spills, fire, etc., or for the purpose of trending in environmental monitoring.

This application demonstrates that LODs can be achieved with the TSQ Series triple stage quadrupole mass spectrometer in real time, without sample pre-concentration or any separation technique. The achieved LOD values are lower than the regulatory limits for the respective compounds.

The custom configuration of the TSQ Series triple stage quadrupole mass spectrometer is well-suited for installation in mobile laboratories (Figure 11). Such configuration demonstrates, in addition to the reliability and ruggedness of the TSQ instrumentation, applicability of the system to on-site environmental analysis. In emergency situations, like fires or chemical spills, these mobile facilities are essential for real-time ambient air analysis.

Figure 11: The mobile laboratory of MDDEP Québec, Canada, containing the rugged and reliable TSQ Series triple quadrupole system functioning dynamically on-board.

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Application Note: 521

The Analysis of Bisphenol A-diglycidyl Ether (BADGE), Bisphenol F-diglycidyl Ether (BFDGE) and Their Derivatives in Canned Food and Beverages by LC-MS/MS

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Key Words
- BADGE
- BFDGE
- LC-MS/MS
- Food Safety

Introduction

As an attempt to reduce food spoilage and prevent degradation of the container, epoxy-based lacquers or vinylic organosol (PVC) materials are commonly used as coating material in food cans. These lacquers are epoxy phenolic resins based on polymerization products of bisphenol A-diglycidyl ether (BADGE) or bisphenol F-diglycidyl ether (BFDGE). Chlorinated derivatives can be generated during the coating thermal treatment, since BADGE and BFDGE are also used as additives to remove the hydrochloric acid formed in this process. Moreover, hydrolyzed derivatives such as BADGE.2H_2O, BADGE.H_2O, and BFDGE.2H_2O can be produced during storage when the coating comes into contact with aqueous and acidic foodstuffs. The European Union has set specific migration limits (SML) for these compounds: 9 mg/kg for the sum of BADGE and its hydrolyzed derivatives and 1 mg/kg for the sum of BADGE.HCl, BADGE.2HCl and BADGE.HCl.H_2O.[1,2] The presence of this family of compounds in food has received much attention lately due to its suspected mutagenic, genotoxic, and anti-androgenic effects.[3-6]

Goal

To develop a fast and sensitive LC-MS/MS method for the simultaneous quantitative analysis of BADGE, BFDGE, and their derivatives in canned food and beverages.

Experimental

Sample Preparation

Canned Food:

The whole can content was homogenized. A sample of 3 g was mixed with 6 mL of ethyl acetate. The resulting mixture was shaken for 20 minutes and sonicated for 30 minutes in an ultrasonic bath. The mixture was then centrifuged at 4000 rpm for 15 minutes. Five (5) mL of supernatant was transferred to an 8-mL vial and evaporated to dryness under a stream of nitrogen. The extract was reconstituted in 1 mL of MeOH:H_2O (1:1) and filtered before injection (10 µL injection).

Beverages:

A 20-mL sample of beverage was degassed by sonication for 20 min. Then, 3 mL was loaded into a polymeric SPE cartridge that was previously conditioned with 3 mL of MeOH and 3 mL of H_2O. Finally, the analytes were eluted with 4 mL of MeOH. The collected fraction was evaporated to dryness and the extract reconstituted with 1 mL of MeOH:H_2O (1:1) and filtered before injection (10 µL injection).

LC Conditions

Solvent A	Formic acid-ammonium formate (25 mM, pH 3.75, 50 °C)
Solvent B	Methanol
Flow Rate	600 µL/min
Analytical Column	Fused Core™ Ascentis Express C18 150 x 2.1 mm i.d., 2.7 µm (Supelco)

The gradient method was started at 30% solvent B (0.25 min) and linearly increased to 50% solvent B in 0.75 min. The gradient was then increased to 60% of solvent B in 0.5 min, and then to 80% in 4 minutes. This composition was maintained for 0.5 min.

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MS Conditions

MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra AM mass spectrometer equipped with a heated electrospray ionization probe. The MS conditions were as follows:

Ion Source Polarity	Positive Ion Mode
Spray Voltage	4000 V
Vaporizer Temperature	475 °C
Sheath Gas Pressure (N_2)	60 units
Auxiliary Gas Pressure (N_2)	40 units
Ion Sweep Gas Pressure	2 units
Capillary Temperature	375 °C
Tube Lens	65 V
Collision Gas (Ar)	1.5 mTorr

Results and Discussion

The family of compounds studied tends to form adducts and clusters in positive ionization mode $[M+NH_4]^+$, $[M+Na]^+$, and $[M+K]^+$. The mobile phase used favored the formation of ammonium adducts ions $[M+NH_4]^+$, which dominated the full scan spectra (base peaks). The cleavage of the phenyl-alkyl bond and the α-cleavage of the ether bond were identified as the most intense and characteristic fragmentation of $[M+NH_4]^+$, and therefore selected for quantification and confirmation purposes (Table 1).

Matrix effects were evaluated by analyzing two samples free of BADGEs and BFDGEs – cola soft drink beverage and red pepper. These samples were analyzed by external and matrix-matched calibration. The results showed similar responses for both methods and matched calibration curves, indicating that no matrix effect occurred in the analysis of BADGEs and BFDGEs using the developed LC-MS/MS method.

To evaluate limits of quantification, blank samples were spiked with the studied compounds at low concentration levels (below 2.5 µg/kg) and submitted to the sample pre-treatment detailed above. The results obtained allowed the analysis of this family of compounds in beverages and canned food, given that the LOQs obtained are below (3 to 4 orders of magnitude) the specific migration limits established by the European Union (Table 2).

Good linearity ($r^2 > 0.999$) was observed for calibration curves for standard solutions ranging from 0.5 µg/kg to 5,000 µg/kg.

Run-to-run precision was evaluated by analyzing six replicates of a red pepper sample and a cola sample spiked at two concentration levels. In addition, the ion ratios (quantitative versus confirmatory) were calculated and errors (compared with standards) were always below 10%. Finally, recoveries were calculated by addition of different amounts of the studied compounds (between the LOQ and 250 µg/kg) to blank samples, which were analyzed by external calibration.

Sample Analysis

The LC-MS/MS method developed for the analysis of BADGEs and BFDGEs in canned food and soft-drinks was employed to analyze six aqueous-based canned foods and seven soft-drink samples (Figure 1). In canned soft-drink beverages only BADGE·$2H_2O$ was detected, at concentrations ranging from 2.3 µg/L to 5.1 µg/L, while other BADGEs and BFDGEs were not detected. As an example, Figure 2 shows the LC-MS/MS chromatogram of two canned soft-drinks samples where BADGE·$2H_2O$ was found. In contrast, several BADGEs were found in canned food samples. BADGE·$2H_2O$ was found in all food samples at concentrations between 2.7 µg/kg and 675 µg/kg, with the highest concentration level being

Table 1. Transitions monitored for the analysis of BADGEs and BFDGEs

Compound	Precursor ion *(m/z)*, $[M+NH_4]^+$	Quantitation		Confirmation		Ion Ratio ± SD[b]
		Product Ion *(m/z)*	CE[a] (V)	Product Ion *(m/z)*	CE[a] (V)	
BADGE·$2H_2O$	394.2	209.1	31	135.1	31	1.7 ± 0.1
BADGE·H_2O	376.2	209.1	29	135.1	29	1.9 ± 0.1
BADGE·HCl·H_2O	412.2	227.0	33	135.1	33	1.4 ± 0.1
BADGE	358.2	191.0	30	135.1	30	4.3 ± 02
BADGE·HCl	394.2	227.0	13	135.1	13	2.6 ± 0.3
BADGE·2HCl	430.2	227.0	30	135.1	30	2.0 ± 0.1
BFDGE·$2H_2O$	366.2	133.1	22	181.1	22	1.5 ± 0.1
BFDGE	330.2	163.1	12	189.1	12	1.3 ± 0.1
BFDGE·2HCl	402.1	199.1	20	181.1	20	1.7 ± 0.2

[a]CE: collision energy

[b]SD: Standard deviation (n = 5)

Table 2. MLOQs, run-to-run precision, recoveries, and ion ratio of the LC-MS/MS method

Compound	MLOQ (µg/L)	Soft-drinks and canned food			
		Precision (RSD %)		Recovery (%)	Ion ratio[c]
		Low concentration[a]	Medium concentration[b]		
BADGE·2H$_2$O	0.13 – 1.0	7	3	70 - 95	1.8
BADGE·H$_2$O	0.14 – 1.1	12	3	60 - 83	1.8
BADGE·HCl·H$_2$O	0.14 – 1.1	20	9	69 - 95	1.5
BADGE	0.16 – 1.2	12	10	80 - 86	4.3
BADGE·HCl	0.16 – 1.3	3	11	60 - 70	2.4
BADGE·2HCl	1.6 – 3.4	14	10	80 - 82	2.1
BFDGE·2H$_2$O	1.5	16	8	85 - 90	1.3
BFDGE	0.7 – 4.0	20	10	70 - 89	1.6
BFDGE·2HCl	1.6	13	4	74 - 95	1.9

[a]Low concentration level: Cola sample (0.15 µg/L to 2.0 µg/L) and red pepper (2.0 µg/kg to 15.0 µg/kg).
[b]Medium concentration level: Cola sample (1.5 µg/L to 20 µg/L) and red pepper (20 µg/kg to 150 µg/kg).
[c]Ion ratio calculated at medium concentration level.

in the asparagus sample. Other BADGEs detected in these samples were BADGE·H$_2$O at concentrations ranging from 35 µg/kg to 53 µg/kg, BADGE·HCl·H$_2$O (3.4 – 274 µg/kg) and BADGE·2HCl at concentrations between 0.9 µg/kg and 2.8 µg/kg. In contrast, the original monomer (BADGE) was not found in the samples, probably because it was easily hydrolyzed in these water-based samples. In addition, none of the BFDGEs were found, confirming the decrease in use of BFDGE-based coatings.

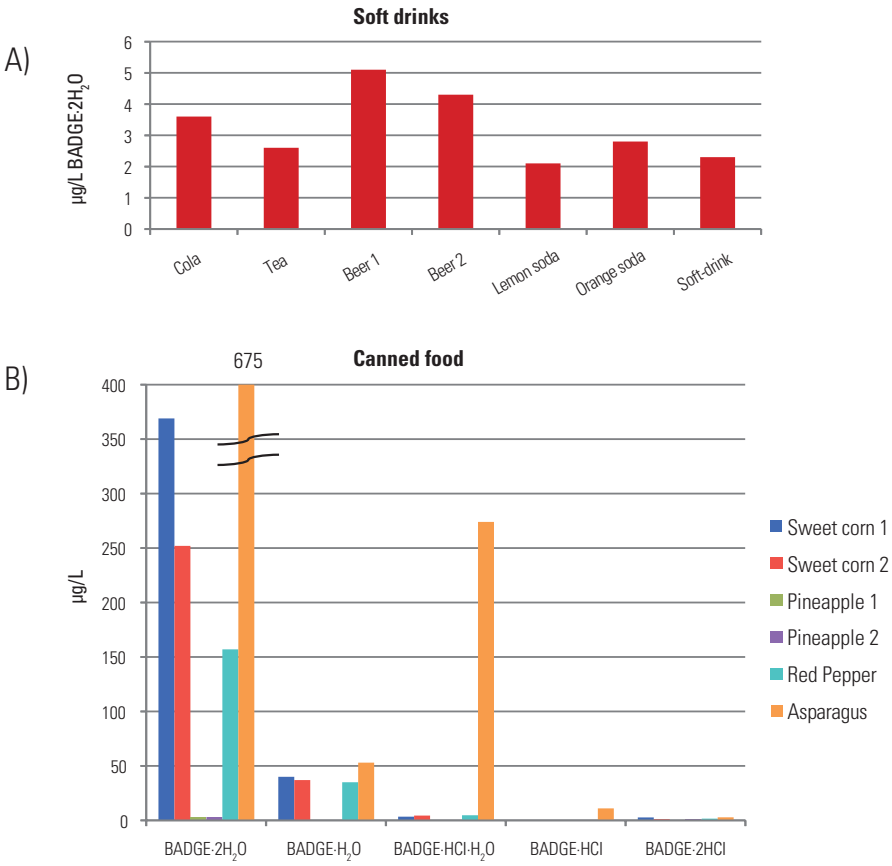

Figure 1. Canned soft-drinks (A) and food samples (B) analyzed using the developed LC-MS/MS method

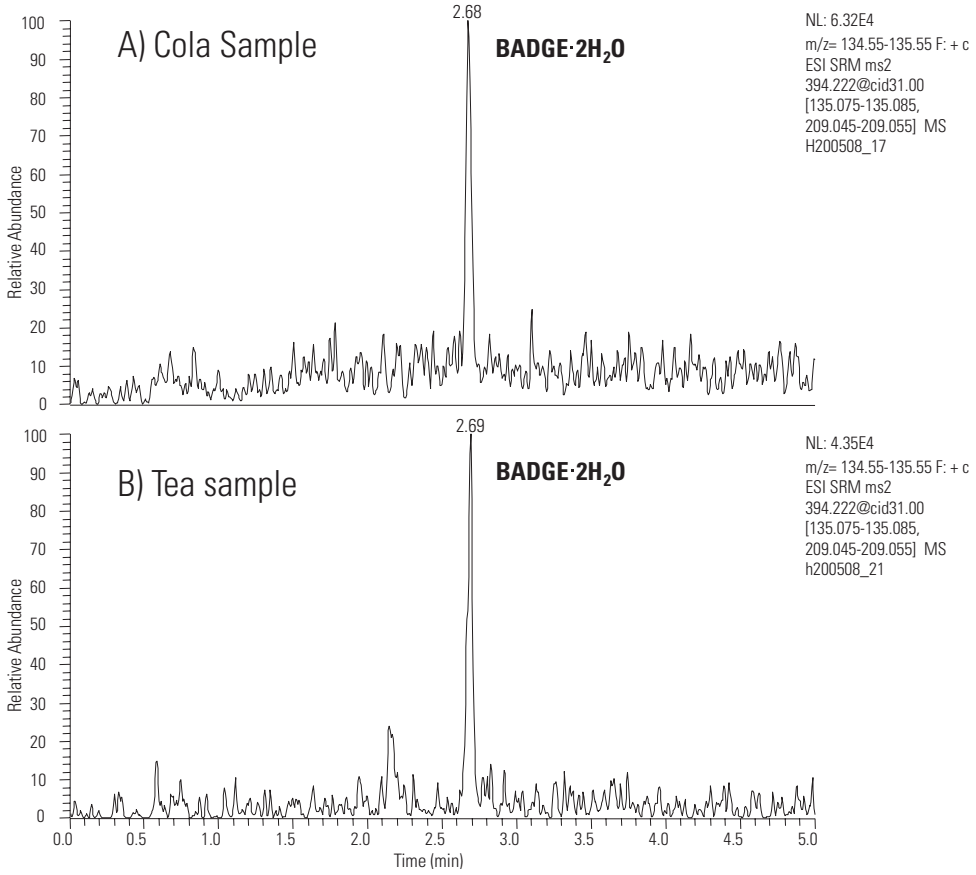

Figure 2. LC-MS/MS chromatograms for cola (A) and tea (B) samples

Conclusions

A fast and sensitive method for the simultaneous analysis of BADGEs and BFDGEs in canned food and beverages is proposed. The limits of quantification of the method vary between 0.13 and 1.6 µg/L for beverages and between 1.0 and 4.0 µg/kg for foodstuff. The method has been applied to real samples. BADGE.2H_2O was detected in all samples at levels between 2.1 and 675 µg/kg. Other derivatives of BADGE were also detected and quantified. No BFDGE or its derivatives were detected.

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Application Note: 560

Analysis of Illegal Dyes in Food Matrices using Automated Online Sample Preparation with LC/MS

Yang Shi, Catherine Lafontaine, Matthew Berube, John Fink, François Espourteille
Thermo Fisher Scientific, Franklin, MA

Key Words

- Transcend TLX-1
- TurboFlow Technology
- Exactive
- Accucore HPLC Columns

Introduction

Sudan dyes are red dyes used for coloring solvents, oils, waxes, petrol, or as additives in shoe and floor polish. In addition, they have been found in a number of food products such as chili or chili-containing products. Sudan dyes are banned as food additives in the USA[1], the EU[2,3] and many other countries due to links to cancer and other negative health effects.

Liquid chromatography-ultraviolet-visible (LC–UV–vis) and liquid chromatography-mass spectrometry (LC/MS) are currently the most popular methods for analysis of Sudan dyes.[4] Traditional sample preparation methods, especially solid phase extraction (SPE), have also been widely used in the determination of Sudan dyes. However, these procedures can be labor-intensive, time-consuming and costly, resulting in low sample throughput when performed manually. Lower recoveries have also been noticed associated with SPE cleanup.[4] There is consensus that one of the major scientific challenges in the analysis of Sudan dyes is to achieve high sensitivity and selectivity while minimizing sample clean up.[5]

In this study we describe an easy, comprehensive LC method using a Thermo Scientific Transcend TLX-1 system powered by TurboFlow™ technology coupled to a Thermo Scientific Exactive MS to analyze five illegal dye residues in a variety of sauces.

Goal

Develop a rapid and sensitive automated online sample preparation LC-MS/MS method to detect and quantify multiple Sudan dyes in a variety of food matrices and also to shorten assay time and increase throughput.

Experimental

The Matrix Standard Curve

Five analytes, Sudan I, Sudan II, Sudan III, Sudan IV and Para Red (Figure 1) were obtained from Sigma-Aldrich (St. Louis, MO). A total of four different food products purchased from local grocery stores were used in this study: Chili Sauce I; Chili Sauce II; Hot Sauce I; Hot Sauce II.

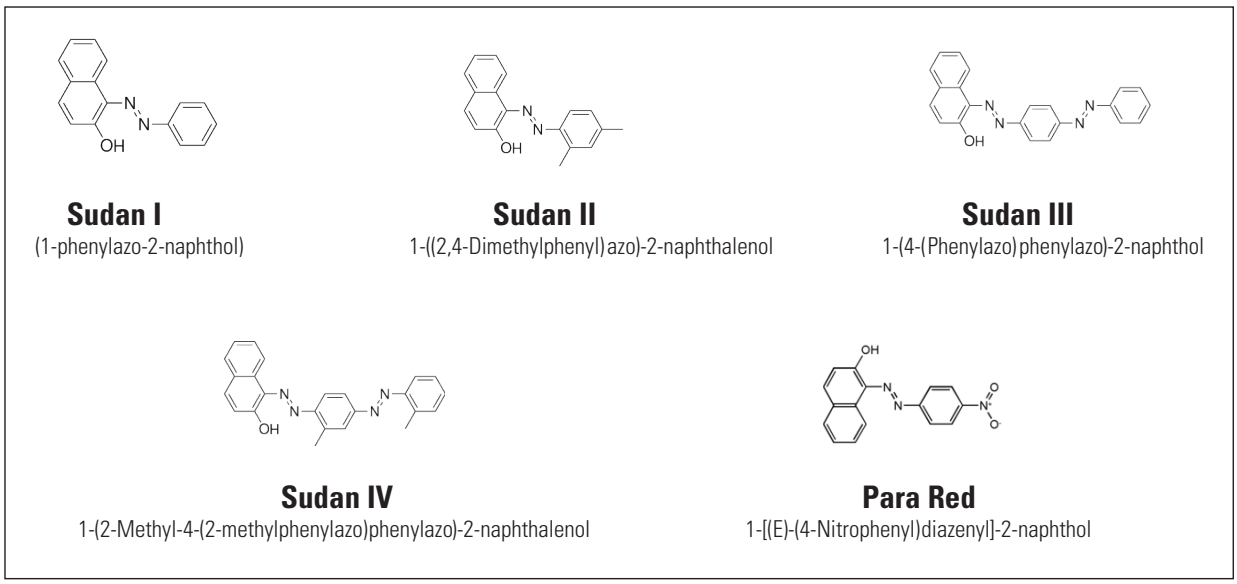

Figure 1. Chemical structure of test compounds

Three grams of each homogenized matrix were weighed into a 50-mL centrifuge tube, followed by the addition of 30 mL of acetonitrile (ACN). The tube was vortexed for 10 minutes and then sonicated for another 60 minutes. The resulting solution was centrifuged at 10,000 RPM for 15 minutes. The supernatant was then filtered through a 0.45-mm syringe filter. No additional clean up of the sample solution was performed. Each milliliter of supernatant corresponds to 0.1 g semi-solid food matrix as the unit of conversion.

