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Direct quantitative determination of cyanamide by stable isotope dilution gas chromatography–mass spectrometry

Syuntaro Hiradate [a,*], Tsunashi Kamo [b], Eri Nakajima [a], Kenji Kato [b], Yoshiharu Fujii [a]

[a] *Department of Biological Safety Science, National Institute for Agro-Environmental Sciences, 3-1-3 Kan-nondai, Tsukuba, Ibaraki 305-8604, Japan*
[b] *Department of Bioscience and Biotechnology, Faculty of Agriculture, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano 399-4598, Japan*

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Abstract

Cyanamide is a multifunctional agrochemical used, for example, as a pesticide, herbicide, and fertilizer. Recent research has revealed that cyanamide is a natural product biosynthesized in a leguminous plant, hairy vetch (*Vicia villosa*). In the present study, gas chromatography–mass spectrometry (GC–MS) equipped with a capillary column for amines was used for direct quantitative determination of cyanamide. Quantitative signals for ($^{14}N_2$)cyanamide, ($^{15}N_2$)cyanamide (internal standard for stable isotope dilution method), and *m*-(trifluoromethyl)benzonitrile (internal standard for correcting errors in GC–MS analysis) were recorded as peak areas on mass chromatograms at *m*/*z* 42 (A_{42}), 44 (A_{44}), and 171 (A_{IS}), respectively. Total cyanamide content, ($^{14}N_2$)cyanamide plus ($^{15}N_2$)cyanamide, was determined as a function of $(A_{42}+A_{44})/A_{IS}$. Contents of ($^{14}N_2$)cyanamide and ($^{15}N_2$)cyanamide were then calculated by multiplying the total cyanamide content by $A_{42}/(A_{42}+A_{44})$ and $A_{44}/(A_{42}+A_{44})$, respectively. The limit of detection for the total cyanamide content by the GC–MS analysis was around 1 ng. The molar ratio of ($^{14}N_2$)cyanamide to ($^{15}N_2$)cyanamide in the injected sample was equal to the observed A_{42}/A_{44} value in the range from 0.1 to 5. It was, therefore, possible to use the stable isotope dilution method to quantify the natural cyanamide content in samples; i.e., the natural cyanamide content was derived by subtracting the A_{42}/A_{44} ratio of the internal standard from the A_{42}/A_{44} ratio of sample spiked with internal standard, and then multiplying the resulting difference by the amount of added ($^{15}N_2$)cyanamide (SID-GC–MS method). This method successfully gave a reasonable value for the natural cyanamide content in hairy vetch, concurring with the value obtained by a conventional method in which cyanamide was derivatized to a photometrically active compound 4-cyanimido-1,2-naphthoquinone and analyzed with reversed-phase HPLC (CNQ-HPLC method). The determination range of cyanamide in the SID-GC–MS method was almost the same as that in the CNQ-HPLC method; however, the SID-GC–MS method was much simpler than the CNQ-HPLC method.

Keywords: Capillary gas chromatography–mass spectrometry; Cyanamide; ($^{15}N_2$)Cyanamide; 4-Cyanimido-1,2-naphthoquinone; Isotope dilution method; Hairy vetch; *Vicia villosa*

1. Introduction

Calcium cyanamide (Ca=NC≡N) was first artificially synthesized in 1898 in Germany [1]. Since then, it has been used worldwide in many roles, including as nitrogen fertilizer, defoliant, herbicide, fungicide, and anthelmintic [1–3]. When calcium cyanamide comes in contact with water molecules, cyanamide ($H_2NC{\equiv}N$) is spontaneously generated as shown in the following reactions [4]:

$$2Ca{=}NC{\equiv}N + 2H_2O \rightarrow Ca(HNC{\equiv}N)_2 + Ca(OH)_2$$

$$Ca(HNC{\equiv}N)_2 + CO_2 + H_2O \rightarrow 2H_2NC{\equiv}N + CaCO_3$$

Cyanamide is the active ingredient causing the herbicidal and pesticidal effects of calcium cyanamide. Cyanamide itself is also toxic to mammals, irritating the eyes, skin, and respiratory system, and inducing allergic contact dermatitis, miosis, salivation, lacrimation, and twitching [1–5]. In soils, however, cyanamide is detoxified and transformed into ammonia, possibly by enzymatic and catalytic reactions through urea or dicyandiamide, whereby it works as a nitrogen fertilizer [6]. Therefore, cyanamide is an excellent multifunctional agrochemical with low risk of environmental toxicity in agricultural fields.

