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Identification of melamine derivative in powdered milk samples by gas chromatography-mass spectrometry

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Abstract: An identification method for the analysis of melamine in powdered milk using gas chromatography-mass spectrometry (GC-MS) has been developed. Melamine analyte was extracted by homogenization of powdered milk in the homogeneous mixture of acetonitrile, water and diethylamine solvent. The homogenate was centrifuged and an aliquot of supernatant was diluted with acetonitrile, concentrated and fortified with a stable isotope-labeled analogue of melamine. Analyte was detected using atmospheric pressure chemical ionization and multiple reaction monitoring. Identification of positive samples was performed using the internal standard method. This method was successfully used for routine diagnosis of melamine toxicosis in powdered milk samples.

Keywords: dairy, contamination, analyte, acetonitrile, GC-MS, Bangladesh

Introduction

Melamine has been used in the production of melamine–formaldehyde resins for surface coatings, laminates and adhesives and in the production of flame retardants. Melamine is produced by the thermal condensation of dicyandiamide at over 300°C. During this process, some de-ammonia condensation derivatives, including melame and meleme, are produced.

These melamine derivatives have already been reported since 1834 [1, 2, 3]. However, there have been few studies on their physical and chemical properties because these melamine derivatives are chemically inert but show remarkably low solubility in aqueous solutions and organic solvents. Takimoto *et al*. [4, 5] studied the synthesis, isolation of melame and meleme and their characterization based on their IR and UV absorption spectra about 30 years ago. These derivatives could be separated using cation-exchange chromatography. However, the elution of melame and meleme in this system took 9–17 h and their determination was achieved only after isolation. Thus, their rapid and quantitative analysis has not been done satisfactorily. Due to these analytical problems, the fundamental study and the application of these derivatives are still unexplored.

Several methods have been developed for the analysis of melamine. These include methods utilizing gas chromatography (GC) [6], gas chromatography-mass spectrometry (GC/MS) [7], liquid chromatography (LC) [8, 9, 10], capillary electrophoresis [11] and immunoassay [12]. Among the methods available, gas chromatography-mass spectrometry (GC-MS) has been shown to be the most accurate, reproducible and the least time consuming technique [13].

In this paper, we describe the simple extraction and identification method for melamine in powdered milk samples by using GC-MS and establish the fairly comprehensive chromatographic finger print of melamine in the samples.

Materials and Methods

Chemicals and reagents

A standard sample of melamine was purchased from Merck (Darmstadt, Germany). Solvents used for chromatography were acetonitrile (GC grade), water (GC grade), diethylamine (GC grade) obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical grade or GC grade.

Sample collection

There are different brands of powdered milk samples available in Bangladeshi markets which are imported from different milk producing countries in the world. For our experiment we used 12 different varieties of powdered milk samples collected from local markets of Dhaka, Bangladesh.

Melamine extraction

Powdered milk (1 g) was weighed into a centrifuge tube and 40 ml of mixture solvent (1:4:5; diethylamine/water/acctonitrile) was added. The whole mixture was shaken vigorously (by hand). The solution was mixed for 30 minutes using a decon FS minor ultrasonic bath (England). The sample was then centrifuged for 15 min at 5000 rpm (ABBOTA^R Corporation, USA). The extract was filtered with a 0.45 µm polyamide membrane. 200 µl of the filtrate was transferred into a GC vial and evaporated under a freeze drier. After complete evaporation, 200 µl of pyridine and 200 µl of sylon BFT were added to the GC vial and finally 100 µl of additional pyridine as an internal standard was added. The cover on the vial was screwed, shaken and placed it in an incubator at 70°C for 45 minutes. The sample was then ready for injection.

