**Sensitive determination of melamine in milk samples by high-performance
liquid chromatography using dabsyl chloride derivatization followed by
dispersive liquid–liquid microextraction**

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ABSTRACT

A new and sensitive pre-column derivatization with dabsyl chloride followed by dispersive liquid-liquid microextraction was developed for the analysis of melamine in liquid milk and powdered infant formula by high performance liquid chromatography (HPLC) with visible detection. Derivatization with dabsyl chloride leads to improving sensitivity and hydrophobicity of melamine. Under optimum conditions of derivatization and microextraction, the method yielded a linear calibration curve ranging from 1.0 to 500 μL^{-1} with a determination coefficient (R^2) of 0.9995. Limit of detection and limit of quantification were 0.1 and 0.3 $\mu\text{g L}^{-1}$, respectively. The relative standard deviation percent (RSD%) for intra-day and inter-day extraction and determination at 25 and 100 $\mu\text{g L}^{-1}$ levels of melamine was less than 7.0% ($n = 6$). Finally, the proposed method was successfully applied for the preconcentration and determination of melamine in different liquid milk and powdered infant formula, and satisfactory results were obtained (relative recovery $\geq 94\%$).

Keywords: Melamine, Dabsyl chloride, High performance liquid chromatography, Milk, Dispersive liquid-liquid microextraction.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine, MEL) is an organic compound often used with formaldehyde to produce MEL resin, a synthetic fire resistant and heat tolerant polymer (Kim et al, 2008; Andersen et al., 2008). Melamine (MEL) is not approved as an ingredient in foods, but some manufacturers illegally use it as an adulterant, because of its high nitrogen level (66% by mass) and low price, to increase the apparent protein content. In 2008, a large-scale MEL contamination incident was made public in China and many other countries (Sun et al., 2010; Yan, Zhou, Zhu, & Chen, 2009).

The maximum level allowed for melamine residue is regulated and set 1.0 mg kg⁻¹ for powdered infant formula and 2.5 mg kg⁻¹ for other foods and animal feed (FAO, 2010; European Commission, 2009). Higher concentrations of melamine above the safety regulation level can cause tissue injury such as acute kidney failure, urolithiasis, bladder cancer, and even death above (Skinner, Thomas, & Osterloh, 2010).

In order to detect food adulteration and evaluate food safety several analytical methods have been reported for quantitative determination of melamine in different matrices, including spectrophotometry (Liu et al., 2011), spectroflurometry (Zhou, Yang, Liu, Wang, & Lu, 2010; Zeng, H., Yang, R., Wang, Q., Li, J., & Qu, 2011), high performance liquid chromatography with UV or fluorescence detection (HPLC–UV) (Sun, Wang, Ai, Liang, & Wu, 2010; Venkatasami, & Sowa, 2010; Zhang et al., 2014; Muniz-Valencia et al., 2008; Zheng, Yu, Li, & Dai, 2012; Filazi, Sireli, Ekici, Can, & Karagoz, 2012), liquid chromatography–tandem mass spectrometry (LC/MS/MS) (Ibanez, Sancho, & Hernandez, 2009; Deng et al., 2010; Zhang et al., 2010; Wu et al.,

2009; Gpscinny, Hanot, Halbardier, Michelet, & Van Loco, 2011), gas chromatography–mass spectrometry (GC/MS) (Xu et al., 2009; Pan et al., 2013; Li, Qi, & Shi, 2009; Li, Zhang, Meng, Wang, & Wu, 2010), gas chromatography–tandem mass spectrometry (GC/MSⁿ) (Miao et al., 2009; Tzing, & Ding, 2010), and capillary zone electrophoresis (Xia et al., 2010).