Because cyanamide had not been found in nature, it had long been regarded as an artificial product. However, because

* Corresponding author. Tel.: +81 29 838 8246; fax: +81 29 838 8199.
E-mail address: hiradate@affrc.go.jp (S. Hiradate).

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natural cyanamide-catalyzing enzymes show high specificity to cyanamide, there had been some speculation as to the ecological functions and roles of these enzymes [6]. Recently, however, a leguminous plant, hairy vetch (*Vicia villosa*), has been found to contain cyanamide as a major plant growth inhibitor (a possible allelochemical) [7]. It was confirmed that hairy vetch biosynthesizes cyanamide from nitrate [8]. Since then, many questions, such as the distribution of cyanamide in the plant kingdom and the cyanamide biosynthetic pathway, have been raised. To answer these questions, a highly sensitive and precise method for determining cyanamide with high accuracy is necessary. Contents of cyanamide in cyanamide-rich samples have been determined by titration [3] and high-performance liquid chromatography (HPLC) without derivatization [7,9]. However, these determination methods are not satisfactory when applied to cyanamide-poor plant samples because of the low sensitivity and low selectivity of these methods. The only applicable method was the derivatization of cyanamide to the photometrically active compound 4-cyanimido-1,2-naphthoquinone (CNQ) and analysis with reversed-phase HPLC (CNQ-HPLC method) [10], although this method requires many procedures. In the present study, we report a new quantitative method for determining cyanamide using gas chromatography–mass spectrometry (GC–MS) which allows the application of the stable isotope dilution method, making possible the precise and accurate quantification of cyanamide with a simple procedure (SID-GC–MS method).

2. Experimental

2.1. Standard reagents

Cyanamide (99%, fine granules) with natural isotope abundance and ($^{15}N_2$)cyanamide (51.5% water solution) were purchased from Aldrich (Milwaukee, WI, USA) and Isotec (Miamisburg, OH, USA), respectively. These cyanamide reagents were used without further purification. Mass chromatograms at *m*/*z* 42 and 44 in the GC–MS analysis (see Section 2.3) revealed that the peak area ratio at *m*/*z* 42 (A_{42}) and 44 (A_{44}) was 98.3:1.7 for the cyanamide with natural isotope abundance and 2.0:98.0 for the ($^{15}N_2$)cyanamide. The cyanamide with natural isotope abundance was used as the primary standard, and the concentration of the ($^{15}N_2$)cyanamide was standardized in terms of the primary standard as follows: we prepared a mixed solution containing 5.0 mg l^{-1} cyanamide with natural isotope abundance and ca. 5 mg l^{-1} ($^{15}N_2$)cyanamide, determined A_{42} and A_{44} for the mixed solution by GC–MS analysis, and corrected the ($^{15}N_2$)cyanamide concentration by multiplying 5.0 mg l^{-1} by A_{44}/A_{42}. In the GC–MS analyses, *m*-(trifluoromethyl)benzonitrile (Wako Pure Chemical, Osaka, Japan) was used as an internal standard.

2.2. Plant materials

Seeds of hairy vetch (Takii, Kyoto, Japan) were sown on 6 October 2003 in an experimental field of the National Institute for Agro-Environmental Sciences (Tsukuba, Japan) where no agrochemical had been applied in the previous 10 years. After 7 months of growing without any agrochemical application, the shoots of the hairy vetch were harvested (17 May 2004). Pure ethanol (800 ml) was added to the plant material (100 g), and the mixture was homogenized with a Physcotron homogenizer (Microtec, Chiba, Japan). The homogenized mixture was extracted for 24 h in the dark, filtered through a No. 2 filter paper (Advantec Toyo, Tokyo, Japan), and subjected to cyanamide content analyses.

