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Analysis of trace dicyandiamide in stream water using solid phase extraction and liquid chromatography UV spectrometry

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ABSTRACT

An improved method for trace level quantification of dicyandiamide in stream water has been developed. This method includes sample pretreatment using solid phase extraction. The extraction procedure (including loading, washing, and eluting) used a flow rate of 1.0 mL/min, and dicyandiamide was eluted with 20 mL of a methanol/acetonitrile mixture (V/V = 2:3), followed by pre-concentration using nitrogen evaporation and analysis with high performance liquid chromatography–ultraviolet spectroscopy (HPLC–UV). Sample extraction was carried out using a Waters Sep-Pak AC-2 Cartridge (with activated carbon). Separation was achieved on a ZIC®-Hydrophilic Interaction Liquid Chromatography (ZIC-HILIC) (50 mm × 2.1 mm, 3.5 μm) chromatography column and quantification was accomplished based on UV absorbance. A reliable linear relationship was obtained for the calibration curve using standard solutions (R^2 > 0.999). Recoveries for dicyandiamide ranged from 84.6% to 96.8%, and the relative standard deviations (RSDs, n = 3) were below 6.1% with a detection limit of 5.0 ng/mL for stream water samples.

Introduction

Dicyandiamide (DCD) is a chemical compound used broadly as a nitrification inhibitor, which mixes with urease inhibitors and urea to prevent nitrogen loss in the soil and keep nitrogen in the stable ammoniacal form for a longer period of time (Willison and Anderson, 1991; Di and Cameron, 2011; Zhang et al., 2009). It has also been applied for promoting the growth of grass in cow pastures. However, contaminated grass and drinking water may result in the production of milk with trace levels of dicyandiamide residues. Low levels of dicyandiamide residues in 10 out of 100 samples of Fonterra dairy milk products from the North and South Islands of New Zealand in 2012 have been reported (Lucas, 2013). Investigation of the toxicology (Jia et al., 2008) suggested that when DCD was given to mice at the highest dose, after 16 hr the mice began to show restlessness, jumping, and shortness of breath, and later died within a few minutes. According to the dosage and the mortality of mice, the dicyandiamide LD_{50} was greater than 5000 mg/kg, and its acute toxicological properties were similar to those of melamine. Therefore, DCD residue in food has become a severe concern in milk and dairy products due to the potential risk to children's health. Dicyandiamide (Fig. 1) is a strong polar amine compound (Stockel, 1969) which can be stable in water streams. The source of drinking water for pasture animals in many New Zealand farms is contaminated with DCD because of its stability in water. Therefore, there is a growing desire to monitor trace dicyandiamide in water streams located near pastureland where DCD has been applied.

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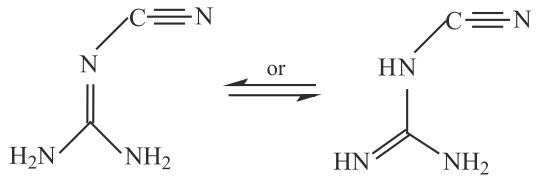

Fig. 1 – Structure of dicyandiamide.

A paper chromatographic method (Milks and Janes, 1956) was first developed to detect dicyandiamide in 1956, but the method provides only qualitative analysis. Spectroscopic (Pretorius et al., 1991) and additional chromatographic methods (Zhou et al., 2009; MacMahon et al., 2012; Hiradate et al., 2005; Xu et al., 2013) have been applied for DCD analysis in the food and medical industries. Turowski et al.'s study (Turowski and Deshmukh, 2004) demonstrated detection limits at 100 ng/mL in an aqueous solution, and another method (Chen et al., 2013) obtained a limit of quantification (LOQ) up to 0.5 ng/g DCD in dairy products using high performance liquid chromatography–diode array detector (HPLC–DAD). A high performance liquid chromatography–tandem mass spectrometry (HPLC–MS–MS) method was developed (Wang et al., 2012) that produced a limit of detection (LOD) of 0.3 ng/mL in food samples. However, most of these methods are expensive (tandem mass spectrometer needed). They have low sensitivity and reproducibility, and require complex sample preparation (pre-column derivatization). Therefore, there is a growing need to develop a fast, simple and cheap method for analyzing trace dicyandiamide in environmental water mixtures.

