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Application News

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Liquid Chromatograph Mass Spectrometry

Ultra-High-Speed Analysis of Melamine in Powdered Milk Using LDTD-MS/MS

The deliberate contamination of powdered milk and pet food with melamine has become a serious social issue. If melamine is contained in food at high concentrations together with cyanuric acid, which is produced in the manufacturing process of melamine, contamination can lead to kidney stones and even kidney failure. In many cases, melamine is added for producing adulterated products, and when added, is done so at very high concentrations. In order to stop these sorts of adulterated products at the border, high-speed screening analysis that can be performed together with easy sample preparation is required. A widely reported analysis technique for melamine in powdered milk involves using LCMS and GCMS after performing pretreatment to remove impurities. This article describes an ultra-high-speed analysis of melamine in powdered milk without column separation by using a laser diode thermal desorption (LDTD) ion source together with the LCMS-8060.

An ion source for ultra-high-speed screening analysis developed by Phytronix Technologies Inc. (https://phytronix.com/) in Canada was employed as the LDTD ion source. Mass spectrometry can be completed within a few seconds by sample vaporization using laser irradiation and subsequent APCI ionization. By applying samples to 96-well plates, up to 10 plates can undergo consecutive analysis. When using the LDTD ion source together with a Shimadzu LCMS-8060, each instrument can be utilized as necessary, such as for direct analysis using LDTD or for LC/MS analysis with column separation, simply by loading a method file with no need to disconnect the LDTD ion source from the LCMS-8060 (Fig. 1). This allows for MRM optimization of the compound for analysis on the LCMS-8060 and then ultra-high-speed analysis with LDTD using the determined MRM transitions. Conversely, polyspecimen analysis screening using ultra-high-speed analysis with LDTD can be performed first, and then using the results, LC/MS analysis can be performed with respect to a particular sample. In this way the combination of the LDTD ion source and LCMS-8060 can be used to switch between two completely different analysis methods according to the purpose of analysis.

In this research, we connected an LDTD ion source, performed MRM optimization of melamine using DUIS (dual ion sources of ESI and APCI), and then used the obtained MRM transitions in ultra-high-speed analysis by LDTD-MS. In performing ultra-high-speed analysis by LDTD-MS, we used a mass spectrometry system comprising an LDTD ion source and the LCMS-8060 and used samples prepared by adding melamine to powdered milk and collecting the melamine using liquid-liquid extraction. The following introduces an example of analyzing melamine in powdered milk by switching between the two analysis systems of LCMS and LDTD-MS.

MRM Optimization Using LC-MS with an LDTD System Connected

First, MRM optimization was performed in DUIS mode using a standard sample of melamine. The LC conditions used in optimization were the MRM optimization conditions used for general flow injection analysis (FIA). Fig. 2 shows the MS/MS spectrum (CE: −25 V) obtained when optimizing melamine in DUIS mode. Of the MRM transitions (*m/z* 126 > 85, 127 > 68, and 127 > 43) identified under these conditions, the MRM transition (*m/z* 127 > 68) with low background noise in LDTD-MS analysis was used to perform the analysis of melamine in powdered milk with LDTD-MS.

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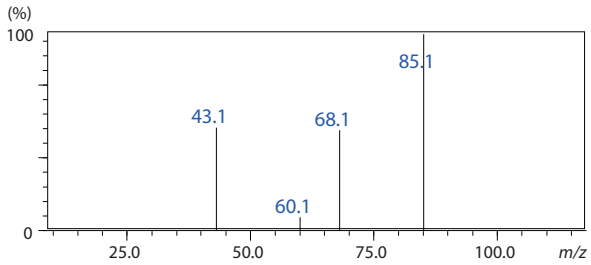
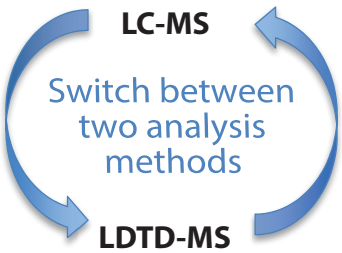


Fig. 2 MS/MS Spectrum of Melamine Using DUIS Mode



While independently utilizing the three ionization methods of ESI, APCI, and DUIS, MRM optimization of target components can be performed to ensure a smooth start to ultra-high-speed analysis using LDTD, and in cases of complex analysis samples, detailed analysis by LC/MS can be performed following the LDTD analysis.

Easy application of samples to 96-well plates for LDTD-MS allows ultra-high-speed analysis (four second ionization) of multiple components by LDTD-MS.