Due to the small and polar nature of MEL, GC-based methods need a further derivatization procedure (Xu et al., 2009; Pan et al., 2013; Li, Qi, & Shi, 2009; Li, Zhang, Meng, Wang, & Wu, 2010; Miao et al., 2009; Tzing, & Ding, 2010). On the other hand, HPLC-based methods need polar reversed-phase columns (Sun, Wang, Ai, Liang, & Wu, 2010; Zheng, Yu, Li, & Dai, 2012) or general C18 and C8 reversed-phase columns with the mobile phase containing ion-pair reagent (Filazi, Sireli, Ekici, Can, & Karagoz, 2012; Ibanez, Sancho, & Hernandez, 2009). Another challenge in MEL analysis is related to unsuitable sensitivity of conventional GC and HPLC detectors. In contrast, MS and MS/MS detectors introduce a high sensitivity, but instruments are expensive and the running cost is high. In addition, complicated instruments and skilled operators are required, which makes their popularization difficult. Therefore, in order to generalize techniques and to overcome the mentioned problems, development of novel sample preparation methods before injection to HPLC or GC is necessary.

Derivatization with suitable fluorophores or chromophores can enhance HPLC sensitivity and improve the chromatographic behavior of many compounds. For the first time, Zhang et al. in 2014 tried derivatization of melamine in order to enhance HPLC sensitivity (Zhang et al., 2014). They developed a sensitive HPLC method with fluorescence (FL) detection for the

analysis of melamine by derivatizing with 10-methyl-acridone-2-sulfonyl chloride (MASC), a compound with excellent fluorescence property. Their results showed that HPLC sensitivity of melamine was greatly enhanced. Meanwhile, the hydrophobicity of melamine was also greatly increased. According to this approach, other labeling reagents containing sulphuryl chloride group could be used for quantification of melamine. Amongst available reagents, dabsyl chloride is a well established UV-labeling agent that has been primarily used for covalent bonding to and quantitation of amino acids (Jansen, Van den Berg, Both-Miedema, & Doorn, 1991), imidazole-containing compounds (Handley et al., 1998; Sormiachi, Ikeda, Akimoto, & Niwa, 1995), and polyamines (Koski, Helander, Sarvas, & Vaara, 1987; Romero, Gazquez, Bagure, & Sanchez-Vinas, 2000). Although dabsyl chloride has never been used to quantify melamine, such derivatization is associated with some advantages. Dabsylation procedure is fast, and dabsyl derivatives are very stable. Moreover, dabsyl derivatives show absorbance in the range of 436–460 nm. In that way, interferences from UV-absorbing biological compounds present in food extracts are mostly avoided (Aboul-Enein, 2003) in comparison with MASC reagent. Further, dabsylation leads to improving hydrophobicity of the compounds (Handley et al., 1998).

The objective of the present work was development and optimization of a simple and fast method for determining melamine in some milk samples based on derivatization with dabsyl chloride and dispersive liquid-liquid microextraction with octanol (as a compatible solvent with RP-HPLC) in combination with HPLC-UV, for the first time. Several experimental parameters which influence melamine derivatization and extraction

performance of the proposed method were investigated and optimized. Finally, figures of merit of the proposed method were compared with previous published methods.

2. Material and methods

2.1. Chemicals and reagents

HPLC-grade acetonitrile was obtained from Merck Company (Darmstadt, Germany). Analytical grade melamine, 1-octanol, sodium acetate, triethyl amine, acetone, methanol, ethanol and NaCl were purchased from Merck Company (Darmstadt, Germany). 4-(4-Dimethylaminophenylazo)benzenesulfonyl chloride (Dabsyl chloride) was provided from Sigma-Aldrich Company (Steinheim, Germany). Dabsyl chloride reagent was dissolved in acetonitrile at 4 mg mL⁻¹ and sonicated for 5 min. Water was purified using a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA). The correct amount of melamine was dissolved in acetonitrile to obtain the stock solution of analyte with a concentration of 1000 mg L⁻¹. Fresh working solutions were prepared by mixing stock solutions and diluting with water.