A calibrant stock solution was prepared at a final concentration of 1 mg/mL of each analyte in ACN. A range of calibration solutions from 0.5 to 100 ng/mL (equal to 5 to 1000 ng/g) was made by serial dilutions using individually produced supernatants.

LC/MS Methods

Thermo Scientific TurboFlow Method Parameters

Column:	TurboFlow XL C8 column 0.5 x 50 mm
Injection Volume:	25 µL
Solvent A:	0.1% formic acid in water
Solvent B:	0.1% formic acid in ACN
Solvent C:	1:1 ACN: isopropanol

HPLC Method Parameters

Analytical Column:	Thermo Scientific Accucore Phenyl-Hexyl 50 x 3 mm, 2.6 µm particle size
Solvent A:	0.1% formic acid in water
Solvent B:	0.1% formic acid in ACN

Thermo Scientific Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of the particles results in much lower backpressures than typically seen with sub-2 µm materials.

Mass Spectrometer Parameters

MS:	Thermo Scientific Exactive high performance benchtop Orbitrap™ MS
MS Ionization Source:	Heated Electrospray Ionization (H-ESI)
Ionization Mode:	Positive
Scan Range:	*m/z* 240.0 to 390.0
Resolution:	50,000
Spray Voltage:	4 KV
Sheath Gas Pressure (N_2):	70 arbitrary units
Auxiliary Gas Pressure (N_2):	40 arbitrary units
Heater Temperature:	400 °C
Capillary Temperature:	350 °C
Capillary Voltage:	27.5 V
Tube Lens Voltage:	95 V
Skimmer Voltage:	22 V

The interference molecules from the matrix were unretained and moved to waste during the loading step of the TurboFlow column, while the analyte of interest was retained on the extraction column. This was followed by organic elution of the analytes to the analytical column and gradient elution to the MS. The system was controlled by Thermo Scientific Aria OS. Data acquisition was performed using Thermo Scientific Xcalibur software. The resulting data were processed with Thermo Scientific LCQUAN quantitative software. The accurate masses of the analytes are listed in Table 1.

Table 1. Testing compounds

	Formula	Exact Mass	$[M+H]^+$
Para Red	$C_{16}H_{11}N_3O_3$	293.080041	294.087841
Sudan I	$C_{16}H_{12}N_2O$	248.094963	249.102763
Sudan II	$C_{18}H_{16}N_2O$	276.126263	277.134063
Sudan III	$C_{22}H_{16}N_4O$	352.132411	353.140211
Sudan IV	$C_{24}H_{20}N_4O$	380.163711	381.171511

Results and Discussion

Figure 2 shows the representative chromatograms of the 5 analytes at 20 ng/g (2 ng/mL) in Hot Sauce II extract. For the concentration range studied (5-1000 ng/g), all limits of quantitation (LOQs) were estimated from triplicate injections (coefficient of variation < 15%) of standard solutions. The area precision and mean accuracy were below 20% at LOQ. As shown in Table 2, the LOQs ranged from 5-20 ng/g for all analytes except Para Red in four of the sauces studied. A lower LOQ could possibly be achieved by increasing sample injection volume because TurboFlow columns can handle larger injections (up to a few hundred microliters) while regular high performance LC (HPLC) or Ultra HPLC (UHPLC) columns cannot. Good linearity was observed over the entire tested range of each analyte. The correlation coefficients obtained using weighted (1/x) linear regression analysis of standard curves were greater than 0.99 for all analytes.

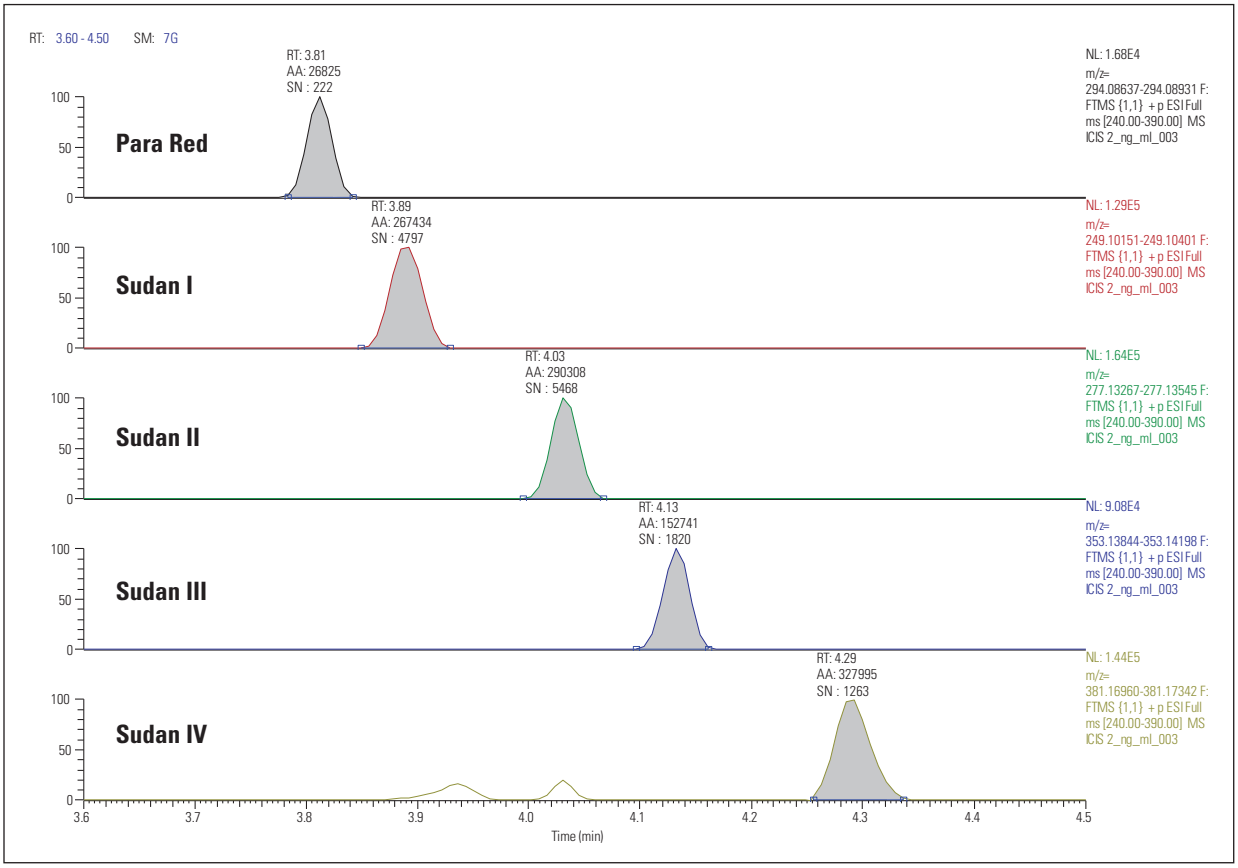

Figure 2. Representative chromatogram (20 ng/g in Hot Sauce II)

To further assess the reproducibility of the present methodology, a relative standard deviation (%RSD) test was performed on all matrices fortified with analytes at 100 ng/g. Table 2 indicates that the RSDs of six replicate injections were less than 10% for the majority of analytes. These results show the feasibility of the current approach for Sudan dyes determination in food matrices.

Table 2. Quantitation limit, linearity and relative standard deviation (%RSD) of analytes in four tested matrices

Chili Sauce I

	LOQ (ng/g)	R^2	%RSD (n=6 at 100 ng/g)
Para Red	20	0.9955	9.64
Sudan I	10	0.9960	3.64
Sudan II	10	0.9936	6.24
Sudan III	10	0.9937	7.45
Sudan IV	5	0.9911	4.55

Chili Sauce II

	LOQ (ng/g)	R^2	%RSD (n=6 at 100 ng/g)
Para Red	50	0.9900	4.50
Sudan I	10	0.9906	6.26
Sudan II	10	0.9920	10.82
Sudan III	10	0.9942	10.29
Sudan IV	The data for Sudan IV was not quantifiable.		

Hot Sauce I

	LOQ (ng/g)	R^2	%RSD (n=6 at 100 ng/g)
Para Red	20	0.9952	4.92
Sudan I	5	0.9980	2.88
Sudan II	10	0.9969	5.11
Sudan III	10	0.9959	2.21
Sudan IV	20	0.9980	4.35

Hot Sauce II

	LOQ (ng/g)	R^2	%RSD (n=6 at 100 ng/g)
Para Red	20	0.9973	7.62
Sudan I	5	0.9981	2.66
Sudan II	5	0.9976	5.36
Sudan III	5	0.9970	6.23
Sudan IV	5	0.9974	2.95

A recovery study was performed on the four matrices fortified with analytes at 100 ng/g. The recovery was assessed by comparing the detector response of a post-extracted spiked sample with that determined from a spiked neat standard sample at the same concentration. As shown in Figure 3, recoveries were 80%-120% for most analytes in all matrices except chili sauce II extract, which indicates no significant matrix effects for the majority of analytes. These matrix-matched calibration curves can be used to overcome matrix effects and calculate concentrations of these illegal dyes in routine lab work.

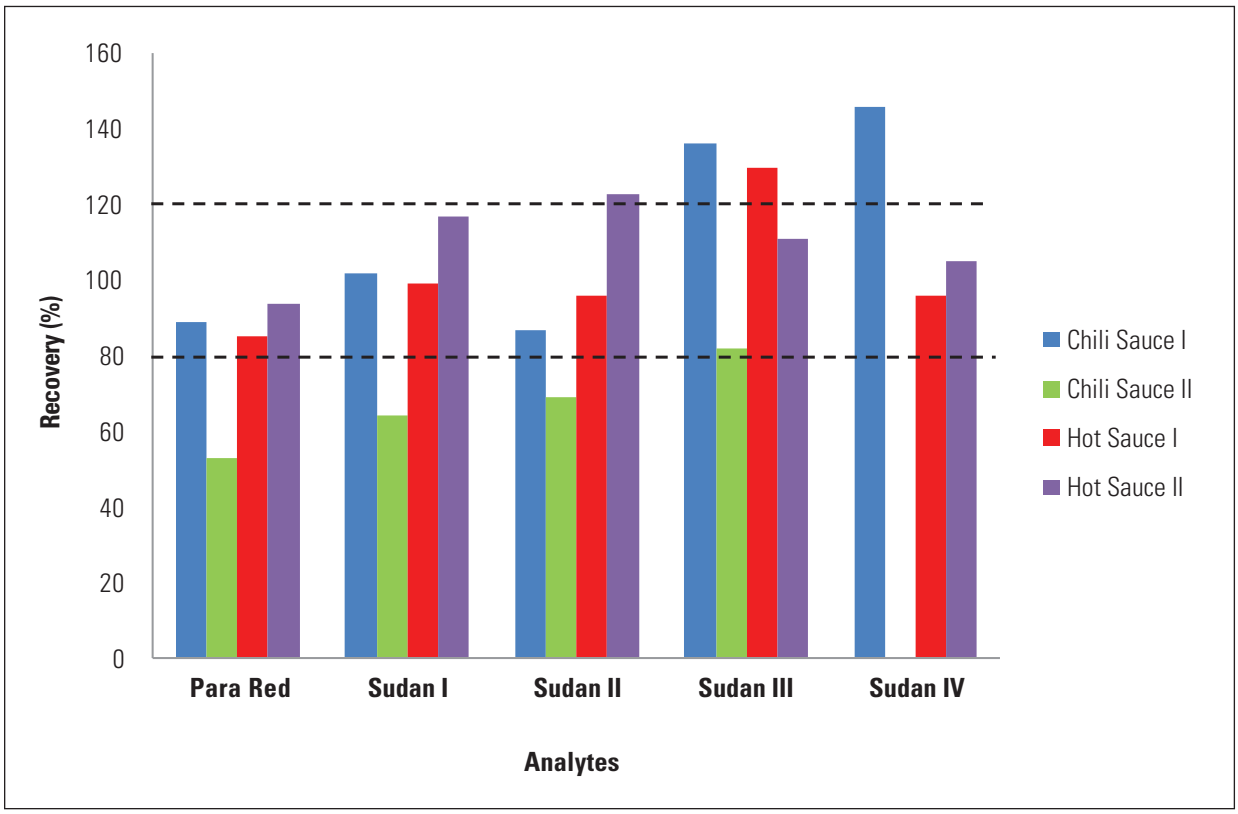

Figure 3. Recoveries of 5 analytes fortified in all tested matrices at 100 ng/g

Conclusion

The current method has been tested with four different sauces. Linearity, specificity, recovery and repeatability of the method have been established. Sample preparation time of this strategy was minimal. Not including sonication and centrifugation times, the sample preparation only took 15 minutes.

Additionally, since all analytes were eluted within less than one minute of a total six-minute LC run, multiplexing with a Transcend TLX-4 system would further reduce total LC-MS/MS run time four-fold and enable screening of more than 30 samples per hour. Future work could involve screening a larger range of illegal dyes, thus combining a screening method with accurate quantification.

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Application Note: 379

Analysis of Microcystins from Blue-green Algae Using the TSQ Quantum Ultra LC-MS/MS System

Mihoko Yamaguchi, Thermo Fisher Scientific, Yokohama, Japan

Key Words

- TSQ Quantum Ultra™
- Blue-green algae
- LC-MS/MS
- Microcystin
- SRM

Introduction

Overgrowth of algae is a common problem in many wetlands with advanced stages of eutrophication (the enrichment of chemical nutrients containing nitrogen or phosphorus in an ecosystem). This often results in a thick, colored layer on the water's surface, known as an algal bloom. Some of the algae that grow in these bodies of water, known as Cyanobacteria or blue-green algae, produce toxic compounds known as microcystins.

Microcystins have a ring peptide structure consisting of seven amino acids, and more than 80 homologs are known. One of the most widely studied of the microcystins is known as Microcystin-LR, and is shown in Figure 1. Many of the microcystins are particularly toxic to the liver. (*See References.*) Among them are Microcystin-LR, YR and RR, which have been detected in wetlands in Japan. This application note reports on the analysis of these microcystins by using LC-MS/MS.

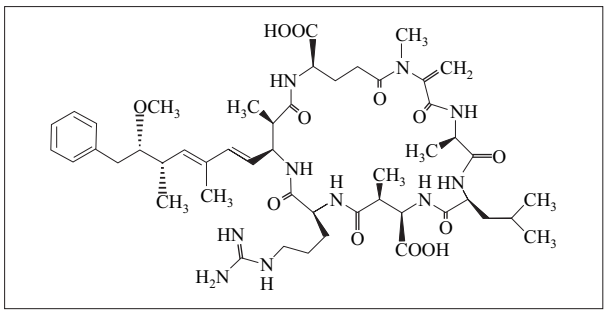

Figure 1: Microcystin-LR

Method

HPLC: HTC PAL Autosampler and Surveyor™ MS pump
Column: HyPURITY™ C18 2.1×50 mm, 5 µ (Thermo Scientific)
Mobile Phase A: Water with 0.1% Formic Acid
Mobile Phase B: Acetonitrile
Gradient: 30%B (0.5 min) → 80%B (in 3 min) → 80%B (2 min hold) → 30%B (7 min hold)
Injection Volume: 20 µL
Flow: 0.2 mL/min
Column temperature: Room temperature
MS: TSQ Quantum Ultra
Ionization: Positive ESI
Spray voltage: 5000 V
Sheath gas: 45 arbitrary units
Auxiliary gas: 15 arbitrary units
Sweep gas: 2 arbitrary units
Capillary T: 350°C
Source CID: Off
Collision gas: Ar, 1.2 mTorr
Scan Time: 0.15 sec
SRM setting: 519.9 → 135.0 @ 32 V (RR)
995.7 → 135.0 @ 65 V (LR)
1045.8 → 135.0 @ 70 V (YR)

SRM Chromatogram (STD 1.0 ppb)

The SRM chromatograms for 1.0 ppb standards are shown in Figure 2. The linear calibration curves of the standards (0.1 ppb–1.0 ppm) are shown in Figure 3.

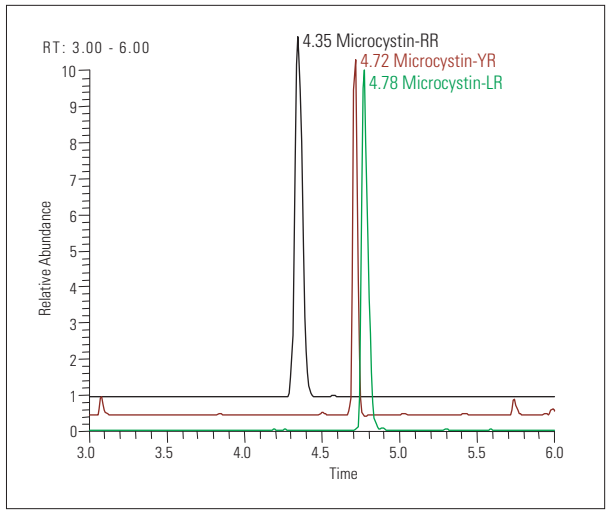

Figure 2: SRM Chromatogram (RT 4.35: Microcystin-RR, RT 4.72: Microcystin-YR, RT 4.78: Microcystin-LR)

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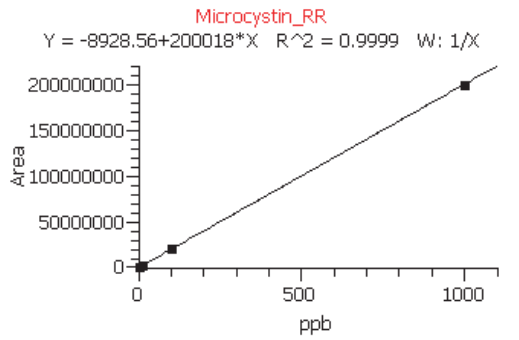

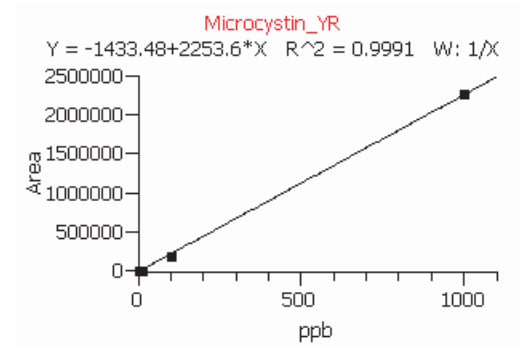

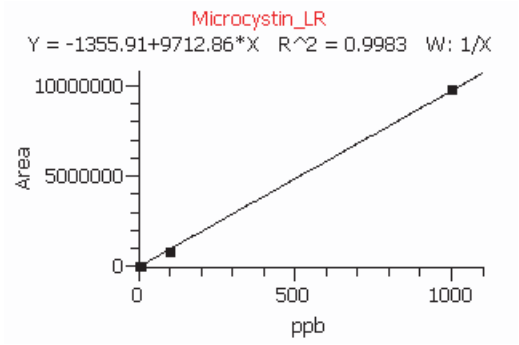

Figure 3: Calibration Curves 0.1 ppb – ~1.0 ppm

Conclusion

Microcystin-LR, YR and RR can be quantitatively analyzed over four orders of dynamic range (0.1 ppb–1.0 ppm) by using the TSQ Quantum Ultra triple quadrupole LC-MS/MS system from Thermo Fisher Scientific.

