Journal of Industrial and Engineering Chemistry 21 (2015) 138–144

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

Short communication

Electrospray ionization tandem mass spectrometry for rapid, sensitive and direct detection of melamine in dairy products

Suresh Kumar Kailasa [a,b,c], Hui-Fen Wu [a,b,d,e,f,*]

[a] Department of Chemistry, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan
[b] Center for Nanoscience and Nanotechnology, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan
[c] Department of Chemistry, S. V. National Institute of Technology, Surat 395007, India
[d] Institute of Medical Science and Technology, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan
[e] School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 806, Taiwan
[f] Doctoral Degree Program in Marine Biotechnology, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan

ARTICLE INFO

Article history:
Received 10 October 2013
Accepted 5 March 2014
Available online 13 March 2014

Keywords:
Melamine
Milk
Fruit juice
ESI–MS

ABSTRACT

A simple, rapid, and sensitive method for detection of melamine in dairy products by electrospray ionization mass spectrometry (ESI–MS) without any sample preparation is demonstrated. The ESI–MS/MS on the protonated melamine at m/z 127 produced the characteristic fragment ions at m/z 110, 85, 68, 60. This method has a good linearity in the range of 0.5–10.0 μg/mL, with detection limit 0.1 μg/mL and RSD 8.9% (n = 5). Together with existing recent methods on mass spectrometry and NP-based sensing methodologies for melamine detection, the present method provides an efficient platform for detection of melamine in milk products without any sample preparation steps or post-separation procedures.

1. Introduction

Melamine (2,4,6-triamino-1,3,5-triazine, MW 126 Da) is widely used as raw material in various industries including plastics, papers and fertilizers. Due to its fire resistance and heat tolerance, it is used as a flame retardant in the manufacture of wrinkle-free textiles. It is illegally added into adulterate protein-rich diets to increase the apparent protein content [1]. Several melamine analogues were the causes of a major outbreak of renal disease and associated deaths in cats and dogs in the USA which is due to its use in pet foods [2]. Besides, it has been found in several dairy products including yogurt and biscuit. Subsequently, it is also one of the contaminant in the dessert products of various countries such as China, Netherlands, New Zealand, Australia and America [3]. Recent clinical reports revealed that the large numbers of insoluble melamine cyanurate crystals are found in kidney and caused renal tissues damages in animals and humans [4,5]. After the scandal involving melamine tainted infant formula in China in September 2008 [6], the US Food and Drug Authority has fixed melamine concentration at ~1 ppm for infant's safety formula [3]. However, ~3300 ppm of melamine was detected in adulterated infant formula food products in China [3]. Kuang's and Xu's groups well reviewed the available analytical methods for the detection of melamine in various dairy products [7]. Furthermore, several analytical tools including HPLC [8], immunoassays [9], nanomaterials-based colorimetric methods [10–13] and capillary electrophoresis [14] have been used for determination of melamine in various samples. Even though these methods can effectively quantify melamine in food samples, they are time consuming [8,9] labor-intensive [9] and required lengthy procedures. Thus, it is necessary to develop sensitive analytical techniques for accurate quantification of melamine from food samples.

Recently, several chromatographic methods coupled with mass spectrometric techniques have been used to effectively detect melamine and its analogs in various food samples [14–18]. Briefly, it was efficiently extracted and detected by using ambient mass spectrometry without any chromatographic separation [17]. However, some methods required sample preparation steps such as isotope internal standards [18] or required special anionic ion-pair reagent (tridecafluoroheptanoic acid) for its satisfactory retention and peak shape in HPLC–MS [16]. Therefore, a continuous growing interest in identification/quantification of melamine

* Corresponding author at: Department of Chemistry, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan. Tel.: +886 7 5252000x3955; fax: +886 7 5253908.
E-mail address: hwu@faculty.nsysu.edu.tw (H.-F. Wu).

