Direct analysis of melamine in complex matrices using a handheld mass spectrometer†‡

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A low temperature plasma ambient ionization source, coupled to a portable mass spectrometer (Mini 10.5), is used for the determination of melamine contamination in whole milk and related materials. Thermally assisted desorption and ionization of the analyte was achieved with the plasma probe. The small size, low power consumption and capability for direct sampling without pretreatment makes plasma ionization an appropriate ionization method for use with a handheld mass spectrometer. The standard discontinuous atmospheric pressure interface used to connect atmospheric pressure ion sources to mass spectrometers (Gao *et al.*, *Anal. Chem.*, 2008, **80**, 4026–4032) was modified by using supplementary pumping to increase the ion transfer efficiency. Whole milk, fish, milk powder and other complex matrices spiked with melamine were placed on glass slides close to the vacuum inlet and analyzed without sample pretreatment. Quantitation in complex matrices was achieved using MS/MS of protonated melamine *m*/*z* 127 to yield the characteristic fragment ion of *m*/*z* 85. Analysis rates of two samples per minute, levels of melamine as low as 250 ng/mL in whole milk (below the regulatory level in the US of 1 ppm (1 μg/mL) or the European level of 2.5 ppm (μg/mL)), a linear dynamic range of 0.5–50 μg/mL and a relative standard deviation of *ca.* 7.6–16.2% were achieved. The importance of melamine to public health and the prior lack of a rapid, sensitive and yet highly specific field analysis method add to the relevance of this study.

Introduction

Melamine, a nitrogen-rich (66.7% by weight) industrial chemical, has been deliberately added into various foods to artificially increase the apparent protein content as judged by total nitrogen measurement (the Kjeldahl nitrogen determination[1]). In 2007, pet food adulteration with melamine leading to kidney toxicity in cats and dogs was reported, and in September of 2008, melamine-contaminated milk resulted in kidney stones and renal failure in infants.[2–4] Melamine reacts with its metabolite cyanuric acid to form a poorly soluble stable complex which can precipitate in renal tubules and lead to kidney failure.[5] Instrumental methods for melamine analysis in food have been developed rapidly in response to the public health alarm: they include immunoassays,[6] capillary zone electrophoresis with diode array and mass spectrometry detection,[7,8] high performance liquid chromatography coupled with ultraviolet absorption[9,10] and with mass spectrometry,[11–15] gas chromatography/mass spectrometry[10,16] and ultra-performance liquid chromatography with tandem mass spectrometry.[17] However, all these methods require tedious sample preparation, for example, the US Food and Drug administration (FDA) has published a method for screening melamine in pet food using GC/MS,[18] which takes about 3 h for a single detection although by using parallel operations the time per sample can be decreased. The continuing need for rapid screening of melamine has led to the application of several of the new ambient ionization mass spectrometric techniques, specifically low temperature plasma (LTP),[19,20] ultrasound-assisted extractive electrospray ionization mass spectrometry (EESI),[21] direct analysis in real time (DART)[22] and desorption atmospheric pressure chemical ionization (DAPCI)[23] for the rapid, sensitive and quantitative analysis of melamine in complex mixtures. The key characteristic of ambient ionization MS is that samples can be analyzed *in situ*, in their native environment and without sample preparation. These ionization methods greatly increase analysis speed but they have only been implemented on conventional laboratory instruments.

Based on a rectilinear ion trap (RIT) mass analyzer,[24] a handheld (10 kg) tandem mass spectrometer known as the Mini 10.5, was developed at Purdue University.[25] In previous studies, this mass spectrometer was applied to the direct analysis of trace amounts of explosives,[26] hydrocarbons[27] and various environmentally relevant compounds.[28,29] Although various ionization methods have been used in conjunction with miniature mass spectrometers, appropriate ionization sources for direct analyte detection in complex matrices are still in a rudimentary stage of

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development. Ambient ionization methods[30–36] are particularly appropriate for use with handheld instruments. They include desorption electrospray ionization (DESI),[30–32] DART,[37–39] electrospray laser desorption ionization,[40] laser ablation electrospray ionization,[41] and DAPCI[42] as well as a group of closely related plasma-based methods (flowing afterglow-atmospheric pressure glow discharge,[43,44] plasma-assisted desorption ionization,[45] dielectric barrier discharge ionization[46] and the present low temperature plasma ionization.[19]) These ionization methods allow the direct mass spectrometric analysis of compounds present in condensed phase samples.

