Supporting materials for ultrasound-assisted EESI – Melamine

1. Experimental section

Ultrasound-assisted EESI, DESI and mass spectrometry

A solvent mixture (methanol / water / acetic acid 40% / 40% / 20%) was electrosprayed at a flow rate of 5μL / min infused by a syringe pump (Harvard Apparatus, Holiston, MA, USA). The quadrupole time-of-flight (Q-TOF) mass spectrometer (QTOF UltimaTM, Micromass / Waters, Manchester, UK) was run in positive ion detection mode, while other parameters were maintained at default values as suggested by the manufacturer. The ESI voltage was + 3 kV and the cone voltage was set according to the required mass range. No further optimization was performed. The rate for acquiring mass spectra was 0.5 seconds. The spectra were recorded continuously, whether the ultrasonic transducer was switched on or not, followed by background subtraction over the m/z range of interest (MassLynx 4.0, Waters, Manchester, UK). The detailed procedure for background subtraction has been described elsewhere[1]. Several parameters needed to be optimized for higher m/z range measurements, such as cone voltage and mass profile settings. Collision induced dissociation (CID) was performed at a collision energy of 5–25 arbitrary units, as defined by the manufacturer.

Sample preparation. A stock solution of milk (different fat contents) spiked with melamine was prepared by dissolving 3 mg of melamine into 6 mL milk (500 ppm). The stock solution was diluted 1:5 times, 1:50 times and 1:500 times with pure milk to obtain milk spiked with 100 ppm, 10 ppm and 1 ppm melamine, respectively.

In the study of melamine in different extraction solvents, 3 mg melamine was dissolved in 3 mL water. This aqueous melamine solution was then diluted 1:100 times by water-miscible solvents (water, acetonitrile, 2-propanol, ethanol and methanol), respectively.

In the study of powders, melamine (3 mg) well ground together with either wheat gluten (1 g) or milk powder (1 g) was extracted by 3 mL methanol, using a simplified recipe published by

FDA[2]. Rather than sonication for 30 minutes, followed by centrifugation (10 mins.) and filtration as recommended by the FDA[2], the mixture was sonicated for only 1 minute in this work, and the top phase of the mixture was taken as the extract, without further treatment. It was further diluted 1:10 times, 1:100 times and 1:1000 times by a methanol extract from an unspiked wheat gluten or milk powder sample, respectively.

Samples and Reagents. Melamine (> 99.0% purity), Nile blue (>= 70% Dye content) and Methyl red (pure) were bought from Fluka (Buchs, Switzerland). The pure milks (UHT milk, 0.1 %, 1.5%, 3.5% fat content, Coop Zurich) and milk powder (Nestlé, 8 months) were obtained from a local supermarket, and the wheat gluten (X-treme whey protein, 80% content, vanilla flavour) was bought from a local fitness center. Chemicals such as methanol, ethanol, acetonitrile, 2-propanol, carbon tetrachloride were obtained from Fluka (Buchs, Switzerland) with HPLC purity. The acetic acid (HPLC grade) was obtained from Carlo ERBA reagent SPA (Rodono, MI). Ultra-pure water was available in our lab.