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Application Note: 533

Analysis of Perchlorate in Infant Formula by Ion Chromatography-Electrospray-Tandem Mass Spectrometry (IC-ESI-MS/MS)

Charles Yang[1], Leo Wang[2], and William Schnute[2]
[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Sunnyvale, CA

Key Words

- TSQ Quantum Access
- Ion chromatography
- Food safety
- Sensitivity

Introduction

Ion chromatography-mass spectrometry (IC-MS/MS) can be used to detect, quantify, and confirm a variety of analytes in environmental and food matrices, including haloacetic acids, bromate, and percholorate. Perchlorate, a naturally occurring and man-made contaminant, is widely found in the environment in surface water, groundwater, and soil. Through environmental contamination, perchlorate has entered the food supply chain and has been detected in a wide variety of foods including eggs, milk, vegetables, and fruits. In humans, perchlorate interferes with the ability of the thyroid gland to take up iodine, which is needed to produce hormones that regulate many body functions after they are released into the blood. Because these thyroid hormones are essential for normal growth and development, infants and children could more likely be affected by perchlorate than adults.

A recent study by the Centers for Disease Control and Prevention examined various types of commercially available powdered infant formulas to determine if perchlorate could be found at measurable levels.[1] All of the powdered infant formulas tested contained perchlorate. Significantly higher concentrations of perchlorate were found in cow's milk-based formulas with lactose than in other types.

A simple and ultra-sensitive IC-MS/MS technique for the quantitation of perchlorate in powdered infant formula, liquid infant formula, and milk is described here. Unlike conventional detection methods that require labor intensive solid phase extraction (SPE) sample enrichment, only a simple sample preparation is necessary.

Goal

To develop a simple and ultra-sensitive IC-MS/MS method to separate and quantitate perchlorate without time-consuming sample preparation.

Experimental Conditions

Sample Preparation

Samples of commercially available liquid infant formula, reconstituted powdered infant formula, and milk were prepared for analysis. A 4-mL sample of formula or milk was mixed with 4 mL of ethanol (pre-cooled at 4 °C) and 0.4 mL of 3% acetic acid. The sample was spiked with 40 µL (100 ng/mL) isotope-labeled internal standard. The mixture was vortexed and then centrifuged at 5000 rpm for 30 minutes under refrigeration (-5 °C). The supernatant was run through a syringe filter that had been preconditioned with 5 mL ethanol and 15 mL of deionized water. The filtrate was collected in 10-mL plastic autosampler vials and readied for IC-MS/MS analysis.

Ion Chromatography

IC analysis was performed on a Thermo Scientific Dionex ICS-3000 Reagent-Free ion chromatography (RFIC) system. The IC conditions were as follows:

Column set:	Thermo Scientific Dionex AG16 / AS16 hydroxide selective anion exchange columns
Suppressor:	Thermo Scientific Dionex ASRS 300 self-regenerated suppressor (external water mode)
Column temperature:	30 °C
Injection volume:	100 µL
Eluent:	Isocratic 45 mM hydroxide
Eluent Source:	EGC III KOH
Solvent:	150 µL/min acetonitrile delivered by an AXP-MS pump
Flow rate:	300 µL/min
Detection:	First detector: Suppressed conductivity Second detector: Thermo Scientific TSQ Quantum Access mass spectrometer

Mass Spectrometry

MS analysis was carried out on a TSQ Quantum Access™ triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) source. The MS conditions were as follows:

Ion source polarity:	Negative ion mode
Spray voltage:	4000 V
Sheath gas pressure:	40 arbitrary units
Ion sweep gas pressure:	15 arbitrary units
Auxiliary gas pressure:	5 arbitrary units
Capillary temperature:	300 °C
Collision gas pressure:	1.8 mTorr
Scan mode:	Selected reaction monitoring (SRM)

Thermo SCIENTIFIC

Selected reaction monitoring allowed the following fragmentation patterns to be observed:

m/z 99 ($^{35}ClO_4^-$) → *m/z* 83 ($^{35}ClO_3^-$)	primary transition for native perchlorate (quantitative)
m/z 101 ($^{37}ClO_4^-$) → *m/z* 85 ($^{37}Cl^{18}O_3^-$)	secondary transition for native perchlorate (confirmative)
m/z 107 ($^{35}Cl^{18}O_4^-$) → *m/z* 89 ($^{35}Cl^{18}O_3^-$)	primary transition for labeled IS (quantitative)

Results and Discussion

Figure 1 shows a representative SRM chromatogram for a perchlorate standard of 20 pg/mL at the low end of the calibration range. Even with such a low concentration, perchlorate shows a well-defined peak that can be accurately quantified.

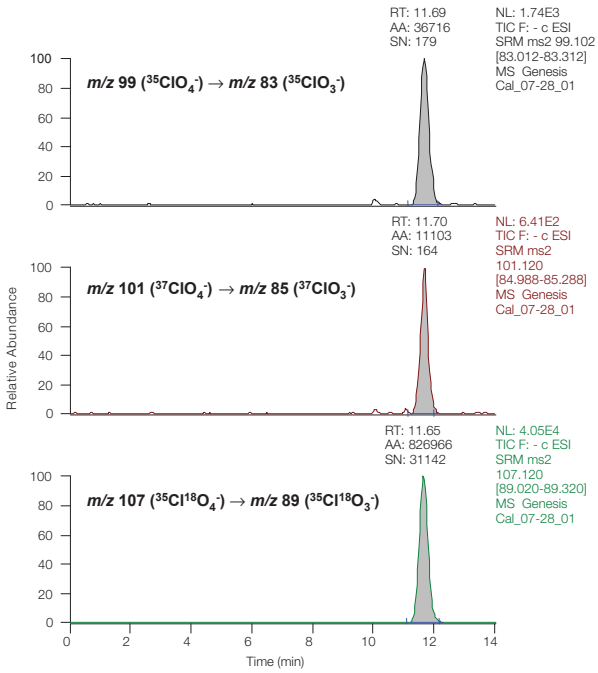

Figure 1: SRM chromatograms of a perchlorate standard at 20 pg/mL

Calibration curves generated on the TSQ Quantum Access mass spectrometer show excellent linearity (Figure 2). For quantitative analysis, the 99 → 83 SRM transition of perchlorate was used (ClO_4^- Quan), and for qualitative analysis, the 101 → 85 SRM transition of perchlorate was used (ClO_4^- Qual). The labeled IS was used with the quantitative and qualitative ions to calculate the squared correlation coefficients (r^2) of the 99 → 83 and 101 → 85 SRM transitions of perchlorate, which were were 0.9996 and 0.9998, respectively. The data was weighted by 1/X to ensure better quantification accuracy for low level samples. The calibration range was 20–10,000 pg/mL.

The instrument lower detection limit was determined to be 5 pg/mL with S/N > 10, which is significantly below the lowest reported value (30 pg/mL)[1]. The upper calibration limit was set at 10 ng/mL, which covered the highest reported value (5.05 ng/mL)[1]. The practical limit of detection (LOD) was determined by $Amt_{Blank} + 3 \times S_0$ = 28.9 pg/mL. The systematic factor Amt_{Blank} was calculated by running deionized water instead of real sample through all sample preparation and instrument analysis procedures.

The IC-MS/MS system provides excellent chromatographic performance and allows separation of perchlorate from complex matrix components. Figure 3 displays the SRM chromatograms for perchlorate in unspiked infant formula and milk samples.

The recovery was evaluated by comparing the differences between unspiked and spiked (5 ng/mL) samples (n=2, n=3). Excellent recoveries were achieved as shown in Table 1.

Table 1. Recovery of perchlorate in infant formula and milk

	Unspiked	Spiked	Recovery
Liquid Infant Formula	1.74 (2.68)	6.91 (4.95)	103%
Milk	2.21 (2.34)	7.93 (2.71)	114%†

†*Reported amounts are in the units of ng/mL with %RSD included in parenthesis.*

The instrument accuracy and precision were evaluated by repeat injections of standards at three levels, as shown in Table 2.

Table 2. Method performance – Accuracy and precision

Specified Amount	Quantified Amount	%RSD	%Deviation	$\%RSD_{R.T.}$
100 pg/mL	98.18 pg/mL	3.05	1.82	0.13
1 ng/mL	1.04 ng/mL	1.62	3.52	0.08
10 ng/mL	10.74 ng/mL	1.45	7.36	0.11

Perchlorate was detected in every sample tested in this study. The method precision, evaluated by repeat assays of each unknown sample, was excellent as shown in Table 3. The powdered infant formula sample was quantified at 2.44 ng/mL, and the milk sample was quantified at 4.64 ng/mL. No interference was detected for any of the samples analyzed.

Table 3. Method performance – Real samples

Sample	Quantified Amount (ng/mL)	%RSD
LIF-1	1.74	2.68 (n=3)
LIF-2	2.21	2.34 (n=3)
LIF-3	1.05	3.63 (n=7)
PIF-1	2.44	1.74 (n=3)
MLK	4.64	1.96 (n=7)

LIF: Liquid infant formula; PIF: Powdered infant formula; MLK: Milk

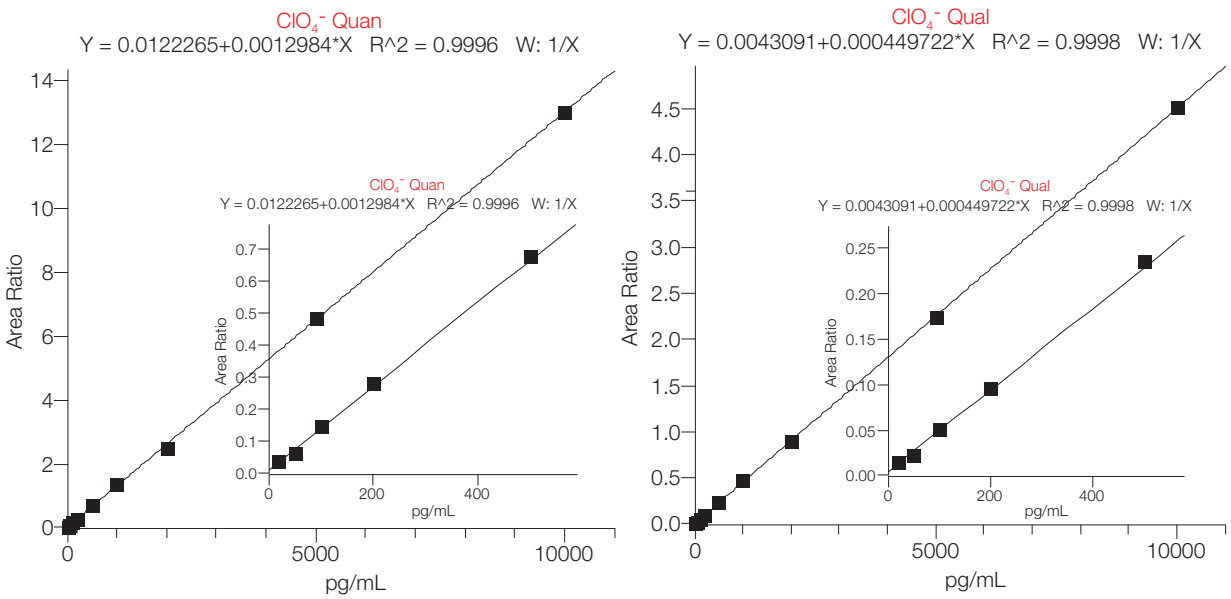

Figure 2. Calibration curves for quantitative and qualitative analysis of perchlorate

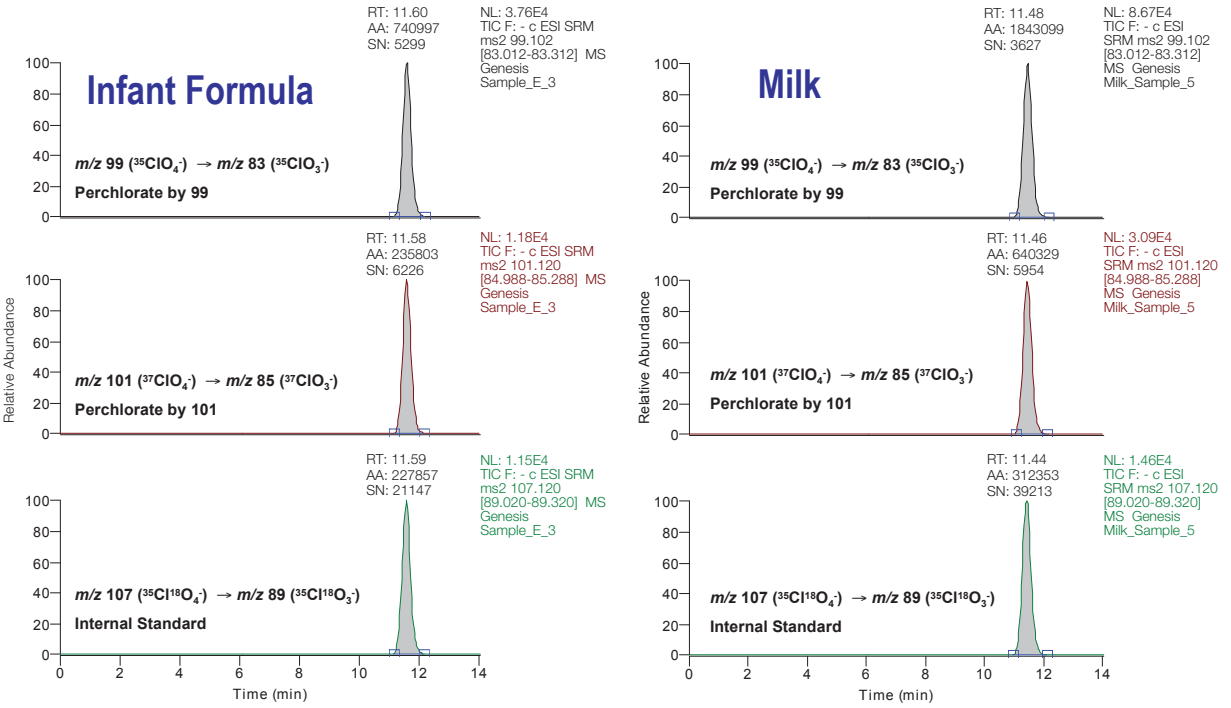

Figure 3. Perchlorate in unspiked infant formula and milk samples. The calculated concentrations are shown in Table 3.

Conclusion

A selective and ultra-sensitive IC-MS/MS method has been successfully applied for the quantification of perchlorate in infant formula and milk. Because the simple sample preparation was deployed and not the long SPE enrichment method, several hours of sample preparation time was saved. The wide linear range covers the reported perchlorate levels in infant formula. Excellent reproducibility (%RSD=3.63, n=7), accuracy, and precision were achieved.

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Application Note: 474

Determination of Bisphenol A in Infant Formula by Automated Sample Preparation and Liquid Chromatography-Mass Spectrometry

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Key Words

- Aria TLX-1
- TurboFlow Technology
- TSQ Vantage
- Infant Formula
- Food Safety

Introduction

2,2-bis(4-hydroxyphenyl) propane, commonly known as Bisphenol A (BPA), is one of the primary chemicals used to make plastics. It is also heavily used in the production of various types of food and drink containers. Because BPA has been known to leach from the plastic lining of metal canned food, the potential risks of exposure to BPA have been a great concern over the past few years. Higher bisphenol A levels are significantly associated with heart disease, diabetes, and abnormally high levels of certain liver enzymes. There is a consensus that infants are at the greatest risk of harm due to exposure to extremely low levels of BPA.[1] The maximum acceptable or "reference" dose for BPA is 50 µg/kg body weight/day, as established by the U.S. Environmental Protection Agency.[2]

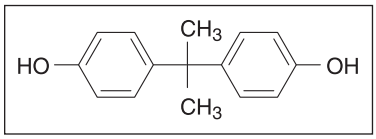

Figure 1: Chemical Structure of Bisphenol A

A liquid chromatography-mass spectrometry (LC-MS) technique has been recently described for the determination of BPA in food.[3] Current strategies for the detection of BPA in canned infant formula employ sample preparations that involve complicated extraction steps such as solid phase extraction, solvent-based extraction, and some micro-extraction techniques. All of these techniques require additional sample concentration and reconstitution in an appropriate solvent. Such sample preparation methods are time-consuming and are more vulnerable to variability due to errors in manual preparation. To offer a high sensitivity (low ppb) BPA detection method and timely, automated analysis of multiple samples, our approach is to use Thermo Scientific TurboFlow technology coupled to the detection capabilities of a high-sensitivity Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Goal

Develop a six-minute LC-MS/MS method using automated sample preparation for the assay of BPA in canned infant formula powder by negative ion atmospheric pressure chemical ionization (APCI) using a deuterated internal standard (BPA-d_{16}).

Experimental

Sample Preparation

Canned infant formula powder, used in this analysis for preparation of blanks, QCs, and standards, was obtained from a local supermarket in Massachusetts. The lid lacquer is low-density polyethylene and the body is polyester. BPA and BPA-d_{16} were obtained from Sigma-Aldrich, US (St. Louis, MO). The diluent (AmAcACN solution) was made using 3% ammonium acetate in acetonitrile-water (70:30, v/v). A BPA working solution was prepared in AmAcACN solution at 10 µg/mL. The infant formula solution was prepared by adding 10 mL of AmAcACN solution to 1 g of infant formula powder and then centrifuging at 10,000 RPM for 30 minutes. BPA standards and QC standards were serially diluted to the target concentrations with the resulting supernatant containing 25 ng/mL BPA-d_{16} as the internal standard. Target standard concentrations ranged from 0.78 ng/mL to 1000 ng/mL. The injection volume was 25 µL.

Method

The extract clean-up was accomplished using a TurboFlow™ method run on a Thermo Scientific Aria TLX-1 LC system using a TurboFlow Cyclone P polymer-based extraction column. Large molecules were not retained and were moved to waste during the loading step while the analyte of interest was retained on the extraction column. This was followed by organic elution to a Thermo Scientific Hypersil GOLD aQ end-capped, silica-based C18 reversed phase analytical column and gradient elution to a TSQ Vantage™ MS with an APCI source. The BPA precursor *m/z* 227 > 133 and 212 high-resolution selective reaction monitoring (H-SRM) transitions were monitored in negative ionization mode. The 133 *m/z* product ion for BPA was used for quantitation, and the 212 *m/z* product ion was used as confirmation. The precursor *m/z* 241 > 223 H-SRM transition was monitored for BPA-d_{16} because BPA-d_{16} is transformed into BPA-d_{14} (MW 242) in water. The total LC-MS/MS method run time was 5.6 minutes.