In a prior short communication, we have reported LTP probe ionization for melamine screening using conventional benchtop mass spectrometers.[20] LTP is a particularly appropriate ionization method for use in conjunction with a miniature mass spectrometer, given the following characteristics: (1) direct sampling occurs without sample pretreatment (except for optional heating), (2) no solvents are used, (3) air can serve as the plasma support gas, (4) low power consumption (~3 W), (5) small size, and (6) rapid analysis. In the present paper, we report the characteristics of the LTP/miniature mass spectrometer combination for the detection and quantification of melamine. A modified discontinuous atmospheric pressure interface (DAPI)[47] was used to increase the ion transfer efficiency into the mass spectrometer. The interface includes a compact version of the LTP probe, a heater which focuses the heat and the plasma onto a 5 mm × 5 mm surface area, and supplementary pumping to transfer the ions effectively into the ion trap. The LTP was used to desorb and ionize the analyte from a glass surface with thermal assistance (up to 200 °C). Analysis speeds can reach the rate of two samples per minute. Results show that levels of melamine as low as 250 ng/mL (250 ppb) spiked into whole milk can be detected and that the linear dynamic range is 0.4–50 μg/mL. The detection limit is well below the US regulatory level of 1 ppm and the European level of 2.5 ppm.

Experimental

Chemical and reagents

All the foods were randomly bought in local supermarkets and were used directly without further treatment. All chemicals, including melamine, cyanuric acid and methanol, were purchased from Sigma-Aldrich (USA) and used without purification. Synthetic urine was purchased from CST Technologies, Inc. (NY, USA). The deionized water used for preparing standard solutions was obtained using a Milli-Q purification system (Millipore, Bedford, MA, USA). Melamine in methanol and water (v:v 1:1) at a concentration of 1000 μg/mL served as stock solution. Spiked milk samples were made by diluting the melamine solution with whole milk using a dilution ratio of 1:20 (melamine solution:milk). For each measurement, 3 μL of solution was placed on a glass slide giving a sample spot of *ca.* 2 mm × 2 mm, then the glass slide was placed directly under the heated LTP probe to execute the analysis. Fish meat was first ground, then 2 g of the fishpaste was mixed with selected amounts of melamine solution (500 μg/mL in methanol/water) to achieve different concentrations. The fishpaste was vortexed for 20 min to assure homogeneous mixing and allowed to stand for another 20 min before analysis (these steps were used in preparing a sample representative of contaminated fish, they are not needed for analysis). For each analysis, 5 mg fishpaste was used. All samples were prepared and measured at room temperature (15–25 °C).

LTP ionization source

The LTP probe consists of a glass tube (o.d. 6.35 mm and i.d. 3.75 mm) with an axial grounded electrode (stainless steel; diameter, 1.57 mm) and an outer electrode (copper tape) wrapped onto the tube, as reported in a previous study.[20] The wall of the glass tube serves as the dielectric barrier. An alternating high voltage, 2.5–5 kV at a frequency between 2 and 5 kHz, was applied to the outer electrode with the center electrode grounded to generate the dielectric barrier discharge. The discharge AC voltage was provided by a custom-built power supply with total power consumption below 3 W. The power supply generated a square waveform with adjustable frequency and amplitude using a digital circuit. The square waveform was amplified by a power amplifier and an automobile engine ignition coil to generate the AC with an amplitude as high as 5 kV. A discharge gas – either helium, argon, nitrogen or air – is fed through the glass tube to facilitate the discharge and to assist in the transport of ions to the mass spectrometer.

Miniature mass spectrometer and its ambient interface

A previously described handheld rectilinear ion trap mass spectrometer (Mini 10.5)[25] was used for the experiments reported in this paper. To confirm the miniature mass spectrometer results, a parallel study was conducted using an LTQ (Thermo Fisher Scientific, Inc., San Jose, CA, USA). A miniature rough pump together with a miniature turbo pump was used to achieve an ultimate vacuum below 1×10^{-5} Torr in the Mini 10.5. A two-stage KNF Neuberger diaphragm pump (1091-N84.0-8.99) with a pumping speed of 5 L/min was used to provide a backing pressure below 2 Torr for the turbo pump. The latter was a 10 L/s Pfeiffer TPD 011 (Pfeiffer Vacuum Inc., Nashua, NH, USA) and it constituted the main vacuum pump of the system. All components of the Mini 10.5, including the electronics and vacuum systems, are assembled in an aluminium case, length 34 cm, width 22 cm and height 19 cm. The total weight of the instrument is 10 kg. A discontinuous DAPI[47] interface is used to transfer the ions created by the LTP source into the vacuum chamber of the Mini 10.5 for detection. The discontinuous interface acts as a mechanical switch, which opens the ion introduction channel briefly (10–30 ms) and then closes it during the subsequent periods of each scan cycle (ion cooling, mass analysis, ion clearance and reset). The pressure inside the vacuum chamber increases significantly (up to 10 mTorr) when the channel is open for ion (and accompanying air/sample vapor) introduction. It is appropriate to shut off all high voltages and maintain only a low RF voltage during this period. After ion introduction, the channel is closed to allow the pressure to decrease over a period of time (300–500 ms) until it reaches a value (normally 1 mTorr) that allows further ion manipulation and mass analysis. At this point, the high voltage is turned on and the RF is scanned to perform mass analysis. Each cycle takes around 1 s and all mass spectra were averaged over 3 cycles and are reported with background