2.2. Apparatus

The chromatographic analysis was carried out in a high-performance liquid chromatography from Knauer of Germany model EuroChrom consisting of a degasser, quaternary pump (model K1100), manual sample injector with a 20-μL loop size, and UV detector (model K2600) was controlled by EZChrom software. The HPLC operating mode was gradient, injection volume was 20 μL, and column temperature was adjusted to room temperature. The chromatography column was a Supelcosil LC-18: 25cm × 4.6 mm, 5μm (Supelco, Bellefonte, PA, USA). The mobile phase used was a combination of phase A (ACN: 20 mM sodium acetate, pH = 5.0, containing 0.2% triethylamine, 25:75, v/v) and

phase B (ACN 100%). Elution was performed as follows: from 0 to 5 min, 100% A; from 5 to 6 min, a linear gradient from 0 to 100%B; from 6 to 14 min, 100%B; from 14 to 15, a linear gradient from 0 to 100%B; from 15 to 20 min, 100%A. The flow rate was 1 mL min⁻¹. The UV-visible detector was adjusted to 460 nm. The mobile phase was filtered through a 0.45-μm pore size filter (Merck Millipore, Billerica, Massachusetts, USA) and degassed by vacuum prior to use. A 40 kHz and 0.138 kW ultrasonic water bath with temperature control (Tecno-GazSpA, Italy) was applied for ultrasonication of the samples. All of the pH measurements were performed with a WTW Inolab pH meter (Weilheim, Germany).

2.3. Sample preparation

8.0 mL of 5% trichloroacetic acid solution and 1.0 mL of 2.2% lead acetate solution were added to 1.0 g of each homogenized milk or powdered infant formula in a 25-mL glass beaker in order to eliminate protein and extract the analyte. The mixture was placed in ultrasonic cleaner for 10 min to mix well, standing for 2 min. Then, the mixed solution was centrifuged for 10 min at 10,000 rpm. For dabsyl derivatization, 200 μL of the supernatant was transferred to a 10.0-mL conical glassware vial. Then, 50 μL of 1.5 mol L⁻¹ sodium carbonate buffer pH = 9.0 and 100 μL of 4 mg mL⁻¹ of dabsyl chloride were added to the vial. The vial was vortexed for 1 min and then allowed to react at 70°C for 10 min in a water bath. Afterward, to stop derivatization reaction, appropriate cool acetonitrile (-18 °C) was added till the final volume of 1.0 mL. For DLLME, 60 μL of 1-octanol (extraction solvent) was added to the vial and mixed. Then, 5.0 mL of deionized water was rapidly injected into the acetonitrile phase. In this step, melamine was extracted into fine droplets of 1-octanol. Subsequently, to separate the organic phase, the liquid mixture was centrifuged for 5 min at 4000 rpm. After this process, the dispersed fine droplets of 1-octanol floated on the aqueous sample. The lower-aqueous phase was separated using a syringe and 20 μL of the floated phase was injected directly into the HPLC using a microsyringe.

3. Results and Discussion

3.1. Optimization of melamine dabsylation conditions

3.1.1. Optimization of pH of sample

pH control of sample solution is of utmost importance since it greatly influences the extension of derivatization reaction and, as a rule of thumb, the sample pH has to be above the pK_a of analyte so that it could be deprotonated. Apart from the analyte, dabsylation occurs at alkaline conditions usually between pH 8.5 and 9.0 (Jansen, Van den Berg, Both-Miedema, & Doorn, 1991; Handley et al., 1998; Sormiachi, Ikeda, Akimoto, & Niwa, 1995; Koski, Helander, Sarvas, & Vaara, 1987; Romero, Gazquez, Bagure, & Sanchez-Vinas, 2000; Aboul-Enein, 2003). In order to evaluate the effect of pH on the extraction efficiency, pH of the sample solutions was adjusted in the range of 7.0–11.0 and the recommended procedure was applied. According to obtained results, maximum intensity was obtained at pH 9.0. For pH below 7, there is hardly any detection of melamine, which is in agreement with the previous assumption (Maseda, Fukui, Kimura, & Matsubara, 1983), while higher pH values ($pHs > 10.0$) would cause smaller recoveries of melamine because a strong basic condition may lead to decomposition of the analyte and the derivatizing reagent (Maseda, Fukui, Kimura, & Matsubara, 1983). Thus, pH of the sample solutions for derivatization was adjusted at 9.0 by using carbonate-bicarbonate buffer in subsequent experiments.

