

Determination of Dicyandiamide in Milk Powder

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Key Words

Polar Compound, Dionex IonPac ICE-AS1 Analytical Column, Ion-Exclusion Chromatography (IEC), HPLC, Food Safety

Goal

To develop an efficient and simple high-performance liquid chromatography (HPLC) method for the determination of dicyandiamide in milk powder using an IEC column

Introduction

Dicyandiamide (structure shown in Figure 1) is a compound used by farmers to minimize the environmental impact of livestock on land. Dicyandiamide reduces the rate at which soil microbes convert ammonia from animal urine into nitrates and nitrous oxide, thus slowing nitrate leaching from the pasture.¹ Overuse of dicyandiamide can lead to its appearance in dairy products. Use of nitrogen-rich compounds such as dicyandiamide to make the protein content of food appear higher (i.e., protein adulteration) can also lead to dicyandiamide contamination in milk and milk powder. Recent reports about residues of dicyandiamide in dairy products highlight the need for fast and reliable analytical techniques to monitor dicyandiamide levels in milk and milk-derived products.

Both reversed-phase HPLC and liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been used to quantify dicyandiamide in various matrices.^{2,3} Dicyandiamide is a polar compound and thus is not well retained on commonly used C18 reversed-phase columns. Ion-pairing agents can be added to improve retention; however, use of ion-pairing agents is not recommended due to their irreversible damage to the columns. Hydrophilic interaction liquid chromatography (HILIC)

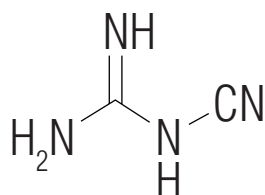


Figure 1. Structure of dicyandiamide.



also has been used for dicyandiamide determination;³ however, the high organic solvent (e.g., acetonitrile) consumption of this mode of chromatography opposes the trend toward green chemistry.

Because of the complex milk matrix, an off-line solid-phase extraction (SPE) method with a specific dicyandiamide cleanup column is frequently used to test milk products. In addition to significant cost in time and labor, this off-line cleanup method often fails to provide satisfactory separation of dicyandiamide and other peaks in the sample.⁴

Equipment

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system, including:
 - LPG-3400RS Quaternary RS Pump (P/N 5040.0036) with SRD-3400 Solvent Rack (P/N 5035.9230)
 - WPS-3000TRS RS Well Plate Autosampler (P/N 5840.0020), equipped with a 25 µL sample loop and a 25 µL syringe
 - TCC-3000RS RS Thermostatted Column Compartment (P/N 5730.0000)
 - DAD-3000RS Diode Array Detector without Flow Cell (P/N 5082.0010)
 - Semi-Micro Flow Cell, 2.5 µL (P/N 6082.0300)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.1 or above
- Thermo Scientific™ Sorvall™ ST16 Centrifuge (P/N 7500.4240)

Consumables

Thermo Scientific™ Target2™ Polypropylene Syringe Filters, 0.45 µm, 30 mm (P/N F2502-9)

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity, generated by the Thermo Scientific™ Barnstead™ GenPure™ Pro ultrapure water system with UV-photo-oxidation and TOC (total organic carbon) monitor (P/N 50131948)
- Acetonitrile (CH₃CN), HPLC Grade (Fisher Scientific P/N AC610010040)
- Formic Acid (HCOOH), Optima™ LC/MS Grade (Fisher Scientific P/N A117-50)

Chromatographic Conditions

Column:	Thermo Scientific™ Dionex™ IonPac™ ICE-AS1 Analytical, 9 × 250 mm (P/N 043197)
Mobile Phase:	3 mM formic acid-acetonitrile (3:2, v/v)
Flow Rate:	0.8 mL/min
Injection Volume:	10 µL (partial loop injection)
Temperature:	30 °C
Detection:	UV, absorbance at 220 nm

Preparation of Standard Solutions

Stock Standard Solution 1

Weigh 10 mg of dicyandiamide into a 10 mL volumetric flask and bring to volume with DI water. The concentration of dicyandiamide in the stock standard solution will be 1.0 mg/mL.

Stock Standard Solution 2

Dilute Stock Standard Solution 1 10-fold to make a 0.1 mg/mL solution.

Stock Standard Solution 3

Dilute Stock Standard Solution 2 10-fold to make a 0.01 mg/mL solution.

Working Standard Solutions for Calibration

Prepare six working standard solutions for calibration with 0.05, 0.5, 1.0, 5.0, 10.0, and 50.0 µg/mL concentrations by adding the correct amounts of stock standard solutions and diluting with DI water.

Sample Preparation

Weigh 1.0 g of milk powder into a 10 mL centrifuge tube, add 2.0 mL of DI water, vortex for 2 min, then add 8.0 mL of acetonitrile and vortex again for 20 min. Centrifuge the mixture for 10 min at 10,000 rpm. Store the supernatant at 4 °C and filter it through a 0.45 µm filter prior to analysis.

To prepare the spiked sample, add 50 µL of Stock Standard Solution 2 (dicyandiamide, 0.1 mg/mL) to 1.0 g of the milk powder sample. Prepare the sample as described above. The spike concentration will be 0.5 µg/mL.

Results and Discussion

Column Selection

The polar character of dicyandiamide makes it difficult to retain using a C18 stationary phase, and the results are poor when using ion-pairing agents to improve retention. An alternate approach for analyzing polar compounds, HILIC, is a frequently used method to determine dicyandiamide. Because milk powder is a complex matrix, it is possible that some compounds may coelute or elute near the dicyandiamide peak—an occurrence that has been observed in applications using a HILIC column.⁴

In contrast, IEC is capable of processing samples with very complex compositions. Strong acids that are completely dissociated and undissociated molecules both will elute in the column's exclusion volume and/or the sum of the column's inner volume and its exclusion volume. Only weakly ionized acids/bases with dissociation constants from 10⁻² to 10⁻⁷ can be separated by IEC.⁵ The Dionex IonPac ICE-AS1 IEC column can provide fast analysis of polar compounds such as aliphatic organic acids and alcohols in complex or high-ionic-strength samples including food and beverage products, biological samples, fermentation process samples, industrial process liquors, and wastewater.⁶ Therefore, the Dionex IonPac ICE-AS1 IEC column was used for this analysis.