GC–MS Instrument and program

The GC–MS analysis of the crude extract of powdered samples was performed using a Varian GC–MS (Model Varian CP 3800, USA) equipped with a VF-5 fused silica capillary column (30 m x 0.25 i. d. mm film thickness 0.25 µm, Varian, USA). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant low rate of 1 ml/min. Injector and mass transfer line temperature were set at 250 and 300^{o}C, respectively. The oven temperature was programmed from 100^{o}C to 200^{o}C at 10^{o}C/min, and then held for 1 min and finally raised to 300^{o}C at 10^{o}C/min. Diluted samples (1/100 v/v, in pyridine medium) of 0.2 µl were manually injected in the split mode. Identification of compounds of the crude extract was based on GC retention time on VF-5 capillary column and computer matching of mass spectra with standards (Mainlab, Replib and Tutorial data of GC–MS systems).

Melamine peak identification

The mass spectrum of the 3-TMS derivative of melamine, as shown in Figure 2, exhibits the molecular ion at m/z = 342, a methyl group loss at m/z = 327 (base peak for melamine) and the significant ions at m/z = 285, 197 and 73. The standard of melamine should be prepared (and TMS-derivatized) and analyzed along with the sample on the same day to confirm the identification.

Results and Discussion

Many different brands of imported powdered milk are available in markets in Bangladesh, thus increasing the chances for prohibited or dangerous additives. Powdered milk is usually fed to infants who, as has been shown in China, are a vulnerable group. Testing methods thus need to be developed to determine if such additives are present and at what levels. In our present experiment, all the tested samples (12) were collected from Dhaka city. The milk samples were collected in aluminum foil sealed packs.

The GC–MS modification method was applied for the analysis of melamine present in the powdered milk samples. In the present study, a programmed method was used for simultaneous assay of the authentic marker. The standard was determined in a single GC–MS run. The standard was resolved and eluted at 14.47 min, with respect to melamine (Fig. 1).

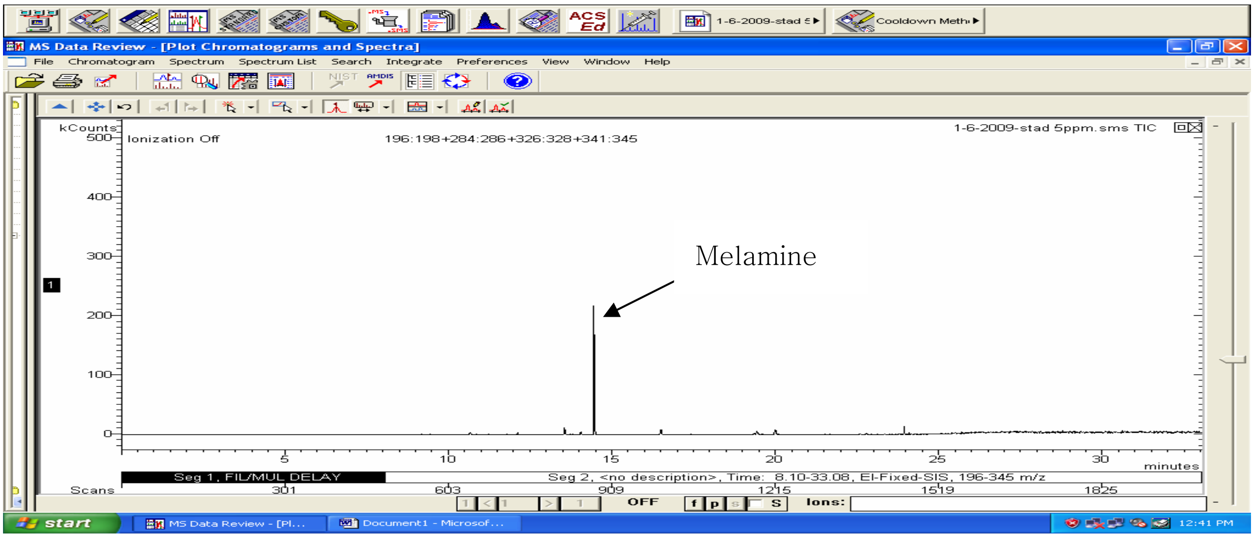

Figure 1. Chromatogram of the reference marker, melamine,

The reference marker was not present in the chromatographic profiles of the different brands of powdered milk samples when analyzed by GC–MS (Fig. 2).

