

Analysis of Melamine and Its Related Substances in Fertilizers

Recently, it has been confirmed that some granulated products of hydrated calcium cyanamide, which are created by adding water to calcium cyanamide, contain a significant amount of melamine. Given this fact, while there are at present no standard values for melamine in fertilizer, regulations are being discussed, and investigations are progressing with respect to the dynamics of melamine in soil and its transition to crops.

This article introduces an example of the analysis of melamine and related substances in fertilizers using an HPLC system, with reference to the fertilizer test methods (2012) under the supervision of the Japan's Food and Agricultural Materials Inspection Center (FAMIC).
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■ Analysis of Standard Solution

Fig. 1 shows the analysis results of a mixed standard solution containing melamine, ammeline, and ammelide (10 mg/L each, dissolved in a mixed solution of acetonitrile / water / diethylamine = 5/4/1 (v/v/v)) using an amide column. Table 1 shows the analytical conditions.

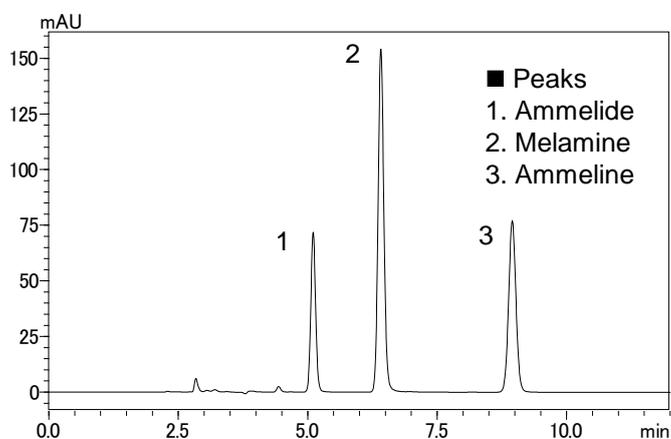


Fig. 1: Chromatogram of a Standard Mixture

Table 1: Analytical Conditions

Instrument:	Prominence HPLC system
Column:	Inertsil Amide (GL Sciences Inc., 250 mmL. × 4.6 mmI.D., 5 μm)
Mobile phase:	A) 5 mmol/L Phosphate buffer (pH 6.7) B) Acetonitrile A/B = 25/75 (v/v)
Flow rate:	1.0 mL/min
Column temp.:	40 °C
Detection:	SPD-20AV at 214 nm
Injection volume:	10 mL

■ Linearity and Repeatability

Fig. 2 shows the calibration curves for 3 components in the range of 0.1 mg/L to 20 mg/L. The contribution ratio (R^2) was 0.999 or higher in all cases, indicating that favorable linearity was obtained. Calculating the detection limit and quantitation limit based on the absolute calibration curve for melamine in Fig. 2 yields 0.0005 mg/L and 0.0015 mg/L, respectively.

Table 2 shows the relative standard deviation (%RSD) for peak area in a 6-cycle repeated analysis of a mixed standard solution of melamine, ammeline, and ammelide (0.1 mg/L each). A favorable repeatability of 0.5 % max. was obtained for all components.

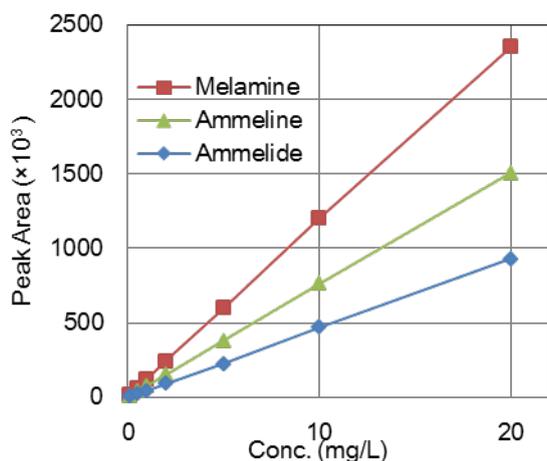


Fig. 2: Linearity (0.1 to 20 mg/L)

Table 2: Repeatability

	Melamine	Ammeline	Ammelide
1st	11921	7414	4590
2nd	11886	7364	4609
3rd	11899	7401	4603
4th	11906	7389	4649
5th	11915	7437	4625
6th	11910	7468	4606
%RSD	0.103	0.494	0.446