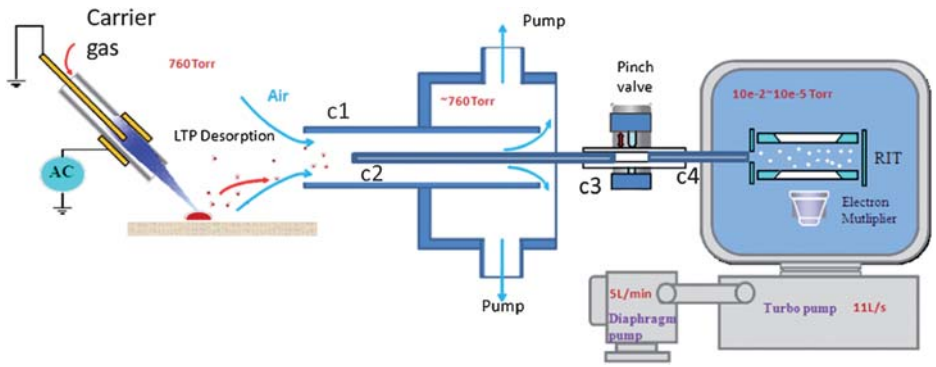

Fig. 1 Schematic diagram of LTP/Mini 10.5 system.

subtraction. For this study an improved version of the interface (described in the next section) was employed. This optimized interface (Fig. 1) is configured as follows: (1) c1: stainless steel tubing, 1/4″ OD, 4 mm ID, 10 cm long; (2) c2: stainless steel capillary, 1/16″ OD, 250 μm ID, 10 cm long; (3) c3: conductive silicon tubing, 1/8″ OD, 1/16″ ID, 3 cm long; and (4) c4: stainless steel capillary, 1/16″ OD, 1 mm ID, 5 cm long.

Results and discussion

LTP and Mini 10.5 interface

The LTP ambient ionization source can be directly combined with a benchtop mass spectrometer.[19,20] However, due to the loss of sensitivity associated with the requirement for small gas (and analyte ion) introduction from an external source imposed by the low pumping speed of these systems, the direct combination of an LTP and miniature mass spectrometer suffers from low sensitivity. The interface between ambient ionization sources and miniature mass spectrometer gives improved performance when it includes discontinuous operation (the DAPI system[47]) based on control of gas introduction with a pinch valve. However, even this arrangement has a low sampling duty cycle (~10 ms per second) and the performance is still much poorer than for benchtop instruments (see E)

The interface between LTP and Mini 10.5 was improved further in this study by using an extra pumping system (shown in Fig. 1). A miniature sample pump (model: NMP015M, KNF