The peak of melamine was confirmed by comparison of the retention times with reference standard.

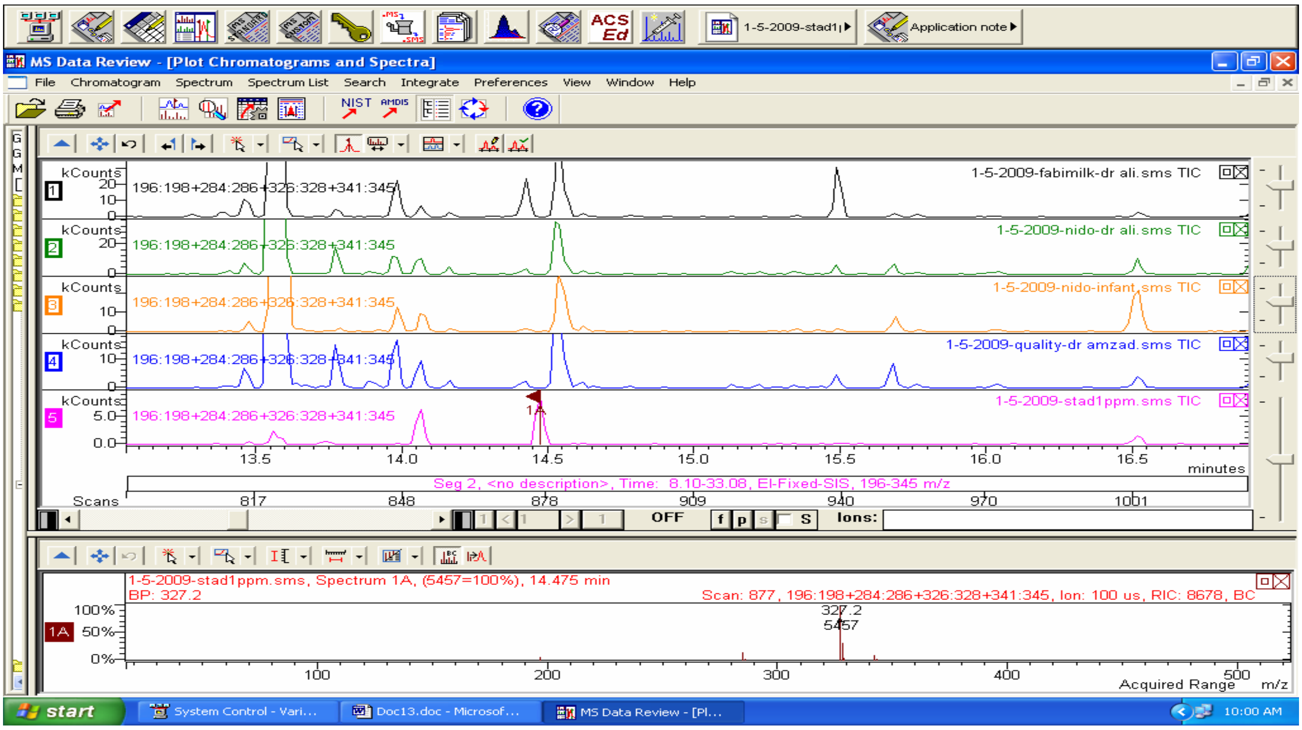

Figure 2. A typical chromatogram of the different brands of powdered milk samples and the standard.

Conclusion

The GC–MS chromatographic profiles of the powdered milk samples collected from the markets of Dhaka City were qualitatively similar but there was no melamine found in the powdered milk samples. This GC-MS method for analyzing melamine in the powdered milk sample is cost-effective, fast and sensitive.

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