J. Dairy Sci. 96:1–6
http://dx.doi.org/10.3168/jds.2013-6881

Microwave-assisted extraction and determination of dicyandiamide residue in infant formula samples by liquid chromatography-tandem mass spectrometry

Yan Shen,*[1] Chao Han,† Xiujin Zhou,‡ Xiangzhun Chen,† Fuzhen Huang,† and Zhenou Zhu†
*College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou 325035, China
†Wenzhou Entry-Exit Inspection and Quarantine Bureau of P.R.C, Wenzhou 325027, China
‡Zhoushan Entry-Exit Inspection and Quarantine Bureau of P.R.C, Wenzhou 325027, China

ABSTRACT

A simple, precise, accurate, and validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the determination of dicyandiamide residue in infant formula samples. Following microwave-assisted extraction with 5% formic acid and clean-up on a Sep-Pak AC-2 SPE cartridge, samples were separated on a ZIC-HILIC HPLC column (150 × 2.1 mm i.d., 5-μm film thickness; Merck KGaA, Darmstadt, Germany) with 20 m*M* ammonium acetate solution-acetonitrile as mobile phase at a flow rate of 0.25 mL/min. A linear calibration curve was obtained in the concentration range from 1.0 to 50 ng/mL. Infant formula samples were fortified with dicyandiamide at 3 levels, producing average recovery yields of 83.6 to 95.7%. The limits of detection and quantification of dicyandiamide were 3 and 10 μg/kg, respectively. Due to its simplicity and accuracy, the straightforward method is particularly suitable for routine dicyandiamide detection.

Key words: dicyandiamide, infant formula, microwave-assisted solvent extraction, liquid chromatography-tandem mass spectrometry

INTRODUCTION

Dicyandiamide (**DCD**), or cyanoguanidine, is produced on a large scale from Nitro-chalk via the intermediate compound cyanamide in a 2-step process. In addition to its various industrial applications, its potential as a nitrification inhibitor is of high importance in agriculture (Schwarzer and Haselwandter, 1996). The nitrification inhibitor DCD reduces the rate of nitrification in soils, subsequently limiting N losses. Dicyandiamide's bacteriostatic mode of inhibiting ammonia-oxidizing bacteria has raised concerns about the efficacy of frequent DCD use (Clough et al., 2007; Wakelin et al., 2013). Because of residual DCD on pastures and dairy cow eating these pastures, it has been reported that DCD was detected in infant formula. Thus, a sensitive method to measure residue levels of DCD in infant formula is needed to detect and evaluate whether low levels of this compound are present in products intended for infant consumption.

Dicyandiamide has been analyzed by various methods, including Raman chemical imaging (Qin et al., 2013), liquid chromatography-tandem mass spectrometry (**LC-MS/MS**; MacMahon et al., 2012), and LC (Nagumo et al., 2009). Very few reports exist about the determination of DCD in infant formula.

Currently, the application of advanced LC-MS/MS to analyze residues has allowed a broad range of compounds to be determined, and this has permitted the comprehensive assessment of food contaminants. Low maximum residue limits have fostered the development of more powerful and sensitive analytical methods to meet the requirements of complex samples, such as food. In this sense, LC-MS/MS with a triple quadrupole in multiple reaction monitoring mode has become the most widely used technique for the quantitation of (polar) residues in food, as reported extensively in the literature.

For isolation of the DCD, microwave-assisted solvent extraction (**MAE**) seems particularly attractive, because of the rapid heating of aqueous samples (Han et al., 2011). The principal of the method is that microwave energy is absorbed by the extractant, which in turn transfers it to the sample in the form of heat. Partitioning of the analytes from the sample matrix to the extractant depends mainly on the temperature and the nature of the extractant. Unlike classical heating, microwaves heat the entire sample simultaneously (Camel, 2000). Microwave-assisted solvent extraction can reduce both extraction time and solvent consumption compared with conventional methods. Many cases

Received April 1, 2013.
Accepted July 27, 2013.
[1]Corresponding author: shenyan@wzu.edu.cn

have already proven that MAE is a viable alternative to conventional techniques for many kinds of samples (Wang et al., 2012).