3.1.2. Effect of derivatization temperature and time

Reaction temperature provides the necessary activation energy to accelerate derivatization reaction to completion, increasing the yield of the derivatives. Derivatization using dabsyl chloride is usually carried out with medium reaction times (5–15 min) at a relatively high temperature (70°C) (Jansen, Van den Berg, Both-Miedema, & Doorn,

1991; Handley et al., 1998; Sormiachi, Ikeda, Akimoto, & Niwa, 1995; Koski, Helander, Sarvas, & Vaara, 1987; Romero, Gazquez, Bagure, & Sanchez-Vinas, 2000; Aboul-Enein, 2003; Maseda, Fukui, Kimura, & Matsubara, 1983). Derivatization has been shown to occur even at 25 °C, but for adequate recovery an extended incubation time, i.e. 30 min, has been required and formation of by-product could be increased [36]. Therefore, in the present study, several derivatization temperatures (60 and 70 °C) were tested. The highest extraction efficiency for melamine was obtained at 70 °C temperature. Therefore, 70 °C was selected as optimum derivatization temperature for further experiments. Also, different reaction time (5, 10, 15, 20, 30 min) were examined to find the most optimum condition for derivatization of melamine. According to obtained results, 10 min is suitable for maximum dabsylation of melamine. This result is in agreement with previous studies (Jansen, Van den Berg, Both-Miedema, & Doorn, 1991; Handley et al., 1998; Sormiachi, Ikeda, Akimoto, & Niwa, 1995; Koski, Helander, Sarvas, & Vaara, 1987; Romero, Gazquez, Bagure, & Sanchez-Vinas, 2000). Increasing the incubation time more than 15 min did show further gain in the recovery of dabsyl derivative, but led to partial hydrolysis of dabsyl melamine (Maseda, Fukui, Kimura, & Matsubara, 1983).

An important point in derivatization is repeatability of reaction; it can be improved by immediately stopping reaction at an exact time (10 min). Lacroix and Saussereau declared that dabsyl derivatization reaction could be stopped by adjusting pH to below 6.0 with buffer, or dabsylation could be stopped by placing the bottom of Eppendorf vials under fresh water resulting in the drop in temperature (Lacroix & Saussereau, 2012). Therefore, in order to improve repeatability of the derivatization reaction, dabsylation was stopped by adding very cold acetonitrile (-20 °C).

3.1.3 Stability of melamine-dabsyl derivatives

One of the distinct features of dabsyl derivatives is their excellent stability (Jansen, Van den Berg, Both-Miedema, & Doorn, 1991) which is very important in sample analysis. Therefore, the stability of dabsyl derivative of melamine was investigated. Melamine derivatives at the concentration of 200 $\mu\text{g L}^{-1}$ were repeatedly analyzed by HPLC after being placed at room temperature for 0, 4, 8, 12, 24, 48, 72 h respectively. Results indicated that the responses of melamine derivatives were stable with peak area deviations of less than 3.6%. Thus, the stability of melamine-dabsyl derivative was sufficient enough for HPLC analysis.

A stability for at least 7 days has also been observed for dabsyl amino acid derivatives. Furthermore, it has been demonstrated that THC and CBN, when crystallized by dabsylation, were unchanged at least for one year (Maseda, Fukui, Kimura, & Matsubara, 1983).