Aria™ TLX-1 System Parameters

TurboFlow Cyclone P column (0.5 x 50 mm)
Hypersil GOLD™ aQ (4 x 50 mm, 3 µm particle size)

Loading Pump Mobile Phases

Mobile Phase A: 10 mM Ammonium bicarbonate pH 10
Mobile Phase B: 0.1% Formic acid in ACN
Mobile Phase C: 20:40:40 Acetone: Acetonitrile: Isopropanol

Elution Pump

Mobile Phase A: H_2O
Mobile Phase B: Methanol

MS analysis was carried out on a TSQ Vantage triple stage quadrupole mass spectrometer. The MS conditions were as follows:

Mass Spectrometer Parameters

Ion Polarity:	Negative ion mode
Discharge Current:	4.0 V
Vaporizer Temperature:	60 °C
Capillary Temperature:	275 °C
Sheath Gas Pressure (N_2):	30 units
Auxiliary Gas Pressure (N_2):	5 units
Ion Sweep Gas Pressure (N_2):	2 units
Scan Type:	Highly Selective Reaction Monitoring (H-SRM)
Chrom Filter Peak Width:	7.0 s
Collision Gas Pressure:	1.2 mTorr
Declustering Voltage:	0 V
Scan Width:	0.002 *m/z*
Scan Time:	0.05 s
Q1:	0.200 Da
Q3:	0.700 Da
S-Lens (*m/z* 321):	77 V
Collision Energy (*m/z* 227 > 133):	27 V
(*m/z* 227 > 212):	19 V

The entire experiment was controlled by Aria operating software 1.6.2. The data were processed using Thermo Scientific LCQUAN 2.5.6 quantitative software after subtracting background using Thermo Scientific Xcalibur 2.0.7 SP1 data system software.

Results and Discussion

Because BPA exists in air (2 - 208 ng/m^3), dust (0.2 - 199 ng/g), water (5 - 320 ng/L) and in many other sources, it is almost impossible to obtain a real blank of BPA in the laboratory.[3] Therefore, we subtracted the pre-standard double blank peaks from all quantified data using the Xcalibur™ built-in background subtraction tool. Figure 2 shows comparison chromatography of BPA and BPA-d_{16} at the lower limit of quantitation (LLOQ) (0.78 ng/mL) and the upper limit of quantitation (ULOQ) (100 ng/mL). The data were processed using LCQUAN™ 2.5.6 data quantitation software. Matrix-matched calibration standards of BPA showed a linear response at greater than 2 orders of magnitude with r^2 = 0.9921 (Figure 3). All %CVs (n=3) were less than 20% for the LLOQ and less than 10% for all other points of the curve. As shown in Figure 4, the comparison between the pre-blank and post-high blank (before subtraction)

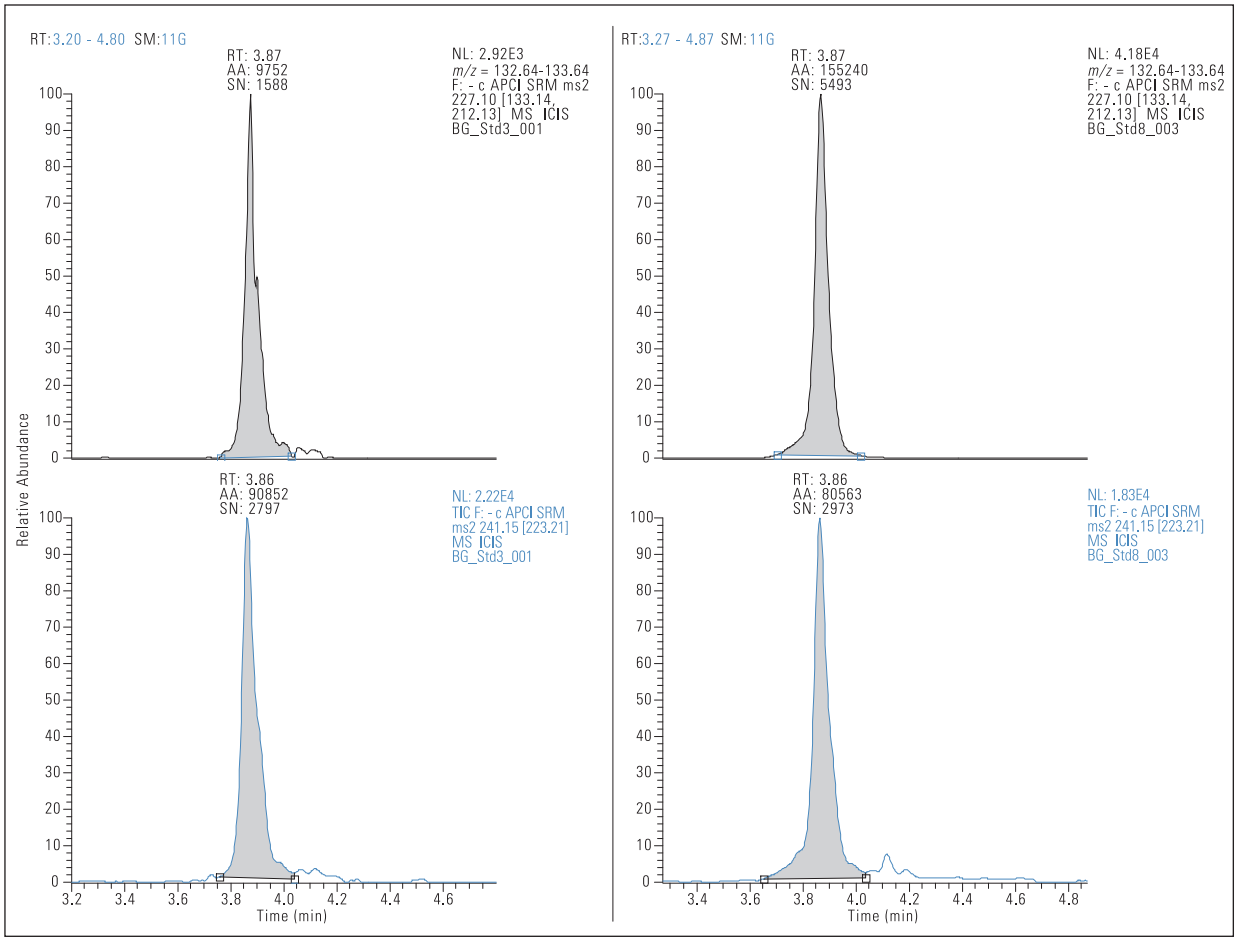

Figure 2: Chromatography comparison of BPA H-SRM *m/z* 133 transition (upper traces) and BPA-d_{16} (lower traces) at LLOQ of 0.78 ng/mL (left panel), and at ULOQ of 100 ng/mL (right panel)

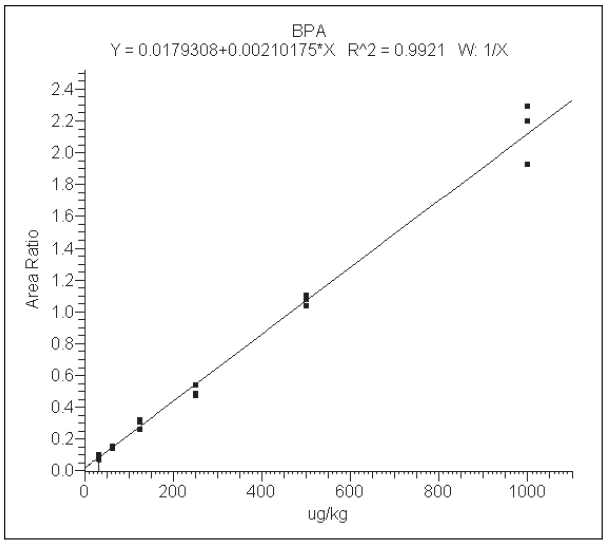

Figure 3: Linear regression curve of BPA standards based on area ratio with internal standard BPA-d_{16}

demonstrated the carryover could be ignored. The matrix interference was evaluated by comparing the chromatogram of the same concentration of BPA spiked in matrix and water. Figure 5 shows such a comparison at 12.5 and 25 ng/mL. As illustrated, the matrix interference was minimal.

We also compared the results of this TurboFlow technology LC-MS/MS study to another popular online solid phase extraction method.[4] Sample preparation times were very close due to few required offline sample treatment steps. The TurboFlow LC-MS/MS method run time, though, was four times faster. Because of differences in food matrices and the number of analytes, it is hard to compare the detection and quantitation limits directly. However, this comparison shows the benefits of using TurboFlow technology in the determination of BPA in food matrices.

Conclusion

A quick, automated sample preparation LC-MS/MS method has been developed that is sensitive enough to detect 7.80 µg/kg (ppb) dry powder (limit of detection) and quantify 31.3 µg/kg (ppb) dry powder (LLOQ) of BPA (background-adjusted) in infant formula powder for screening purposes. Compared to offline liquid/liquid or solid phase extractions, this method eliminates the need for time-consuming sample preparation procedures. The TurboFlow method also shows the advantage of fast separation over other online sample treatment techniques. The LC-MS/MS method run time is only 5.6 minutes, and the sample throughput can be improved by multiplexing on an Aria TLX-2 (or TLX-4) system.

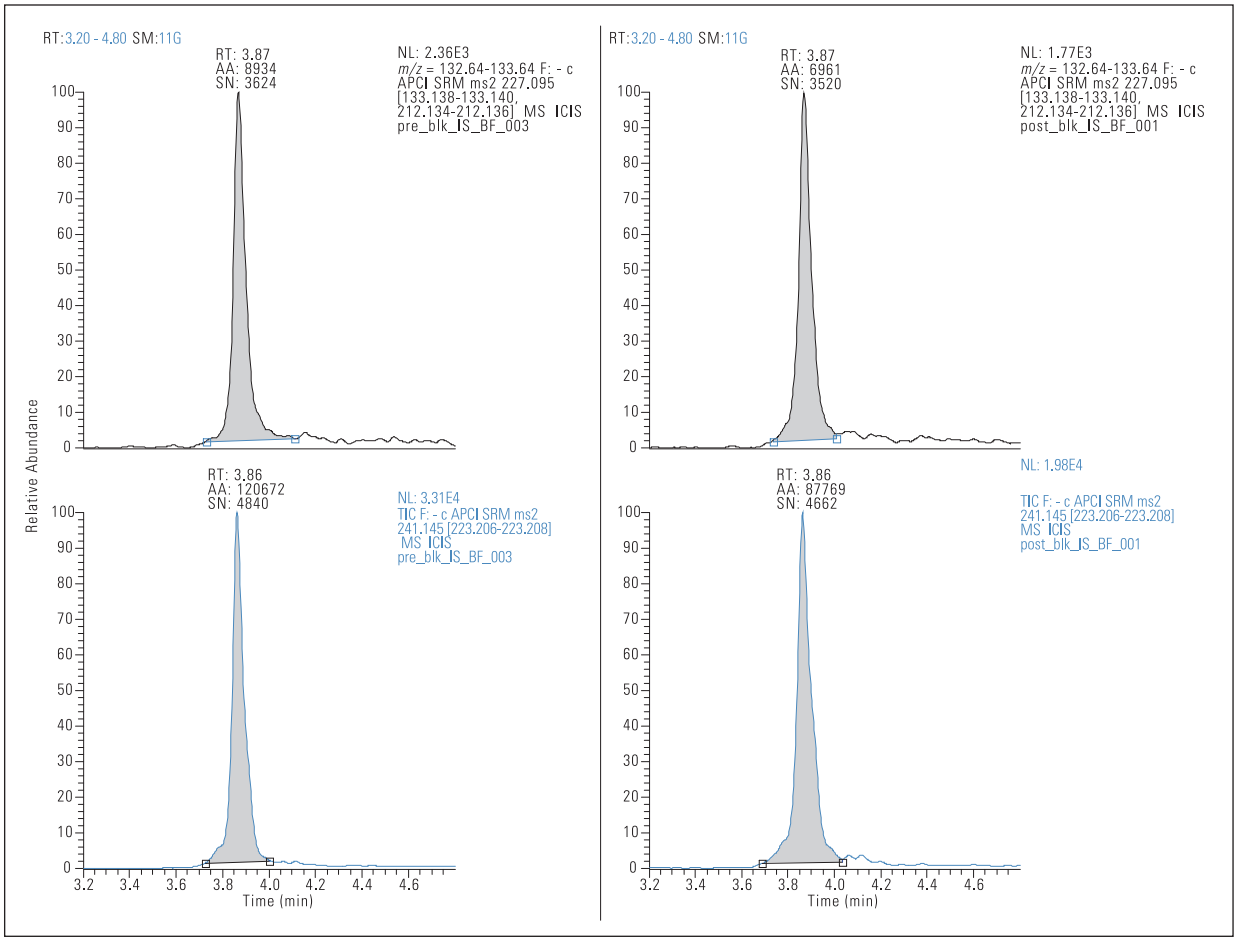

Figure 4: Chromatography comparison of BPA H-SRM *m/z* 133 transition (upper traces) and BPA-d_{16} (lower traces) in pre-blank infant formula matrix (left panel), and in post-high blank (right panel)

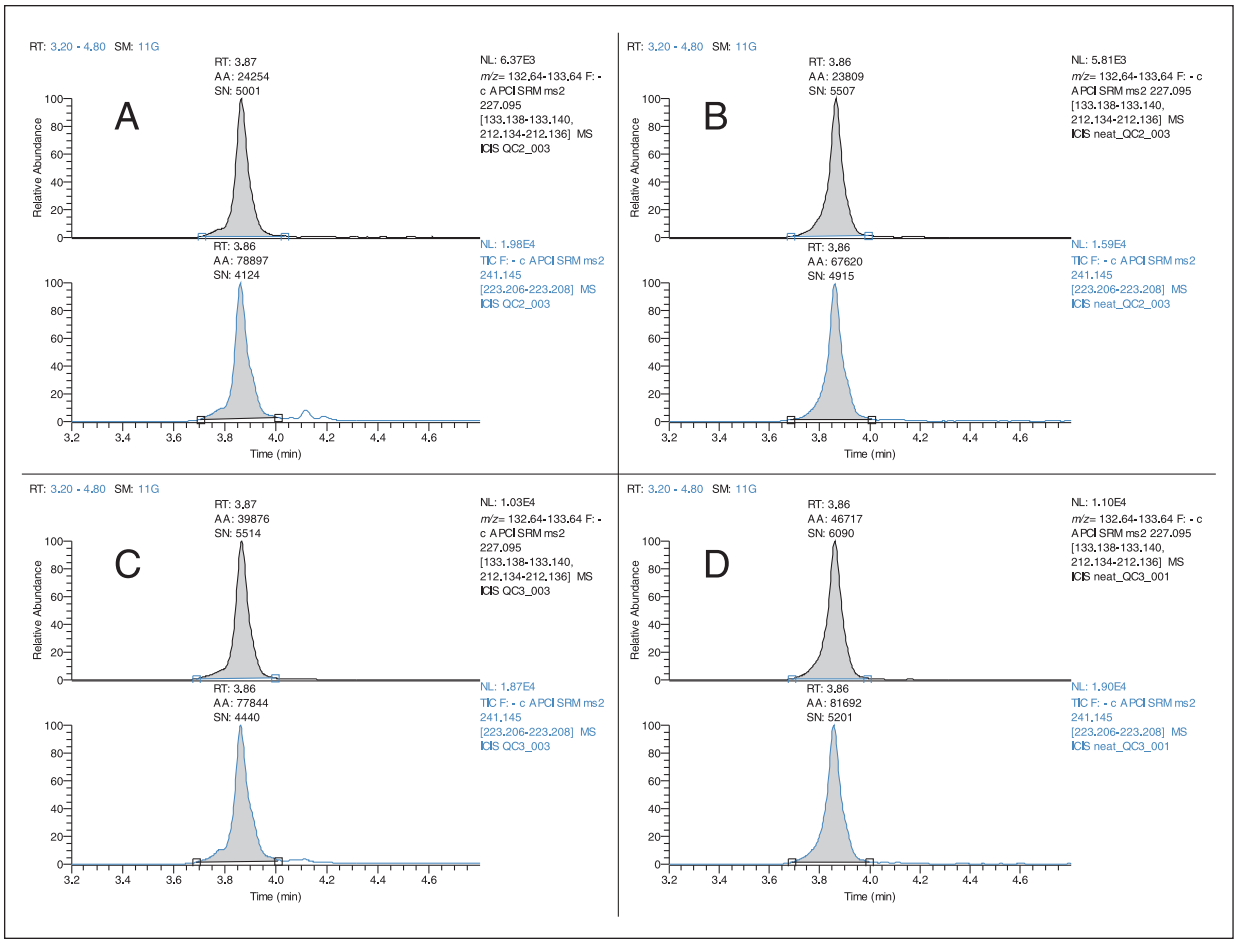

Figure 5: Chromatography comparison of BPA H-SRM *m/z* 133 transition (upper traces) and BPA-d_{16} (lower traces) at 12.5 ng/mL in matrix (panel A), at 12.5 ng/mL in water (panel B), at 25 ng/mL in matrix (panel C), and at 25 ng/mL in water (panel D)

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Application Note: 523

Detection of Mycotoxins in Corn Meal Extract Using Automated Online Sample Preparation with LC-MS/MS

Yang Shi, Catherine Lafontaine, François Espourteille
Thermo Fisher Scientific, Franklin, MA

Key Words

- Transcend TLX-1
- TurboFlow Technology
- TSQ Vantage
- Food Safety

Introduction

Since the discovery of aflatoxin in 1960, mycotoxin research has received considerable attention. Mycotoxins are a group of naturally occurring toxic substances produced by certain molds, which can contaminate food and feed. The inhalation or absorption of mycotoxins into the body may cause harm, including kidney or liver damage, cancer, or even death in man or animals.[1] From a food safety perspective, the aflatoxins, ochratoxin A, patulin, fumonisins, trichothecenes, and zearalenone are the mycotoxins of major concern.

Many countries now monitor mycotoxin levels in food and feed products. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is currently a common analytical approach for the quantification of mycotoxin contamination.[2] Sample preparation for LC-MS/MS analysis can be time and labor intensive, often involving pH modification, solid phase or immunoaffinity column clean-up extraction, multi-step extract clean-up, and pre-concentration.[3] The strict regulation published by the European Union in 1999 asking for lower detection limits and higher method reliability presented a new analytical challenge.[4]

In this study we describe an easy, comprehensive, LC-MS/MS method using a Thermo Scientific Transcend TLX-1 system powered by Thermo Scientific TurboFlow technology to analyze multiple mycotoxin residues in corn meal extract. Figure 1 illustrates a typical Transcend™ TLX-1 system with the Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Goal

Develop a rapid and sensitive automated, online sample preparation LC-MS/MS method to detect and quantify multiple mycotoxins in corn meal extract resulting in a shorter assay time and increased throughput.

Experimental

The matrix standard curve

Five grams of corn meal purchased from a local grocery store were extracted using 25 mL of 70% methanol in water followed by 60 minutes of ultra-sonication. The extract sat overnight at room temperature. The resulting solution was then centrifuged at 6000 RPM for 20 minutes. The supernatant was used to prepare the matrix calibrators and QC samples. Each milliliter of supernatant corresponds to 0.2 g solid corn meal powder as the unit of conversion.

