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Analytical Methods

Determination of cyanamide residue in 21 plant-derived foods by liquid chromatography-tandem mass spectrometry

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ABSTRACT

Cyanamide is widely used in agriculture, and has a modest toxicity in humans. In the present study, a simple, sensitive, and widely applicable method for detecting cyanamide in 21 plant-derived foods was developed. In the proposed method, after the samples were homogenized and extracted, the method employs clean-up with multi-walled carbon nanotubes (MWCNTs) and derivatization with dansyl chloride. The derivatized sample extracts were quantified with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The mean recoveries were in the range of 67.4%–107.1%, and the RSDs were between 1.0% and 17.8%. The quantification limit in shiitake, green tea and chinese pepper was 0.05 mg/kg, and in others 18 plant-derived foods was 0.01 mg/kg. Among the data of 5 different laboratories, the repeatability limits (r) ranged from 0.0010 to 0.0941, and the reproducibility limits (R) ranged from 0.0031 to 0.2667. Moreover, the repeatability among different testing personnels in the same laboratory also has been examined.

1. Introduction

Cyanamide (Fig. 1) is a multifunctional agrochemical such as plant growth regulator, pesticide, and fertilizer (Cai et al., 2012; Hiradate, Kamo, Nakajima, Kato, & Fujii, 2005). It has been used for a number of years on fruit crops to induce uniform bud break, break the winter rest period, enhance production efficiency, and optimize harvest timing and management (Pramanik, Dutta, & Bhattacharyya, 2009). Additionally, in soils cyanamide is detoxified and transformed into ammonia, possibly by enzymatic and catalytic reactions through urea or dicyandiamide, whereby it works as a nitrogen fertilizer (Estermaier, Sieber, Lottspeich, Matern, & Hartmann, 1992). However, cyanamide is modest toxic to humans and causes respiratory irritation, contact dermatitis, headache, and gastrointestinal symptoms of nausea, vomiting, or diarrhea (Hiradate et al., 2005; Nagumo et al., 2009; Schep, Temple, & Beasley, 2009). Therefore, the low maximum-residue limits (MRLs) of cyanamide in agricultural products have been established, such as 0.02 mg/kg in apples and 0.05 mg/kg in grapes in Australia (New Zealand Food Safety Authority, 2011), and 0.01 mg/kg in fruit in European Union (EU) (European Commission, 2014). However, determination of cyanamide in plant-derived foods is difficult, because its solubility in water is higher than those in almost all organic solvents, and cyanamide is a highly volatile compound (Cai et al., 2012). In the previous studies, before 2009 a series of methods for detecting cyanamide have been described, but these may be insufficiently sensitive, narrowly applicable or time consuming (Chen, Ocampo, & Kucera, 1991; Hiradate et al., 2012; Nair, 1994). In 2009, two simple methods for determining cyanamide in grape and hairy vetch have been reported respectively (Nagumo et al., 2009; Pramanik et al., 2009). The cyanamide residue in grapes is extracted with water. After evaporation, the residue is redissolved in aqueous acetic acid solution, and then, directly analyzed by HPLC with ultraviolet detection (UV) (Pramanik et al., 2009). In the other method, cyanamide from hairy vetch (Vicia villosa) plants, which contain cyanamide as a major plant-growth inhibitor (a possible allelochemical), is extracted and diluted with aqueous acetic acid solution, and then reacted with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). Then, the derivative is analyzed using UPLC with UV (Nagumo et al., 2009). However, the LOQ of the two methods are both 0.1 mg/kg, higher than the MRLs in EU. In 2012, Cai et al. developed a sensitive and simple method with the LOQ of 0.01 mg/kg in grape, kiwi, and peach. However, the applicability of all these methods is not extensive. Therefore, it is necessary to develop a widely applicable method.

QuPPe method was developed by the EU Reference Laboratories for residues of highly polar pesticides involving simultaneous extraction and LC–MS/MS determination directly (European

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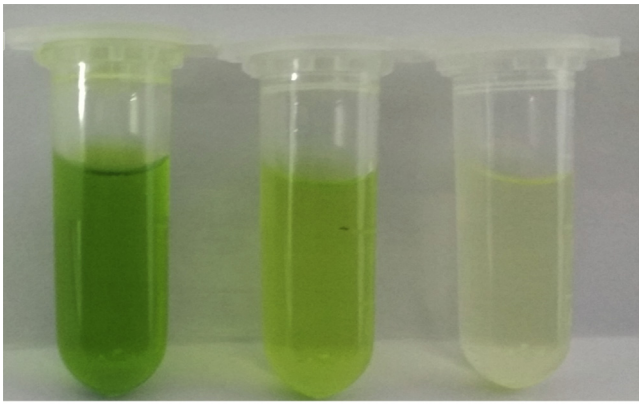

(A) (B) (C)

Fig. 1. Photography of cleanup performance of (A) extract for leek with 50 mg PSA, (B) extract for leek with 10 mg MWCNTs and (C) extract for leek with diatomite SPE.

Commission, 2015). However, we found the results were not entirely satisfactory when completely according to this method, mainly reflected in the purification process. As we all know, the corn and soybean contain a lot of starch and oil, the fruits and vegetables are full of sugar and pigments, and green tea is rich in chlorophyll and alkaloids (Han et al., 2016). These matrix interferences can affect the ionization of the target compound. So the cleanup procedure was necessary and of great significance.

