Journal of Chromatography B, 878 (2010) 2333–2338

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Molecularly-imprinted microspheres for selective extraction and determination of melamine in milk and feed using gas chromatography–mass spectrometry

Mu Li [a], Liying Zhang [a,*], Zihui Meng [b,**], Zongyi Wang [a], Hui Wu [a]

[a] State Key Laboratory of Animal Nutrition, China Agricultural University, 2 Yuanmingyuan West Road, Beijing 100193, PR China
[b] School of Chemical and Environmental Engineering, Beijing Institute of Technology, Beijing 100081, PR China

ARTICLE INFO

Article history:
Received 26 April 2010
Accepted 5 July 2010
Available online 12 July 2010

Keywords:
Molecularly-imprinted polymers
Microsphere
Melamine
Solid-phase extraction
Gas chromatography–mass spectrometry

ABSTRACT

Molecularly-imprinted polymers in the form of microspheres were synthesized using the dispersion polymerization protocol; cyromazine was used as dummy template, while methacrylic acid, ethylene glycol dimethacrylate and acetonitrile (MeCN) were used as functional monomer, cross-linker, and porogen, respectively. When compared with the non-imprinted polymer, the molecularly-imprinted polymers (MIPs) showed outstanding affinity toward melamine in MeCN with a maximum binding concentration (B_{max}) of 53.20 nmol mg^{-1} MIPs, imprinting effect of 4.6, and a dissociation constant (K_d) of 90.45 μM. After optimization of the molecularly-imprinted solid-phase extraction conditions, a new method was developed to determine the melamine in milk and feed with gas chromatography–mass spectrometry. The performance of this method has been evaluated in the tainted milk and feed in terms of recovery, precision, linearity, the limit of detection (LOD) and limit of quantitation (LOQ). Recovery ranged in samples from 93.1 to 101.3% with intra-day and inter-day relative standard deviation values below 5.34%. The LOD and LOQ of melamine in milk and feed were 0.01 μg mL^{-1} (μg g^{-1}) and 0.05 μg mL^{-1} (μg g^{-1}), respectively.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine) is a triazine-based chemical used in the manufacture of plastics and flame retardants (Fig. 1A). Ingestion of melamine may lead to the formation of melamine cyanurate crystals in kidney and consequently obstruct and damage renal tubules and cause renal failure in animals [1]. Melamine became a hot topic after pet food contamination in America in 2007, and melamine-tainted-milk powder incident in China in 2008. Therefore, determination of melamine is urgent to ensure public health. Currently, many methods including high performance liquid chromatography (HPLC) [2,3], gas chromatography with mass spectrometry (GC–MS) [4,5], and liquid chromatography tandem mass spectrometry (LC–MS/MS) [6,7] have been developed for the detection of melamine. In these methods, solid-phase extraction was used for preconcentration and clean-up in the analysis of melamine due to the advantages of simplicity, and rapidness. However, the classical solid-phase extraction (SPE) sorbent (C_8, C_{18}, etc.) retained analytes by non-selective hydrophobic interaction which lead to the coextraction of interfering substances and further purification was still required to remove coextractant.

Molecular imprinting is a useful technique for the preparation of polymeric materials as specific molecular recognition receptors [8,9]. Molecularly-imprinted polymers (MIPs) are prepared by the copolymerization of a cross-linking agent with the complex formed from a template and monomers that have functional groups specifically interacting with the template through covalent or non-covalent bonds. After the template is removed from the resulting polymer matrices, binding sites having the size and shape complementary to the template are generated. These MIPs are synthesized with "tailor-made" binding sites for a template and strongly interact with the template. Due to their favorable molecular recognition capability and stability, potential application of MIPs has been investigated in a broad scientific area, such as ligand binding assays [10], SPE [11], sensors [12], catalysis [13]. Among these applications the one most widely applied is the molecularly-imprinted solid-phase extraction (MISPE), which has been used for the extraction of a broad range of analytes such as aromatic compounds (polycyclic aromatic hydrocarbons) [14] and pharmaceutics (β-agonist [15], chloramphenicol [16]).