Figure 1. Thermo Scientific Transcend TLX system with TSQ Vantage triple quadrupole mass spectrometer

The stock mix solution of analytes was prepared in methanol. Table 1 lists selected reaction monitoring (SRM) transitions and stock concentrations for individual analytes. Eight mycotoxins were analyzed under positive electrospray ionization (ESI) mode. The remaining three compounds, deoxynivalenol (DON), nivalenol (NIV), and 3-acetyl-DON (3-AcDON), were analyzed under negative electrospray ionization (ESI) mode.

Table 1. Analytes list

Compounds	Parent *(m/z)*	Primary *(m/z)*	Secondary *(m/z)*	Stock concentration (µg/mL)
Aflatoxins B1	313	241	285	0.050
Aflatoxins B2	315	259	287	0.015
Aflatoxins G1	329	243	283	0.050
Aflatoxins G2	331	245	275	0.015
Zearalenone (ZEA)	319	187	185	10.000
Ochratoxin A (OTA)	404	239	221	1.000
Fumonisins B1 (FB1)	722	334	352	2.500
Fumonisins B2 (FB2)	706	336	318	2.500
Deoxynivalenol (DON)	295	138	265	20.000
Nivalenol (NIV)	311	281	205	20.000
3-Acetyl-DON (3-AcDON)	337	307	173	20.000

LC/MS Methods using positive ESI mode (Method A):

TurboFlow™ Method Parameters

Column:	TurboFlow Cyclone-P 0.5 x 50 mm
Injection Volume:	10 µL
Solvent A:	10 mM ammonium acetate in water
Solvent B:	0.1% formic acid in acetonitrile (ACN)
Solvent C:	1:1:1 ACN: isopropanol: acetone (v:v:v) with 0.3% formic acid

HPLC Method Parameters

Analytical Column:	Thermo Scientific Hypersil GOLD 2.1 x 100 mm, 1.9 µm
Solvent A:	0.1% formic acid in water
Solvent B:	0.1% formic acid in ACN

Mass Spectrometer Parameters

MS:	TSQ Vantage™ triple stage quadrupole mass spectrometer
MS Ionization Source:	Heated Electrospray Ionization (H-ESI)
Spray Voltage:	5 KV
Sheath Gas Pressure (N_2):	50 arbitrary units
Auxiliary Gas Pressure (N_2):	20 arbitrary units
Vaporizer Temperature:	209 °C
Capillary Temperature:	270 °C
Collision Gas Pressure:	1.5 mTorr

LC/MS Methods using negative ESI mode (Method B):

TurboFlow Method Parameter

Column:	Research column A 0.5 x 50 mm
Injection Volume:	10 µL
Solvent A:	water
Solvent B:	methanol
Solvent C:	0.1% ammonium hydroxide
Solvent C:	45:45:10 ACN: isopropanol: acetone (v:v:v)

HPLC Method Parameters

Analytical Column:	Hypersil GOLD™ 2.1 x 50 mm, 1.9 µm
Solvent A:	0.1% formic acid in water
Solvent B:	0.1% formic acid in ACN

Mass Spectrometer Parameters

MS:	TSQ Vantage triple stage quadrupole mass spectrometer
MS Ionization Source:	H-ESI
Spray Voltage:	4.5 kV
Sheath Gas Pressure (N_2):	50 arbitrary units
Auxiliary Gas Pressure (N_2):	20 arbitrary units
Vaporizer Temperature:	250 °C
Capillary Temperature:	270 °C
Collision Gas Pressure:	1.5 mTorr

The LC method views from Thermo Scientific Aria Operating Software are shown in Figures 2 and 3.

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	0.00	45	2.00	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0
2	0.75	5	0.10	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0
3	0.83	120	0.10	Step	100.0	-	-	-	T	in	0.30	Step	98.0	2.0
4	2.83	5	2.00	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0
5	2.92	90	2.00	Step	-	-	100.0	-	====	out	0.30	Ramp	60.0	40.0
6	4.42	220	2.00	Step	-	100.0	-	-	====	out	0.30	Ramp	30.0	70.0
7	8.08	220	2.00	Step	-	-	100.0	-	====	out	0.30	Ramp	2.0	98.0
8	11.75	45	2.00	Step	-	100.0	-	-	====	in	0.30	Step	2.0	98.0
9	12.50	180	2.00	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0

Figure 2. Method A view in Aria OS software

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	00:00	45	1.50	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0
2	00:45	60	0.07	Step	100.0	-	-	-	T	in	0.50	Step	98.0	2.0
3	01:45	45	1.50	Step	-	100.0	-	-	====	out	0.30	Ramp	20.0	80.0
4	02:30	45	1.50	Step	-	100.0	-	-	====	out	0.30	Ramp	10.0	90.0
5	03:15	45	1.50	Step	-	50.0	50.0	-	====	out	0.30	Ramp	2.0	98.0
6	04:00	30	1.50	Step	-	-	-	100.0	====	out	0.30	Step	2.0	98.0
7	04:30	15	1.50	Step	-	100.0	-	-	====	out	0.30	Step	2.0	98.0
8	04:45	30	1.50	Step	-	5.0	95.0	-	====	in	0.30	Step	98.0	2.0
9	05:15	150	1.50	Step	100.0	-	-	-	====	out	0.30	Step	98.0	2.0

Figure 3. Method B view in Aria OS software

Results and Discussion

Figure 4 shows the comparison of chromatograms of eight analytes at 1:100 dilutions in methanol and corn meal extract, indicating excellent chromatographic separation in both solvent standard and matrix. Matrix-matched calibration standards showed linear response of two orders of magnitude ($r^2 > 0.99$) for six of them (Table 2). Significant signal enhancement was observed for FB1 and FB2 due to matrix-induced ionization variability, which was previously reported by other researchers.[5] In future work, the isotope-labeled internal standard might be used to compensate for the matrix interference.

Because DON, NIV, and 3-AcDON have a better signal response under negative ionization mode, a separate LC-MS/MS method was developed. Figure 5 shows the chromatograms of DON, NIV, and 3-AcDON identified at 100 ng/mL fortified in the corn meal extract.

Figure 6 presents the linear fit calibration curves for DON and NIV, indicating excellent linear fits over the dynamic range. Table 3 summarizes detection, quantitation limits, and standard curve linearity for three analytes analyzed in negative ion mode. For all analytes, the quantitation limits obtained using the present methodology comply with the maximum levels in foods defined by European Union.[6] To the best of our knowledge, this is the first application of its type to detect these three compounds using an automated online sample preparation technique coupled to tandem mass spectrometry.

In addition, a lower limit of quantitation (LOQ) could be achieved by increasing sample injection volume since TurboFlow columns can handle larger injections (up to a few hundred microliters) while regular HPLC or UHPLC columns can not.

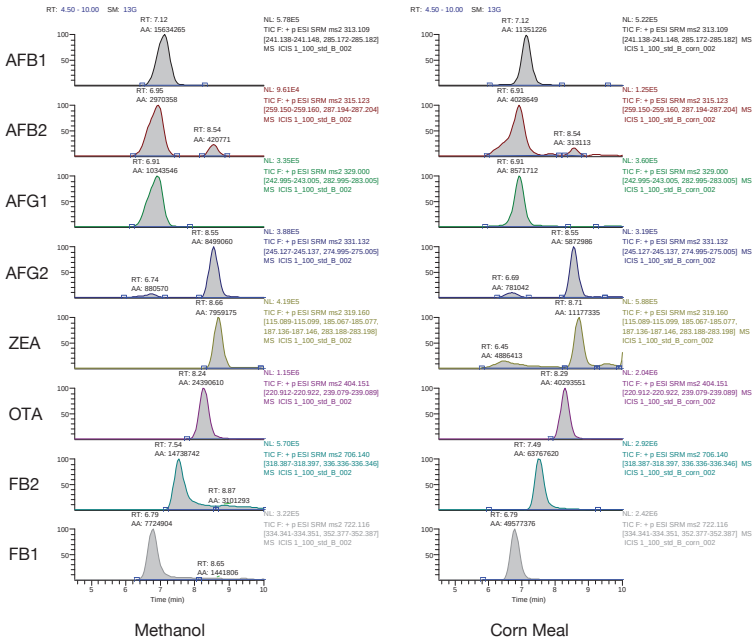

Figure 4. Comparison of chromatograms of 8 SRM analytes in methanol and corn flour extract (1:100 dilution of stock mixture)

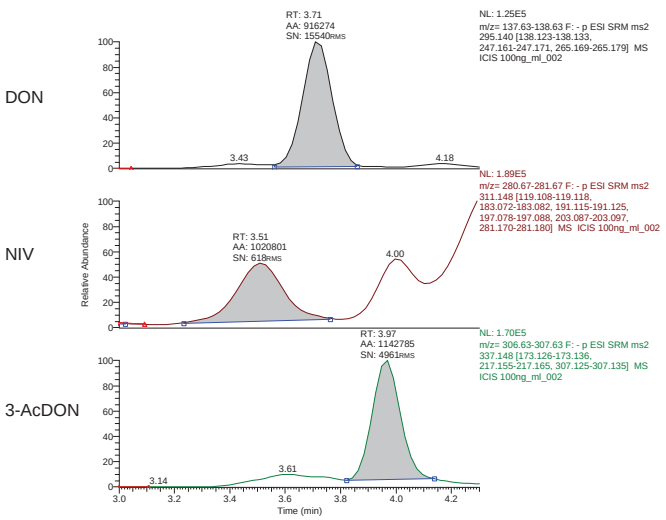

Figure 5 Selected chromatograms of DON, NIV, and 3-AcDON detected at 100 ng/mL fortified in the corn meal extract

Table 2. Limit quantitation (LOQ) and standard curve linearity (r^2) for analytes detected in positive ion mode

Compounds	LOQ (ng/g)	r^2
B1	0.50	0.9956
G1	0.50	0.9910
OTA	5.00	0.9937
ZEA	50.00	0.9955
FB1	12.50	0.9984
FB2	12.50	0.9965

Table 3. LOQ and standard curve linearity for analytes detected in negative ion mode

Compounds	LOQ (ng/g)	r^2
Deoxynivalenol (DON)	25.00	0.9934
Nivalenol (NIV)	25.00	0.9933
3-Acetyl-DON (3-AcDON)	25.00	0.9925

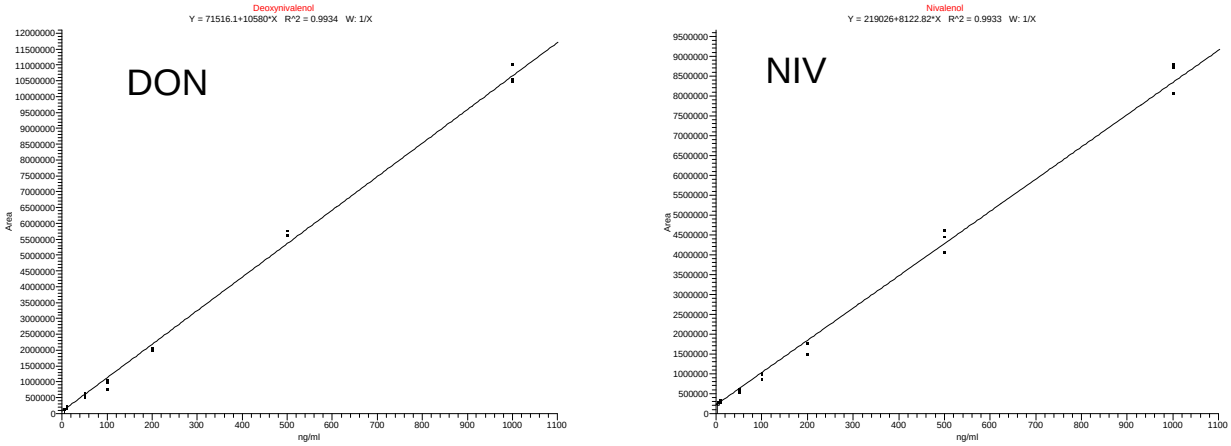

Figure 6. Calibration curves for DON and NIV

Conclusion

Developing a rapid and sensitive quantitative method is always a major goal for mycotoxins analysis.[7] Two quick, automated online sample preparation LC-MS/MS methods have been developed that are sensitive enough to detect mycotoxins in corn meal extract. By eliminating manual sample preparation, the reliability of this methodology was improved significantly. The sample throughput could be improved by multiplexing the two methods on different LC channels using a Transcend TLX-2 (or TLX-4) system. Future work will focus on the application of this methodology on various food matrices and references.

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Multi-mycotoxin Screening and Quantitation Using UHPLC, High Resolution and Accurate Mass

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Key Words

- Exactive LC-MS
- Beer
- Cereals
- Mycotoxins

Introduction

Mycotoxins are the toxic secondary metabolites produced by many species of microscopic filamentary fungi occurring on field cereals, including barley. The most abundant fungal genera affecting the malting barley are *Alternaria, Aspergillus, Penicillium* and *Fusarium*, which simultaneously showed relatively high-producing potential for a wide range of mycotoxins.[1] In addition to the relatively common micro mycetes mentioned above, *Claviceps purpurea* which causes ergot disease, belongs to numerous barley pathogens.

Although the carry-over of aflatoxins, ochratoxin A, zearalenone, fumonisins, and ergot alkaloids from malted grains into beer was documented, the main research in this area focused on deoxynivalenol, the most frequent Fusarium mycotoxin.[2,3] In recent years, the presence of deoxynivalenol's main metabolite, deoxynivalenol-3-glucoside, has been reported at relatively high levels in malt and beer. This fact was further confirmed in the follow-up study, in which both deoxynivalenol and its glucoside were identified as the main contaminants of beers retailed on the European market.[4] As beer is a significant dietary constituent to a large portion of the population, control of mycotoxins in this commodity is very important. For this purpose, reliable analytical methods for fast and effective monitoring of mycotoxins during the beer production chain are needed.

There is a trend toward the simplification of sample preparation procedures as much as possible. Full spectral data acquisition techniques are also preferred because of their ease of usage, along with the possibility of retrospective archived data mining. Until now, the most common full spectral mass-spectrometric approach has been the time-of-flight technology (TOF-MS), with typical resolving power of approx. 12,500 FWHM (full width half maximum). However, in complex food matrices such as beer, this rather limited mass resolving power leads to the risk of inaccurate mass measurements caused by unresolved background matrix interferences.[5,6] Mass spectrometry systems based on the Thermo Scientific Orbitrap technology routinely achieve mass resolving power of up to 100,000 FWHM and maintain excellent mass accuracy up to <5 ppm without the use of internal mass correction.[7]

The aim of this study was to introduce a multi-mycotoxin method for analysis of 32 mycotoxins in beer based on very simple sample preparation and ultra high performance liquid chromatography coupled with full spectral Orbitrap™ MS detection.

Mycotoxin standards of (i) Fusarium toxins, major conjugate and other products of transformation (nivalenol, deoxynivalenol, deoxynivalenol-3-glucoside, deepoxydeoxy - nivalenol, fusarenon-X, neosolaniol, 3-acetyldeoxynivalenol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, verrucarol, zearalenone, α-zearalenole, β-zearalenole); (ii) aflatoxins (aflatoxin G1, aflatoxin G2, aflatoxin B1, aflatoxin B2), (iii) sterigmatocystin; and (iv) ochratoxins (ochratoxin A, and ochratoxin α) were purchased from Biopure (Tulln, Austria), standards of (v) alternaria toxins (altenuene, alternariol, and alternariol-methylether) were obtained from Sigma-Aldrich (Taufkirchen, Germany), and standards of (vi) ergot alkaloids (ergosine, ergocornine, ergocryptine, ergocristine) were provided by The Czech Agricultural and Food Inspection Authority. The purity of standards was declared in the range 96–98.9%. Solid standards of nivalenol, deoxynivalenol, fusarenon-X, neosolaniol, 3-acetyldeoxynivalenol, T-2 toxin, verrucarol, zearalenone, α-zearalenole, β-zearalenole, sterigmatocystin, ochratoxin A, altenuene, alternariol and alternariol-methylether were dissolved in acetonitrile. Liquid standards of deepoxyde-oxynivalenol, diacetoxyscirpenol, HT-2 toxin, alfa-zear-alenole, beta-zearalenole, ochratoxin α, and ergot alkaloids were supplied in acetonitrile, and deoxynivalenol-3-glucoside was delivered in acetonitrile:water (1:1, v/v) solution. All of the standards were stored at -20 °C. For spiking experiments and calibration purposes, a composite working standard solution in acetonitrile (1000 µg L^{-1}) was prepared. All of the standards were brought to room temperature before use. The organic solvents acetonitrile and methanol (HPLC grade) were obtained from Sigma-Aldrich (Taufkirchen, Germany). Ultra-pure water was produced by Milli-Q system (Millipore Corporation, Bedford, MA, USA).

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Sample Preparation

The aliquot of 4 mL of beer sample in PTFE cuvette was degassed in the ultrasonic bath, and after addition of 16 mL acetonitrile, the content was vigorously shaken for approximately 1 min. The dark colored matrix precipitated under these conditions and was then separated by centrifugation (10 min, 11,000 rpm). In the next step, the 5 mL aliquot of the supernatant was evaporated to dryness and reconstituted in 1 mL of methanol:water (50:50, v/v). To avoid obstruction of the UHPLC system, microfiltration was performed prior to injection (centrifugation through the 0.2 µm microfilter, (PVDF Zentrifugenfilter, Alltech, USA)).

To control potential losses due to partition between precipitate and aqueous phase, aliquots of ^{13C}-labelled deoxynivalenol and ^{13C}-labelled zearalenone standard solution were added as the surrogates prior to processing (^{13C}-deoxynivalenol and ^{13C}-zearalenone for correction of more and less polar analytes, respectively).

Instrument Setup and Conditions

The Thermo Scientific Accela UHPLC system was used for the separation of target analytes. Detection was carried out using a Thermo Scientific Exactive benchtop single stage mass spectrometer, powered by Orbitrap technology and operated in full scan mode at different resolution settings. The use of internal mass axis calibration (lock mass) was not necessary. Conditions used are summarized in Table 1. The capillary and tube lens were set for ±45 and ±115 V respectively.

For the mass accuracy estimation, the mass at the apex of the chromatographic peak, obtained as the extracted ion chromatogram, was used. The calculated (exact) masses of quantification ions are summarized in Table 2.

Results and Discussion

Considering the current trend of analyzing for multiple food contaminants while maintaining high throughput and simplified sample preparation, direct analysis of a liquid sample may seem like the preferred option. However, in this case, direct injection of the matrix directly on the chromatographic column was not feasible because of its very high complexity. Direct injection also provided poor detectability of target analytes due to high matrix interference. In addition to this limitation, direct injection also lowered the analytical column lifetime and rapidly contaminated the ion source. Because of the complex properties of the 32 mycotoxins and their metabolites, neither adsorption nor immunoaffinity chromatography represented a feasible sample preparative strategy. The only simple approach to eliminating at least part of the matrix components, while keeping target analytes in solution, was by reducing the polarity of beer sample by addition of water-miscible solvent – acetonitrile.