2.3. Determination of cyanamide content by the SID-GC–MS method developed in the present study

The plant extract (0.4 g fresh weight equivalent) was mixed with 10, 30, or 90 μl of 1176 mg l^{-1} ($^{15}N_2$)cyanamide standard solution, which was the internal standard used for determining the natural cyanamide content by the stable isotope dilution method and for calculating the recovery rate. Because cyanamide is a volatile compound (melting point, 45–46 °C; boiling point at 49 kPa, 83 °C; vapor density, 1.45 (air = 1.00) [2,4,11]), 10 μl of dimethylsulfoxide was added to the sample solution as a solvent to trap the cyanamide before evaporation. After evaporating at 35 °C in vacuo in a rotating evaporating container (CVE-200D; Tokyo Rikakikai, Tokyo, Japan), the residue was redissolved in a small amount of acetone (<0.3 ml) and transferred into a normal-phase solid-phase extraction column (Bond Elut SI, 500 mg/3 ml; Varian, Palo Alto, CA, USA) previously filled with 2 ml of hexane, followed by successive elutions with 3.0 ml each of 20, 30, 40, and 50% acetone in hexane. Preliminary experiments showed that almost all of the cyanamide was eluted in the 30 and 40% acetone in hexane fractions (data not shown). Therefore, we mixed 1/3 portions (1.0 ml) of the 30 and 40% acetone in hexane-eluted fractions (to achieve 2.0 ml volumes), and subjected them to GC–MS analysis. A 2-ml sample of the standard cyanamide solution was also prepared. Before injection, a 10-μl portion of 1000 mg l^{-1} *m*-(trifluoromethyl)benzonitrile in methanol was added to the 2.0 ml of test solution as an internal standard for GC–MS analysis. The prepared solution was then set in an auto injector (AOC-17; Shimadzu, Kyoto, Japan). The analytical conditions were as follows: GC–MS instrument, QP-5000 (Shimadzu); analytical column, CP-Sil 8 CB for amines (0.25-mm I.D., 30-m length, 0.25-μm thickness; GL Sciences, Tokyo, Japan); injector temperature, 250 °C; interface temperature (ion source temperature), 250 °C; ionizing voltage, 70 eV (EI/MS); signal sampling rate, 0.2 s; injection mode, split-less mode with 30-s sampling time; injection volume, 2.0 μl; column temperature, 50 °C for the initial 5 min followed by an increase in temperature at 15 °C min^{-1} up to 250 °C and kept at 250 °C for 3 min (total analytical time, 21.3 min); carrier gas, He; total flow rate, 50 ml min^{-1}; column flow rate, 1 ml min^{-1}. Cyanamide and *m*-(trifluoromethyl)benzonitrile (internal standard for correcting errors in GC–MS analysis) were detected at retention times of 8.5 and 9.7 min, respectively (Fig. 1). Quantitative signals of ($^{14}N_2$)cyanamide, ($^{15}N_2$)cyanamide, and *m*-(trifluoromethyl)benzonitrile were recorded as the corresponding peak areas on the mass chromatograms at *m*/*z* 42 (A_{42}), 44