The use of MIPs for the determination of melamine has been previously described in some matrixes [17–20]. However, the MIPs used for separation and analysis of melamine in the literature were still prepared by bulk polymerization. Using this procedure, grinding and sieving steps are unavoidable, the obtained particles

* Corresponding author. Tel.: +86 10 6273 3588; fax: +86 10 6273 3688.
** Corresponding author. Tel.: +86 10 6891 3065; fax: +86 10 6891 3065.
E-mail addresses: Zhangliying01@sina.com (L. Zhang), Mengzihui@hotmail.com (Z. Meng).

1570-0232/$ – see front matter.
doi:10.1016/j.jchromb.2010.07.003

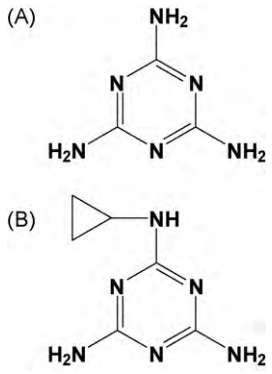

Fig. 1. Chemical structure of melamine (A) and cyromazine (B).

possessed invariably an heterogeneous particle size distribution with poor binding site accessibility for the target analyte [21]. Furthermore, to satisfy the special analytical applications, MIPs with well controlled physical properties, such as particle size distribution and morphology are highly required [22]. Considering these issues MIPs in the form of microspheres with high selectivity along with control on particle shape and size are demanded.

Therefore, in this study, we reported on preparation of molecularly-imprinted microspheres by dispersion polymerization and developed a MISPE-GC–MS method to determine the melamine in the milk and feed.

2. Experimental

2.1. Reagents and chemicals

Melamine, cyromazine, citric acid (CA), and ethylene glycoldimethacrylate (EGDMA) were purchased from Sigma (St. Louis, MO, USA). 1-Heptanesulfonate sodium salt (HSS) was obtained from Dikma (Beijing, China). Methacrylic acid (MAA) was purchased from Tianjing Guangfu Research Institute (Tianjing, China). The initiator α,α′-azobisisobutyronitrile (AIBN) was obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). HPLC-grade acetonitrile (MeCN) and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The derivatization reagent *N*,*O*-bis (trimethylsiyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Supelco (Bellefonte, PA, USA). Scanning electron micrograph of the MIPs was obtained with an S-4800 SEM (Hitachi, Japan). Adsorption/desorption analyses were carried out using a nitrogen surface area analyzer (ASAP 2010c, Micromeritics, USA). All the other chemicals were of analytical grade from Beijing Chemical Reagent Co., Ltd (Beijing, China).

2.2. Preparation of MIPs

The pre-polymerization mixture composed of cyromazine (0.5 mmol), MAA (3 mmol), the cross-linker EGDMA (15 mmol), and the initiator AIBN (20 mg), was dissolved in 18 mL MeCN in a 50 mL flask. The mixture was incubated into an ice-bath and purged with nitrogen gas for 5 min in order to get rid of oxygen. Afterwards, the tube was sealed, placed in a water bath at 60 °C for 24 h for the polymerization to proceed. The solid polymer was collected by centrifugation, washed extensively with 30 mL of methanol-acetic acid (80:20, v/v) continuously overnight, followed by five washing steps with 30 mL of ethanol for 1 h, and dried. The corresponding non-imprinted polymers (NIPs) were prepared in the same manner in the absence of template.

2.3. Binding of melamine

Binding studies were carried out in MeCN. A preweighed amount of MIPs suspended in 1.8 mL of MeCN was mixed with 0.2 mL of different concentration of melamine in methanol while stirring at 300 rpm for 60 min. The polymers were then removed by centrifugation at 8000 rpm for 5 min, and 1 mL of supernatant was removed for HPLC assay. The binding site capacity (B_{max}) and dissociation constant K_d were determined by fitting the equation $y = B_{max} \times C_f/(K_d + C_f)$ (where y is the nmols of target adsorbed per mg of polymer, and C_f is the concentration of free target in μM, both at equilibrium) to the binding isotherm data at 25 °C using Prism 4 program from GradPad Software Inc (San Diego, CA, USA).