3.2. Optimization of DLLME parameters

In this study DLLME was done for two purposes: (1) for the preconcentration melamine-dabsyl derivatives to improve sensitivity, and (2) for omitting excess amounts of dabsyl chloride reagent before injection to HPLC as result of very low extraction yield of the reagent to octanol phase (the excess amount of dabsyl chloride in presence of water is converted to methyl orange which is not dissolved in extraction phase). So, in order to achieve maximum extraction efficiency, several parameters affecting the DLLME of melamine-dabsyl derivatives, including the volume of extraction solvent, volume of dispersive solvent, and salt effect, were optimized using one-variable-at-a-time optimization method.

Selection of extraction solvent is very important for DLLME processing. Primary requirements for an adequate extraction solvent include low solubility in water, larger density

than water, and high extraction efficiency for the analytes of interest (Rezaee et al., 2006; Rezaee, Yamini & Faraji, 2010). So, 1-octanol was selected as extraction solvent in this study due to its accessibility and compatibility with RP-HPLC method without need to a further evaporation/reconstitution step (when using chlorinated solvents such as dichloromethane and chloroform).

The suitable volume of extraction solvent was investigated using 1.0 mL acetonitrile (melamine-dabsyl phase) with different volumes of 1-octanol (60, 80, 100, 120 μ L). As can be seen in Fig. 1, the peak area of melamine-dabsyl was decreased by increasing the extractionsolvent volume. This trend can be interpreted by decreasing enrichment factor due to dilution effect. Consequently, 60 μ L of 1-octanol was chosen for further experiments.

In DLLME, disperser solvent plays a crucial role, as it allows the dispersion of extraction solvent into the aqueous sample where it is immiscible (Rezaee, Yamini & Faraji, 2010). In this study, because of using acetonitrile as dabsyl chloride solvent and also as cool diluents, acetonitrile was chosen as disperser solvent, and further optimization of nature of disperser solvent was not done. Furthermore, the volume of acetonitrile was optimized by varying the volume between 0.75 and 1.25 mL at a constant volume of 60 μ L of 1-octanol (extraction solvent). Extraction efficiency of DLLME was significantly increased by the increase in the volume of ethanol up to 1.0 mL and tends to decrease after 1.0 mL. It appears that at a low volume, acetonitrile's cloudy state is not well formed, making recovery slow (Rezaee, Yamini & Faraji, 2010). On the other hand, solubility of extraction solvent and also melamine-dabsyl in aqueous phase increased when alarger amount of disperser solvents was used (above 1.0 mL). Therefore, 1.0 mL of acetonitrile was selected as the optimum volume of disperser solvent for further experiments.

Generally, addition of salt enhances extraction of analytes, because the salting-out effect can reduce the solubility of analytes in water and force more of them onto the organic

phase (Razmara, Daneshfar & Sahrai, 2011). On the other hand, in DLLME methods, by increasing ionic strength, volume of the sediment phase increases because of the decrease insolubility of the extraction solvent (Rezaee, Yamini & Faraji, 2010). To investigate the effect of salt on the extraction efficiency of DLLME, NaCl was added in the range of 0–15% (w/v). Results revealed that salt addition had a significant effect on the extraction efficiency of DLLME, as the peak response was found to decrease as the ionic strength increases. These results revealed that the second phenomenon is predominant. Therefore, no salt was added in further experiments.

