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Application News

No.L513

High Performance Liquid Chromatography

Analysis of Nitrous Acid and Ammonium Thiocyanate in Fertilizers

After it is spread onto agricultural land, nitrogen fertilizer converts to nitrate-nitrogen or nitrite-nitrogen and leaches into subsoil and river water polluting the groundwater. Drinking water with a high nitrate-nitrogen or nitrite-nitrogen content is a potential public health risk. It causes methemoglobinemia that inhibits the oxygen-carrying capacity of blood and has caused deaths in infants outside Japan.
Fertilizers with high concentrations of nitrous acid and ammonium thiocyanate have a negative effect on plant growth, therefore maximum content levels (permitted content levels) for toxic substances are prescribed in official specifications for commercial fertilizers according to the Fertilizer Control Law[1].
An example of simultaneous analysis of the nitrous acid and ammonium thiocyanate content of fertilizer by HPLC is described. Analysis was performed in conformance with the test method that appears in Testing Methods for Fertilizers (5.8.b and 5.9.a, 2016)[2], published by the Food and Agricultural Materials Inspection Center (FAMIC).

Analysis of a Standard Mixture

Analytical conditions are shown in Table 1. A chromatogram of a standard mixture of nitrous acid and ammonium thiocyanate (20 mg/L each) is also shown in Fig. 1. Please refer to the test method[2] for details on mobile phase preparation. Standard solution was prepared by dissolution and dilution with water. An NH2 column was used for analysis.

Table 1 Analytical Conditions

System	: Prominence
Column	: Shodex Asahipak NH2P-50 4E (250 mm L. × 4.6 mm I.D., 5 µm)
Guard Column	: Shodex Asahipak NH2P-50G 4A (10 mm L. × 4.0 mm I.D., 5 µm)
Mobile Phase	: (Sodium) phosphate buffer containing sodium perchlorate
Flowrate	: 1.0 mL/min
Column Temp.	: 40 ˚C
Detection	: UV-VIS detector (SPD-20AV) at 210 nm
Injection Vol.	: 10 µL

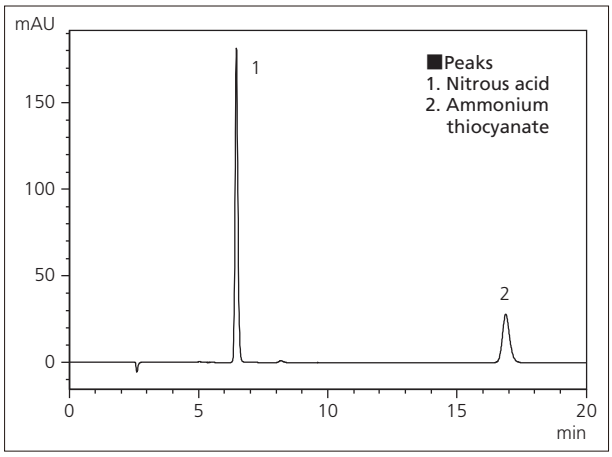

Fig. 1 Chromatogram of Standard Mixture (20 mg/L each)

Linearity of Calibration Curves

Fig. 2 shows calibration curves for nitrous acid and ammonium thiocyanate analyzed under the conditions shown in Table 1. The range used for calibration curves was 1 to 20 mg/L. Good linearity was obtained for both compounds with contribution rates (R^2) of 0.9999 or higher.

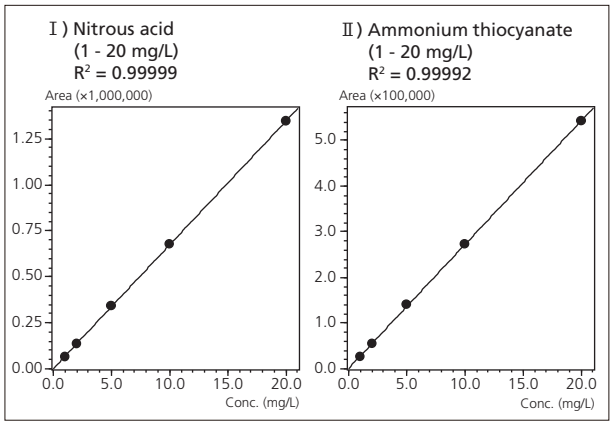

Fig. 2 Linearity of Calibration Curves
Ⅰ) Nitrous acid (1 - 20 mg/L),
Ⅱ) Ammonium thiocyanate (1 - 20 mg/L)