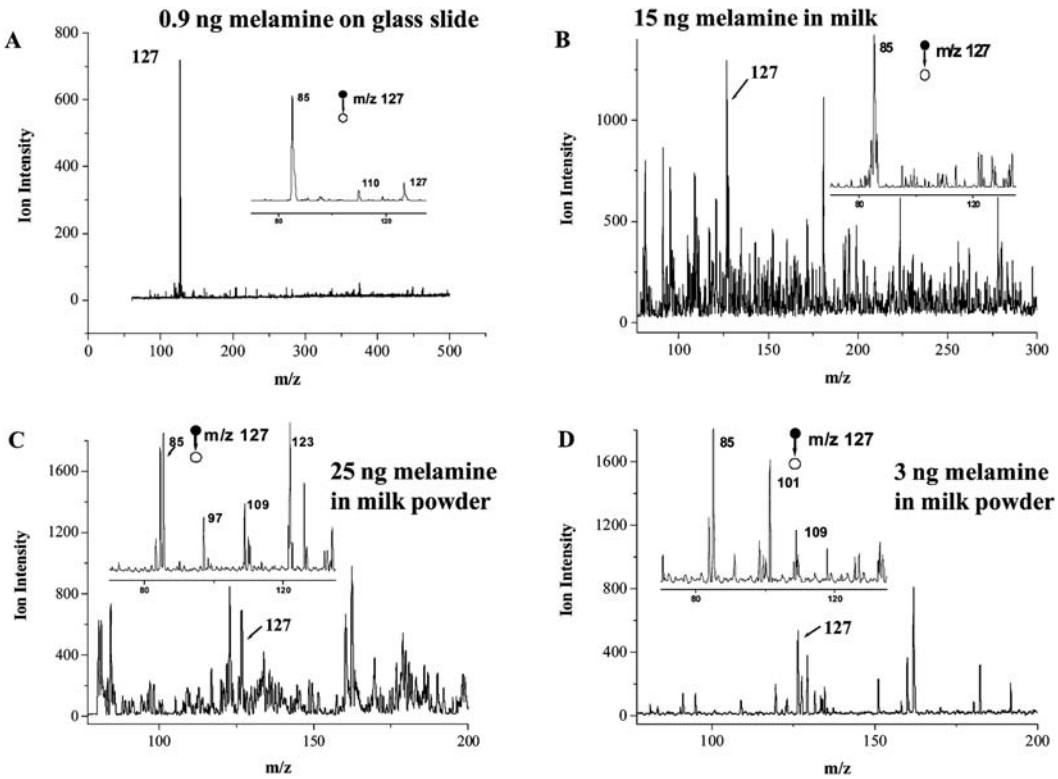

Fig. 2 Typical mass spectra of melamine in different matrices using LTP/Mini 10.5. Samples: (A) MS spectrum of 300 ng/mL melamine in water/methanol (v:v = 1:1), loading volume 3 μL (absolute melamine amount of 0.9 ng); (B) 5 μg/mL melamine in whole milk, loading volume 3 μL (absolute melamine amount of 15 ng); (C) 5 μg/g melamine in milk powder, loading volume 5 mg (absolute melamine amount of 25 ng); (D) 1 μg/mL melamine in synthetic urine, loading volume 3 μL (absolute melamine amount of 3 ng). Inserts: MS/MS product ion spectrum of the protonated molecule.

Neuberger, Trenton, NJ, USA) was used to provide an air flow rate of 2.5 L/min toward the vacuum inlet. Ions created by the LTP were sucked into the quarter inch capillary (c1 in Fig. 1) using the miniature sample pump. When the pinch opens (10 ms each second), ions are sucked into mass spectrometer inlet capillary (c2 in Fig. 1), and then pass into the ion trap for *m*/*z* distinction and detection. After optimization of the LTP/Mini 10.5 interface, ion abundance was improved by an order of magnitude (see Supplementary Materials‡).

Direct detection of melamine in complex matrices

With the LTP/Mini 10.5 described above (Fig. 1), pure melamine in water/methanol (v:v 1:1), at concentrations as low as 300 ng/mL (0.9 ng in 3μL) provides mass (Fig. 2A) and MS/MS spectra (insert in Fig. 2A) which readily confirm the presence of melamine.

Direct detection of melamine in milk. Soon after the 2008 melamine scandal,[2–4] several groups developed rapid melamine detection methods for complex samples using benchtop mass spectrometers operated with various ambient ionization methods, including LTP,[19,20] EESI,[21] DART[22] and DAPCI.[23] The first study to detect melamine using a miniature mass spectrometer (Mini 10.5) is presented in the present study. Organic milk spiked with melamine to a concentration of 5 μg/mL (sample dilution and operational details described in the Experimental section) gave a mass spectrum using the LTP/Mini 10.5 from which the presence of melamine could be detected (Fig. 2B). It should be noted that for milk samples, a peak at *m*/*z* of 127 appeared in the control samples. This is likely due to glucose in the milk sample which can be converted into 5-hydroxymethylfurfural (MW 126) upon heating. Although protonated melamine and 5-hydroxymethylfurfural ions have the same *m*/*z* as 127, the MS/MS spectrum of protonated 5-hydroxymethylfurfural shows a fragment ion at *m*/*z* 83 while the characteristic fragment for protonated melamine has *m*/*z* 85. To avoid interference, the protonated ion *m*/*z* 127 and the MS/MS fragments at *m*/*z* 85 and 110 (as shown in Fig. 2A insert) were used to identify melamine for the rest of the study, while quantitative measurements were based on the characteristic fragment ion of *m*/*z* 85. The detection speed of melamine in the whole milk is as fast as 30 s per sample, which is similar to the preliminary work done with a lab instrument.[20]

Direct detection of melamine in milk powder. Detection of melamine in milk powder could become a quality control activity in dairy plants. Due to the unique advantages of the LTP source,

