

Application News

No.L514

High Performance Liquid Chromatography

Analysis of Melamine and Its Related Substances in Fertilizers

Calcium cyanamide is effective as a fertilizer, pesticide, soil amendment, and for many other uses, and it is essential compound for producing high quality vegetables. Recently, high levels of melamine were discovered as a byproduct in some calcium cyanamide hydrate products, pelletized by adding water to calcium cyanamide. Due to the risk of agricultural products absorbing the melamine from the soil, it has been identified as a potential public health risk. For example, if both melamine and its related substance cyanuric acid are ingested at the same time, they can form crystals that can impede kidney function.¹⁾

As a result, the Food Safety and Consumer Affairs Bureau in the Ministry of Agriculture, Forestry and Fisheries in Japan issued a notice specifying a 0.4 % provisional maximum allowable concentration of melamine in calcium cyanamide.²⁾

This article describes an example of pretreating and analyzing melamine and its related substances, namely ammeline, ammelide, and cyanuric acid, in fertilizer, in accordance with the testing methods supervised by the Food and Agricultural Materials Inspection Center (FAMIC) in Japan for fertilizers and other substances (2016, 8.1.c).^{3), 4), 5)}

■ Analysis of Standard Solution

The structure of melamine and its related substances is shown in Fig. 1. The analytical conditions are indicated in Table 1. The chromatogram of the standard mixture solution of melamine and its related substances (1 mg/L each) is shown in Fig. 2. For more details regarding the procedures used to prepare the standard solution and mobile phase, refer to the applicable test methods. Calibration curves for melamine and its related substances are shown in Fig. 3. Calibration curves were prepared for a concentration range of 0.05 to 5 mg/L. The results indicated good linearity, with a contribution rate (R^2) over 0.9999.

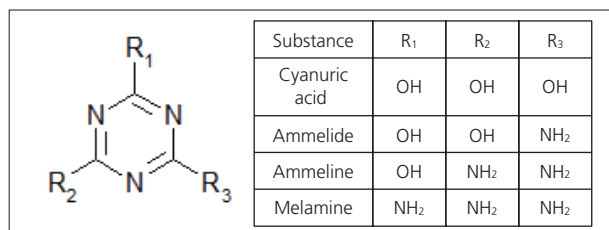


Fig. 1 Chemical Structure of Melamine and Its Related Substances

Table 1 Analytical Conditions

System	: Prominence
Column	: TOSOH, TSKgel Amide-80 (250 mm L. × 4.6 mm I.D., 5 μm)
Guard Column	: TOSOH, TSKgel guardgel Amide-80 (15 mm L. × 3.2 mm I.D.)
Mobile Phase	: (Sodium) phosphate buffer pH 6.7±0.2 / Acetonitrile = 1/4 (v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 10 μL
Detection	: UV-VIS detector (SPD-20A) at 214 nm

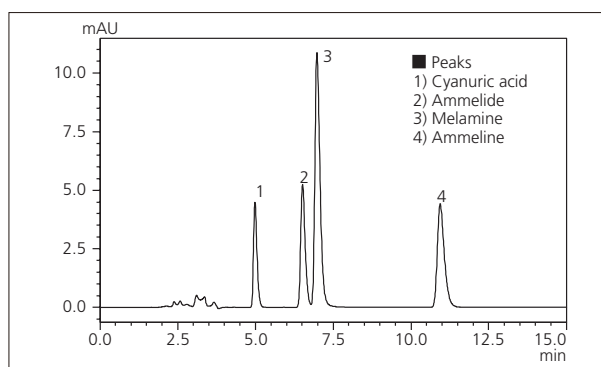


Fig. 2 Chromatogram of Standard Mixture (1 mg/L each)

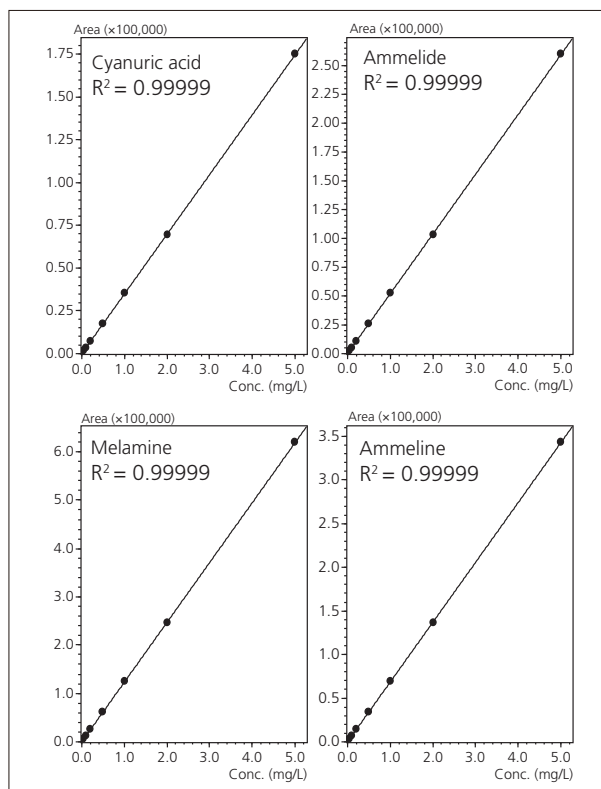


Fig. 3 Linearity (0.05 to 5 mg/L)

■ Repeatability

The relative standard deviation (%RSD) results for peak area after analyzing the standard solution (0.1 mg/L) six consecutive times were very good, with 0.41 % for cyanuric acid, 0.42 % for ammelide, 0.52 % for melamine, and 0.56 % for ammeline. When pretreated as indicated in Fig. 4, the 0.1 mg/L concentration of the standard solution is equivalent to a 0.02 % concentration of melamine and other related substances in fertilizer.