Repeatability

The relative standard deviation (%RSD) of retention times and peak areas obtained from an analysis of each compound at 0.1 mg/L repeated six times is shown in Table 2 and 3. The concentration analyzed (0.1 mg/L) was equivalent to 1/10 the lowest concentration on the calibration curve of either compound. Good repeatability was obtained for the retention times and peak areas of both compounds.

Table 2 Repeatability of Retention Time and Peak Area for Nitrous Acid Analysis

	R.T. (min)	Area
1st	6.452	6,752
2nd	6.452	6,801
3rd	6.450	6,722
4th	6.452	6,794
5th	6.452	6,727
6th	6.451	6,823
Ave.	6.452	6,770
%RSD	0.012	0.62

Table 3 Repeatability of Retention Time and Peak Area for Ammonium Thiocyanate Analysis

	R.T. (min)	Area
1st	16.868	2,551
2nd	16.870	2,534
3rd	16.882	2,524
4th	16.881	2,519
5th	16.885	2,546
6th	16.868	2,553
Ave.	16.876	2,538
%RSD	0.046	0.57

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The method used to prepare fertilizer samples is shown in Fig. 3. The method of pretreatment differed depending on whether the sample was a powder or liquid, and on extraction liquid pH. Standard additions of nitrous acid and ammonium thiocyanate were made to the samples for analysis (fertilizer) before being further prepared according to the procedure shown in Fig. 3[3), 4)]. The results of analysis are shown in Fig. 4.
Testing Methods for Fertilizers (2016) describes a method that uses ion chromatography for the analysis of ammonium thiocyanate (5.8.a). However, because this method produces a complex eluent, it takes some time for the baseline to stabilize. Another problem with the method is that it has resulted in multiple system peaks and peaks close to the elution position of ammonium thiocyanate. Nevertheless, on this occasion, the results show that good separation was achieved, including for contaminating constituents in the fertilizer.