2. MS/MS spectrum of m/z 343 from milk

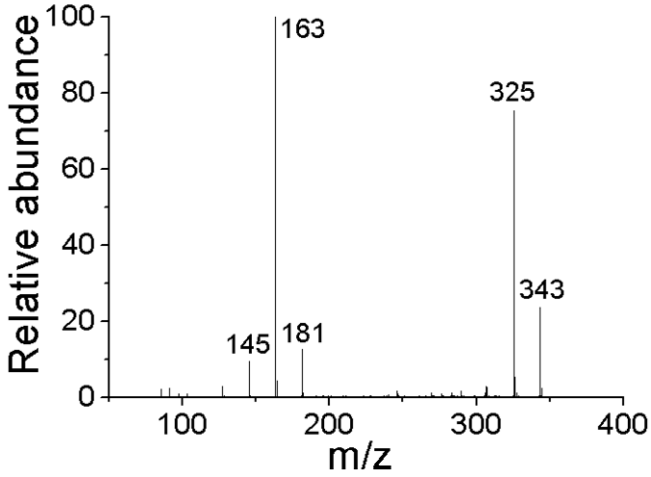

Figure s1. MS/MS spectrum of the m/z 343 from milk, using ultrasound-assisted EESI. The fragment ion at m/z 325 could arise from the loss of H_2O from the molecular ion. Lactose is a disaccharide that consists of β-D-galactose and β-D-glucose fragments bonded through a β1-4 glycosidic linkage. Upon collision with CID gas, the lactose ions can form protonated glucose (m/z 181) and m/z 163 which is a fragment from galactose formed by loss of H_2O. Thus, m/z 343 could be identified as lactose (present at a level of ~ 3% in pure milk).

3. Solvent effects for droplet desorption and subsequent mass measurements

A critical step in developing the protocol is identifying the best solvent for desorption and subsequent ionization for mass spectrometry. In order to determine the solvent, solutions of the same melamine concentration (10 ppm) in different water-miscible solvents (water, acetonitrile, 2-propanol, ethanol and methanol) were prepared, as described in the experimental section. Afterwards, aliquots from each melamine solution were measured in series (Fig s2). It is clear that melamine extracted by methanol yields the best response, ethanol is the second best, while water, acetonitrile and 2-propanol show comparably poor results. To explain the difference between these solvent, the first property we notice is the surface tension[3]. All organic solvents utilized herein have similar surface tensions. However, water exhibits a much higher surface tension[3]. It is well known that finer droplets are generated from solvents with lower surface tension[4].

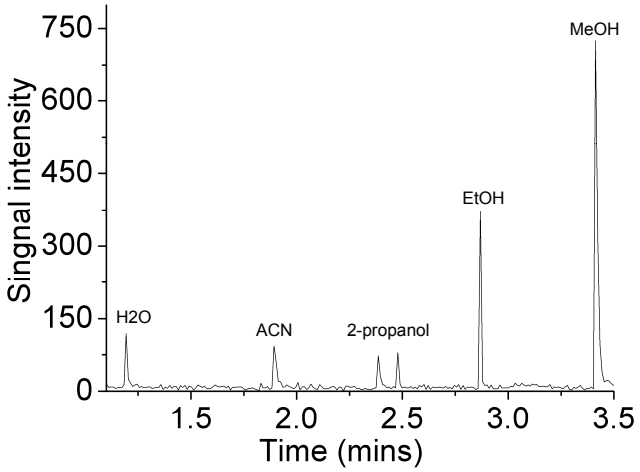

Figure s2. Responses of melamine of the same concentration (10 ppm) in several different solvents. Melamine extracted by methanol and ethanol yield higher signal levels, which is possibly due to their lower surface tension and higher evaporation rate. The behaviour of 2-propanol solution is restricted by its low evaporation rate and high viscosity. For acetonitrile, the high acid dissociation constant (pKa value) diminishes the melamine signal compared to other organic solvents.

The differences in nebulization efficiency caused by solvents with surface tension are shown in Fig. s3, which visualizes the distribution of aerosols produced by ultrasonical desorption