■ Analysis of Melamine and its Related Substances in Fertilizer

The analytical sample (fertilizer) was pretreated in accordance with the test method by adding a standard quantity of melamine and its related substances. The pretreatment procedure is indicated in Fig. 4 and the analytical results are shown in Fig. 5. In this example, five types of samples were tested, including two types of commercially available nitrolime, a synthetic fertilizer that contains calcium cyanamide, a synthetic fertilizer, and ammonium sulfate. The quantities of the substances added to the analytical samples, as a percentage of mass, were equivalent to about 0.035 to 2.8 % melamine, about 0.035 to 1.6 % ammelide, about 0.035 to 1.1 % ammeline, and about 0.037 to 1.2 % cyanuric acid. These results demonstrate that the Prominence system provides more than adequate performance for measuring the provisional 0.4 % melamine limit issued by the Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries for calcium cyanamide and fertilizers that contain calcium cyanamide as an ingredient.

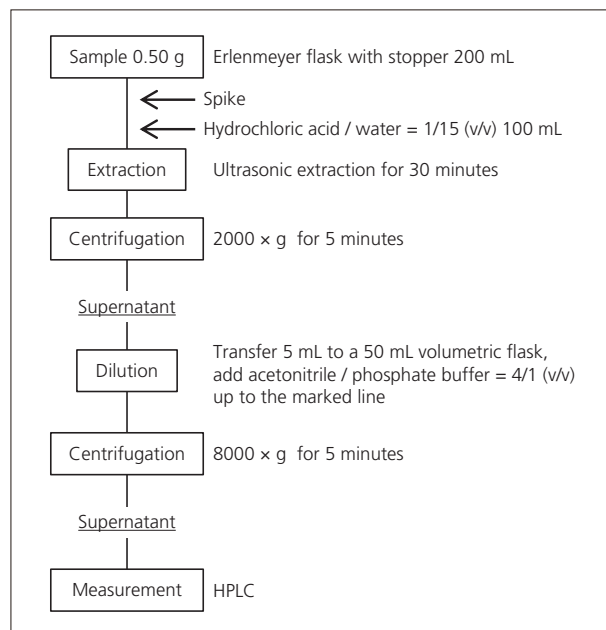


Fig. 4 Pretreatment Procedure

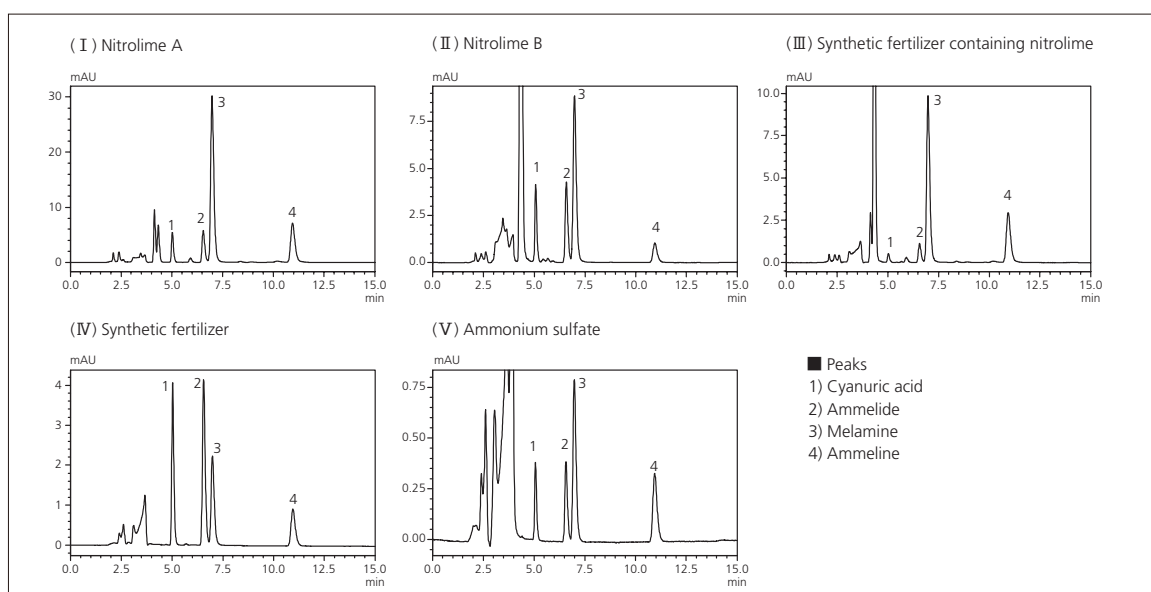


Fig. 5 Chromatograms for (I) Nitrolime A, (II) Nitrolime B, (III) Synthetic Fertilizer Containing Calcium Cyanamide, (IV) Synthetic Fertilizer, and (V) Ammonium Sulfate

References

- 1) Health effects of melamine, etc.: Food Safety Commission of Japan, October 9, 2008, updated April 30, 2009
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- 3) Testing Methods for Fertilizers (2016), Food and Agricultural Materials Inspection Center (FAMIC): http://www.famic.go.jp/ffis/fert/obj/shikenho_2016.pdf (in Japanese)
- 4) Etsuko Bando and Yuji Shirai: Validation of High Performance Liquid Chromatography (HPLC) for Determination of Melamine and Its Related Substances in Fertilizer, Research Report of Fertilizer Vol. 6, pp. 27 to 35 (2013)
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