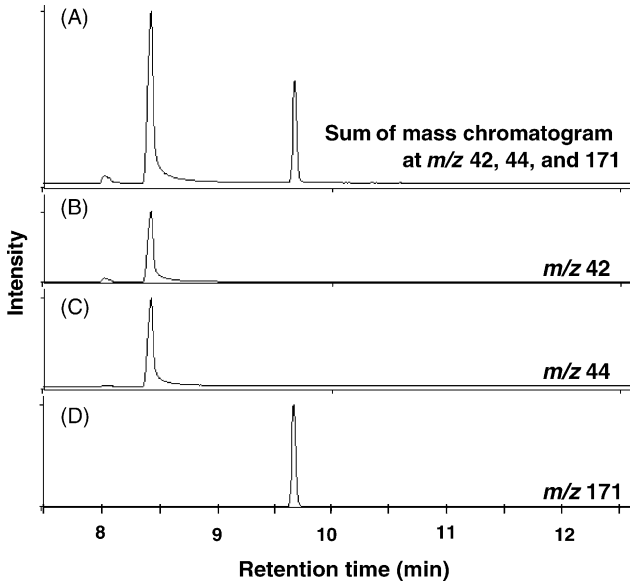

Fig. 1. Chromatograms of a mixed standard solution containing cyanamide with natural isotope abundance, ($^{15}N_2$)cyanamide (internal standard for the stable isotope dilution method), and *m*-(trifluoromethyl)benzonitrile (internal standard for correcting errors in GC–MS analysis) as determined by GC–MS equipped with a capillary column for amines (CP-Sil 8 CB for amines, GL Sciences). (A) signals recorded as the sum of mass chromatograms at *m*/*z* 42, 44, and 171; (B) mass chromatogram at *m*/*z* 42 for the determination of ($^{14}N_2$)cyanamide; (C) mass chromatogram at *m*/*z* 44 for the determination of ($^{15}N_2$)cyanamide; (D) mass chromatogram at *m*/*z* 171 for the determination of *m*-(trifluoromethyl)benzonitrile. The *y*-gain values of all chromatograms were adjusted to the same value. The injected amounts of cyanamide with natural isotope abundance, ($^{15}N_2$)cyanamide, and *m*-(trifluoromethyl)benzonitrile were 20.0, 23.5, and 20.0 ng, respectively. For other conditions, see Section 2.3.

(A_{44}), and 171 (A_{IS}), respectively. Determination of cyanamide in the plant extract by the SID-GC–MS method was conducted in triplicate for each treatment.

2.4. Determination of cyanamide by the CNQ-HPLC method [10]

For comparison, cyanamide content was also determined by a conventional method, the CNQ-HPLC method [10], with complementary modification. The plant extract (5.0 g fresh weight equivalent) was mixed with or without 0.5 ml of 500 mg l^{-1} primary cyanamide standard solution for determining the recovery rate. The mixture was evaporated at 35 °C in vacuo in a rotary evaporator to remove the ethanol (not dried) and ca. 10 ml of H_2O was added. The sample was mixed with 10 g of EXtrelut (Merck, Darmstadt, Germany), transferred to a glass extraction column (19-mm I.D., 300-mm length) previously filled with 50 ml of ethyl acetate, and eluted further with 90 ml of ethyl acetate. After the addition of 40 ml of H_2O to the eluate, ethyl acetate was evaporated at 35 °C in vacuo in the rotary evaporator and the volume of the sample solution was adjusted to 50 ml with H_2O. A 10-ml portion of the sample solution was passed through a reversed-phase solid-phase extraction column (Bond Elut C18, 500 mg/3 ml; Varian), which had been preconditioned with ca. 20 ml of pure methanol and then ca. 20 ml of H_2O, and the column was washed with an additional 6 ml of H_2O. The eluate (ca. 10 + 6 ml) was mixed and adjusted to a volume of 20 ml with H_2O. The sample solution (20 ml) was mixed with 10 ml of a 0.2 mol l^{-1} sodium bicarbonate (Wako Pure Chemical) solution (pH 10.5) and 2 ml of a 10 g l^{-1} 1,2-naphthoquinone-4-sulfonic acid potassium salt (Aldrich) solution, and then allowed to stand for 20 min at 60 °C to derivatize cyanamide to 4-cyanimido-1,2-naphthoquinone. After addition of 1 ml of 0.1 mol l^{-1} tetrabutylammonium hydrogen sulfate in methanol (total volume, 33 ml), a 10 ml portion of the reacted solution was applied to a reversed-phase solid-phase extraction column (Bond Elut C18, 500 mg/3 ml; Varian) preconditioned as described earlier, followed by elutions with 5 ml each of H_2O, 20% methanol in H_2O, and pure methanol. The pure methanol-eluted fraction (final volume was adjusted to 5.0 ml) was subjected to HPLC analyses (626 pump, 996 photodiode array detector, and 717 plus autosampler; Waters, Milford, MA, USA), with the HPLC equipped with a reversed-phase column (analytical column: Inertsil ODS-3, 5 μm, 4.6-mm I.D., 250-mm length, GL Sciences; guard column: Inertsil ODS-3, cartridge guard column E, 5 μm, 4-mm I.D., 20-mm length, GL Sciences) and eluted with a mixed solution of (MeOH): (0.1 mol l^{-1} tetrabutylammonium hydrogen sulfate in methanol): (0.05 mol l^{-1} sodium phosphate buffer [pH 5.5]) with a ratio of 68:2:30 (v/v/v) at a flow rate of 1.0 ml min^{-1}. The injection volume for the HPLC analysis was 10 μl. The derivatized 4-cyanimido-1,2-naphthoquinone was eluted at a retention time of 13.2 min and detected at 485 nm.

