HPLC Analysis of Melamine and Related Substances in Fertilisers

by Azusa Morita, Shimadzu Corporation, Japan
Gesa J. Schad, Shimadzu Europa GmbH
Shirai Yuji, Food and Agricultural Materials Inspection Center, Japan

Calcium cyanamide is a multipurpose nitrogen fertiliser, which is also effective as a pesticide and soil amendment. It plays an important role in crop cultivation by ensuring that plants are supplied with nitrogen. Recently, high levels of melamine were discovered as a by-product in some calcium cyanamide hydrate products, pelletised by added water. Due to the risk of agricultural products absorbing melamine from soil, it has been identified as a potential public health risk.

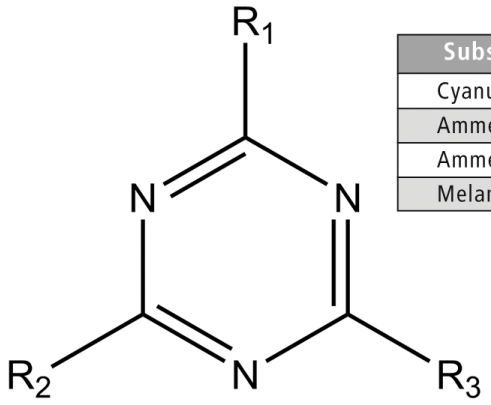
Health issues associated with melamine have been in the public eye since the Chinese milk scandal in 2008. A widely utilised precursor for many applications on the one hand, harmful contamination for feedstock and milk on the other, its use in fertiliser can also facilitate the introduction of melamine into the food chain. Long-term exposure to melamine and its related substance, cyanuric acid, through ingestion can result in bladder or kidney stones which ultimately can lead to bladder cancer [1, 2], concentration of this compound in food and feed needs to be controlled carefully.

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 [3].

In this work, a simple and robust method for sample pretreatment and analysis of melamine and related substances, namely ammeline, ammelide and cyanuric acid in fertiliser, carried out using HPLC with UV detection is demonstrated. Five products were investigated, commercially available in Japan, namely Nitrolime 1, Nitrolime 2, synthetic fertilisers (x 2) and ammonium sulphate.

Materials and Methods

Melamine synthesis can result in a number of by-products by replacement of the amino group '-NH2' in the R1-R3 positions with a hydroxy group '-OH'. The structures of melamine and its related substances are shown in Figure 1.



Substance	R_1	R_2	R_3
Cyanuric acid	OH	OH	OH
Ammelide	OH	OH	NH_2
Ammeline	OH	NH_2	NH_2
Melamine	NH_2	NH_2	NH_2

Figure 1: Chemical structures of melamine and its related substances.

Simultaneous analysis of the compounds of interest was performed using a Shimadzu Prominence™ HPLC system consisting of a DGU-20A degassing unit, an LC-20AD pump, a SIL-20ACHT auto-sampler, a CTO-20AC column oven and an SPD-20A UV-visible detector. Chromatographic separations were performed using a TSKgel® Amide-80 column (250 mm L. x 4.6 mm I.D., 5 µm) at a temperature of 40°C. Analytical conditions are further specified in Table 1.

Sample preparation

Fertiliser samples to be tested were prepared in accordance with the testing methods for fertilisers [4]. Five hundred

Table 1: Analytical conditions of the analysis of melamine and related substances.

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

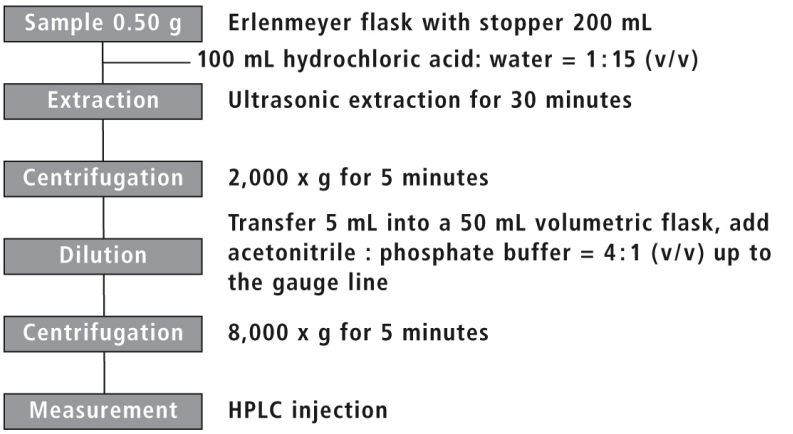


Figure 2: Sample pre-treatment procedure.