Sample pretreatment is very important when carrying out trace analysis, because an effective sample pretreatment method (Zheng et al., 2008; Liu et al., 2014; Tang et al., 2014; Tran et al., 2013) may increase overall method sensitivity. Oasis MCX mixed-mode cation exchange and reversed-phase sorbent has high selectivity for basic compounds and is suitable for compounds with amine groups. This sorbent has active carbon nanoparticles, which possess high surface activity and result in efficient adsorption and desorption of DCD. DCD contains a cyanide and amine groups, therefore, cation exchange mode extraction cartridges such as proElut PXC and Sep-Pak AC-2 (containing activated carbon) could also be used as part of a detection and quantification method.

The goal of this work was essentially pragmatic: to develop a method using simple apparatus that is effective, highly sensitive, cheap and easily implemented in ordinary laboratories for monitoring trace level DCD.

1. Materials and methods

1.1. Reagents, apparatus, equipment and solutions

Dicyandiamide (analytical grade) to prepare the required analytical standards, and acetonitrile and methanol (HPLC grade) were purchased from Sigma Aldrich (USA). Ultrapure water was produced by a Milli-Q water purification system (Direct-Q3, Merck Millipore, Wimereux, France). Calibration standard solutions were diluted with ultrapure water to obtain different concentration solutions in the range of 1.0–100.0 mg/L, then 0.2 mL of each solution was diluted to 2.0 mL with acetonitrile to obtain the final corresponding standard solution, respectively. The eluting solution was prepared by mixing two volumes of methanol with three volumes of acetonitrile.

Sep-Pak AC-2 solid phase extraction (SPE) cartridges (400 mg, Waters Corporation, Milford, MA, USA) were purchased from Waters. A nitrogen evaporator (N-EVAPIII, Organmation Associates Inc., West berlin, MA, USA) and vortex mixer (Standard mixer, Fisher Scientific, Fair Lawn, NJ, USA) were applied. The HPLC–UV system was an Agilent 1200 with UV detector system (HPLC-1200, Agilent Technologies Inc., Santa Clara, CA, USA) and liquid chromatography column ZIC®-Hydrophilic Interaction Liquid Chromatography (ZIC-HILIC) (50 mm × 2.1 mm, 3.5 μm, Merck & Co. Inc., Darmstadt, Germany).

1.2. Instrumental analysis

The analysis of DCD was performed on an Agilent 1200 HPLC system. Separation was achieved with a ZIC-HILIC column (50 mm × 2.1 mm, 3.5 μm). The column temperature was kept at 22 °C, the mobile phase was an acetonitrile aqueous solution (acetonitrile:water = 90:10), the flow rate was 0.1 mL/min and injection volume was 5 μL. Total run time was 6 min and the UV detection wavelength was set at 219 nm.

1.3. Solid phase extraction and preparation of solution for analysis

According to the manufacturer's instructions, Sep-Pak AC-2 cartridges (400 mg) were activated using 10 mL acetonitrile and equilibrated with 10 mL ultrapure water. Water samples (20–100 mL) were then loaded onto the cartridges and adsorbed by activated carbon at a flow rate of 1 mL/min. Subsequently, the cartridge was washed with 10 mL of ultrapure water. Dicyandiamide was eluted immediately with 20 mL of an acetonitrile/methanol mixture (ratio 3:2, V/V) with a flow rate of 1 mL/min.