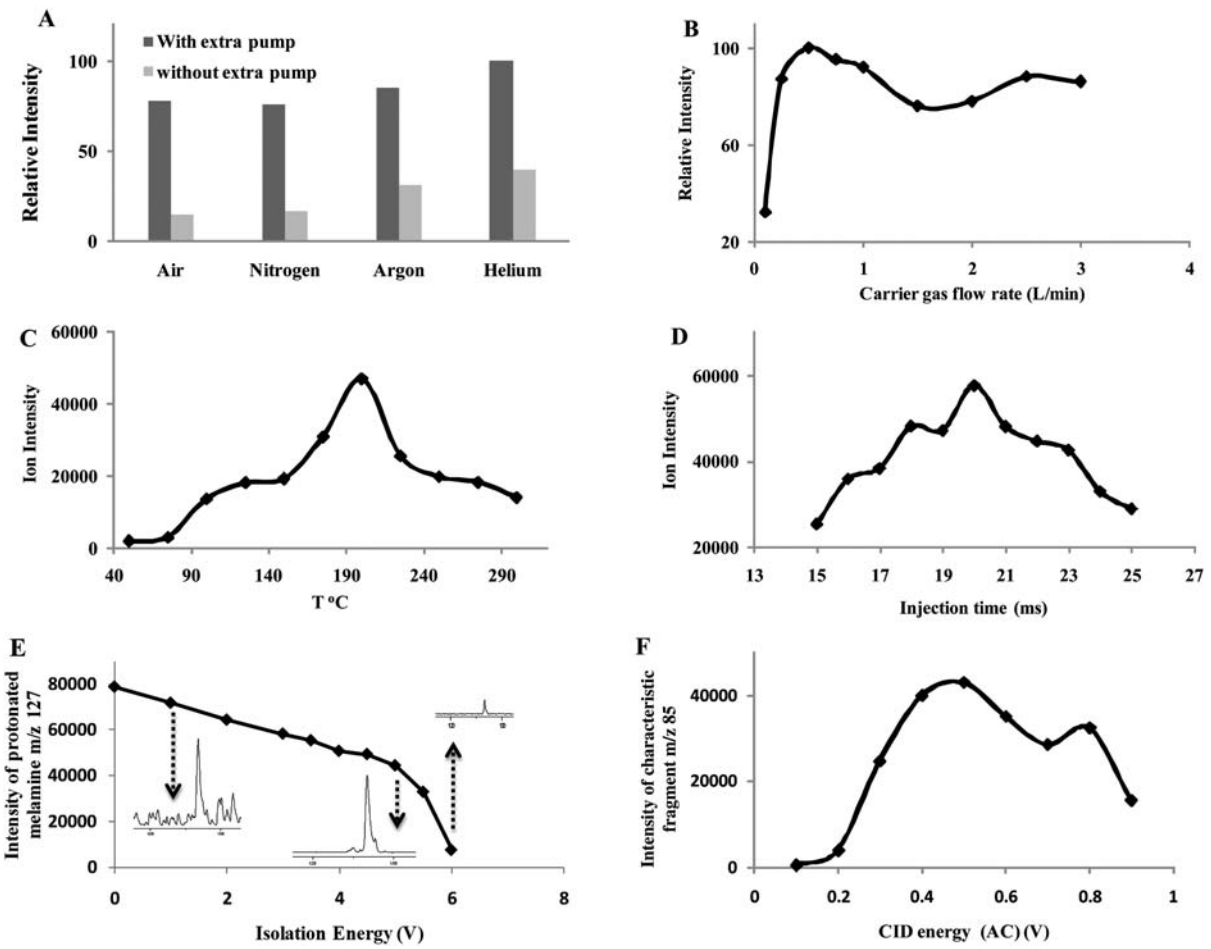

Fig. 3 Optimization of MS conditions for melamine determination. Sample: 500 ng/mL melamine in whole milk (v:v = 1:1), loading volume 3 μL (absolute melamine amount of 15 ng).

i.e. no direct contact with the sample, low carrier gas flow rate and direct analyte desorption from the sample, the LTP/Mini 10.5 procedure can be used to detect melamine directly in milk powder at a concentration of 5 μg/g (Fig. 2C).

