

Analysis of Dicyandiamide in Milk products by LC/MS/MS

In 2012, an incident in which dicyandiamide was detected in dairy products produced in New Zealand was reported. Previous incidents in which melamine and its metabolite cyanuric acid were detected in dairy products have also been reported. These nitrogen-containing low-molecular-mass compounds may be mixed in for the purpose of increasing the protein content of dairy products. In addition to the aforementioned compounds, urea, cyromazine, biuret, and others have been listed by the FDA as substances that require caution. Introduced here is a hydrophilic interaction chromatography (HILIC)-based method for separating these high-polarity compounds. Also described is the addition of dicyandiamide to commercially available milk powder and subsequent analysis after pretreatment.

A TSKgel Amide-80 3- μm column was used. Aqueous ammonium formate/acetonitrile was used as the eluent. Figure 1 shows MRM chromatograms for six representative nitrogen-containing low-molecular-mass compounds. Chromatograms exhibiting good peak shape were obtained for each compound. A good calibration curve was obtained for dicyandiamide over the concentration range 0.1 to 10 $\mu\text{g/L}$ (Figure 3). Under these analytical conditions, the limit of quantitation was 0.07 $\mu\text{g/L}$ ($S/N=10$). When the pretreatment described in Figure 4 was carried out, this value was 0.02 $\mu\text{g/g}$ sample. Figure 2 shows a chromatogram in which dicyandiamide was added to commercially available milk powder to a concentration of 1.0 $\mu\text{g/g}$, after which the sample was pretreated and analyzed. The resulting recovery, assuming an addition of 1.0 $\mu\text{g/g}$, was 87.5%, with an RSD value of 3.2% ($n=5$).

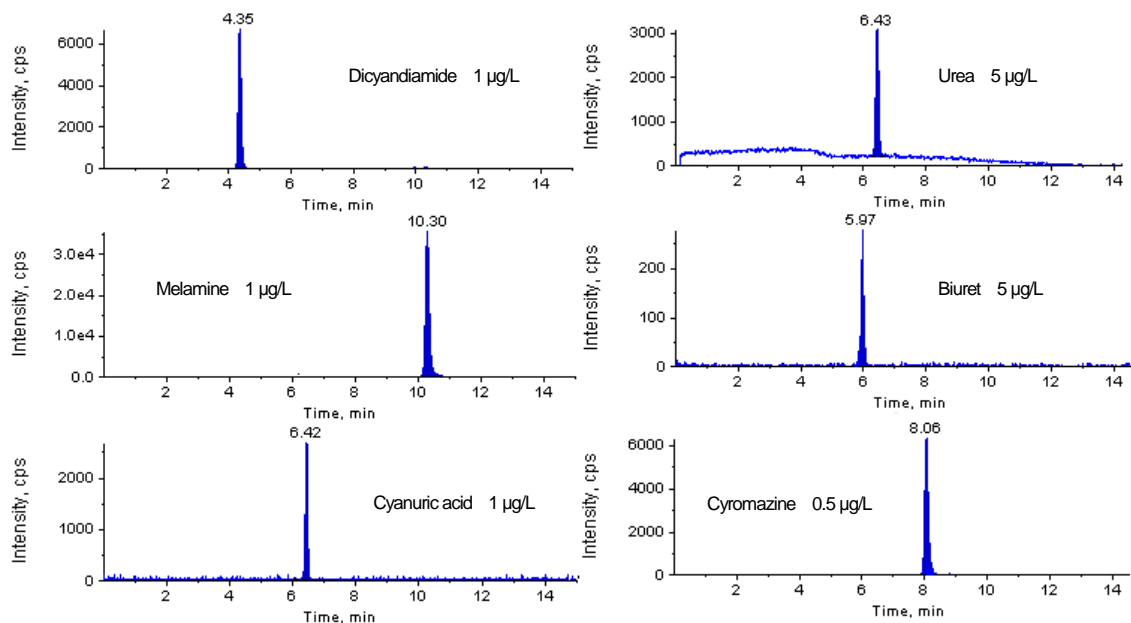


Figure 1 MRM chromatograms of standard reference materials of nitrogen-containing compounds

