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Detection of Melamine in Dairy Products by Mass Spectrometry

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Abstract

Melamine is a nitrogen-rich chemical compound and is illegally added to dairy products by manufacturers to boost the nitrogen content misleading the protein content result of Kjeldahl method (i.e. the internationally recognized method for estimating the protein content in foods). According to U.S. Food and Drug Administration (FDA), when melamine and cyanuric acid are absorbed into the bloodstream, then concentrated and interacted in the renal microtubules to form large amount of crystals (kidney stones) which block and damage the renal cells, causing kidneys damage. In 2008, there is a serious food safety incident related to melamine-tainted infant formula milk powder causing illnesses of about 300,000 Chinese infants and young children and six reported deaths. The melamine-tainted event leads to a need for sensitive and reliable techniques for detection and quantitation of melamine in dairy products. This article reviews the most commonly used mass spectrometric analytical technique that has been used for the analysis of melamine in dairy products.

Keywords: Dairy products; Melamine; Liquid chromatography; Mass spectrometry

Introduction

Melamine (IUPAC name: 1,3,5-triazine-2,4,6-triamine) is an organic molecule with a molecular formula $C_3H_6N_6$ and molar mass 126.12 g/mol. Chemical structure of melamine is shown in Figure 1. It is a white solid at room temperature and is an organic base in nature. Melamine has a wide range of applications, it is used to manufacture many different useful commercial products, such as tableware, polymeric cleaning foam sponge, colorant inks and fire-retardant additives. However, some manufacturers illegally added melamine to food products. Melamine-contaminated pet feed causing over thousands of dogs and cats dead from kidney failure in North America in 2007. And after one year, melamine was illegally added to infant formula milk powder causing illnesses and dead of infant and children in China in 2008. (Figure 1)

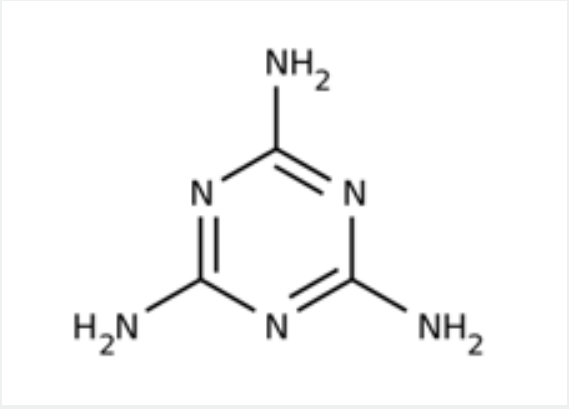

Figure 1: Chemical structure of melamine.

The regulation of melamine in food is that World Health Organization (WHO) set the tolerable daily intake (TDI) for melamine at 0.2 mg/kg of body weight per day. The Chinese Administration of Quality Supervision, Inspection and Quarantine has reported that the melamine content in dairy products determined was up to 6200 mg/kg which is over thirty thousand times of the melamine limit set by WHO [1]. Dairy products are food products that produced from milk. There are various dairy products in the market, such as milk, powdered milk, infant formula milk powder, ice-cream, butter, cheese and yogurt. According to United States Food and Drug Administration [2], the quality control methods for dairy products involve three major tests: Salmonella test, phosphatase test and penicillin test. Negative results must be obtained from these tests to ensure the dairy products are absence of Salmonella, phosphatase and penicillin. Salmonella is bacteria that can cause typhoid fever, paratyphoid fever, foodborne illness and human death [3]. Phosphatase is used as an indicator in dairy products because the destruction of phosphatase in dairy products coincides with the heat treatment used to kill Mycobacterium tuberculosis and other pathogenic organisms [4]. While penicillin is a group of antibiotics derived from penicillium fungi, it can cause penicillin allergy to human such as anaphylaxis, hives, below-the-skin swelling and asthma symptoms [5]. However, melamine test does not involve in the quality control methods for dairy products. The purpose of illegally adding melamine to dairy products by manufacturer is to elevate the apparent nitrogen content to give a false protein content result of Kjeldahl test method. There are various methods available for the detection of melamine in dairy products, for examples, micellar electrokinetic chromatography (MEKC), enzyme-linked immunosorbent assay (ELISA), liquid chromatography with photodiode-array detection (LC-PAD), gas chromatography with mass spectrometry (GC-MS) and liquid chromatography with mass spectrometry (LC-MS) [6].