In order to obtain reasonable extraction efficiency, 10–50 ng/mL standard solutions with increments of 10 ng/mL were selected for the experiment, with three replicates for each concentration. Different concentration water samples were loaded onto a Sep-Pak AC-2 cartridge and eluted with separate 1 mL portions of acetonitrile and methanol mixed solution, respectively.

After the Sep-Pak AC-2 solid phase extraction, the 20 mL eluant solution was evaporated using a nitrogen stream in a 50 °C water bath for 15 min; the residue was then dissolved in 1 mL of a 90% acetonitrile aqueous solution (LC mobile phase solution). Subsequently, the acetonitrile aqueous solution was vigorously shaken using a vortex mixer for 30 sec and filtered by a 0.45 μm filter membrane; the final solution was then ready for the LC-UV analysis.

1.4. Calculation method for recovery

Recovery experiments were also carried out to evaluate the extraction efficiency of the method. 20–100 mL of dicyandiamide-spiked water samples was extracted using the above-mentioned method. Concentrations of the spiked samples were determined

using a calibration curve. Recoveries were calculated as the following Eq. (1):

$$R = (X_1 - X_2)/X_3 \times 100\% \quad (1)$$

where, R is the recovery; X_1 is the observed amount for the spiked sample; X_2 is the observed amount for the unspiked sample; and X_3 is the amount in the spiked sample.

2. Results and discussion

2.1. Selection of chromatographic separation column

DCD is an organic amine compound containing two amine groups that are extremely polar. Following the reported literature (MacMahon et al., 2012; Xu et al., 2013), comparative experiments were carried out using a ZIC-HILIC column (50 mm × 2.1 mm, 3.5 μm) and an Agilent ZORBAX Extend C18 column (150 mm × 4.6 mm, 5 μm). Experimental results showed that with the Extend C18 column, retention was too weak to separate interfering substances, such as urea, from DCD. Although the ZIC-HILIC column required more time to equilibrate than the Extend C18 column, the ZIC-HILIC column had better separation under the method conditions specified. Therefore the ZIC-HILIC column was selected for this method. The mobile phase (acetonitrile:water = 9:1) was optimized at a flow rate of 0.1 mL/min to obtain optimum separation. The chromatograms of dicyandiamide in water samples are shown in Fig. 2.

2.2. Selection of solid phase extraction column

The extraction of the dicyandiamide compound from water by SPE is difficult because of its strong polarity. Therefore we studied the SPE for dicyandiamide precisely using various polymeric adsorbent materials. The strong cation exchange mode-sulfonic acid group SPE cartridges, such as Oasis MCX (Mixed-mode Cation Exchange, Waters Corporation, Milford, MA, USA), proElut PXC (Mixed-mode Cation Exchange, Dikma Technologies Inc., Lake Forest,CA,USA), and proElut SPE (Ion Exchange, Dikma Technologies Inc., Lake Forest, CA, USA), and an activated carbon adsorption SPE cartridge (Sep-Pak AC-2, Waters Corporation, Milford, MA, USA) were investigated in the experiment. According to the conditions for using SPE cartridges and the chemical properties of dicyandiamide, the methanol and acetonitrile can be evaporated quickly and the UV cut-off wavelength is suitable for a UV detector, thus, the mixed solution of methanol and acetonitrile was selected to elute the analyte from SPE cartridges.

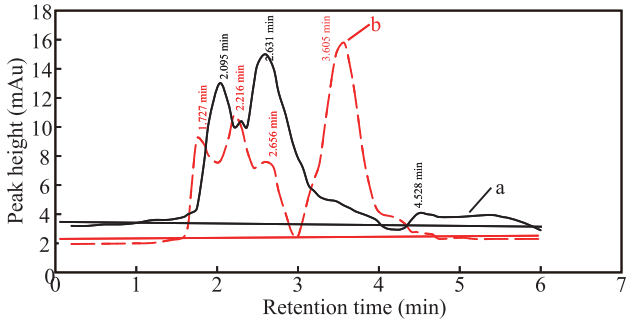

Fig. 2 – Chromatogram of dicyandiamide (DCD) in water sample. (a) No sample pretreatment for DCD aqueous solution of 10 ng/mL; (b) after SPE and concentrated 20 times for DCD aqueous solution from 5 to 100 ng/mL. Peaks at 3.665 and 4.528 min are characteristic of DCD. mAu: 10^3 absorbance unit.