3.3. Method performance

The figures of merit in the proposed method, including linear dynamic range (LDR), limit of detection (LOD), and limit of quantification (LOQ), and intra and inter-day precisions for the extraction of melamine from matrix-matched samples were investigated under optimum conditions. The obtained results are shown in Table 1. Calibration curves were plotted using 8 spiking levels of melamine in concentrations ranging from 1.0 to 500 $\mu\text{g L}^{-1}$ and the good determination coefficient (R^2) of 0.9952 was obtained. For each level, three replicate extractions were performed under optimum conditions. LOD values were calculated based on signal to noise ratio of three ($\text{LOD} = 3 \times S_b/m$, where S_b is the standard deviation of blank, and m is the slope of calibration curve), LOD was 0.1 $\mu\text{g L}^{-1}$ and LOQ based on signal to noise ratio of ten ($\text{LOQ} = 10 \times S_b/m$) was obtained 0.3 $\mu\text{g L}^{-1}$. The intra-day precision of the proposed method was obtained 3.2 and 2.6 at 25 and 100 $\mu\text{g L}^{-1}$ levels of melamine, and inter-day precision of the proposed method was obtained 6.7 and 5.4 at 25 and 100 $\mu\text{g L}^{-1}$ levels of melamine, respectively.

3.4. Sample analysis

In order to evaluate the applicability of the proposed method to analysis of the melamine in real samples, different liquid milk and powdered infant formula samples were prepared and analyzed in triplicate under optimum conditions. Moreover, in order to evaluate accuracy of the method in real sample analysis, samples were spiked at the known level of 50 $\mu\text{g L}^{-1}$. The obtained results are shown in Table 2. Good results were obtained, with average recoveries ranging from 90.0 to 104.2% with RSD% less than 6.7%. Fig. 4 depicts the chromatograms of melamine in the powdered infant formula 1 before (Fig. 4a) and after spike at 50 $\mu\text{g L}^{-1}$ (Fig. 4b).

Evaluation of real sample analysis results showed that between tested samples melamine was only found in powdered infant formula 1 (0.48 $\mu\text{g L}^{-1}$) and powdered infant formula 5 (0.23 $\mu\text{g L}^{-1}$). Results demonstrated that the tested samples are in agreement with the maximum level allowed for melamine residue (1.0 mg kg^{-1} for powdered infant formula and 2.5 mg kg^{-1} for other foods and animal feed) (FAO, 2010; European Commission, 2009).

3.5. Comparison of the applied method with other reported methods

The proposed method was compared with a variety of methods that had recently been reported in the literature for preconcentration and determination of melamine. The distinct features of the proposed method were summarized in Table 3. As can be seen from Table 3, it is evident that the proposed method has a wide dynamic linear range. Moreover, LOD of the method is better than some other methods which even use sensitive detection methods such as LC-MS (Ibanez, Sancho, & Hernandez, 2009; Deng et al., 2010; Zhang et al., 2010), GC-MS (Xu et al., 2009; Pan et al., 2013), and HPLC-FLD (Zhang et al., 2014). Also, in regard with the running cost and complication of instrument, the proposed method has a moderate running cost by using the common instrument of HPLC-UV-Vis which could be applied in routine melamine analysis in food control laboratories.

4. Conclusions

In the present study, for the first time a very sensitive method was developed for analysis of melamine in powdered infant formula and milk samples based on dabsyl chloride derivatization and dispersive liquid-liquid microextraction followed by HPLC-UV-Vis analysis. Dabsylation increases detection sensitivity (low detection limit) and also increases hydrophobicity of polar compound of melamine (good retention of melamine), both of which lead to generalization of melamine analysis with the common instrument of HPLC-UV-Vis and widely used C18 columns. Meanwhile, DLLME of melamine-dabsyl derivatives resulted in further preconcentration and omission of the excess amount of reagent. The proposed method in comparison with the methods which used LC-MS and GC-MS has advantages such as sensitivity, simplicity, and a low cost. The proposed method allows melamine determination in different powdered infant formula and milk samples with good accuracy and reproducibility at levels as low as $1.0 \mu\text{g L}^{-1}$, so it can be used for routine control at maximum level allowed for melamine residue in powdered infant formula and milk samples.

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Figure legends

Fig. 1. Derivatization scheme of dabsyl with melamine.

Fig. 2. Effect of pH on derivatization efficiency (Fig. 2a). Effect of dabsyl chloride volume on
derivatization efficiency (Fig. 2a).