Mass spectrometric methods are widely used to analyze melamine in dairy products in the world. It is because it can provide high sensitivity and high resolution. Mass spectrometry is a powerful analytical technique that analyze sample by ionizing the sample and detecting the ions according to their mass-to-charge ratios [7,8]. The molecular mass, molecular formula and concentration of sample can be determined from the mass spectrum obtained by mass spectrometry. It is excellent for both qualitative and quantitative analysis. Moreover, mass spectrometer is usually coupled with liquid or gas chromatography, LC-MS or GC-MS to analyze sample mixture. The mass spectrometer acts as a detector for the chromatography. Sample components that separated by chromatography are then analyzed by the mass spectrometer. The mass spectrum obtained is a plot of ion intensity against m/z ratio. The m/z ratio of molecular ion and fragment ions can provide structural information of the sample molecule for identification. And the ion intensity is proportional to the concentration of the sample molecule that can be used for quantitative analysis. The results obtained from mass spectrometry are high in accuracy and precision. This review is aimed to present an overview of the detection of melamine in dairy products by mass spectrometry. The most widely used mass spectrometric method, i.e. liquid chromatography with mass spectrometry (LC-MS) is discussed.

Discussion

In order to determine melamine in dairy products, liquid chromatography coupled with mass spectrometry (LC-MS) is the preferred method because this technique can give a high selectivity, sensitivity and reproducibility. The analytical ability of this technique can also be enhanced by using tandem mass spectrometry MS/MS or MS^n which can prevent false positive results, and hence precise, accurate and more reliable identification and quantification results can be obtained [9]. Good adaptability is also one of the advantages that using mass spectrometry to determine melamine in dairy products because mass spectrometry can be applied to the same analyte in different matrices of dairy products [10].

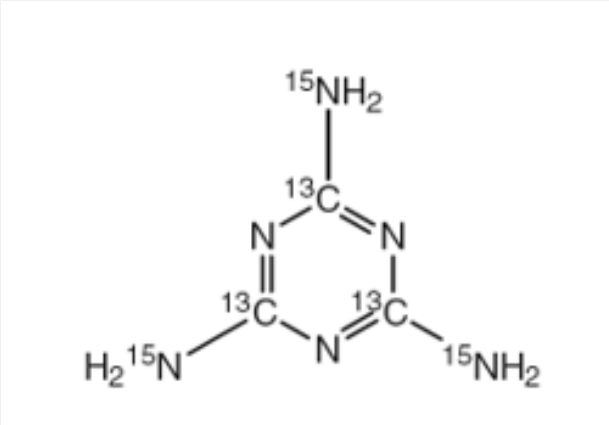

Figure 2: Chemical structure of melamine-13C3 and amino-15N3 labeled melamine.

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Liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis is the most commonly used method for determination of melamine in dairy products. By using internal standard method, melamine-$^{13}C_3$ and amino-$^{15}N_3$ labeled melamine (its chemical structure is shown in figure 2) is used as the internal standard to enhance the sensitivity and accuracy by reducing errors from the matrix effect or enhancement effects during mass analysis [11]. The internal standard used should be a compound with similar chemical and physical properties, but different mass as compared to the compound to be measured. A calibration curve is obtained by measuring samples containing the fixed quantity of the internal standard and increasing quantities of the melamine. The sample to be quantified is then measured with a constant quantity of internal standard added to it. Finally, the unknown concentration of melamine in the dairy products can be calculated from the calibration curve. (Figure 2)

Dairy products such as infant formula milk powder is a challenge to analysis because it is a very complicated biological samples that composed of different nutrients, including carbohydrates, proteins, fats, linoleic acid, vitamins A, C, D, E, K, thiamin (B1), riboflavin (B2), B6, B12, niacin folic acid, pantothenic acid, minerals calcium, magnesium, iron, zinc, manganese, copper, phosphorus, iodine, sodium chloride, potassium chloride and etc [13]. Since dairy products are very complex matrices, it generally needs different sample pre-treatment and cleanup procedures before the melamine analysis [14]. Table 1 showed that the experimental results of LC-MS/MS method for the quantitative determination of melamine in milk-based infant formula. The LC-MS/MS results demonstrated good recovery, relative standard deviation and limit of detection (LOD). It is a very accurate and precise method used to determine melamine in dairy products [15].

Conclusion

Melamine is a useful ingredient that can be synthesized into different materials for different uses. The most commonly used melamine product in the daily life is melamine-formaldehyde tableware which is a durable and hard thermosetting plastic with water and acid resistant properties. However, some manufacturers illegally added melamine to food, such as pet food, dairy products and infant formula milk powder in order to boost the nitrogen content misleading the protein content result of Kjeldahl method. These kinds of illegal action cause illnesses and dead of many pets, infant and children. Acute toxicity of melamine can cause nausea, diarrhea and vomiting. While chronic toxicity of melamine usually results in kidney stone formation and kidneys failure. Melamine usually interacts with its by-product cyanuric acid to melamine cyanurate which is more toxic than either melamine or cyanuric acid alone causing the problem worse. The melamine-tainted events lead to a need for sensitive and reliable techniques for detection and quantitation of melamine in dairy products. The testing method for determining melamine in dairy products based on U.S. FDA LC-MS/MS method is still the most commonly used method for melamine determination in the world until nowadays because of its high sensitivity and high accuracy and precision.

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