It is well recognized that the solution flow rate may affect the extraction efficiency and an appropriate slow flow rate is suitable for extraction. According to the cartridges' recommended values for flow rate (1.0–2.0 mL/min) and saturated capacity, the experiment selected 20–100 mL water samples, which were then loaded onto the cartridges and eluted with a flow rate of 1.0 mL/min under the natural gravitational discharge. Meanwhile, the eluent volume was optimized in order to ensure complete elution of the analyte.

In addition to testing the different cartridges, the effect of varying the pH of the water was studied. DCD is a weakly basic compound; therefore, the water sample should be acidified before SPE because then the analyte is charged and experiences maximum retention, primarily via the ion-exchange mechanism, which is beneficial to be able to capture the basic analyte and then wash out interfering substances aggressively. The optimal pH value of the dicyandiamide solutions for the different cation exchange SPE cartridges was approximately 2.0. However, the effect of varying the pH value when using the activated carbon adsorption SPE cartridge (Sep-Pak AC-2, Waters Corporation, Milford, MA, USA) was extremely small, and the Sep-Pak AC-2 cartridge demonstrated the best extraction efficiency compared to other cartridges without sample pH adjustment.

After the extraction, the cartridges were not allowed to dry. They were washed of interfering compounds using 10 mL H_2O and then immediately eluted with 20 mL of a mixture of methanol and acetonitrile (2:3, *V/V*). The compounds were eluted into 10 mL glass vials. The solvent mixture was evaporated to dryness using a gentle stream of nitrogen in a water bath at 50 °C, then 1.0 mL acetonitrile was used to dissolve the analyte in vials. Absolute recoveries were determined using a calibration curve.

The extraction efficiency was calculated using 5–50 ng/mL DCD standard solutions with three replicates. The average extraction efficiency of these cartridges is shown in Fig. 3, and Table 1 shows that the extraction recovery of DCD was 84.6%–96.8%. It should be noted that the operator technique and different cartridge batches also affect the extraction efficiency in water samples, as shown in Fig. 3.

2.3. Matrix effect

Matrix effects were determined by preparing a calibration curve in acetonitrile and comparing the response to a curve prepared with post-extraction matrix extracts (Matuszewski et al., 2003). The response at each concentration in a matrix extract was divided by the response of the equivalent concentration in the acetonitrile solvent. The linear regression equations of DCD in solvent and matrix were $Y_1 = 0.2633C_1 - 0.8633$ ($R_1 = 0.9999$)

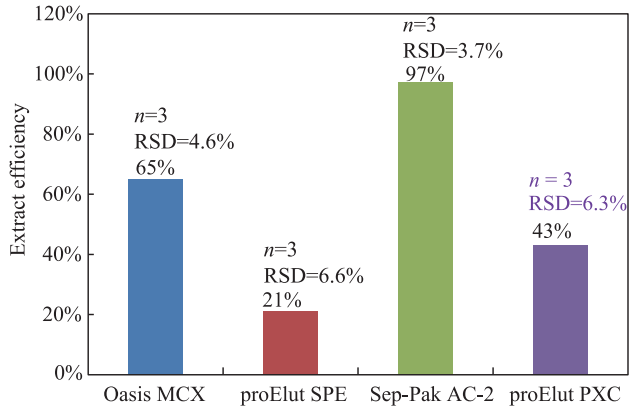

Fig. 3 – Solid phase extraction efficiency. RSD: relative standard deviation; *n*: the number of replicates; Sep-Pak AC-2, Oasis MCX, proElut SPE, proElut PXC are different types of SPE cartridges.