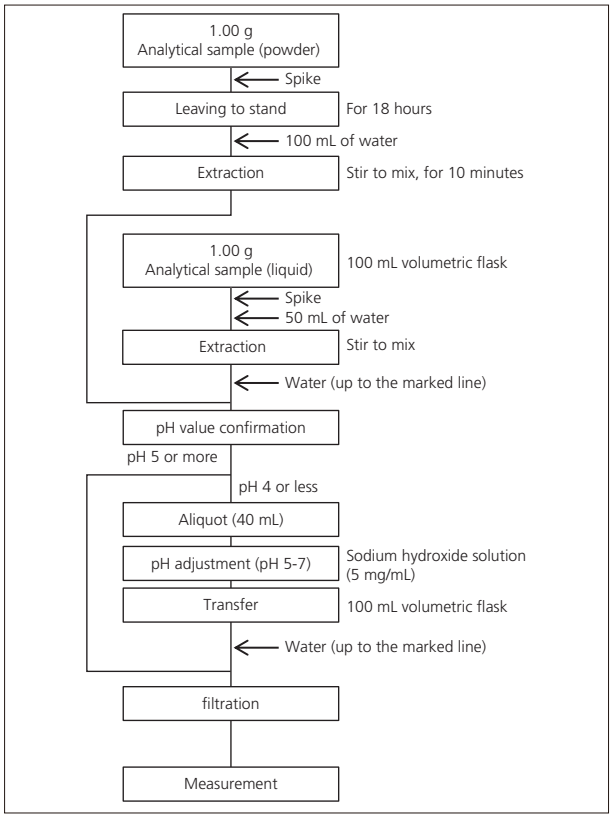

Fig. 3 Pretreatment

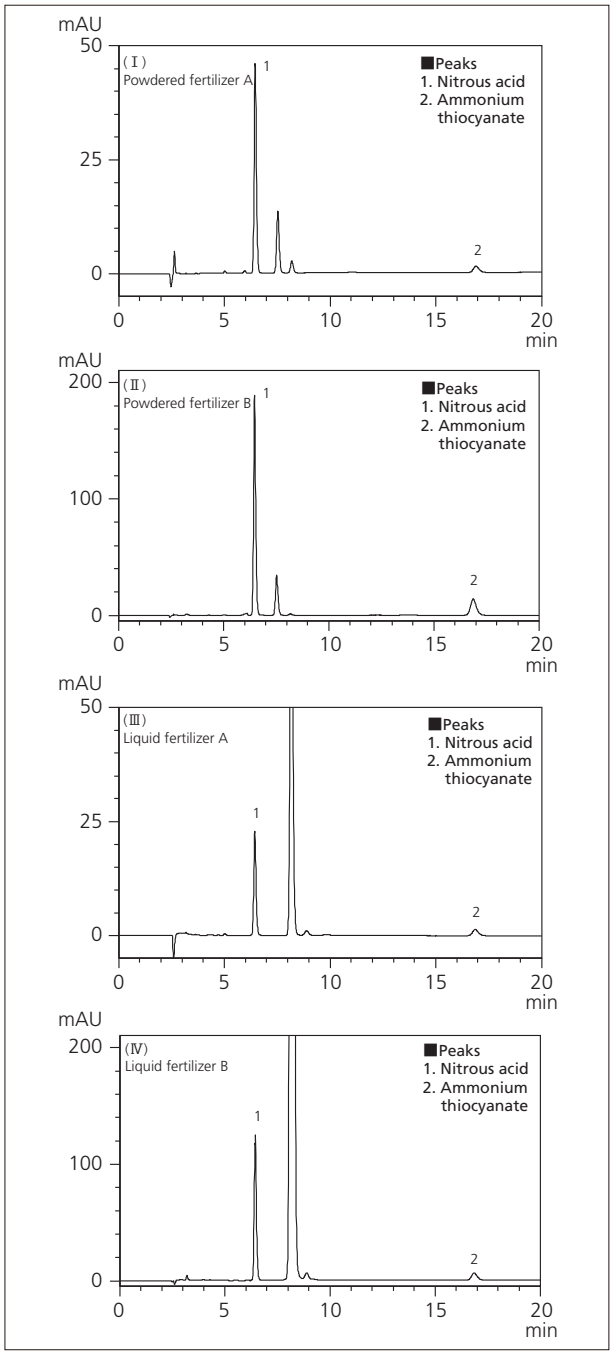

Fig. 4 Chromatograms of Fertilizer Sample Solutions (Ⅰ) Powdered fertilizer A, (Ⅱ) Powdered fertilizer B, (Ⅲ) Liquid fertilizer A, (Ⅳ) Liquid fertilizer B

References
1) Ministry of Agriculture, Forestry and Fisheries notification: Establishment of official specifications for commercial fertilizers according to the Fertilizer Control Law, dated February 22, 1986. Ministry of Agriculture, Forestry and Fisheries notification no. 284: Final revision, dated March 30, 2016. Ministry of Agriculture, Forestry and Fisheries notification no. 884: Enactment, dated April 1, 2016. [In Japanese]
2) 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]
3) Masanori Hasegawa, Yasuharu Kimura: Determination of Nitrous Acid and Ammonium Thiocyanate in Fertilizer by High Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer, vol. 8, 70-78 (2015) [in Japanese]
4) Kohei Ito, Yasuharu Kimura, Masanori Hasegawa, Yuji Shirai: Simultaneous determination of nitrous acid and thiocyanate in fertilizer by HPLC, Japanese Journal of Soil Science and Plant Nutrition, 87 (3), 120-124 (2016) [In Japanese]

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