Direct detection of melamine in urine. If, unfortunately, the above food safety measures are unsuccessful and melamine-contaminated products do get into the food market, rapid and direct analysis of body fluid (such as urine) is important for clinical diagnosis. Given the complexity of the urine matrix, standard methods such as ESI and APCI are not suited to direct melamine detection. Although matrix assisted laser desorption ionization (MALDI) has recently been used to detect melamine in urine,[48] addition of *R*-cyano-4-hydroxycinnamic acid is still needed. The methods reported in this paper allow direct detection of melamine in urine using a portable mass spectrometer. As illustrated in Fig. 2D, as little as 1 μg/mL melamine spiked into synthetic urine can be identified within 30 s.

Optimization and analytical performance

Optimization experiments were conducted to decrease the LOD and increase the linear dynamic range of the LTP/Mini 10.5 combination (Fig. 3). Several types of gases – helium, argon, nitrogen and air – were tested as carrier gases for the LTP (Fig. 3A). Helium was found to produce the highest sensitivity, but air does not require a gas cylinder which would add weight to the 10 kg portable mass spectrometer. The flow rate of the air was optimized as 0.75 L/min for maximum MS intensity (Fig. 3B). As reported in our preliminary study using lab-scale instrumentation,[20] desorption efficiency is increased if the sample is heated. For example, at room temperature, the LOD is 1 μg/mL for pure melamine in methanol/water but when heated to 170 °C, concentrations as low as 120 ng/mL can be detected.[20] Similar results were obtained for the LTP/Mini 10.5 system and, based on the data shown in Fig. 3C, the operating temperature was set at 200 °C. Another important parameter of the DAPI interface is the pinch valve open time: as the open time increases more ions can be sucked into the vacuum chamber resulting in increased signal. However, when the open time exceeds 20 ms, the pressure inside the vacuum chamber rises too much, leading to space charge effects which decrease resolution as well as the peak intensity. Therefore, the pinch open time was set at 20 ms in a total experiment cycle time of 1 s. The Mini 10.5 system used a notched broadband waveform for ion isolation and a sine wave AC for activation of the isolated ion population.[25] The notch of the waveform for isolation of protonated melamine was set to cover 138–142 kHz corresponding to an isolation window of *m*/*z* 127 ± 2. The frequency of the AC for CID was set to 140 kHz to optimize the fragment ion abundance. The amplitudes of the AC for both isolation and activation were optimized and set at 6 V and 0.4 V (Fig. 3E and 3F).

Under these optimized conditions, the linear dynamic range for determination of melamine in whole milk is between 0.4 and 50 μg/mL with a LOD of 250 ng/mL (see Supplementary Materials‡). The detection limit is far below the regulatory level in the US of 1 ppm or the European level of 2.5 mg/kg or 2.5 ppm. As noted in the European regulations, "…*the level of 2.5 mg/kg is the appropriate level to distinguish between the unavoidable background presence of melamine and unacceptable adulteration*…".[49] The analytical performance of the LTP/Mini 10.5 for all matrices tested in the present work is summarized in Table 1 (linear dynamic ranges and LODs are given in both concentration units and in absolute units). Recoveries for melamine in milk, milk powder and fish meat were determined at the concentrations of 1, 1.5 and 2 times LODs, and the values were in the range of 88–97% for milk, 88–104% for milk powder and 85–92% for fish.

Melamine detection in milk and urine in the presence of cyanuric acid

It has been widely reported that melamine itself has low toxicity; however, in the presence of cyanuric acid – one of its metabolites (which include ammeline and ammelide as well as cyanuric acid) – it forms a stable toxic compound known as melamine cyanurate with poor aqueous solubility. For this reason, the detection of melamine in the presence of cyanuric acid in the matrices described above was also investigated. The fragment of *m*/*z* 85 obtained *via* CID from protonated melamine (*m*/*z* 127) was recorded (Fig. 4). Although melamine cyanurate has a poorer solubility than melamine,[5] similar ion intensities for the characteristic fragment *m*/*z* 85 were found in both melamine-spiked samples as in the samples spiked with both melamine and cyanuric acid. However, the peak of melamine with the presence of cyanuric acid is delayed by *ca.* 5–10 s in the heating cycle relative to that of melamine alone (shown in Fig. 4); this might be

Table 1 Analytical performance: melamine detection in different matrices using LTP/Mini 10.5

Matrix	Linear dynamic range (absolute amount)	LOD[a] (absolute amount)	RSD[b] (RE)[c]
Methanol and water[d]	0.05–20 μg/mL (0.15–60 ng)	0.03 μg/mL (0.09 ng)	7.6% (9.2%)
Milk[d]	0.4–50 μg/mL (1.2–150 ng)	0.25 μg/mL (0.75 ng)	15.8% (19.4%)
Milk powder[e]	0.5–50 μg/g (2.5–250 ng)	0.25 μg/g (1.25 ng)	13.8% (16.4%)
Synthetic urine[d]	0.2–50 μg/mL (0.6–150 ng)	0.1 μg/mL (0.3 ng)	10.2% (15.8%)
Fish meat (Tilapia)[e]	0.7– 50 μg/g (3.5–250 ng)	0.5 μg/g (2.5 ng)	16.2% (22.8%)