from a droplet (3 μL) of Rhodamine 6G in an aqueous and methanol solution, respectively. These two pictures were recorded using a piece of white paper, located perpendicularly to the ultrasonic transducer surface, thus giving a cross-sectional view of the nebulization process. As can be seen, the spatial distributions of the aerosol droplets are quite different from these two R6G solutions. The distinct contact angles for aqueous and methanol droplets with the surface of the transducer lead to quite different vertical momentum transfer efficiencies, causing a distinctly different spatial distribution of the aerosol droplets. The methanol droplets are directed mainly upwards (the same findings also apply to other organic solvents), while the water aerosols are ejected in different directions. For our experimental setup, as shown in Fig. 1 in the text, a narrower spatial distribution of aerosols leads to a better overlap between the sample plume and the ESI plume, which is more efficient for extractive electrospray ionization. The second reason for the better performance of methanol and ethanol is their high evaporation rate[5]. As can be seen in Fig. 3a, there are several fairly large methanol droplets after dispersion, which is not advantageous for efficient ion formation. We surmise that its high evaporation rate compensates for the large size droplets. In addition, it is well know that solvents with high evaporation rates are good for ESI. The behaviour of 2-propanol solution appears to be restricted by its low evaporation rate and its high viscosity, compared to methanol and ethanol. For acetonitrile, its relatively high acid dissociation constant (pKa value) of ~ 25 diminishes the analyte signal compared to water (~15.7), methanol (~15.5) and ethanol (~16)[6], since melamine is a weak base. Based on these facts, methanol was chosen as the extraction solvent in the following experiments.

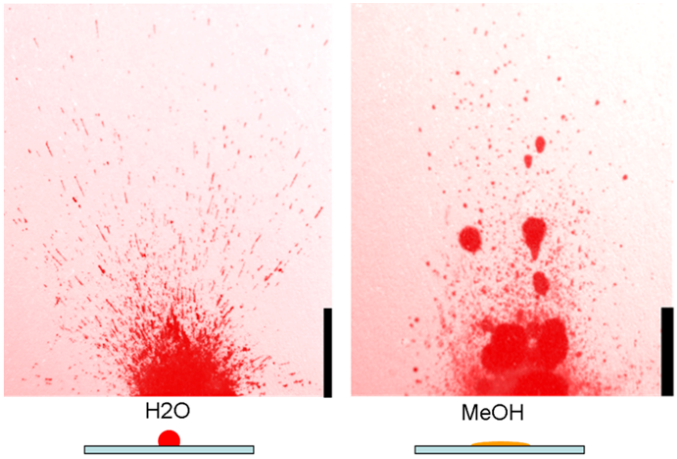

Figure s3. Aerosols produced by ultrasound desorption from a droplet (3 μL) of aqueous solution of Rhodamine 6G and methanol solution of Rhodamine 6G, respectively. These pictures were recorded using a piece of white paper, which is located perpendicular to the ultrasonic transducer surface. As can be seen obviously, the spatial distributions of aerosols were quite different in these two R6G solutions, which might due to the hydrophobic surface of ultrasonic transducer. The distinct contact area for aqueous and methanol droplets lead to quite different vertical momentum transfer efficiency, causing a distinct spatial distribution of aerosols, as shown in the picture above. The black bar in both pictures is 5 mm in scale.

4. Measurements with milk powder

The extraction procedure and subsequent measurements for tainted milk powder is exactly the same as the one for wheat gluten. Fig s5. shows the dependence of the melamine signal on the melamine concentration in these extracts. The data suggests a detection limit of 270 ppb (μg/kg) with a signal to noise ratio of 3, while the S/N is around 11 for 1 ppm melamine methanol extracts.

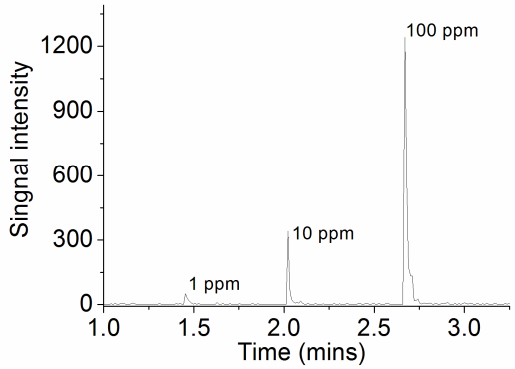

Figure s5. SIC of m/z 127 during measurements of extracted solution of melamine from milk powder at 1 ppm, 10 ppm and 100 ppm concentration, respectively.

References

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