Standard solutions containing 1.2–410 μg of cyanamide in 10 ml of water were also subjected to the procedures described above (i.e., clean-up by the first reversed-phase solid-phase extraction column, derivatization to 4-cyanimido-1,2-naphthoquinone, clean-up by the second reversed-phase solid-phase extraction column, and HPLC analysis). The amounts of cyanamide injected into the HPLC system were equivalent to 0.75–250 ng cyanamide per 10-μl injection volume. The cyanamide content in the sample solution was calculated by comparing the peak area of the HPLC chromatograms for the sample with those of the standard solutions. Determination of cyanamide in the plant extract by the CNQ-HPLC method was performed in triplicate for each treatment.

3. Results and discussion

3.1. Chromatographic behavior of cyanamide in GC–MS analysis

When the GC–MS was equipped with a non-polar-type capillary column (DB-1, 0.25-mm I.D., 30-m length, 0.25-μm thickness, GL Sciences), cyanamide was undetectable as a quantitative peak because of severe peak tailing (data not shown). This could be due to a strong interaction between cyanamide and a liquid-phase in the capillary column. This shortcoming was greatly rectified when the GC–MS was equipped with a capillary column for amines (CP-Sil 8 CB for amines, GL Sciences), which gave only moderate tailing peaks for ($^{14}N_2$)cyanamide and ($^{15}N_2$)cyanamide in the GC–MS analysis (Fig. 1), although such peak tailing is not preferred for quantitative

determination, especially in the low concentration region. On the other hand, the internal standard for the GC–MS analyses, *m*-(trifluoromethyl)benzonitrile, gave an ideal sharp peak (Fig. 1).

3.2. Quantification of ($^{14}N_2$)cyanamide and ($^{15}N_2$)cyanamide by GC–MS analysis

Preliminary experiments indicated that A_{42}/A_{IS} and A_{44}/A_{IS} values were influenced by the presence of ($^{15}N_2$)cyanamide and ($^{14}N_2$)cyanamide, respectively (data not shown). For example, the A_{42}/A_{IS} value for ($^{14}N_2$)cyanamide coexisting with ($^{15}N_2$)cyanamide was larger than that without ($^{15}N_2$)cyanamide. Therefore, it was not appropriate to determine the ($^{14}N_2$)cyanamide and ($^{15}N_2$)cyanamide contents directly from the A_{42}/A_{IS} and A_{44}/A_{IS} values. In the present study, we used the $(A_{42}+A_{44})/A_{IS}$ value to determine the content of ($^{14}N_2$)cyanamide plus ($^{15}N_2$)cyanamide (total cyanamide content). The ($^{14}N_2$)cyanamide and ($^{15}N_2$)cyanamide contents were then determined by multiplying the total cyanamide content by $A_{42}/(A_{42}+A_{44})$ and $A_{44}/(A_{42}+A_{44})$, respectively.

A linear relationship between the amounts of total cyanamide injected and the $(A_{42}+A_{44})/A_{IS}$ values was observed in the range of 10–120 ng of total cyanamide injection (Fig. 2A). In the range lower than 10 ng of total cyanamide injection, the $(A_{42}+A_{44})/A_{IS}$ values changed logarithmically, probably because of the characteristics of cyanamide detected as a tailing peak. For quantifying small amounts of total cyanamide, a logarithmic regression model would be more suitable (Fig. 2B).