[a] Quantitative detection for melamine was achieved by measuring solid phase and liquid phase samples at various concentrations; the mass spectrometer was scanned to record MS/MS spectra and the characteristic fragment ion (*m*/*z* 85) obtained *via* CID from protonated melamine (*m*/*z* 127) was used for quantitation. The criteria for estimating the LODs were the smallest amount sample that yielded a signal-to-noise ratio for the characteristic fragment ion (*m*/*z* 85) of more than 6 in the chronogram (MS scan). [b] Relative Standard Deviation (RSD): maximum value of the 5 RSDs at different concentrations within the linear dynamic range (7 replicate measurements conducted at each of the five different concentration levels). [c] Relative Error (RE): maximum value of the 35 REs within the linear dynamic range.[d] Melamine spiked in 3 μL matrix. [e] Melamine spiked in 5 mg matrix.

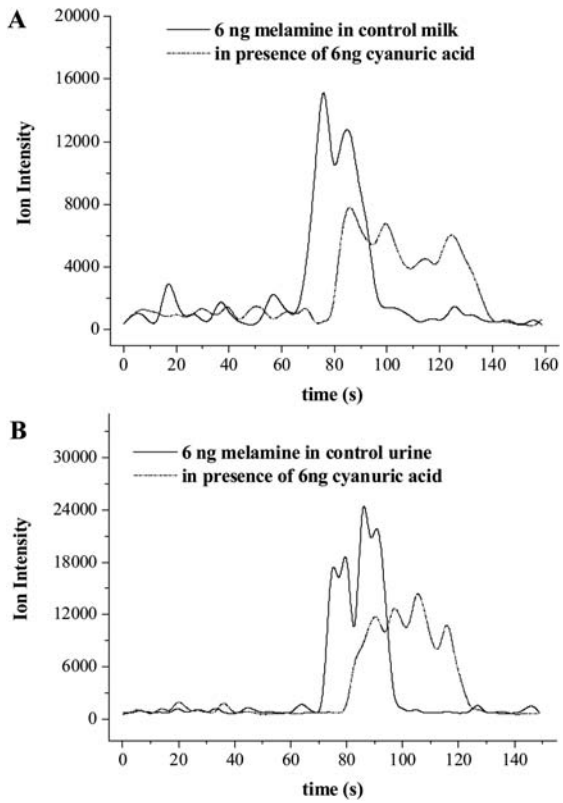

Fig. 4 Melamine detection in the presence of cyanuric acid (chronogram of characteristic fragment (*m*/*z* 85) obtained *via* CID from protonated melamine (*m*/*z* 127)). (A): (solid line) 20–50 s: control whole milk (3 μL); 65–95 s: 2 μg/mL melamine in whole milk (3 μL); (dashed line) 20–50 s: whole milk spiked with 2 μg/mL cyanuric acid (3 μL); 80–140 s: whole milk (3 μL) spiked with 2 μg/mL cyanuric acid and 2 μg/mL melamine. (B): (solid line) 20–50 s: synthetic urine (3 μL); 65–95 s: 2 μg/mL melamine in synthetic urine (3 μL); (dashed line) 20–50 s: synthetic urine spiked with 2 μg/mL cyanuric acid, 80–120 s: synthetic urine (3 μL) spiked with 2 μg/mL cyanuric acid and 2 μg/mL melamine.