The present study proposes a method for the analysis of DCD in infant formula by LC-MS/MS. The method was then used to analyze 100 infant formula samples purchased from local supermarkets.

MATERIALS AND METHODS

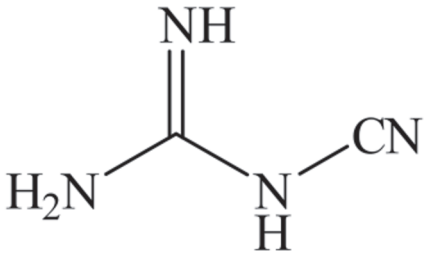
Chemicals, Reagents, and Equipment

All reagents and solvents were analytical grade unless otherwise specified. A certified DCD standard (99% purity) was purchased from National Institutes for Food and Drug Control (Beijing, China); a $^{15}N_4$-labeled DCD (**$^{15}N_4$-DCD**) standard was supplied by Toronto Research Chemicals Inc. (North York, ON, Canada). The structures of DCD and $^{15}N_4$-DCD are shown in Figure 1. The water used was purified with a Milli-Q water purification system (Millipore Corp., Bedford, MA); HPLC-grade dichloromethane, acetonitrile, and methanol were obtained from Merck KGaA (Darmstadt, Germany). Formic acid (99% purity) was from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Ammonium acetate was acquired from Alfa Aesar (Ward Hill, MA). An MAE system (Ethos; Milestone Systems, Wiesbaden, Germany) and LC-MS/MS (Agilent 1200-API 4000; Agilent Technologies Inc., Santa Clara, CA) were used in sample analysis. The LC and MS/MS analytical conditions are given in Tables 1 and 2, respectively.

Preparation of Standards

A stock standard solution of DCD was prepared at concentration of 100 μg/mL in acetonitrile, stored in a refrigerator at 4°C in the dark, and used to prepare working standard solutions by appropriate dilution with acetonitrile. Each standard solution was injected into the LC-MS/MS system 3 times. A specific average peak area was regressed with a specific level to calculate the calibration equation.

(A)



(B)

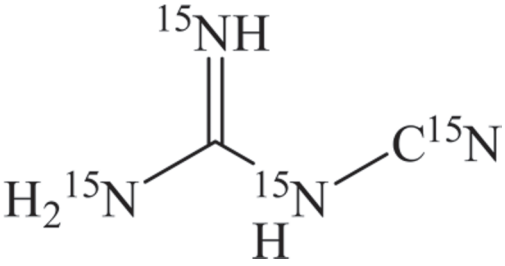


Figure 1. Chemical structure of (A) dicyandiamide (DCD) and (B) $^{15}N_4$-labeled DCD ($^{15}N_4$-DCD).

Sample Preparation

Microwave-assisted extraction was performed with an Ethos MAE system (Milestone Systems) equipped with a 12-vessel carousel, operated in the closed-vessel mode, and a magnetic stirrer. Polytetrafluoroethylene (PTFE)-lined extraction vessels were used and during operation both temperature and pressure were monitored; a fiber optic temperature sensor in the interior of the microwave oven was also used. An accurately weighed 1.00 g powdered sample was placed into an extraction vessel. Next, 500 μL of $^{15}N_4$-DCD (1,000 μg/mL) and 20 mL of 5.0% formic acid solution were placed in an extraction vessel and the vessel was capped. On the basis of a predesigned experimental trial, the temperature was increased to 80°C from room temperature in 5 min and then kept constant at 80°C for 25 min.

Table 1. Analytical conditions for liquid chromatography

Item	Details
Instrument	Agilent 1200 system (Agilent, Germany)
Column	ZIC HILIC HPLC column (150 × 2.1-mm i.d., 5-μm film thickness; Merck KGaA, Darmstadt, Germany)
Mobile phase	A = 20 m*M* ammonium acetate solution; B = acetonitrile; gradient elution used with the ratio of A:B varied as follows: 0 min, 10:90; 6 min, 10:90; 6.1 min, 40:60; 12 min, 40:60; 12.1 min, 10:90; 20 min, 10:90. Flow was diverted to waste from 0 to 3.5 min and from 6.0 to 20 min
Flow rate	0.25 mL/min
Column temperature	30°C