It should be noted, that until now, most published studies concerned with determination of multiple mycotoxins in a single analysis used electrospray source ionization (ESI). However, the detection limits obtained by ESI were still rather poor for several Fusarium toxins, particularly for DON and its conjugate. Due to the importance of reliable analysis of these very common natural beer contaminants, the capability of atmospheric pressure chemical ionization (APCI) was evaluated. The optimal flow rate of mobile phase was determined to be 5 mL min^{-1} and the vaporizer temperature was set to 250 °C. Under APCI conditions, the enhancement in detectability of Fusarium toxins was as high as 1200% of the value achievable by ESI.

UHPLC Conditions		**MS Conditions (APCI)**	
Column	Hypersil GOLD aQ, 100 mm × 2.1 mm i.d., 1.9 µm	Sheath Gas	35 units
Mobile phase A	5 mM NH_4COOH in water	Auxiliary Gas	10 units
Mobile phase B	Methanol	Capillary Temperature	250 °C
Flow Rate	500 µL/min	Vaporizer Temperature	250 °C
Column Temperature	40 °C	Capillary Voltage	+60/-50 V
Injection Volume	5 µL	Discharge Current	5 µA
Gradient Elution Program		Scan Range	100-1000 *m/z*
0.0 min	5% B	Resolution Settings	10,000
6.0 min	50% B	(FWHM)	25,000
10.0 min	95% B		50,000
15.0 min	95% B		100,000
15.1 min	5% B		
18.0 min	5% B		

Table 1: Accela™ UHPLC/Exactive MS settings

Recommended Thermo Fisher Scientific Supplies

- Hypersil GOLD aQ, *p/n 25302-102130*, Thermo Scientific
- Methanol Optima LC/MS Grade, *p/n A456-212*, Fisher Scientific
- Acetonitrile Optima LC/MS Grade, *p/n A955-212*, Fisher Scientific
- Water, *p/n W6-212*, Fisher Scientific
- Ammonium Formate, *p/n A666-500*, Fisher Scientific
- Fisherbrand™ Higher-Speed Easy Reader Plastic Centrifuge Tubes, *p/n 06-443-19*, Fisher Scientific

Analyte	Retention Time (min)	Elemental Formula	Molecular Weight Da	Exact Mass $[M+H]^+$ *m/z*	Exact Mass $[M+NH_4]^+$ *m/z*	Exact Mass $[M-H]^-$ *m/z*	Exact Mass $[M+HCOO]^-$ *m/z*
Nivalenol	2.4	$C_{15}H_{20}O_7$	312.1209				357.1191
Deoxynivalenol	3.3	$C15H_{20}O_6$	296.1260				341.1242
Deoxynivalenol-3-glucoside	3.4	$C_{21}H_{30}O_{11}$	458.1788				503.1770
Deepoxydeoxynivalenol	4.5	$C_{15}H_{20}O_5$	280.1311				325.1293
Fusarenon-X	4.5	$C_{17}H_{22}O_8$	354.1315				399.1297
Neosolaniol	4.9	$C_{19}H_{26}O_8$	382.1628		400.1966		
Verrucarol	5.2	$C_{15}H_{22}O_4$	266.1518		284.1856		
3-acetyldeoxynivalenol	5.7	$C_{17}H_{22}O_7$	338.1366				383.1348
Ochratoxin α	5.7	$C_{11}H_9ClO_5$	256.0139			255.0061	
Aflatoxin G2	6.5	$C_{17}H_{14}O_7$	330.0740	331.0812			
Aflatoxin G1	6.8	$C_{17}H_{12}O_7$	328.0583	329.0656			
Altenuene	7.1	$C_{15}H_{16}O_6$	292.0947				337.0924
Aflatoxin B2	7.2	$C_{17}H_{14}O_6$	314.0790	315.0863			
Aflatoxin B1	7.5	$C_{17}H_{12}O_6$	312.0634	313.0707			
Diacetoxyscirpenol	7.6	$C_{19}H_{26}O_7$	366.1779		384.2017		
Ochratoxin A	8.5	$C_{20}H_{18}ClNO_6$	403.0823	404.0901			
Alternariol	8.7	$C_{14}H_{10}O_5$	258.0528			257.045	
HT-2 Toxin	8.7	$C_{22}H_{32}O_8$	424.2097		442.2435		
β-zearalenol	9.2	$C_{18}H_{24}O_5$	320.1624			319.1546	
T-2 Toxin	9.6	$C_{24}H_{34}O_9$	466.2203		484.2541		
α-zearalenol	9.9	$C_{18}H_{24}O_5$	320.1624			319.1546	
Ergosin	10.2	$C_{30}H_{37}N_5O_5$	547.2795	548.2867			
Zearalenone	10.2	$C_{18}H_{22}O_5$	318.1467			317.1394	
Sterigmatocystin	10.6	$C_{18}H_{12}O_6$	324.0634	325.0712			
Alternariol-methylether	10.7	$C_{15}H_{12}O_5$	272.0685			271.0607	
Ergocornine	10.7	$C_{31}H_{39}N_5O_5$	561.2951	562.3024			
Ergosinine	11.8	$C_{30}H_{37}N_5O_5$	547.2795	548.2867			
Ergocryptine	11.1	$C_{32}H_{41}N_5O_5$	575.3108	576.3180			
Ergocristine	11.2	$C_{35}H_{39}N_5O_5$	609.2951	610.3024			
Ergocorninine	11.8	$C_{31}H_{39}N_5O_5$	561.2951	562.3024			
Ergocryptinine	12.1	$C_{32}H_{41}N_5O_5$	575.3108	576.3180			
Ergocristinine	12.3	$C_{35}H_{39}N_5O_5$	609.2951	610.3024			

Table 2: Overview of the most intensive ions used for quantification by the Exactive

The lone exception was ochratoxin A, which showed better ionization efficiency under the electrospray conditions, APCI was chosen for use because it provided significant improvement of detection limits for most of the tested analytes. The extracted ion chromatograms of individual mycotoxins shown in Figure 1 document very good and fast separation achieved on the Accela™ UHPLC system.

In a routine trace analysis, both high mass resolving power and high mass accuracy play an important role in the unbiased identification and reliable quantification of target analytes.[5] Figure 2 illustrates the benefits of high resolving power setting on the discrimination of isobaric interferences. The importance of optimal choice of extraction window width is demonstrated here mainly for the use of lower mass resolution. While the use of a wide mass window typically results in worsened selectivity, using a narrow mass window presents a risk of removing some analytes from the chromatogram.

As demonstrated in Figure 3, the risk of false negative results occurs, especially for low intensity ions. While 50 µg L^{-1} of deoxynivalenol-3-glucoside was still detectable at the mass resolving power setting of 10,000 FWHM, almost no signal was detected by the same mass resolution at level 5 µg L^{-1}. At resolving power of 25,000 FWHM, the peak shape was improved. When the resolving power of 50,000 and/or 100,000 FWHM was enabled, optimal peak shape of deoxynivalenol-3-glucoside at 5 µg L^{-1} was obtained. As demonstrated, the higher resolving power, the better mass accuracy of deoxynivalenol-3-glucoside is obtained.

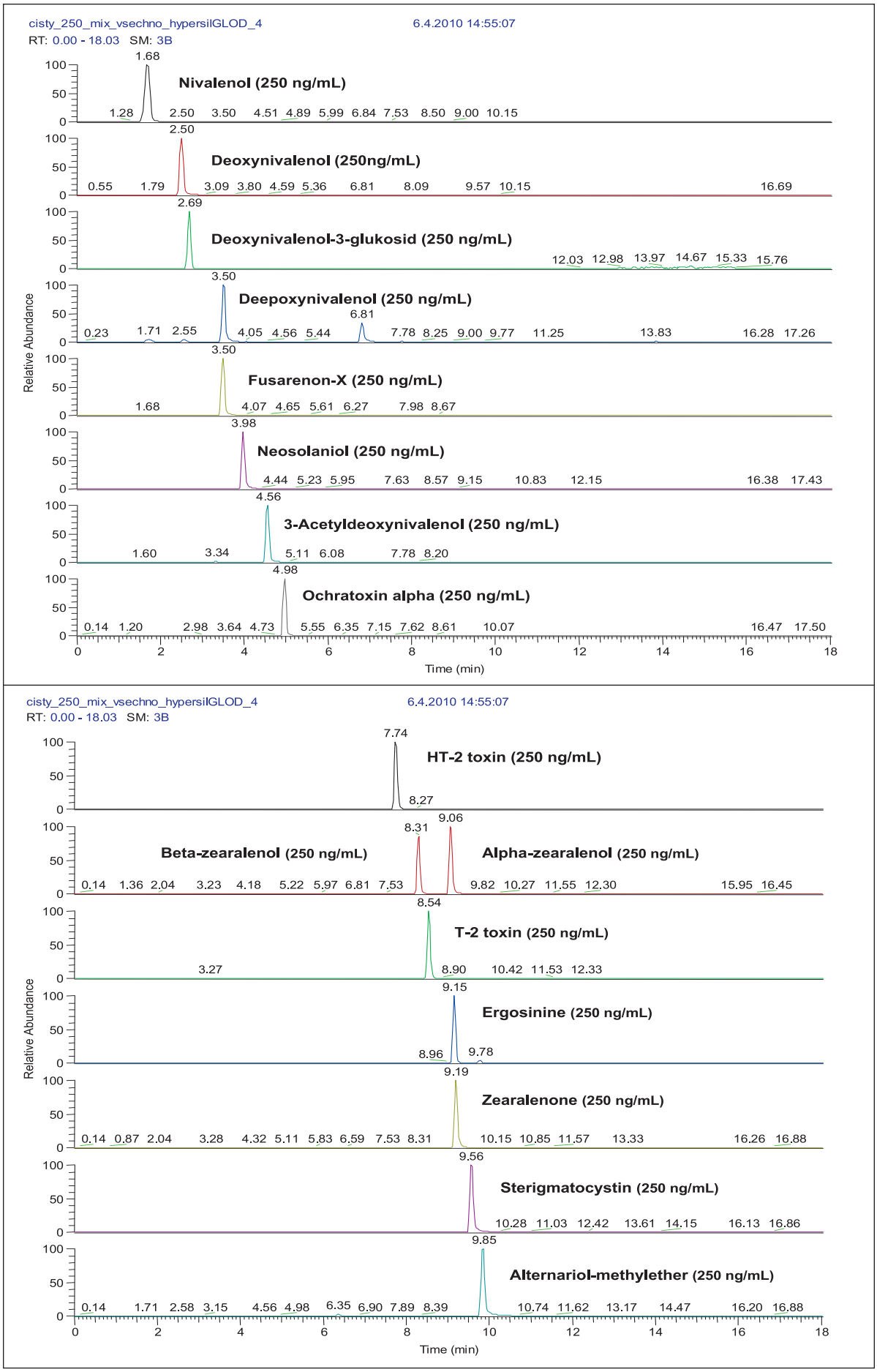

Figure 1: Extracted ion chromatograms of analyzed mycotoxins

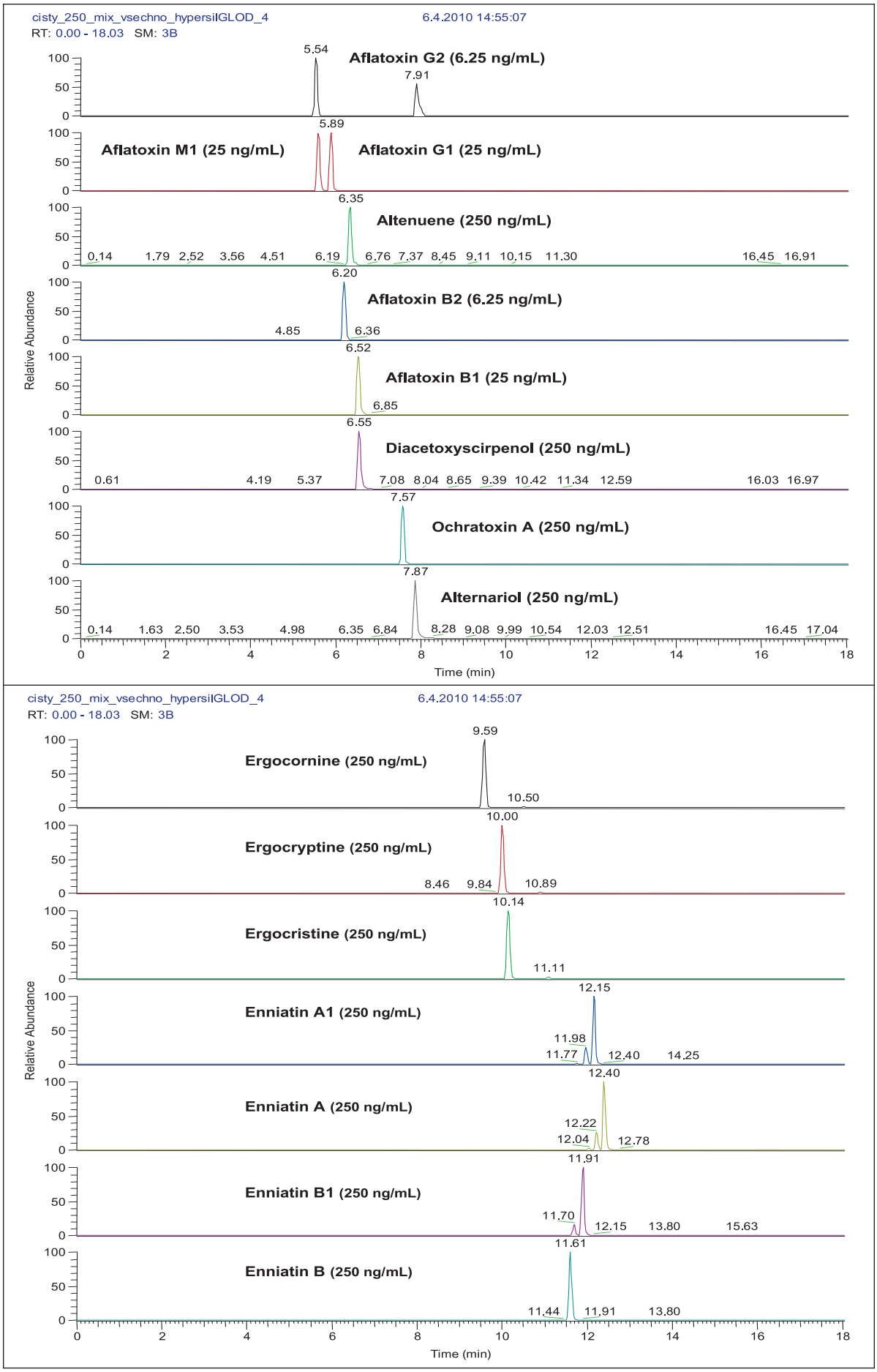

Figure 1 Continued: Extracted ion chromatograms of analyzed mycotoxins

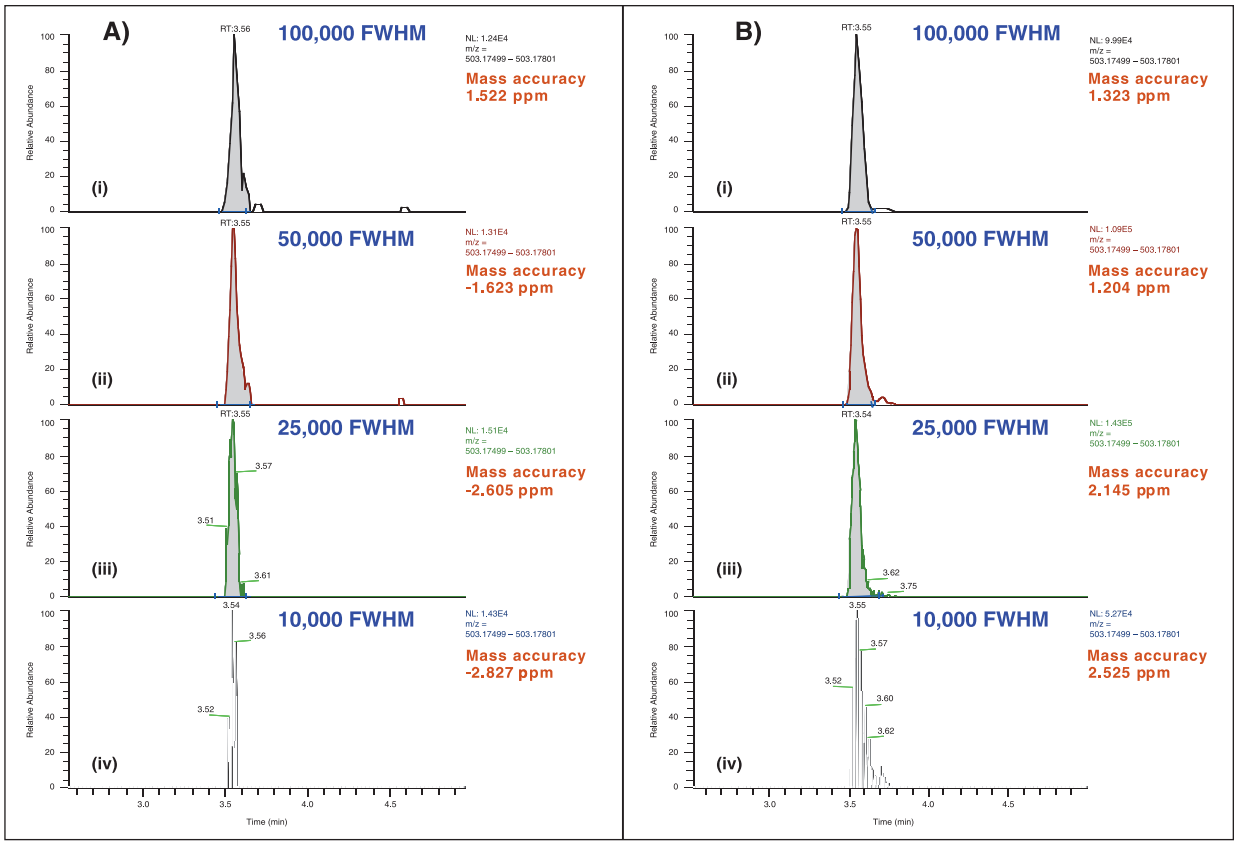

Figure 2: Extracted ion chromatograms of deoxynivalenol-3-glucoside in beer when performing four different resolving power settings (10,000; 25,000; 50,000; and 100,000 FWHM), mass extraction window ±3 ppm. The spiking levels were 5 µg L^{-1} (A) and 50 µg L^{-1} (B).