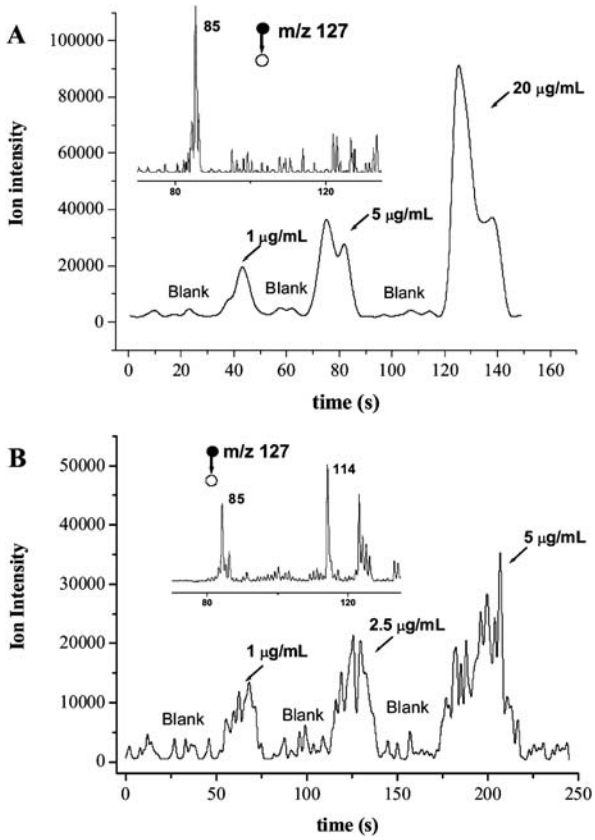

Fig. 5 Melamine screening in milk and fish (tilapia). (A) and (B): Chronogram of characteristic fragment (*m*/*z* 85) obtained *via* CID from the protonated melamine (*m*/*z* 127). Inserts: MS/MS product ion spectrum of the protonated molecule. Sample information: (A) 0–30 s: control sample (whole milk); 30–60 s: milk spiked with 1 μg/mL melamine; 60–90 s: milk spiked with 5 μg/mL melamine; 90–120 s: control sample (whole milk); 120–150 s: milk spiked with 20 μg/mL melamine. (B) 0–50 s: control sample (fishpaste); 50–80 s: fishpaste spiked with 1 μg/mL melamine; 80–110 s: control sample (fishpaste); 110–140 s: fishpaste spiked with 2.5 μg/mL melamine; 140–170 s: control sample (fishpaste); 170–210 s: fishpaste spiked with 5 μg/mL melamine; 210–240 s: control sample (fishpaste). All loading volumes are 3 μL for milk and 5 mg for fishpaste.

due to the extra energy needed to break the complex and generate free melamine. Similar phenomena were observed in parallel experiments using a benchtop MS instrument (LTQ (Thermo Fisher Scientific, Inc., San Jose, CA)) and also when MALDI was used as the ionization source.[48] These results imply that the LTP/Mini 10.5 system can be used to detect melamine in the presence of cyanuric acid, without significant matrix interference.

Melamine screening from complex matrices

Screening of melamine in complex matrices (whole milk and tilapia fish meat) was also tested in this study. For each test, several samples (3 μL for milk and 5mg for fishpaste) were placed on glass slides with each sample covering an area of around 2 mm × 2 mm and the distance between the samples being 1 mm. Control samples (blank) and samples spiked with different concentrations were placed on the same slides, which were attached to a 1-D moving stage and moved past the source at a speed of 8 mm/min. As samples passed under the LTP probe, MS/MS spectra were continuously recorded as shown in Fig. 5. The intensity of the characteristic fragment (*m*/*z* 85) obtained *via* CID from protonated melamine (*m*/*z* 127) was recorded and is shown in Fig. 5A and 5B. For both whole milk and fish matrices, the present method using the handheld mass spectrometer can clearly distinguish the spiked samples down to the level of 1 μg/mL for both whole milk and fish from the control samples (Fig. 5A and 5B). It should be noted that the baseline returns to the blank level within 5 s after each sample, and none of the control samples showed interference due to signals from the spiked samples at melamine concentrations up to 20 μg/mL. This illustrates that the measurement can be conducted continuously, without the need of extra time to flush the capillary as done in extractive electrospray ionization.[21]

It can also be concluded from Fig. 5 that the time required for the experiment is around 30 s per sample, which in principle allows 3000 samples per 24 h per instrument. This throughput is much higher than any of the present HPLC/MS or GC/MS methods, even if the sample pretreatment time is excluded (3 h/sample is suggested by the FDA[12]) although parallel

operation will decrease the last restriction. The ruggedness of the present method was tested by examining around 100 melamine-spiked samples (concentration ranged between 100 ng/mL and 100 μg/mL) per day for over 4 weeks without cleaning the instrument. No significant MS signal decrease was observed, demonstrating that the LTP/Mini 10.5 is robust for melamine screening in complex matrices.

Conclusion

Rapid screening of melamine in various complex matrices (whole milk, milk powder, urine and fishpaste) can be implemented without sample preparation using LTP ionization on a handheld mass spectrometer. The LTP ionization source directly desorbs and ionizes melamine from complex matrices, and tandem mass spectrometry performed in the ion trap provides the sensitivity, specificity and the quantitative accuracy to much exceed regulatory requirements. The LTP/Mini 10.5 combination is shown to be a robust method with a high throughput, around 120 samples/h, which satisfies requirements for realistic *in situ* analysis.

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