Simplified Sample Preparation

The matrix of milk powder is complicated and proteins are the main molecules that interfere with dicyandiamide detection by UV. Use of acetonitrile to precipitate protein is a routine procedure in sample preparation. It is also sometimes necessary to use a cleanup cartridge to further clean a milk sample. This study demonstrates that IEC using the Dionex IonPac ICE-AS1 column can separate interfering peaks from the dicyandiamide peak without requiring the cartridge cleanup procedure. The column's polymeric structure allows use of low-pH mobile phases. Furthermore, HPLC solvents can control hydrophobic retention and provide effective column cleanup.⁶ Therefore, only the acetonitrile precipitation procedure was adopted prior to sample injection.

Reproducibility, Linearity, and Detection Limits

Method reproducibility was estimated by making eight consecutive injections of a milk powder sample spiked with 0.5 µg/mL dicyandiamide standard (0.5 µg/mL). Figure 2 shows the overlay of chromatograms of the eight consecutive injections. Retention time RSD is 0.026 and peak area RSD is 1.01, demonstrating good method reproducibility.

Calibration linearity of dicyandiamide was investigated by making three consecutive injections of the standard prepared at six different concentrations (0.05, 0.5, 1.0, 5.0, 10, and 50 µg/mL; 18 total injections). The external standard method was used to establish the calibration curve and quantify dicyandiamide in a milk powder sample. Good linearity was observed from 0.05 to 50 µg/mL when plotting the concentration versus peak area. The linear regression equation is $A = 1.1862c$ (force to zero axial), where A represents peak area, c represents concentration of the analyte, and the coefficient of determination is 0.9986. This calibration curve was used to quantify dicyandiamide in milk powder samples.

Eight replicate injections of milk powder sample spiked with 0.5 µg/mL dicyandiamide standard were used for estimating method detection limits (MDLs) using the single-sided Student's t test method. The calculated MDL was 10 µg/L.

Sample Analysis

Figure 3 shows the chromatograms of a milk powder sample analysis. No dicyandiamide was detected in the milk powder sample. In the spiked sample, 0.46 µg/mL of dicyandiamide was found; based on that, the recovery of the dicyandiamide was calculated at 92%, demonstrating good method accuracy.

Conclusion

The work shown here describes a simple HPLC method with excellent separation for the determination of dicyandiamide in milk powder samples. The separation power of the Dionex IonPac ICE AS1 column simplifies the sample preparation process and eliminates the cartridge cleanup procedure, thus reducing analysis time and cost.

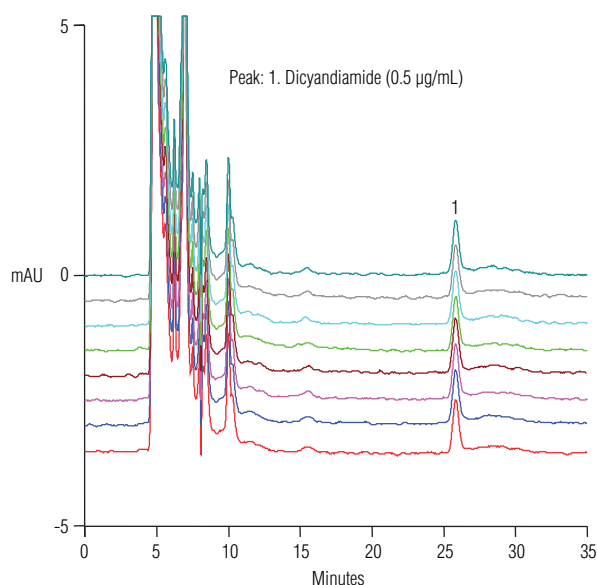


Figure 2. Overlay of chromatograms of eight consecutive injections of a milk powder sample spiked with 0.5 µg/mL dicyandiamide standard.

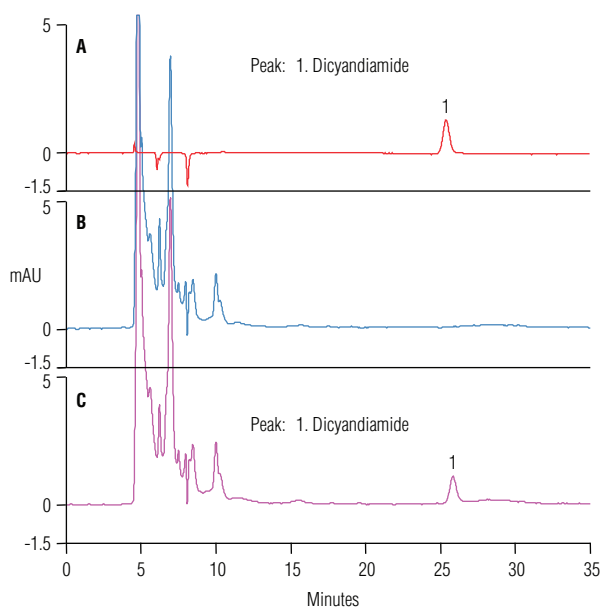


Figure 3. (A) A dicyandiamide standard (0.5 µg/mL), (B) a milk powder sample, and (C) the same sample spiked with dicyandiamide standard (0.5 µg/mL).

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