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Evaluation and single laboratory validation of an on-line turbulent flow extraction tandem mass spectrometry method for melamine in infant formula

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ABSTRACT

This report presents the single-laboratory validation of a method for the determination of melamine in dairy-based products using on-line turbulent flow extraction-tandem mass spectrometry. Liquid or powder test portions were dissolved in water, enriched with $^{13}C_3{}^{15}N_3$-Melamine internal standard, followed by protein precipitation and withdrawal of an aliquot for analysis. The turbulent flow method was validated by analyses of liquid and powdered proficiency test portions containing up to 10 mg/kg melamine. Accuracy of results ranged from 96 to 106% of the assigned values for the 6 proficiency test portions tested with relative standard deviations of 4–8%. Apparent recoveries based on addition of amino-$^{15}N_3$-Melamine to prepared test portions were between 98 and 114%. Based on the repeat analysis of a known blank sample the limit of detection and limit of quantification were determined to be 27 and 87 μg/kg, respectively. Additionally, this report demonstrates that turbulent flow chromatography is significantly faster than traditional LC–MS, with sample analysis times of less than 2 min.

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1. Introduction

A study of the 2004 and 2007 incidents of pet food-associated renal failure in cats and dogs led Brown to report compelling evidence that melamine and cyanuric acid were the causal agents [1]. Analyses for these chemicals in the ingredients used to manufacture pet food and trace back of these ingredients led to their discovery in other food products including animal feeds [2]. Cyanuric acid is permitted as a nitrogen supplement in animal feed [3]. However, its presence with melamine in these products was indicative of their intentional use as economic adulterants.

In response to the adulteration of pet food and feeds with melamine and cyanuric acid, various melamine assays were developed by U.S. Food and Drug Administration (FDA) Scientists. Heller and Nochetto developed a rapid method for the determination of melamine and cyanuric acid in animal feeds [4]. Smoker and Krynitsky collaborated to develop an in-house tandem mass spectrometry (MS) method, which used stable isotope dilution, for the determination of melamine and cyanuric acid residues in tissue [5]. This method was tested against incurred residues of these adulterants in fish and pork and performed well in a Fall 2009 collaborative study conducted by the Food Industry Analytical Chemists Committee (FIACC) of the Grocery Manufacturers Association (unpublished data) as well as the 2009 melamine proficiency test conducted by the Joint Research Centre (JRC) of the European Commission Institute for Reference Materials and Measurements [6].

In 2008, an increased incidence of infant kidney disease in China led Chinese authorities to discover melamine adulteration of infant formula and milk by several Chinese producers [7,8]. Although there had been no indication that US food imports had been contaminated, part of the FDA response was a survey of American infant formulas. The Smoker and Krynitsky method was applied to the analysis of infant formulas for melamine. Every American infant formula product tested was found to be safe (unpublished data), but the manpower, time and resources required to prepare and analyze the extracts were considerable. Consequently, the 2009 report [9] of a 4-min, on-line turbulent flow isolation of melamine from dairy-based food extracts was a significant advance in the methodology for high-throughput melamine measurement. This report formed the basis for the collaborative effort described below, which had the goal of testing and validating turbulent-flow methodology for high throughput foods analysis applications.

In turbulent flow chromatography, high flow rates are used with large (ca 50 μm), chromatographically active, porous polymeric particles. The high flow rates produce a turbulent flow, instead of the laminar flow achieved in traditional chromatographic systems. The small (ca 75–125 Å) pores, turbulent flow and solvent selection allow the retention of small analytes, while the larger matrix compounds, such as proteins, are washed from the column. After elution of matrix compounds, the mobile phase is changed to elute the previously retained small analyte(s) [10]. Since Quinn

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and Takarewski patented turbulent flow chromatography in 1997 [11], it has been used primarily for rapid determinations in pharmacokinetic studies and therapeutic drug monitoring [12–14]. For example, Srinubabu et al. described the development and validation of an automated method for loratadine and deloratadine in human plasma [15]. The extraction, separation and tandem MS quantification were completed within 8 min. On-line turbulent flow chromatography has been applied to environmental matrices such as the determination of pesticides in ground, waste, and drinking water [16,17]. Additionally, the application of turbulent flow chromatography to the analysis of food matrices, such as the determination of veterinary drugs in honey and milk, has been reported [18,19].

We report here the development of an on-line turbulent flow tandem MS method for the determination of melamine in dairy-based foods and its subsequent single laboratory validation. The resulting method is 15 times faster than the LC–MS/MS method of Smoker and Krynitsky and provides comparable results in analyses of proficiency test portions bracketing the Codex maximum level for infant formula [20].

2. Method

2.1. Reagents and materials

Melamine (99+%), acetic acid (99.7%), formic acid (98–100%), ammonium hydroxide (ACS reagent grade), ammonium acetate (99.999%) and HPLC grade acetone (99.9%) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO). $^{13}C_3{}^{15}N_3$-Melamine ($^{13}C_3$, 99%; amino-$^{15}N_3$, 98%) 100 μg/mL in water was purchased from Cambridge Isotopes Laboratories (Andover, MA). Amino-$^{15}N_3$-Melamine was synthesized for FDA in June, 2007, by Goncalo Gamboa, at the FDA National Center for Toxicological Research (Jefferson, AR). Optima LC–MS grade acetone, acetonitrile, methanol, 2-propanol, and water were purchased from Thermo Fisher Scientific (Pittsburgh, PA). Conical 50 mL screwcap polypropylene centrifuge tubes were purchased from VWR Scientific Products (Buffalo Grove, IL). Narrow-mouth 1 L Teflon wash bottles were purchased from Cole Parmer (Vernon Hills, IL) and fitted with narrow mouth solvent reservoir caps purchased from Waters (Milford, MA) for use as solvent reservoirs for basic solutions.