In the GC–MS analysis, the limit of detection for the total cyanamide content, which was determined at a signal-to-noise ratio of 3, was around 1 ng. The relative standard deviation (RSD) for the $(A_{42}+A_{44})/A_{IS}$ value was 5.4% ($n=10$) when 10 ng of cyanamide with natural isotope abundance plus 11.8 ng of ($^{15}N_2$)cyanamide was repeatedly injected. The standard curve for ($^{14}N_2$)cyanamide is substantially the same as that for ($^{15}N_2$)cyanamide over the range tested.

3.3. Determination of stable isotope dilution ratio of cyanamide by GC–MS analysis

The molar ratios of ($^{14}N_2$)cyanamide to ($^{15}N_2$)cyanamide in the injected samples were equal to the A_{42}/A_{44} ratios determined by the GC–MS analysis in the range between 0.1 and 5 (Fig. 3). Therefore, it is possible to determine the natural cyanamide content from the A_{42}/A_{44} ratio by applying the stable isotope dilution method; i.e., the natural cyanamide content is derived by subtracting the A_{42}/A_{44} ratio of the internal standard from the A_{42}/A_{44} ratio of sample spiked with the internal standard, and then multiplying the resulting difference by the amount of added ($^{15}N_2$)cyanamide. The RSD for the A_{42}/A_{44} ratio was 2.8% ($n=10$) when 10 ng of cyanamide with natural isotope abundance plus 11.8 ng of ($^{15}N_2$)cyanamide was repeatedly injected; this value was smaller than the RSD for the $(A_{42}+A_{44})/A_{IS}$ ratio (5.4%). Therefore, in the A_{42}/A_{44} ratio range between 0.1 and 5, it would be better to determine the natural cyanamide content by using the A_{42}/A_{44} ratio (stable isotope dilution method, SID-GC–MS method) for accuracy and simplicity, instead of determining total cyanamide content from the standard curve as a function of $(A_{42}+A_{44})/A_{IS}$ and multiplying it by the $A_{42}/(A_{42}+A_{44})$ ratio.

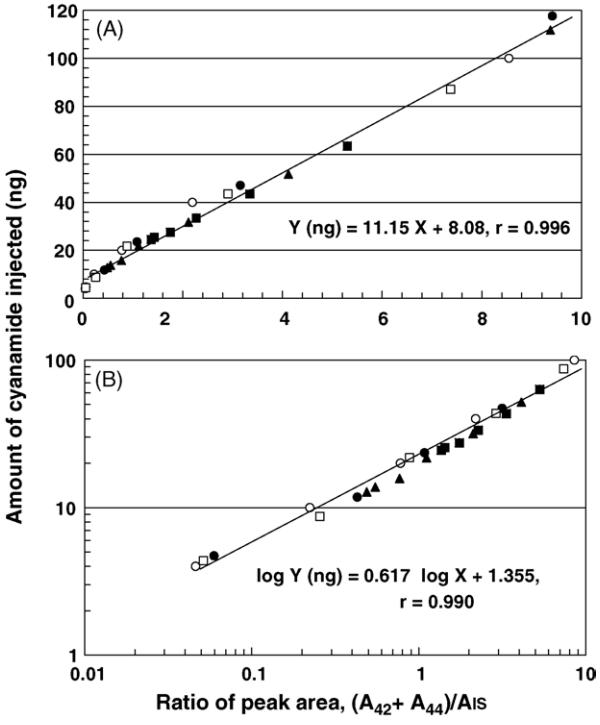

Fig. 2. Standard curves for determining the amount of total cyanamide [($^{14}N_2$)cyanamide plus ($^{15}N_2$)cyanamide] as a function of peak area ratio, $(A_{42}+A_{44})/A_{IS}$: (A) linear regression model; (B) log–log regression model; (◯) cyanamide with natural isotope abundance; (□) ($^{15}N_2$)cyanamide; (●) ratio of cyanamide with natural isotope abundance to ($^{15}N_2$)cyanamide was 0.85; (▲) arbitrary amount of cyanamide with natural isotope abundance plus 11.8 ng of ($^{15}N_2$)cyanamide; (■) arbitrary amount of cyanamide with natural isotope abundance plus 23.5 ng of ($^{15}N_2$)cyanamide.