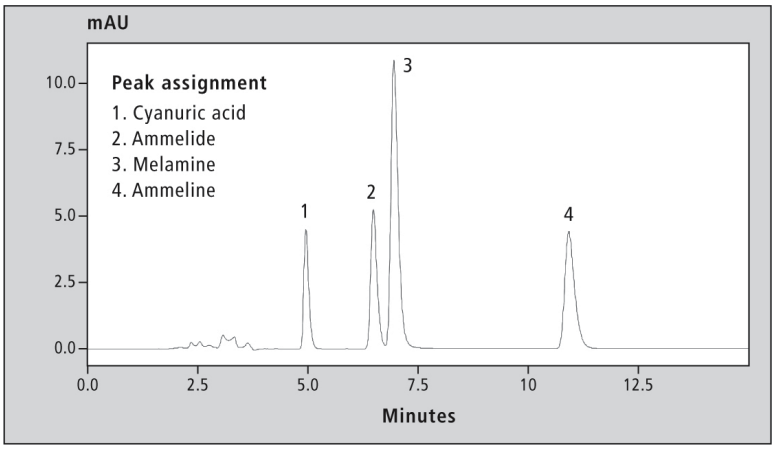


Figure 3: Chromatogram of standard mixture of melamine and related substances (each 1 mg/L).

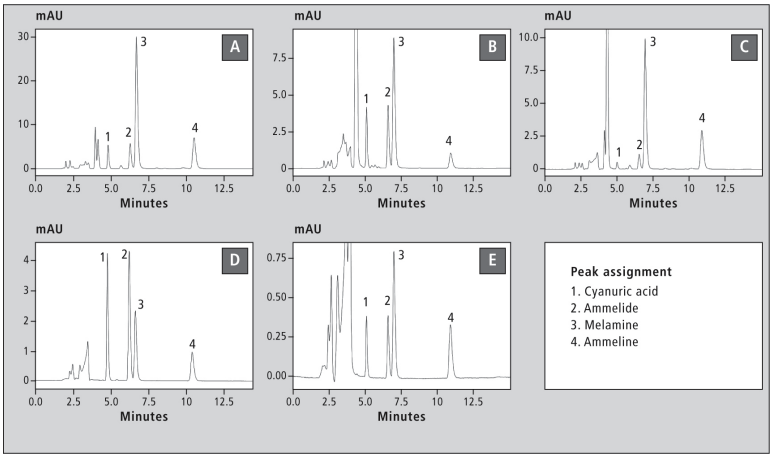


Figure 4: Chromatograms for (A) Nitrolime 1, (B) Nitrolime 2, (C) Synthetic fertiliser containing calcium cyanamide, (D) Synthetic fertiliser and (E) Ammonium sulphate.

milligram fertiliser samples were weighed into an Erlenmeyer flask and extracted in an ultrasonic bath for 30 min, using a mixture of hydrochloric acid / water (1:15 v/v). The samples were then centrifuged at 2000 x g for 5 min, and 5 ml of the supernatant were transferred into a 50 ml volumetric flask and diluted to volume with a mixture of acetonitrile/ phosphate buffer (4:1 v/v). Aliquots of the dilute sample were again centrifuged at 8000 x g for 5 min, and the supernatant was transferred to an HPLC vial for analysis. The sample pretreatment procedure is illustrated in Figure 2 [5-6].

Results

The chromatogram of the standard mixture containing melamine and its related substances (1 mg/L each) is shown in Figure 3. The range of calibration curves are from 0.05 up to 5 mg/L for each compound. The results show good linearity, with R2 ≥ 0.9999. The relative standard deviation (% RSD) for each peak area from six consecutive analyses was 0.41% for cyanuric acid, 0.42% for ammelide, 0.52% for melamine and 0.56% for ammeline respectively.

Five different kinds of fertilisers were analysed as displayed in Figure 4. Quantities of target compounds obtained in the evaluated samples were approximately 0.035 to 2.8%w/w melamine, 0.035 to 1.6%w/w ammeline, 0.035 to 1.1%w/w ammelide and 0.037 to 1.2%w/w cyanuric acid respectively.

Conclusion

A robust, fast and sensitive HPLC method has been developed for the determination of melamine and its related substances in fertilisers. The results demonstrate that the proposed assay can satisfy the provisional 0.4%w/w melamine limit issued by the Food Safety and Consumer Affairs Bureau, Ministry of Agriculture (Japan), Forestry and Fisheries for calcium cyanamide and fertilisers that contain calcium cyanamide as a component.

Acknowledgements

This study was carried out as a collaborative study supported by the Food and Agricultural Materials Inspection Center (FAMIC) in Japan. We would like to give special thanks to all of the members at the Fertilizer and Feed Inspection Department in FAMIC.

References

1. https://www.cdc.gov/niosh/ipcsneng/neng1154.html (Nov. 2018)

2. Food Safety Commission of Japan, October 9, 2008, updated April 30, 2009

3. Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries in Japan, Notice No. 6116, 2012, issued March 25, 2013 and partially revised March 30, 2013

4. Testing Methods for Fertilizers (2016), Food and Agricultural Materials Inspection Center (FAMIC):http://www.famic.go.jp/ffis/fert/obj/TestingMethodsForFertilizers2016.pdf (Nov. 2018)

5. 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 - 35 (2013)

6. Etsuko Bando and Shigehiro Kai: Determination of Melamine and Its Related Substances in Fertilizer by High Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer Vol. 7 pp. 10 - 21 (2014)