Fig. 1 Two Methods of Analysis Using LC-MS and LDTD-MS

Extraction of Melamine Added to Powdered Milk

Commercially-available powdered milk was weighed out (125 mg portions) and transferred to 1.5 mL Eppendorf tubes. Next, 0.5 mL of ultra pure water and 0.5 mL of acetonitrile were added and the mixtures were thoroughly agitated for one minute. Then, 12.5 µL of 0, 5, 10, 25, 50, 100, 500, and 1000 µg/mL melamine solutions prepared in advance were added to each powdered milk suspension. These correspond to the concentrations of 0, 0.5, 1, 2.5, 5, 10, 50, and 100 ppm in the powdered milk. Further agitation was performed for another minute to ensure that the added melamine was sufficiently mixed into each solution. Powdered milk components were precipitated by centrifugal separation (14,000 g, room temperature, 5 min) and 200 µL of supernatant containing melamine was collected and transferred to new tubes. Next, 200 µL of sodium carbonate buffer solution (saturated NaCl, pH 10) was added and thoroughly agitated, and then 1 mL of ethyl acetate was added and sufficiently agitated. Since this separates into an aqueous layer and organic layer, centrifugal separation was performed. From the organic layer which contains melamine, 4 µL was taken and dispensed into a LazWell plate (96 well) and then dried. The LazWell plate was set into the LDTD ion source and batch analysis was performed on each sample.

Table 1 LDTD-MS Analysis Conditions

LDTD Analysis Conditions	
Laser pattern	: 65 % laser power, 2 seconds
Gas flow rate	: 3.0 L/min
MS Analysis Conditions	
Mode	: MRM (pos)
Interface	: APCI
DL temperature	: 250 °C
Heat block temperature	: 400 °C

LDTD-MS Analysis of Melamine Added to Powdered Milk

Table 1 summarizes the LDTD-MS analysis conditions. Fig. 3 shows MRM chromatograms of melamine added to powdered milk (corresponding to 0.5, 5, and 50 ppm concentrations in the powdered milk). It is apparent that the LDTD ion source ionized the melamine within just six seconds (within 0.1 minute). Also, analysis at n = 3 of the samples with melamine added at each concentration resulted in favorable repeatability as shown in Fig. 3. These results indicate that ultra-high-speed analysis by LDTD-MS has unparalleled throughput and is capable of quantitative analysis with high repeatability that is comparable to LCMS analysis. Next, the peak area for each additive concentration of melamine was graphed based on the analysis results of each sample concentration (Fig. 4). A linearity of R^2 = 0.998 was verified from these analysis results. From these results we can see that LDTD-MS enables ultra-high-speed analysis with both high repeatability and linearity, even for samples that contain many impurities, such as melamine in powdered milk.

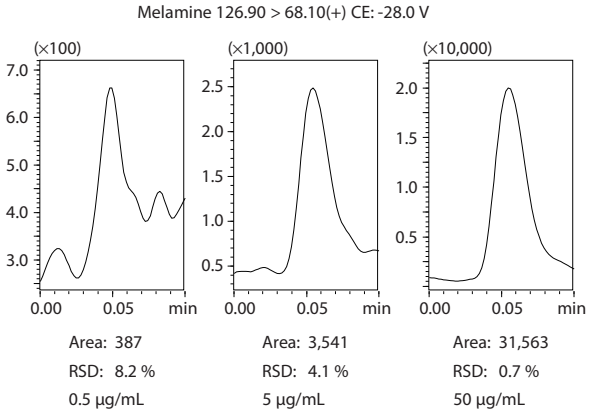


Fig. 3 MRM Chromatograms of Melamine Added to Powdered Milk

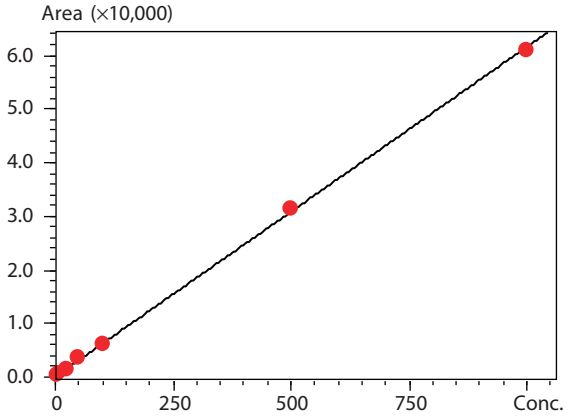


Fig. 4 Linearity of Melamine Added to Powdered Milk

In this research, we performed MRM optimization in DUIS mode on the LCMS-8060 followed by ultra-high-speed analysis using LDTD-MS with respect to melamine added to powered milk, and verified the level of repeatability and linearity. As demonstrated, the combination of the LCMS-8060 with an LDTD ion source allows easy switching of the analysis system according to the purpose of analysis, thereby allowing multicomponent optimization by LCMS, or LCMS analysis of complex analysis samples as necessary based on the results of simple ultra-high-speed screening analysis by LDTD. These two characteristic analysis methods can be utilized as necessary.

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