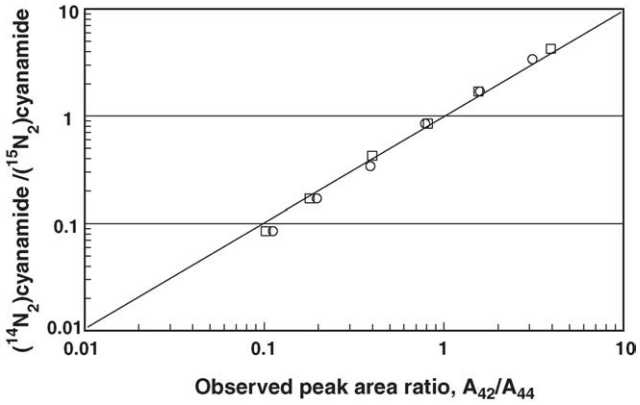

Fig. 3. Relationship between the molar ratio of cyanamide with natural isotope abundance to ($^{15}N_2$)cyanamide in the injected sample and the A_{42}/A_{44} ratio determined by GC–MS analysis. (◯) Arbitrary amount of cyanamide with natural isotope abundance plus 11.8 ng of ($^{15}N_2$)cyanamide; (□) arbitrary amount of cyanamide with natural isotope abundance plus 23.5 ng of ($^{15}N_2$)cyanamide.

Table 1
Natural cyanamide content in hairy vetch as determined by the SID-GC–MS method and the CNQ-HPLC method [10]

Quantification method	Internal standard added[a] (mg kg^{-1} fresh weight)	Recovery (%)	Cyanamide content[b] (mg kg^{-1} fresh weight)	RSD (%)
SID-GC–MS	29.4	104.1	100 ± 7	6.9
	88.2	92.6	103 ± 2	1.7
	264	102.0	100 ± 3	3.1
CNQ-HPLC	50.0	103.2	102 ± 7	7.1

[a] ($^{15}N_2$)Cyanamide was added as the internal standard in the SID-GC–MS method, and cyanamide with natural isotope abundance was added as the internal standard in the CNQ-HPLC method.
[b] Values are average ± standard deviation (n = 3).

3.4. Quantification of cyanamide in a plant extract of hairy vetch by the SID-GC–MS method and comparison with the CNQ-HPLC method

In the CNQ-HPLC method, a good correlation (r = 0.99) was observed between the amount of derivatized cyanamide injected (in the range of 0.75–250 ng injected as cyanamide) and the peak area. The cyanamide added as an internal standard was completely recovered and the cyanamide content in the plant extract of hairy vetch as determined by the CNQ-HPLC method was 102 ± 7 mg kg^{-1} fresh weight (Table 1).

In the SID-GC–MS method, signals of ($^{14}N_2$)cyanamide, ($^{15}N_2$)cyanamide, and *m*-(trifluoromethyl)benzonitrile were well-resolved in the mass chromatograms at *m/z* 42, 44, and 171 in the GC–MS analysis, respectively (Fig. 4). In the SID-GC–MS method, the added ($^{15}N_2$)cyanamide (internal standard for the stable isotope dilution method) was completely recovered at each of three levels of addition (Table 1). The natural cyanamide contents in hairy vetch as determined by the SID-GC–MS method were equal for each of the three levels of added internal standard, and were consistent with the value determined by the CNQ-HPLC method. This indicates that the SID-GC–MS method developed in the present study is as effective as the CNQ-HPLC method in determining the natural cyanamide content.