Carbon nanotubes are novel and interesting carbonaceous materials, which are classified as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) on the principle of carbon atom layers in the wall of nanotubes (Qin et al., 2016). Recently, multi-walled carbon nanotubes (MWCNTs) have been reported to absorb the interfering substances in the fruit and vegetable matrices owing to their extremely large surface area and unique structure, and also the MWCNTs have a good effect for the purification of complex matrix, such as leek, onion, ginger, garlic and tea (Han et al., 2016). There are numerous interferences in plant-derived foods, which may interfere with the determination of cyanamide quantitatively and qualitatively. In addition, the molecular weight of cyanamide is too small to measure. Thus, MWCNTs cleanup and derivatization reaction were used in our study.

In the present study, a simple, sensitive, and widely applicable method for detecting cyanamide in plant-derived foods was developed. This method is applicable on 8 categories of plant-derived foods (cereals, oil plants, vegetables, fruits, edible fungus, vegetable oil, tea, and spices), which include 21 kinds of plant-derived foods (brown rice, wheat, corn, peanut, brussel sprouts, celery, tomato, eggplant, potato, radish, common bean, leek, apple, peach, grape, orange, almond, shiitake, soybean oil, green tea and chinese pepper).

2. Materials and methods

2.1. Chemicals and reagents

Cyanamide (purity ≥ 98.0%) was provided by Alfa Aesar (China). Dansyl chloride (purity ≥ 98.0%) was supplied by J&K Scientific (China). The MWCNTs were purchased from Agela Technologies (Tianjin, China) (5–10 nm), and Beijing Botai Minan Biological Technology Co., Ltd (Beijing, China) (10–20 nm). All analytical grade reagents (acetonitrile, acetone, *tert*-butanol, Sodium carbonate, sodium bicarbonate and so on) were purchased from Yili Fine Chemicals (Beijing, China). Water was purified by a Millipore Purification System (Milli-Q system). The stock solutions of cyanamide (1000 mg/L in acetone) and dansyl chloride (5.0 g/L in acetone) were prepared. The sodium carbonate/sodium bicarbonate buffer was prepared by volumetrically combining 4.0 mL of 0.2 M sodium carbonate with 46 mL of 0.2 M sodium bicarbonate. All solutions were stored at 4 °C.

2.2. Sample preparation

The method on shiitake, green tea and chinese pepper was validated at 0.5, 0.1, and 0.05 mg/kg cyanamide, and on other 18 plant-derived foods was validated at 0.5, 0.05, and 0.01 mg/kg. Each assay was conducted in quintuplication per-concentration. All the samples were crushed separately in a homogenizer except soybean oil. Since cyanamide is easily soluble in water and most of the plant-derived food contains water, so the water originally contained in the plant also should be calculated in the volume of the extraction solvent. According to the quick method for analysis of highly polar pesticide in EU (QuPPe-Method) (European Commission, 2015), the water content of plant-derived foods will be knew. The extraction solvent (Table 1) was added according to the optimized extraction method. Extracts were treated with a vortex mixer for 5 min, and with an ultrasonic bath for 10 min, followed by centrifugation at 4000 rpm for 5 min. In order to clean up, 2.0 mL the extracts of shiitake, green tea and chinese pepper were mixed with 2.0 mL *n*-hexane twice, and then discarded *n*-hexane. (the other 18 extracts of samples were not needed). One milliliter of the extract from each sample was transferred to a 1.5 mL centrifuge tube containing MWCNTs (almond, peanut, and soybean oil were not needed), mixed with a vortex mixer for 1 min, and centrifuged for 1 min at 4000 rpm, followed by filtration (0.22 μm) to remove MWCNTs and impurities. The method of derivatization is based on the EPA method for measurement of cyanamide residues in soil (Environmental protection Agency (EPA), 1993). To each sample, 0.5 mL of the extract was delivered to a 2 mL centrifuge tube and added with 0.5 mL dansyl chloride solution (5.0 g/L) and 0.5 mL sodium carbonate/sodium bicarbonate buffer solution. The sample solutions were briefly vortexed and placed in a water bath cauldron heated at 50 °C for one hour. Then the samples were removed from the cauldron and allowed to cool to room temperature. Finally, the samples were filtered (0.22 μm) and analyzed with LC-MS/MS. Additionally, the details of sample preparation method are showed in Table 1.

2.3. Cyanamide standards and linearity

The control samples of 21 plant-derived foods were extracted by the method as previously described (2.2 sample preparation), and the mixture of each control extract and cyanamide solution was derivatized with dansyl chloride also as the description. The calibration curves on shiitake, green tea, and chinese pepper were constructed using 0.005, 0.01, 0.05, 0.1, and 0.2 mg/L, and on other 18 plant-derived foods were constructed using 0.0005, 0.005, 0.01, 0.05, and 0.1 mg/L.

2.4. Precision of the method

The examination of precision includes the repeatability among different testing personnels in the same laboratory, and the reproducibility among different laboratories. In order to examine the repeatability among different testing personnel, 4 testing personnels repeated the method as previously described at 0.05 mg/kg cyanamide (shiitake, green tea, and chinese pepper at 0.1 mg/kg) in 21 plant-derived foods (n = 3). Data were tested for statistical significance using analysis of variance (ANOVA) followed by SPSS 17.0. The means were considered significantly different when the probability (*P*) was less than 0.05. In addition, chose 11