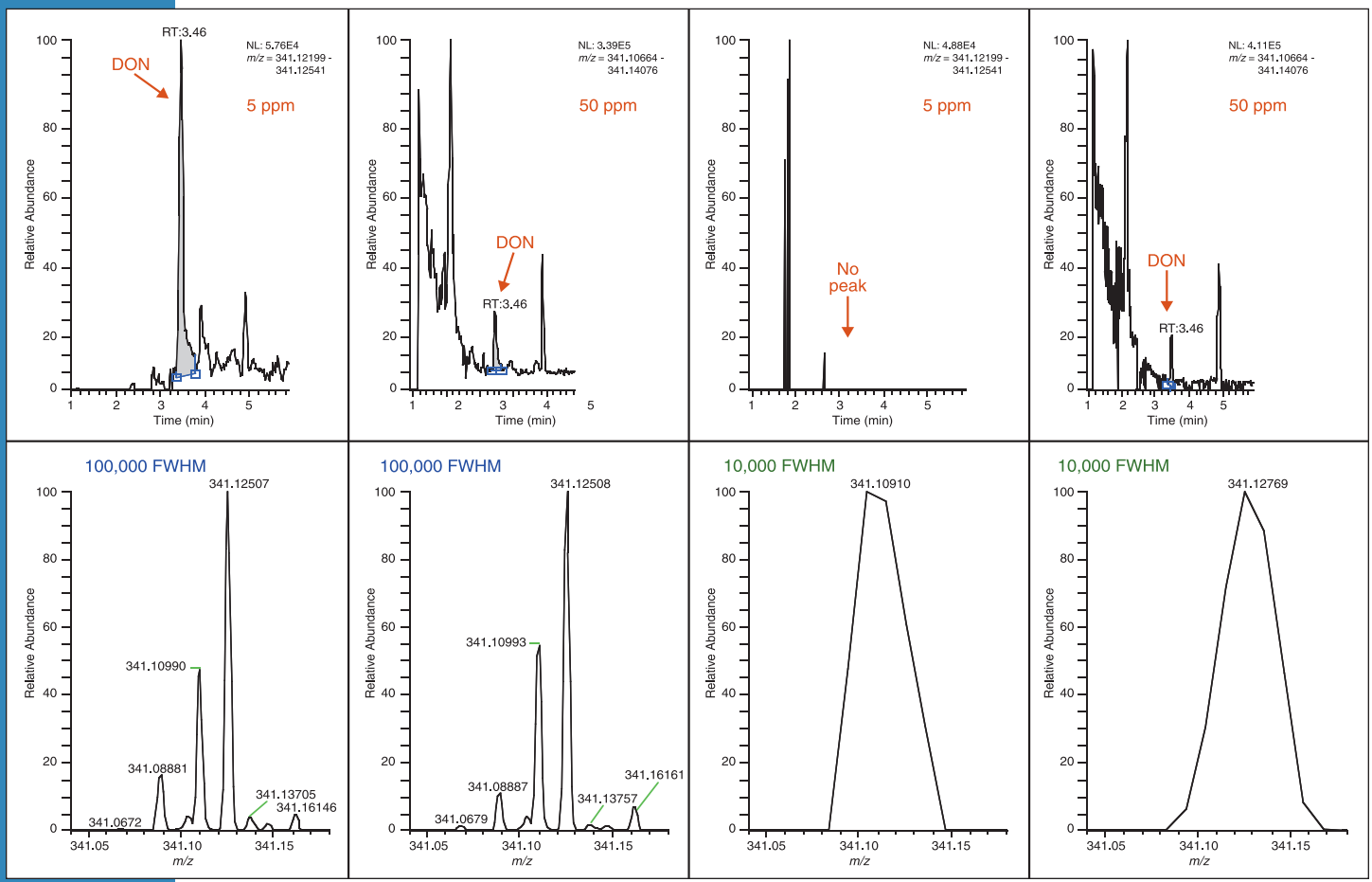

Figure 3: Extracted ion chromatograms and the mass spectra of deoxynivalenol in beer (10 µg L^{-1}) when performing two different resolving power settings (10,000 and 100,000 FWHM) and two different mass extraction windows (±5 and ±50 ppm).

Method Validation

The optimized multi-mycotoxin UHPLC-MS method was thoroughly validated. Prior to analysis of spiked samples, the extent of matrix effects was investigated in order to determine the quantification strategy. For this purpose, two calibration sets were prepared: (i) standards net solvent; (ii) matrix-matched standards. In both cases, the concentration of target mycotoxins was in the range 0.5–250 µg L^{-1}. Although the signal suppression/enhancement (SSE) range was not too broad (63–112%) matrix-matched calibration standards were used.

An important issue to address is calculating an equivalent to limit of quantification (LOQ). Tandem mass spectrometry's classical definition of LOQs based on signal to noise ratio (typically S/N > 6) is not always applicable in high resolution MS because a chemical noise is, in fact, absent in the chromatogram. Due to that fact, lowest calibration levels (LCL) were determined to be the most suitable option. The LCLs of analytes in our study were experimentally established as the lowest concentrations of matrix-matched standards repeatedly identified over time. The relative standard deviations of measurement calculated from nine repeated injections ranged between 11–28% (see Table 3). While these lowest calibration levels for 91% of analytes were at 1-10 µg L^{-1} level, a relatively high LCL level was found for ochratoxin A, which showed much better ionization under electrospray conditions (less than 5 µg L^{-1}).

Mycotoxin	LCL Pure Standard (µg L^{-1})	LCL Matrix-matched Standard (µg L^{-1})	Recovery %			RSD (%) at the Spiking Level 10 µg L^{-1} [1]	RSD (%) at the LCL Level[2]	SSE (%)[3]
			Spike 10 µg L^{-1}	Spike 30 µg L^{-1}	Spike 60 µg L^{-1}			
Nivalenol	2	6	107	97	103	8.9	19	92
Deoxynivalenol	2	3	104	112	99	4.9	24	112
Deoxynivalenol-3-glucoside	2	2	96	103	100	4.3	23	92
Deepoxydeoxynivalenol	4	15	102	116	104	7.2	19	94
Fusarenon-X	2	4	105	113	119	10.3	16	75
Neosolaniol	2	2	99	111	112	10.5	14	93
Verrucarol	3	4	98	99	101	8.4	18	84
3-acetyldeoxynivalenol	4	8	103	96	102	13.7	24	86
Ochratoxin α	4	31	102	98	108	9.8	21	67
Aflatoxin G2	1	2	103	106	99	10.9	25	65
Aflatoxin G1	1	4	117	94	107	8.9	19	63
Altenuene	0.5	1	119	120	113	8.4	22	93
Aflatoxin B2	0.5	1	111	106	104	5.5	12	91
Aflatoxin B1	0.5	2	107	90	92	5.2	13	105
Diacetoxyscirpenol	0.5	1	116	113	124	7.4	17	94
Ochratoxin A[4]	60	60	105	96	97	9.15[5]	26	84
Alternariol	0.5	2	101	107	98	8.5	16	76
HT-2 Toxin	2	4	117	116	104	6.9	19	87
β-zearalenol	1	2	111	92	98	9.1	11	85
T-2 Toxin	1	2	99	119	105	7.9	17	88
α-zearalenol	1	1	114	107	97	8.9	16	84
Ergosin	1	3	111	109	106	12.9	26	78
Zearalenone	1	1	106	117	105	9.4	19	91
Sterigmatocystin	0.5	0.5	118	98	110	11.6	16	107
Alternariol-methylether	1	1	114	109	113	9.1	14	88
Ergocornine	1	2	115	121	102	9.6	20	81
Ergosinine	1	2	98	114	102	8.4	12	91
Ergocryptine	1	2	103	111	101	14.9	23	101
Ergocristine	2	8	95	112	94	6.1	24	81
Ergocorninine	1	2	114	124	104	11.7	15	95
Ergocryptinine	1	2	88	113	101	11.4	26	97
Ergocristinine	2	8	104	119	99	9.1	28	103

Table 3: Validation data for the developed UHPLC-Orbitrap-MS method

1. RSD at the spiking level 10 µg L^{-1} was calculated from 6 spikes
2. RSD at the LCL level was calculated from 11 repeated injections of the particular matrix-matched standard
3. SSE (%) = matrix-matched calibration slope/solvent calibration slope * 100; SSE value of 100% means no effect of matrix on the ion signal
4. The spiking levels of ochratoxin A were 80, 100, and 120 µg L^{-1}
5. The RSD of ochratoxin A was determined at the spiking level of 100 µg L^{-1}

The linearity of the new method was tested for solvent as well as matrix-matched calibration curve constructed in the ranges LCL to 250 µg L^{-1}. The majority of analytes showed linearity in the range 0.9960–0.9999 (R^2). The recoveries of analytes tested at levels 10, 30, and 60 µg L^{-1} ranged from 92–124%, with no losses of analytes during the sample preparation occurred (Table 3).

Conclusion

The UHPLC-MS technology represents the most interesting alternative equivalent to tandem mass spectrometry with the possibility of retrospective data mining. Our UHPLC-MS operated in APCI mode enables rapid determination of trace levels of multiple mycotoxins occurring in complex beer samples. At the highest resolving power setting, 100,000 FWHM, the mass error up to 5 ppm (without the use of internal mass correction) enables the use of a very narrow mass extracting window, ±5 ppm, for the routine work, which significantly improves the selectivity of detection.

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Application Note: 52154

Non-Targeted Screening of Lipophilic Marine Biotoxins by Liquid Chromatography – High-Resolution Mass Spectrometry

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Key Words

- Exactive
- Hypersil GOLD
- Liquid Chromatography
- Marine Biotoxin
- Non-Targeted Screening
- Orbitrap Technology

Introduction

Marine biotoxins are produced by naturally occurring microalgae, whose populations can increase significantly under certain environmental conditions to form a harmful algal bloom (HAB). During the incidence of a bloom, marine biotoxins pose a significant food safety risk when bioaccumulated in shellfish that are ingested by humans. Therefore, adequate testing for biotoxins in shellfish is required to ensure public safety and long-term viability of commercial shellfish markets.

The lipophilic marine toxins class includes the dinophysistoxins, azaspiracids, pectenotoxins, and yessotoxins. The compounds are structurally diverse, as shown in Figure 1, and thus do not contain a common UV chromophore or reactive functional group for fluorescence derivatization. Therefore, LC-MS is the method of choice for their analyses and several MRM-based methods have been reported.[1-3]

In response to the need for non-targeted methods that can potentially detect unknowns, high-resolution LC-MS has been successfully implemented for screening and quantification in food safety applications.[4-6] The lower-cost, higher-mass accuracy, and ease-of-use of modern quadrupole time-of-flight (QTOF) and Thermo Scientific Orbitrap based mass spectrometers have made high-resolution systems viable alternatives to triple-quadrupole systems for routine analysis. After full-spectrum data acquisition, specificity is typically achieved by extracting narrow mass windows (ie. 2–5 ppm) centered around a list of target analytes. Using this approach, it has been demonstrated that a resolving power of 50,000 or greater is required for correct mass assignments in complex matrices.[6] This report describes the use of the Thermo Scientific Exactive benchtop LC/MS system powered by Orbitrap™ technology for screening lipophilic marine biotoxins commonly found in shellfish.[7] The method was optimized using a standard mixture of marine biotoxins, and then applied to a mussel tissue extract.

Experimental

Chemicals and Materials

Certified calibration solutions and mussel tissue reference materials were purchased from the NRC Certified Reference Materials Program (Halifax, Nova Scotia, Canada). Certified calibration solutions were used for the following biotoxins: okadaic acid (OA), dinophysistoxin-1 (DTX1), dinophysistoxin-2 (DTX2), pectenotoxin-2 (PTX2), azaspiracid-1 (AZA1), azaspiracid-2 (AZA2), azaspiracid-3 (AZA3), and yessotoxin (YTX). As a test sample, a mussel tissue containing certified levels of OA and DTX1 was used (CRM-DSP-Mus-b).

HPLC grade acetonitrile and formic acid (98%) were purchased from EMD chemicals (Gibbstown, NJ, USA). Distilled-in-glass grade methanol was acquired from Caledon Laboratories (Georgetown, ON, Canada), and ammonium formate (≥ 99.0%) was from Fluka (St. Louis, MO, USA).

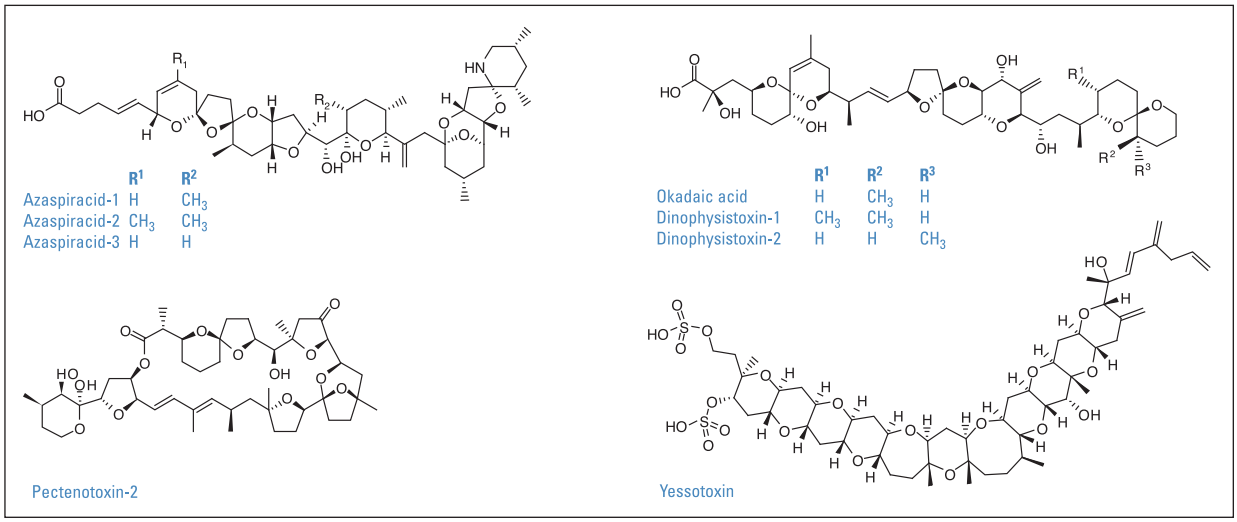

Figure 1: Chemical structures of the primary analogs of the regulated lipophilic marine biotoxins

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Extraction of Lipophilic Toxins From Mussel Tissue

Approximately 4 g of tissue was homogenized with 4 mL of 80% methanol solution using a Polytron PT3000 mixer (Brinkmann, USA) at 10,000 rpm with ice cooling. The sample was then centrifuged at 7,000 rpm for 15 minutes and the supernatant was decanted into a flask. Another 8 mL of 80% was used to clean the mixer by running the homogenizer briefly. The rinsate was centrifuged as before and this supernatant was combined with the first supernatant. 6 mL of 80% methanol was then added to the original pellet, which was homogenized again. After centrifugation, the final supernatant was combined with the previous two. The final volume was made up to 25 mL with 80% methanol solution. Approximately 0.5 mL of this solution was filtered through a 0.45 µm spin-filter (Millipore, Billerica, MA, USA) prior to analysis.

LC-MS Instrumentation and Method

LC-MS analysis was carried out on a Thermo Scientific Accela High-Speed LC coupled to an Exactive™ mass spectrometer, equipped with an Orbitrap mass analyzer and a HESI-II probe for electrospray ionization. The instrument was mass-calibrated daily for positive and negative modes, and the capillary and tube lens voltages were optimized daily, using the automated script within the Exactive acquisition software in both cases. For positive mode, mass calibration was performed with a mixture consisting of caffeine, MRFA tetrapeptide, and Ultramark 1621, while the negative mode calibration was performed with sodium dodecyl sulfate, sodium taurocholate, and Ultramark 1621. All analyses were performed using the 'balanced' automatic gain control (AGC) setting with a 50 ms maximum inject time. Data acquisition was carried out using Thermo Scientific Xcalibur 2.1. Optimal ion source and interface conditions consisted of a spray voltage of 3 kV, sheath gas flow of 50, capillary temperature of 360 °C, and a heater temperature of 250 °C. Alternating positive and negative polarity scans were acquired at a scan rate 2 Hz (50,000 resolution) for an overall cycle time of 1.25 seconds.

Lipophilic toxins were separated on a Thermo Scientific Hypersil GOLD C18 column (2.1 × 100 mm, 1.9 µm particle size), at a flow rate 400 µL/min and using 3 µL injections. Mobile phases were prepared from a stock solution of 1% formic acid solution in water with the pH adjusted to 3.0 using concentrated ammonium hydroxide. This stock solution was then diluted 10-fold with water (A) or acetonitrile (B), resulting in 0.1% formic acid in water for mobile phase A and 0.1% formic acid in 90% acetonitrile for B. Analytes were eluted with a linear gradient from 10 to 90% B from 0 to 2 min, held for 1 min, before returning to the initial conditions of 10% B.

Results

Lipophilic toxins were separated by reversed phase chromatography coupled to the Exactive mass spectrometer. As shown in Figure 2, eight lipophilic toxin standards were baseline separated in just under 6 min and the data shown represents 5 ppm extracted mass chromatograms centered around the masses of the target analytes. As OA, DTX1, DTX2, and YTX ionize significantly better in negative mode, alternative positive and negative polarity scans were acquired to achieve maximum signal for all analytes. To maintain a sufficient number of data points across chromatographic peaks, data was collected at a scan rate of 2 Hz. The scan rate of 2 Hz generates resolution of roughly 50,000, much lower than the maximum resolution possible with the mass spectrometer, but was selected as a reasonable compromise between selectivity and quantitative performance. In addition, it has been demonstrated that a

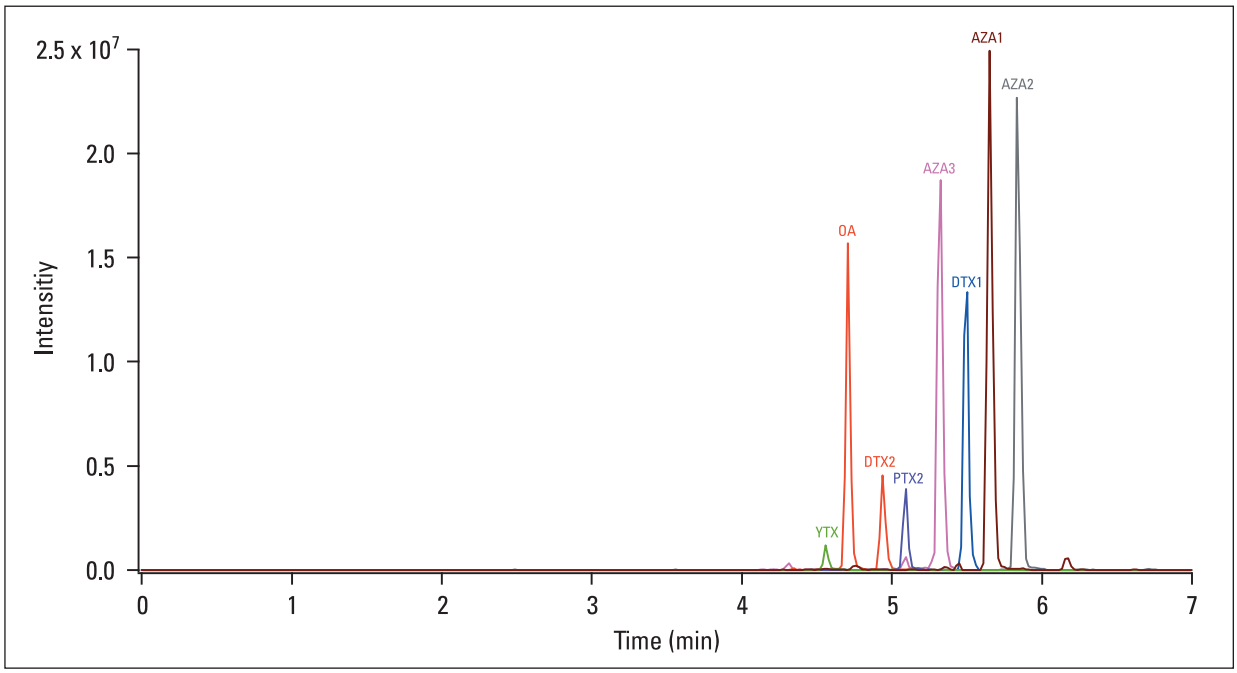

Figure 2: LC-MS chromatograms of eight lipophilic biotoxin standards acquired with alternating positive (PTX2, AZA1,-2,-3) and negative (YTX, OA, DTX1,-2) scans at 2 Hz. Data shown represents 5 ppm mass windows centered around the analyte mass.

resolving power of 50,000 provides sufficient specificity in complex matrices.[6] The ability to rapidly scan both positive and negative polarities allows data collection in a true non-targeted fashion and permits independent optimization of the LC method without consideration of the retention time of positive and negative analytes.