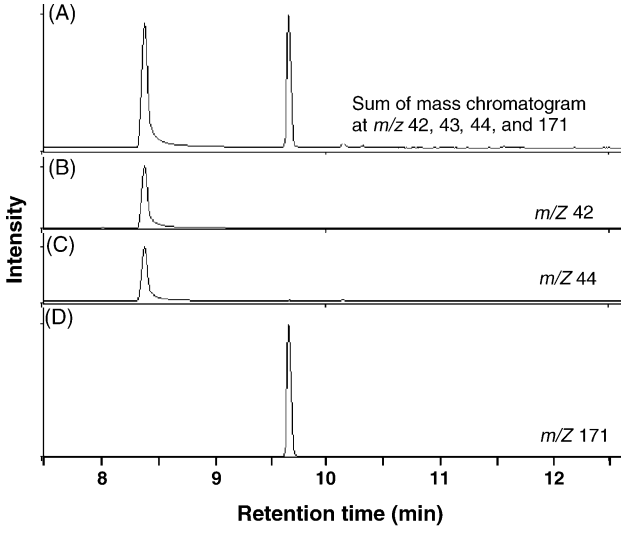

Fig. 4. Representative GC–MS chromatograms of a sample solution prepared from a hairy vetch extract containing natural cyanamide, ($^{15}N_2$)cyanamide (internal standard for the stable isotope dilution method), and *m*-(trifluoromethyl)benzonitrile (internal standard for correcting errors in GC–MS analysis): (A) signals recorded as the sum of mass chromatograms at *m/z* 42, 43, 44, and 171; (B) mass chromatogram at *m/z* 42 for the determination of ($^{14}N_2$)cyanamide; (C) mass chromatogram at *m/z* 44 for the determination of ($^{15}N_2$)cyanamide; (D) mass chromatogram at *m/z* 171 for the determination of *m*-(trifluoromethyl)benzonitrile. The *y*-gain values of all chromatograms were adjusted to the same value. The amount of added ($^{15}N_2$)cyanamide was 88.2 mg kg^{-1} fresh weight of hairy vetch (see Table 1). For other conditions, see Section 2.3.

The determination range of cyanamide in the SID-GC–MS method was almost the same as that in the CNQ-HPLC method. As for the experimental operation, however, the SID-GC–MS method is much simpler than the CNQ-HPLC method. While only one purification step is required in the SID-GC–MS method, three purification steps and one derivatization step are necessary in the CNQ-HPLC method. Furthermore, with the SID-GC–MS method, one set of samples supplies two data (i.e., the quantitative natural cyanamide content and recovery rate), whereas with the CNQ-HPLC method, two sets of samples are required to obtain these two data. Hence, using the SID-GC–MS method would be advantageous for quantifying natural cyanamide contents.

4. Conclusions

It was possible to quantitatively determine cyanamide content with a GC–MS equipped with a capillary column for amines. The total cyanamide content was first determined as a function of $(A_{42} + A_{44})/A_{IS}$, and the ($^{14}N_2$)cyanamide and ($^{15}N_2$)cyanamide contents were then calculated by multiplying the total cyanamide content by $A_{42}/(A_{42} + A_{44})$ and $A_{44}/(A_{42} + A_{44})$, respectively. The limit of detection for the total cyanamide content determined by the GC–MS analysis was around 1 ng. The molar ratio of ($^{14}N_2$)cyanamide to ($^{15}N_2$)cyanamide in each injected sample was equal to the observed A_{42}/A_{44} ratio in the range from 0.1 to 5. It was, therefore, possible to apply the SID-GC–MS method to quantify the natural cyanamide content in samples, i.e., the natural cyanamide content was derived by subtracting the A_{42}/A_{44} ratio of the internal standard from the A_{42}/A_{44} ratio of sample spiked with the internal standard, and then multiplying the resulting difference by the amount of added ($^{15}N_2$)cyanamide. This method successfully gave a reasonable value of natural cyanamide content in hairy vetch, which was consistent with the value determined by the conventional CNQ-HPLC method. The determination range of cyanamide in the SID-GC–MS method

was almost the same as that in the CNQ-HPLC method; however, the SID-GC–MS method was much simpler than the CNQ-HPLC method.

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