Table 1 Conditions

LC	Instrument	: Agilent 1200SL series (Agilent Technologies)	
	Column	: TSKgel Amide-80 3 μ m (2.0 mml.D. x 15 cm)	
	Eluent	: A; 10 mmol/L Ammonium formate (pH 3.75) B; Acetonitrile	
	Gradient	: B conc. (0.0 min) 95 % \rightarrow (10.0 min) 70 % \rightarrow (10.1-15.0 min) 50 % \rightarrow (15.1-18 min) 95 %	
	Flow rate	: 0.2 mL/min	
	Column temp.	: 40 $^{\circ}$ C	Injection vol.
MS/MS	Instrument	: QTRAP (AB SCIEX)	
	Ionization	: ESI-Positive (Dicyandiamide, Melamine, Urea, Biuret, Cyromazine)	
		: ESI-Negative (Cyanuric acid)	
	Mode	: MRM	
	m/z	: 85/68 (Dicyandiamide), 127/85 (Melamine), 61/44 (Urea), 104/61 (Biuret), 167/125 (Cyromazine)	
	: 128/42 (Cyanuric acid)		

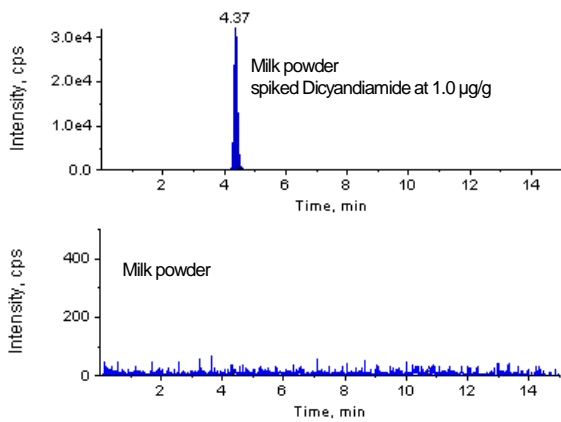


Figure 2 MRM chromatograms of milk powder
Upper: DCDA spiked sample (1 μ g/g) Lower: Control

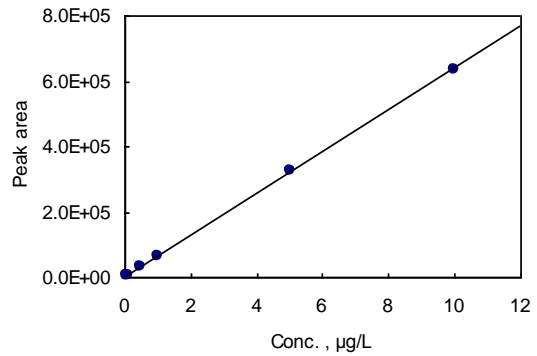


Figure 3 Calibration curve for DCDA

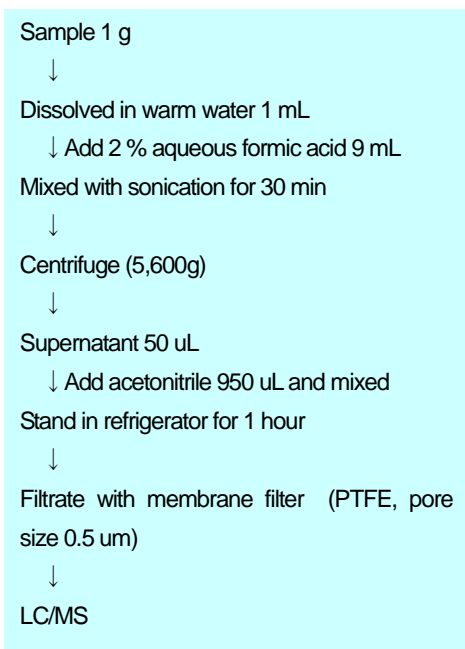


Figure 4 Pretreatment conditions

Conc. Spiked in Milk powder (μ g/g)	Recovery (%)	RSD(n=5)
1.0	88.5	3.2
5.0	95.2	2.7

Table 2 recovery and repeatability