http://dx.doi.org/10.1016/j.jiec.2014.03.012
1226-086X/

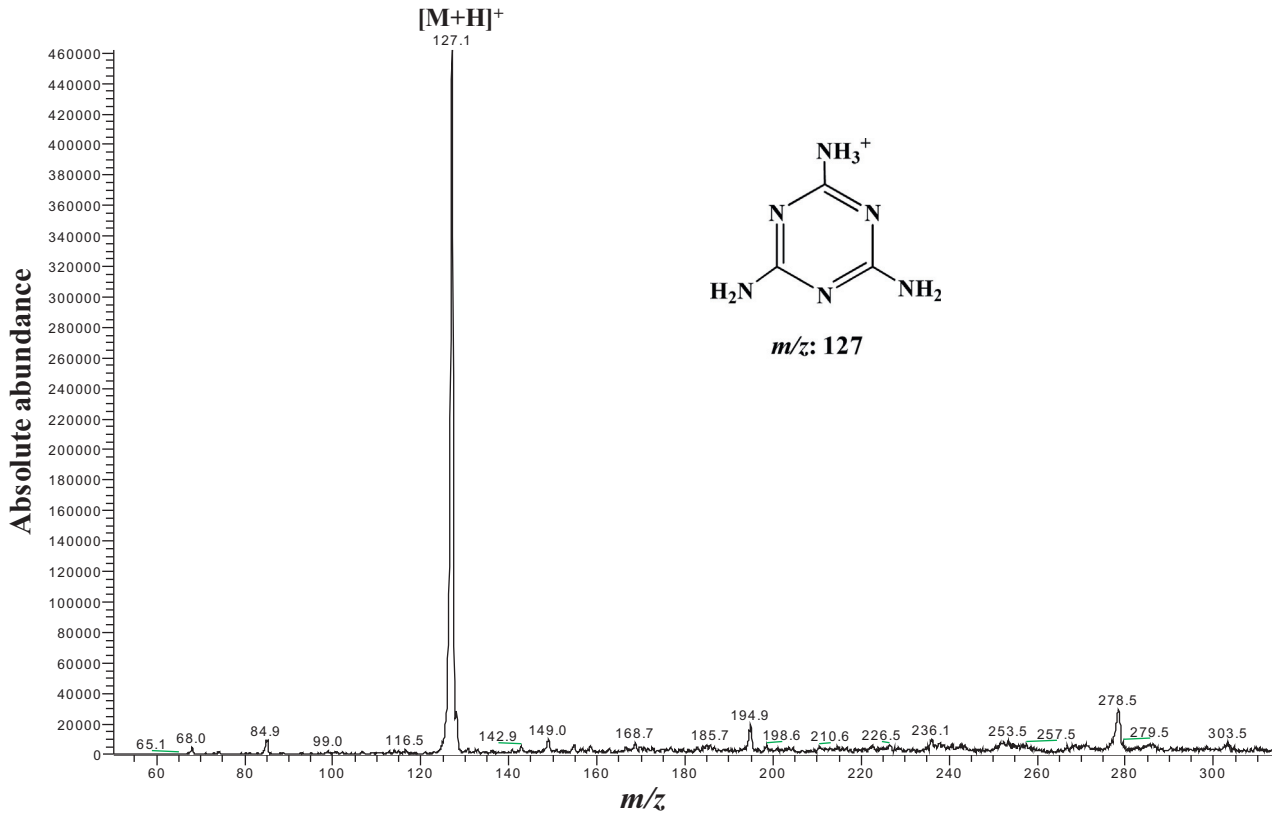

Fig. 1. Positive ion ESI mass spectrum of melamine (5.0 μg/mL) from spiked water.

in various food and biological samples by different mass spectrometric technique including direct analysis in real time (DART) [19], low temperature plasma [20,21], desorption electrospray ionization [22], surface desorption atmospheric pressure chemical ionization (DAPCI) [23], ultrasound-assisted extractive electrospray ionization (EESI) [24], matrix-assisted laser desorption ionization [25,26] and surface-assisted laser desorption ionization mass spectrometry [27] have been reported. These approaches can resolve the difficulties and allowed real time, high-throughput analyses of melamine in ambient/low pressure/atmospheric pressure with minimum or no sample preparation requirements. Herein, we report the use of ESI–MS/MS method for the rapid identification of melamine in milk products.

2. Experimental

Melamine standard was purchased from Alfa Aesar, MA, USA. Standard solutions were prepared by deionized water using a Milli-Q purification system (Millipore, Bedford, MA, USA). Stock solution of melamine was prepared by dissolving 1 mg/mL in methanol:water (1:1, v/v). Fruit juice and milk were purchased from local market in Kaohsiung, Taiwan. Urine sample was collected from a healthy volunteer.

2.1. Sample preparation

Prepared standard solution (1 mg/mL) was used for the quantification of melamine by ESI–MS. Working solutions were prepared by the dilution of standard solution. These solutions were stored at 4 °C and used for the construction of calibration graphs for melamine quantification. Melamine (0.5–10.0 μg/mL) was spiked onto three samples (urine, fruit juice and milk) and these samples were injected into ESI–MS for its identification and quantification in milk and urine samples.

2.2. Instrumentation

A Finnigan MAT ion trap mass spectrometer (Finnigan LCQ-Advantage, San Jose, CA, USA) equipped with an electrospray ionization source was used in this study. The ESI–MS was operated in the positive ion mode and the solution was injected into the ESI source through the pump at solvent flow rate 10 μL/min. The fine droplets were produced by applying tip voltage at 5000 V and the temperature was 250 °C. A good ESI mass spectrum was generated by using a tube lens offset voltage at 80 V and a capillary voltage at 10 V. Collisionally activated dissociation (CAD) was applied to the parent ion at *m*/*z* 127 to yield product ions for the structural characterization of melamine. Helium was used as the collision gas at energy of 20–35 eV. Each ESI mass spectrum was generated by 15 individual scans.

3. Results and discussion

3.1. Identification of melamine by ESI–MS/MS

Melamine structure is shown in Fig. 1. The positive ion mode of ESI–MS was used for the identification of melamine and its fragmented ions. The ESI mass spectra were obtained when a continuous stream of solvent was nebulized as fine droplets (aerosol) by applying a strong electric field. The fine droplets were evaporated and then the charged species were formed in the gas phase. These ions were sent into the ion optics and then separated according to their mass to charge (*m*/*z*). Fig. 1 shows the ESI mass spectrum of melamine (5.0 μg/mL) from aqueous samples. This result revealed that the protonated melamine ion is appeared