Listed in Table 1 are accurate masses and limits of detection for the lipophilic toxins using external calibration exclusively, without any mass correction on an internal standard or a background ion. In general, accurate masses are below 1 ppm error for analytes detected in positive mode, while those detected in negative mode range between 1–3 ppm error. Similarly, limits of detection ranged from 0.052–0.10 µg/L (ppb) for the positive ions, while those detected in negative mode were distinctly higher at 1.6–5.1 µg/L.

The utility of the screening method for lipophilic toxins was evaluated by analyzing a mussel tissue reference material containing certified levels of okadaic acid and DTX1, as shown in Figure 3. The top trace of Figure 3 represents the total ion chromatogram (TIC), revealing the complex matrix of the mussel tissue. Excellent specificity was demonstrated by the minimal background peaks detected in the 5 ppm mass windows associated with OA and DTX1 (lower trace), and OA and DTX1 are clearly discriminated from the complex matrix. Quantification against calibration with toxin standards in methanol yielded levels of OA and DTX1 of 4.1 µg/g and 0.58 µg/g, respectively, with precision of roughly 10% RSD for both analytes. These concentrations represent roughly half of the certified values for OA and DTX1, with ion suppression by the matrix being the likely cause for these discrepancies. Ion suppression effects are generally observed for all types of mass spectrometers employing electrospray ionization, and can be mitigated with the use of matrix-matched standards if accurate quantification is desired.[8]

Toxin	Tret (min)	Chemical Formula	Ion Detected	Calculated (*m/z*)	Observed (*m/z*)	Error (ppm)	LOD (µg/L)
YTX	4.63	$C_{55}H_{82}O_{21}S_2$	$[M-H]^-$	1141.47172	1141.47433	2.3	5.1
OA	4.81	$C_{44}H_{68}O_{13}$	$[M-H]^-$	803.45872	803.45963	1.1	2.8
DTX2	5.04	$C_{44}H_{68}O_{13}$	$[M-H]^-$	803.45872	803.46002	1.6	1.6
PTX2	5.19	$C_{47}H_{70}O_{14}$	$[M+NH4]^+$	876.51038	876.51067	0.33	0.10
AZA3	5.45	$C_{46}H_{69}NO_{12}$	$[M+H]^+$	828.48925	828.48973	0.58	0.062
DTX1	5.59	$C_{45}H_{70}O_{13}$	$[M-H]^-$	817.47427	817.47639	2.6	2.0
AZA1	5.78	$C_{47}H_{71}NO_{12}$	$[M+H]^+$	842.50490	842.50477	0.15	0.052
AZA2	5.96	$C_{48}H_{73}NO_{12}$	$[M+H]^+$	856.52055	856.52062	0.080	0.064

Table 1: Accurate masses and LODs for the lipophilic marine biotoxins

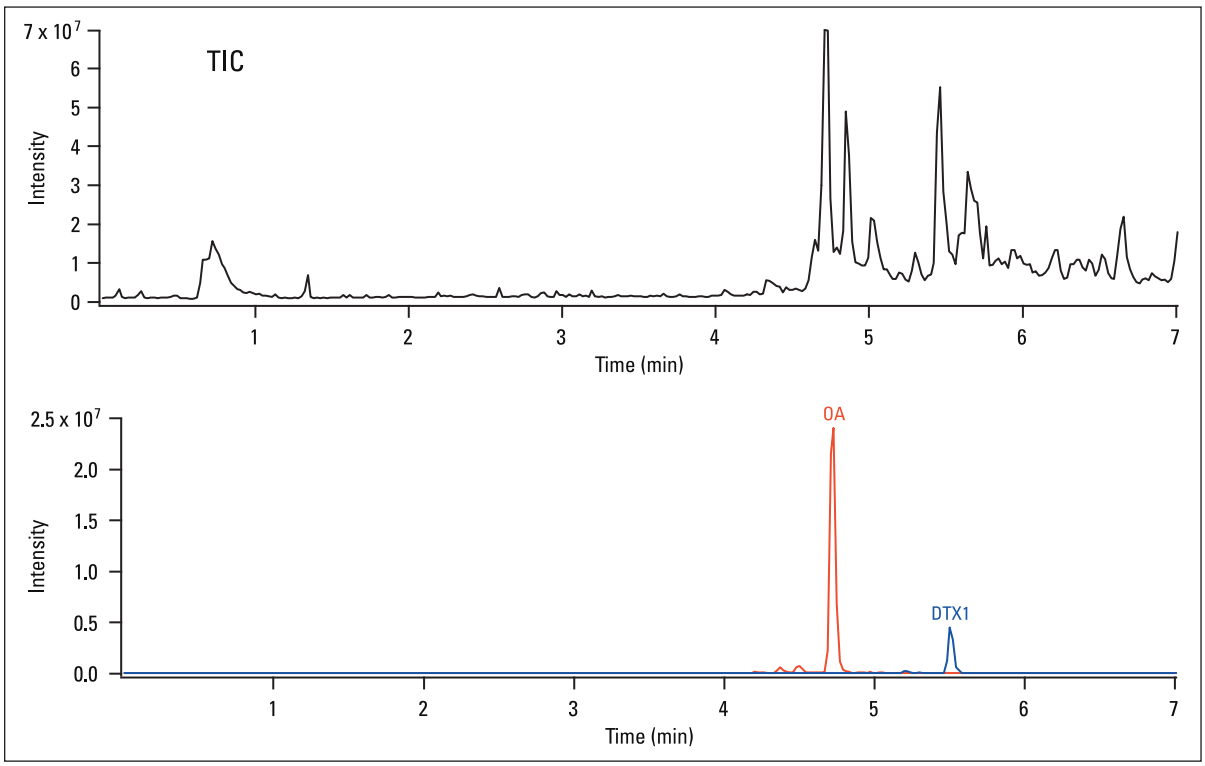

Figure 3: Exactive analysis of a mussel tissue extract showing the total ion chromatogram (TIC; top trace) and 5 ppm mass chromatograms for okadaic acid and DTX1 (lower trace)

Conclusions

The Exactive benchtop LC-MS system was successfully applied to the screening of lipophilic marine biotoxins commonly found in shellfish. This non-targeted approach provides high-resolution data over the entire chromatographic separation, allowing detection of new or unknown compounds in addition to those of interest. Furthermore, the approach requires little method development, as settings are not tuned for individual analytes. Although the results described above were limited to a relatively small subset of biotoxins for which calibration standards are available, extending the approach to other toxins or toxin analogues can be simply accomplished by expanding on the target list of analyte masses during data processing.

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Application Note: 479

Quantification of Polyphosphonates and Scale Inhibitors in High Ionic Strength Matrix Effluents Using IC-MS/MS

Charles Yang[1], Stacy Henday[2], Leo Wang[2], and Bill Schnute[2]
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Key Words

- TSQ Quantum Access
- SRM
- Ion chromatography
- Water analysis

Introduction

Scale deposits and corrosion formation in aqueous industrial cooling systems reduce the efficiency of heat transfer and can lead to equipment failure and increased operating costs. The addition of scale and corrosion inhibitors to cooling tower water streams helps to minimize corrosion formation by allowing dissolved minerals to remain soluble in water instead of depositing as scale. In turn, these additives permit the repeated cycling of water in cooling systems.

Before scale and corrosion inhibitors were commonly used, all cooling systems were "once-through" systems. Copious amounts of water were removed from lakes and streams by the cooling systems, greatly stressing aquatic life and negatively affecting the environment. By adding polyphosphonate compounds, such as HEDP (1-hydroxy ethylidene-1, 1-diphosphonic acid) and PBTC (2-phosphonobutane-1,2,4-tricarboxylic acid), to cooling water, corrosion and scale are minimized so that the cooling water can be cycled repeatedly through the system before it is released back into the environment.

When the cooling water is released back to the lake or stream, it must meet the standards of the United States Environmental Protection Agency (US EPA) Clean Water Act (CWA). Section 316(b) requires industrial plants to employ the best technology available to protect fish and aquatic life.[1] With the increased use of scale and corrosion inhibitors, polyphosphonates are now an emerging environmental contaminant and few quantitation methods exist. The ion chromatography – mass spectrometry (IC-MS/MS) technique described here provides robust quantitation in less than 20 minutes for five common scale and corrosion inhibitors in cooling water effluents – ATMP (amino trimethylene phosphonic acid), HEDP, PBTC, HPMA (hydrolyzed polymaleic anhydride), and PSO (a proprietary phosphinosuccinic oligomer)[2-4].

Goal

To develop a robust IC-MS/MS method for the quantitation of scale and corrosion inhibitors in a high anionic matrix.

Experimental Conditions

Ion Chromatography

IC analysis was performed on a Dionex ICS 3000 ion chromatography system (Dionex Corporation, Sunnyvale, CA). The polyphosphonate and scale inhibitor samples were directly injected and no sample pre-treatment was required. The IC conditions were as follows:

Column set: IonPac® AG21 (2.1 × 50 mm) / AS21 (2.1 × 250 mm); guard and separator columns (Dionex)
Suppressor: ASRS® 300, 2 mm; operated at 38 mA (Dionex)
Column temperature: 30 °C
Injection volume: 100 µL
Mobile phase: Potassium hydroxide, electrolytically generated with an EGC-KOH cartridge
Gradient: 0–7 min: 20 mM KOH
7–12 min: 20–60 mM KOH
12–17 min: 60 mM KOH
17.1 min: 20 mM KOH
Flow rate: 300 µL/min

Eluent generation technology allows automatic in-situ production of high-purity IC eluent (Figure 1). The pump delivers water to an eluent generator cartridge (EGC) that converts the water into a selected concentration of potassium hydroxide eluent using electrolysis. After separation on the column, the eluent enters the ASRS suppressor, which produces hydronium ions to exchange with potassium in the eluent and neutralizes the hydroxide. This makes the mobile phase compatible with an atmospheric ionization source as featured on LC-MS and LC-MS/MS systems.

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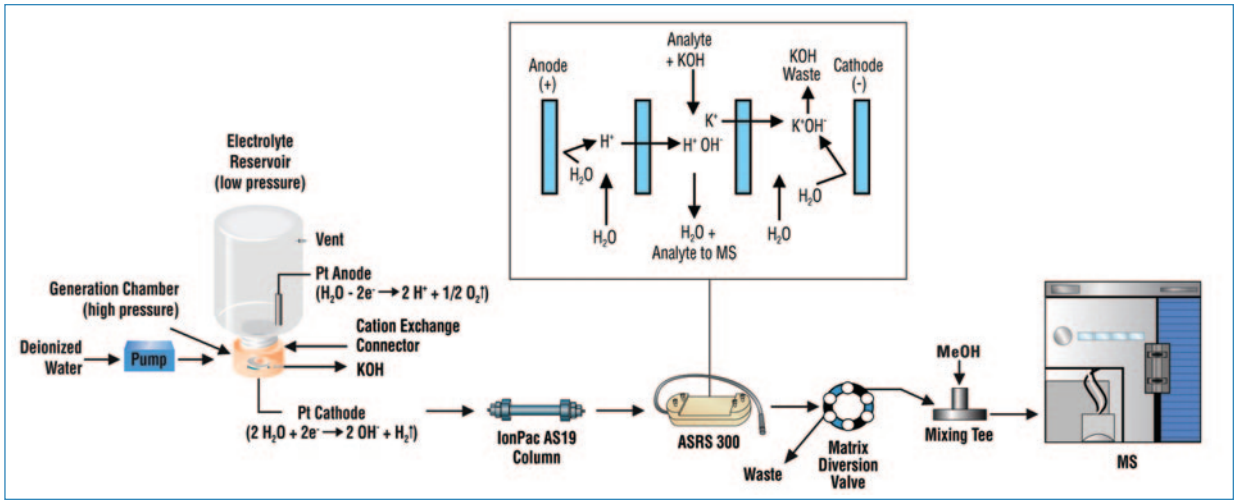

Figure 1. The flow schematic for an IC-MS application shows how an eluent generator cartridge produces potassium hydroxide. The eluent then passes through a suppressor, making it compatible with a mass spectrometer.

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific TSQ Quantum Access triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) source. The MS conditions were as follows:

Ion source polarity:	Negative ion mode
Spray voltage:	4000 V
Sheath gas pressure:	40 arbitrary units
Ion sweep gas pressure:	1 arbitrary unit
Auxiliary gas pressure:	2 arbitrary units
Capillary temperature:	300 °C
Collision gas pressure:	1.2 bar
Skimmer offset:	0 V
Detection mode:	Selective reaction monitoring (SRM); see Table 1 for details.

The cooling water matrix ions eluted prior to the analytes; therefore, the first 7.5 minutes of elution were diverted from the mass spectrometer to decrease source fouling. While the eluent was diverted, a make up flow of methanol was supplied to the mass spectrometer.

Table 1. SRM conditions

Name	SRM Transitions	Collision Energy (V)	Scan Width (Da)	Scan Time (s)	Tube Lens (V)
HEDP	204.580 → 168.860	16	0.01	0.5	49
	204.580 → 186.855	13			
PBTC	268.910 → 188.925	20	0.01	0.5	45
	268.910 → 206.911	16			
ATMP	297.770 → 197.896	26	0.01	0.5	54
	297.770 → 215.870	20			
PSO*	296.850 → 118.749	27	0.01	0.5	34
	296.850 → 146.832	19			
HPMA	337.490 → 318.829	16	0.01	0.5	60
	318.960 → 230.997	13			

*PSO is a proprietary molecule. For this oligomer, m/z 296.85 was found to be a consistent marker ion.

Results and Discussion

Calibration curves generated on the TSQ Quantum Access™ mass spectrometer show excellent linearity using only external quantitative measurements with no internal standard correction. The detection range for all compounds was 5-5000 ppb (Figure 2 and Table 2).

The method detection limit (MDL) in matrix was calculated by seven replicate injections of 100 ppb in a simulated matrix of fluoride (20 ppm), chloride (30 ppm), nitrate (100 ppm), phosphate (150 ppm), and sulfate (150 ppm). Using the equation $MDL = t_{99\%} \times S_{(n-1)}$, where t equals the Student's t test at 99% confidence intervals ($t_{99\%, (6)} = 3.143$) and S is the standard deviation, the MDLs for all compounds were calculated (Table 2). Figure 3 shows the response of the analytes spiked in the simulated matrix. The recoveries of all of the compounds were within 15% of the 100 ppb spike. The reproducibility of all the matrix-spiked samples was within 5%, without internal standard correction.

Table 2. Linearity and calculated detection limits of the analytes

Compound	R^2	MDL in matrix (ppb)
HEDP	0.9979	8.3
PBTC	0.9975	3.7
ATMP	0.9998	16.5
HPMA	0.9985	16.5
PSO	0.9965	8.8

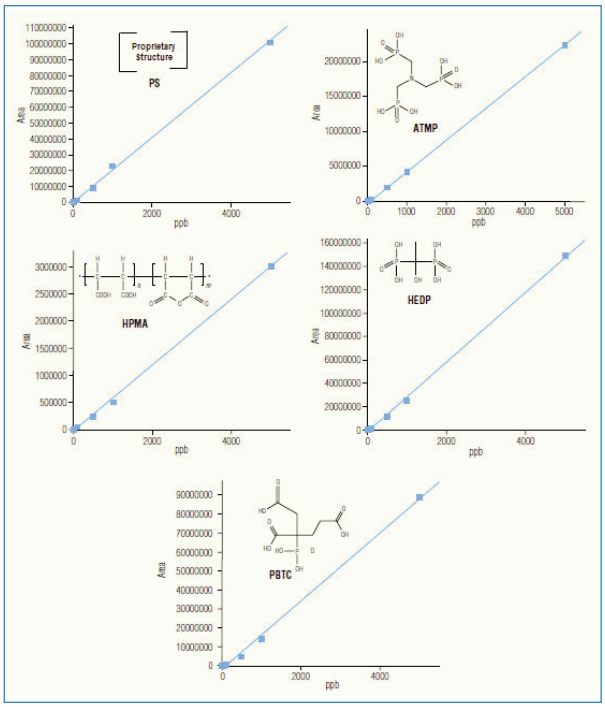

Figure 2. Calibration curves from 5 ppb to 5000 ppb for the analytes of interest, determined by linear regression analysis with equal weighting of the data.

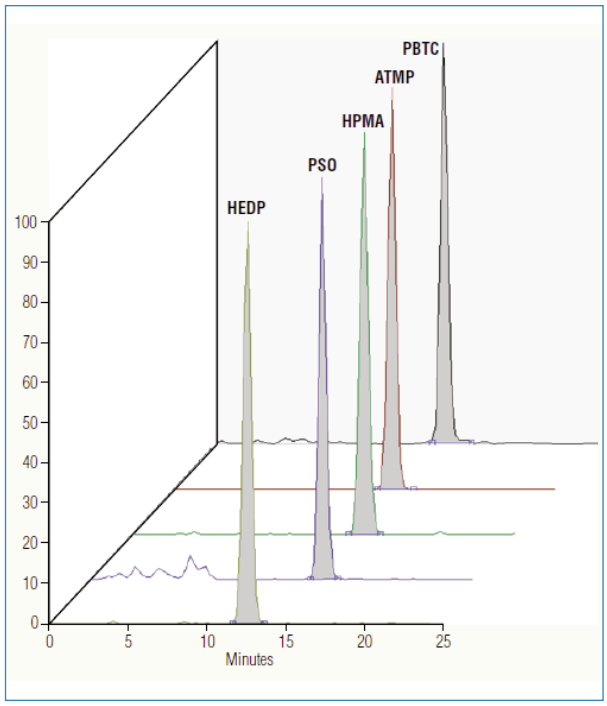

Figure 3. The response of 100 ppb analytes spiked into a high ionic strength matrix. The analytes showed excellent recoveries (within 15% of the 100 ppb spike) when spiked into the matrix.

Water treatment chemistry is a specialized field that often uses proprietary technology. As such, it is difficult to evaluate methods to reduce the environmental impact from the operation of cooling water systems. The method described here can detect the scale and corrosion inhibitors at sub-part-per-billion levels, although most cooling streams have part-per-million levels of scale and corrosion inhibitors. Any adverse matrices are diluted when the sample is diluted into the calibration range of 5-5000 ppb.

Compounds such as PSO and HPMA are proprietary blends with many components. When issued, they are sold in controlled, blended formulations. In the sample we received, one main marker and its transitions were examined. These marker ions, of *m/z* 297 and *m/z* 337, respectively for PSO and HMPA, showed excellent linearity over the quantitation range (Table 2).

Conclusion

The addition of scale and corrosion inhibitors to the water stream in industrial cooling systems reduces corrosion and allows repeat water cycling. While there is no current EPA guideline for the amount of corrosion and scale inhibitors released into the environment, interest in the quantification of these products in released water has increased. The minimum detection level established by this method shows that low-level quantitation of scale and corrosion inhibitors is possible, even in a high concentration of laboratory-simulated matrix.

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