and $Y_2 = 0.2421C_2 + 4.9498$ ($R_2 = 0.9997$), respectively. In this study, we used Eq. (2) to calculate the matrix effect.

$$\text{Matrix effect} = (X_4 - X_5)/X_4 \times 100\% \quad (2)$$

where, X_4 and X_5 are slopes of the calibration curves of dicyandiamide in pure solvent and matrix, respectively. A calculated matrix effect with a positive value indicates ion suppression, whereas a negative value indicates ion enhancement. An efficient SPE step may eliminate some of the co-eluting substances and result in reduced matrix interferences. The experiment result shows that the matrix effect for the pretreatment matrices was 8.1% (<12%). Research workers have emphasized that matrix effects are not significant if the matrix effect value is less than 12% for trace organic compounds in environmental water (Moreno-Bondi et al., 2009). Since the extraction method used for this study reduced matrix effects, the preparation of a matrix-matched calibration curve was not necessary. Linear regression for the two calibration curves was 0.999. In addition, the experiment achieved acceptable recoveries (84.6%–96.8%) using pure solvent calibration curves.

2.4. Detection limits and linear range

This method has enrichment and concentration factors of 100 fold, where the water sample pretreatment procedure should be considered. The method limit of detection (LOD) and limit of quantitation (LOQ) were determined with a blank post-extraction matrix with known standard concentrations, measuring the concentrations where the signal to noise ratios of quantitative transition for dicyandiamide were greater than 3:1 (LOD) and 10:1 (LOQ). Subsequently the LOD and LOQ of the method were calculated using seven replicates to obtain a standard deviation. In the present study, a method LOD 1.5 ng/mL and LOQ 5.0 ng/mL were successfully achieved in the matrix water samples.

In addition, the linearity of the present method was established using different working concentrations of dicyandiamide. Results showed that this method has a good linear range of 5.0–1000 ng/mL with R^2 of 0.9997.

2.5. Recovery and dicyandiamide concentration of water samples

In this study, experimental water samples were collected from New Zealand pasture streams in the southland province of New Zealand. Three different classes of water samples were selected including New Zealand stream water, simulated water samples with matrix chemicals, and New Zealand blank water samples. The recoveries, which ranged from 84.6% to 96.8%, are summarized in Table 1. Relative standard deviations were less than 6.1%. Higher than 100% recovery is the result of enrichment or operation factors, whereas low R would occur if a fraction of the analyte is lost during extraction. The method is considered to be reliable for quantification of DCD in stream waters, and it has relatively low relative standard deviations based on three replicate analyses.

3. Conclusions

The method was optimized to ensure fast and sensitive determination of trace dicyandiamide by combining solid phase extraction with HPLC-UV. The solid phase extraction method significantly reduced about 70% of the matrix interference. A low limit of quantification of 5 ng/mL was achieved using a relatively small sample volume of 20–100 mL. The experimental results suggested that the method is reliable and consistent, as the method produced acceptable recoveries in the range of 84.6%–96.8% with 1.8%–6.1% RSDs.

Table 1 – Results of water samples and recovery experiment.

Sample describe	Amount of unspiked sample (ng/mL)	Spiked amount (ng/mL)	Enrichment factor	Amount of spiked sample (ng/mL)	Recovery	Relative standard deviations
Mixed water 1# from	13.5	10.0	20	450	95.7%	6.1%
New Zealand stream	13.5	10.0	20	399	84.6%	
	13.5	10.0	20	426	90.6%	
Mixed water 2# from	50.5	50.0	10	986	96.2%	1.8%
New Zealand stream	50.5	50.0	10	962	91.4%	
	50.5	50.0	10	953	89.6%	
Simulated water of matrix	0.0	50.0	20	942	94.2%	3.7%
from New Zealand	0.0	20.0	50	916	91.6%	
blank water	0.0	10.0	100	968	96.8%	

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