Fig. 3. Effect of octanol volume on extraction efficiency of DLLME method (Fig. 3a). Effect of acetonitrile volume on extraction efficiency of DLLME method (Fig. 3a).

Fig. 4. HPLC-UV-Vis chromatogram ($\lambda = 460$ nm) of the powdered infant formula 1 for (a) non-spiked and (b) spike of $50 \mu\text{g L}^{-1}$ of melamine.

Table 1. Figures of merit of the proposed method for extraction and determination of melamine.

RSD%				LOQ	LOD	Linear Range	R ²
Intra-day (n=6)		Inter-day (n=6)		(µg L ⁻¹)	(µg L ⁻¹)	(µg L ⁻¹)	
25	100	25	100				
(µg L ⁻¹)	(µg L ⁻¹)	(µg L ⁻¹)	(µg L ⁻¹)				
3.2	2.6	6.7	5.4	0.3	0.1	1.0-500	0.9952

Table 2. Determination of melamine in different powdered infant formula and milk samples

Sample ^a	C _{added} (µg L ⁻¹)	C _{found} (µg L ⁻¹)	Recovery%	RSD (%) (n = 3)
Powdered infant formula 1	-	48	-	1.8
	50	102	104.2	2.3
Powdered infant formula 2	-	N.D ^b	-	4.6
	50	46	92.0	3.7
Powdered infant formula 3	-	N.D	-	2.5
	50	45	90.0	6.4
Powdered infant formula 4	-	N.D	-	3.8
	50	52	104.0	4.3
Powdered infant formula 5	-	23	-	6.6
	50	70	95.9	3.8
Milk 1	-	N.D	-	2.7
	50	46	92.0	5.4
Milk 2	-	N.D	-	1.9
	50	48	96	3.4
Milk 3	-	11		4.8
	50	58	95.1	3.2
Milk 4	-	N.D	-	4.5
	50	49	98.0	5.3
Milk 5	-	N.D	-	1.5
	50	47	94.0	2.6

^bNot detected

Table 3. Comparison of the proposed method with other developed methods to determine melamine in powdered infant formula and milk samples

Method type	Matrix	LOD ($\mu\text{g L}^{-1}$)	LR	Running cost	Ref.
GC-MS	Dairy products	50	0.05-2 mg L^{-1}	High	Xu et al. 2009
GC-MS	Dairy product	25	50-800 $\mu\text{g L}^{-1}$	High	Pan et al. 2013
LC-MS	Milk-based products and beverage products	100	Not reported	High	Ibanez et al. 2009
LC-MS	Different foodstuff	39.4	0-500 $\mu\text{g L}^{-1}$	High	Deng et al. 2010
LC-MS	Human urine	6	10-5000 $\mu\text{g L}^{-1}$	High	Zhang et al. 2010
HPLC-UV	Liquid milk	18	0.1-50 mg L^{-1}	moderate	Sun et al. 2010
HPLC-UV	Infant formula	100	1.0-80 mg L^{-1}	moderate	Venkatasami & Sowa et al. 2010
UV	Milk	12	0.4-4 mg L^{-1}	Low	Liu et al. 2011
FL	Tainted milk	300	0.25-7.57 mg L^{-1}	Low	Zhou et al. 2011
FL	milk-based products	120	0.2-80 mg L^{-1}	Low	Zeng et al. 2011
HPLC-FL	Melamine leached from tableware	0.005-0.4	0.5-200 $\mu\text{g L}^{-1}$	moderate	Zhang et al. 2014
HPLC-UV	Liquid milk, infant formula	0.1	1.0-500 $\mu\text{g L}^{-1}$	moderate	This work

Abbreviations: LR, linear range; LOD, limit of detection;

^aData not reported

Fig. 1

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Fig .2a

Fig .2b

Fig. 3a

Fig. 3b

Fig. 4