2.2. Sample preparation

Liquid concentrate and powder dairy products were prepared according to manufacturer's instructions prior to sampling. Liquid (2 mL) or solid test portions (1 g or less) of baking products were weighed into 50 mL centrifuge tubes. Liquid test portions were spiked with 200 ng $^{13}C_3{}^{15}N_3$-Melamine internal standard (200 μL), 1.8 mL 2% acetonitrile in water, and vortexed (0.5 min). For solid matrices, water (2 mL) was added to solid portions and vigorously vortexed for 0.5 min prior to and after addition of spiking solutions. To precipitate proteins a crash solution consisting of 30% aqueous solution of 50 mM ammonium acetate mixed with 70% acetonitrile (25 mL) was added to each test portion and vortexed for 1 min. The portions were then centrifuged at 9500 rpm for 10 min in a Beckman Coulter Allegra 21 centrifuge (Palo Alto, CA) to separate the precipitated solids from the supernatant. A 1 mL aliquot of clarified supernatant was withdrawn and transferred to an LC sample vial for analysis.

2.3. Instrumental analysis

Turbulent flow chromatography of the test portions was performed using a Transcend TLX-2 two-channel, two-dimensional LC system configured for turbulent flow on-line solid phase extraction and fast liquid chromatography (Thermo Fisher Scientific, San Jose, CA). Each channel, designated loading or eluting, utilized a quaternary LC pump to combine and pump the appropriate solvents. For this analysis, 3 solvents were used from the loading channel and 2 were used in the eluting channel. Only loading pump flow passed through the 100 μL sample injection loop. The eluting pump flow entered the LC system downstream from the injection loop. A prototype Cyclone™ MCX-2 cation exchange TurboFlow™ column was used for on-line extraction.

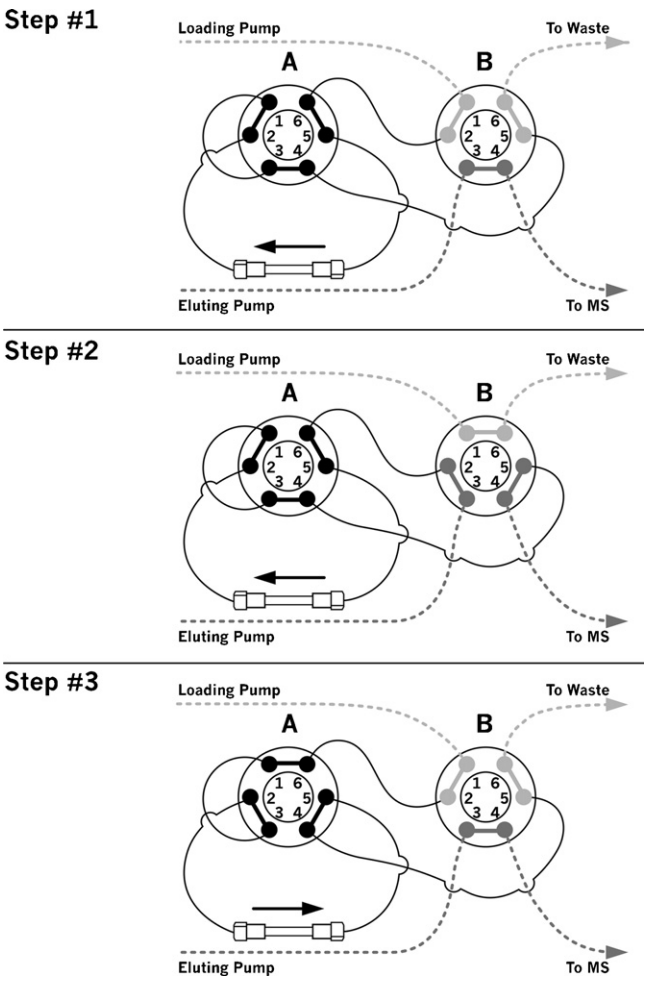

Fig. 1. Paths of loading and eluting pump flows during loading (step 1), elution (step 2), and backwash (step 3) of the TurboFlow™ column.

The first step of on-line extraction sequence (Table 1) began with a 5 μL injection loaded onto the MCX-2 column with 100% loading solvent A (0.1% formic acid and 5% methanol) at a flow rate of 2 mL/min for 1 min. The column effluent was directed to waste during this step (Fig. 1a). In the second step, melamine was eluted from the column, into the mass spectrometer, with 100% eluting solvent A (0.1% NH_4OH and 5% methanol) at 1 mL/min for 1 min. Simultaneously, loading solvent B (100 mM $NH_4C_2H_3O_2$ and 5% methanol, pH 9), was directed to waste at a flow rate of 1 mL/min (Fig. 1b). In the third step, the MCX-2 column was backwashed with loading solvent B at 2 mL/min for 0.5 min (Fig. 1c). Eluting solvent flow was reduced from 1 mL/min to 0.5 mL/min and stepped from 100% eluting solvent A to eluting solvent A/B (methanol, 20/80). In the fourth step the column was backwashed with loading solvent C (2-propanol and acetone 50/50) for 1 min at 2 mL/min while eluting solvent (20/80) continued at 0.5 mL/min to the mass spectrometer. In the fifth step the MCX-2 column and valve system