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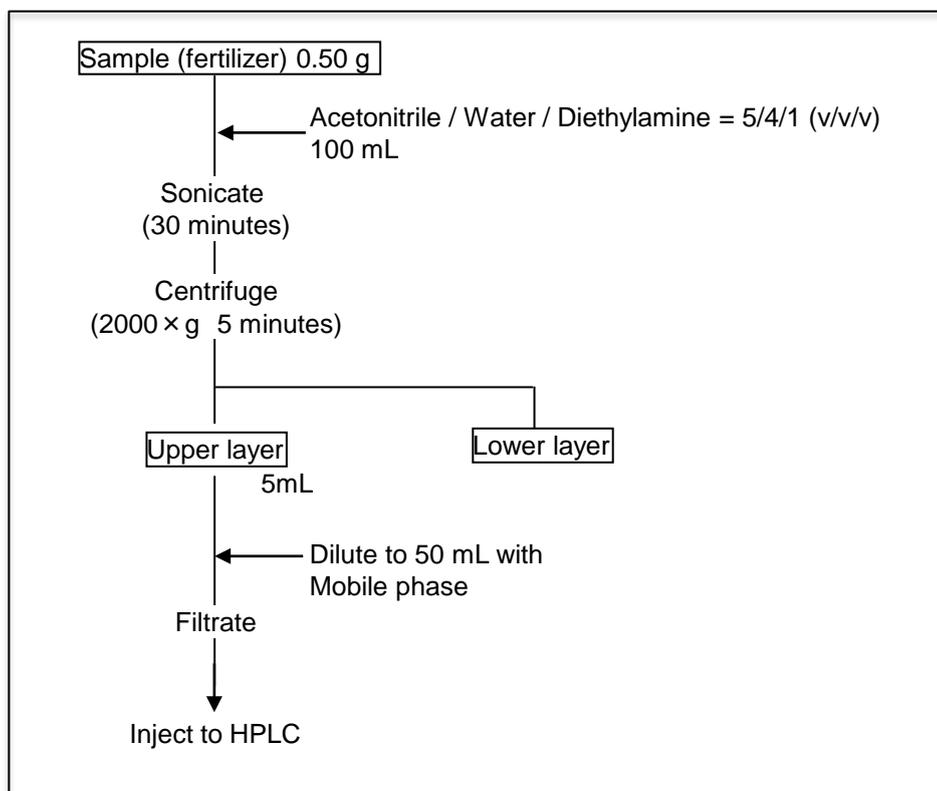


Fig. 3: Sample Preparation

Fig. 3 shows the sample preparation process described in the fertilizer test method. Fertilizer certified reference materials A and B* were treated in accordance with the sample preparation procedure described in Fig. 3. The final solutions from the sample preparation were then spiked with melamine, ammeline, and ammeline with the standard addition technique so that the respective concentrations of these substances reached 10 mg/L. Figures 4 & 5 show the chromatograms obtained. The analysis conditions are the same as in Table 1. (In accordance with the sample preparation procedure in Fig. 3, if 100 % of the melamine and related substances were recovered, the content of these substances would be calculated as equivalent to a 2 % mass fraction with respect to the fertilizer.)

*Fertilizer certified reference material A (FAMIC-A-10) is a high-analysis compound fertilizer containing urea. Fertilizer certified reference material B (FAMIC-B-10) is a general-compound fertilizer not containing urea. Both are commercially available from FAMIC.

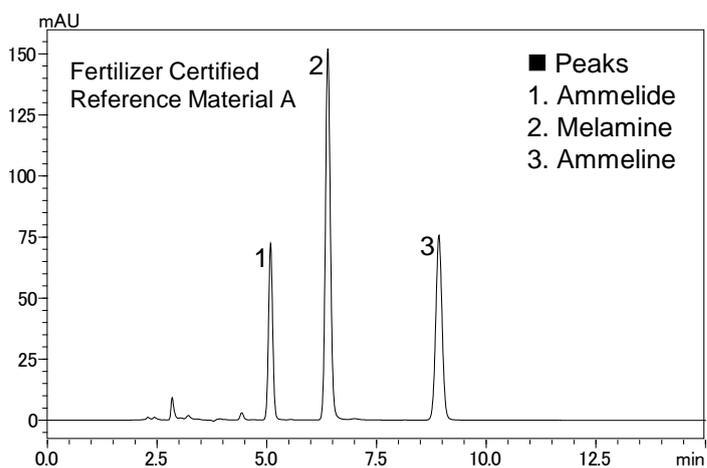


Fig. 4: Chromatogram of Fertilizer Certified Reference Material A (spiked with 10 mg/L melamine, ammeline and ammeline)

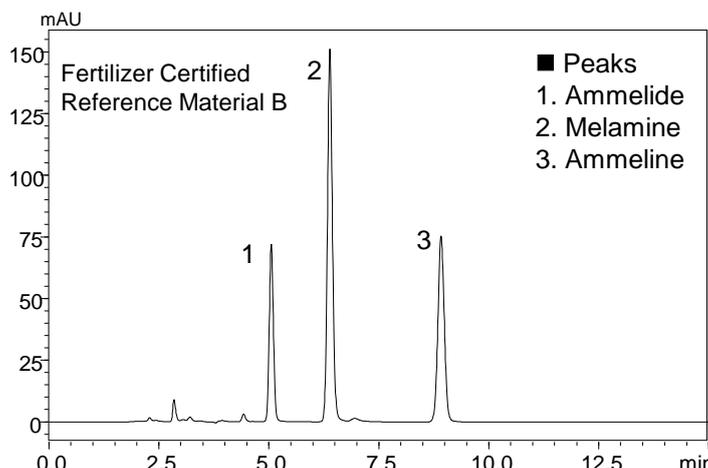


Fig. 5: Chromatogram of Fertilizer Certified Reference Material B (spiked with 10 mg/L melamine, ammeline and ammeline)