2.4. MIPSE and sample preparation

The MIPs and NIPs MISPE cartridges were packed at Agela Technologies (Beijing, China). To ensure all the particles of MIPs could be retained on the frits of the SPE columns, a 0.45 μm nylon membrane was placed on the bottom frit. Samples were added to 50 mL methanol and extracted by ultrasonic extraction for 30 min. Then samples were centrifuged at 10,000 rpm for 10 min and the supernatant was transferred to a clean tube before loading. The cartridge was conditioned with methanol. After loading 3 mL sample, the cartridge was washed with MeCN (3 mL each). The cartridge was fully dried again and eluted with 3 mL methanol-acetic acid (80:20, v/v). The extract was evaporated under gentle nitrogen to dryness and reconstituted in 0.2 mL acetonitrile-BSTFA (50:50 (1% TMCS), v/v). Then the samples were derivatized at 70 °C for 30 min before GC–MS analysis.

2.5. Chromatographic evaluation

Two different instruments and conditions were used in this work. HPLC was used for evaluation while GC–MS for method development.

2.5.1. HPLC

An HPLC (Waters Alliance 2695 HPLC) with Model 996 PDA detector and Millenium 4.0 software (Waters Corp. Milford, MA) was used. The column was a Dikma Plastisil™ ODS-C_{18} (250 mm × 4.6 mm, 5 μm particle size). The mobile phase was MeCN-H_2O (12.5:87.5 (0.01 mol L^{-1} HSS, 0.01 mol L^{-1} CA), v/v) with a flow rate of 1 mL/min. The injection volume was 30 μL. Before injection, all the samples were filtered with a 0.22 μm nylon filter.

2.5.2. GC–MS

Analysis of melamine was performed on an Agilent 6890 Plus GC equipped with an auto sampler and an Agilent 5973N mass selective detector (Agilent Technologies, Atlanta, GA) operated in the electron impact (EI) ionization mode. The GC was fitted with an AB-5ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Agilent J&W Scientific (Folsom, CA). The oven was maintained at the initial temperature of 70 °C for 1 min, heated to 300 °C at the rate of 30 °C min^{-1}, and then held at 300 °C for 1 min. The inlet temperature was 250 °C and transport line 280 °C. Helium was used as carrier gas and the flow rate was 1 mL min^{-1}. Ion source and quadrupole temperature were 230 and 150 °C, respectively. Electron energy was 70 eV. All injections of 1 μL were made in splitless mode. Selected ion-monitoring (SIM) was used for qualitative and quantitative measurement of melamine at *m*/*z* 99, 171, 327, 342 with a solvent delay of 5 min. The molecular ion *m*/*z* 327 was used as quantitative ion.

Table 1
The ratio of adsorption between MIPs and NIPs under the same concentration.

Item	Template/monomer/cross-linker (molar ratio)	MIPs adsorption[a] (%)	NIPs adsorption[a] (%)	$Adsorption_{MIPs}/adsorption_{NIPs}$
1	1/4/12	45.95	22.86	2.01
2	1/6/18	66.31	24.93	2.66
3	1/8/24	66.51	26.71	2.49
4	1/10/30	68.05	27.89	2.44
5	1/4/20	63.49	23.96	2.65
6	1/6/30	87.44	25.42	3.44
7	1/8/40	77.43	26.98	2.87
8	1/10/50	71.49	28.37	2.52

[a] The % of the melamine adsorbed under same concentration (100 μM).

3. Results and discussion

3.1. Synthesis and morphology of MIPs

A well documented drawback of MIPs is the residual template leaching or bleeding that may occur from the MIPs even after extensive washing [23]. In any case, the best manner of preventing the bleeding problem is the use of an analogue of the target analyte as template. Therefore, the bleeding of the template does not interfere in the quantification of the target analyte. Triazine as a template molecule in MAA based molecular imprinting has been reported by several groups [24–26]. Dual-hydrogen bonds are expected to be formed between melamine and MAA as a key interaction necessary for binding site construction, whereby a carboxylic group of MAA works as both a hydrogen bond acceptor and a donor interacting with a hydrogen atom of the amino group and a nitrogen atom of the triazine body, respectively (Fig. 2A) [27]. Therefore, dummy templates should possess two or more dual-hydrogen bondable functional groups. Cyromazine has a cyclopropyl alkyl substituting the hydrogen of the amino group of the melamine (Fig. 1B). In this sense it was expected that the dummy molecules form melamine-like complexes with MAA (Fig. 2B) [19,20].

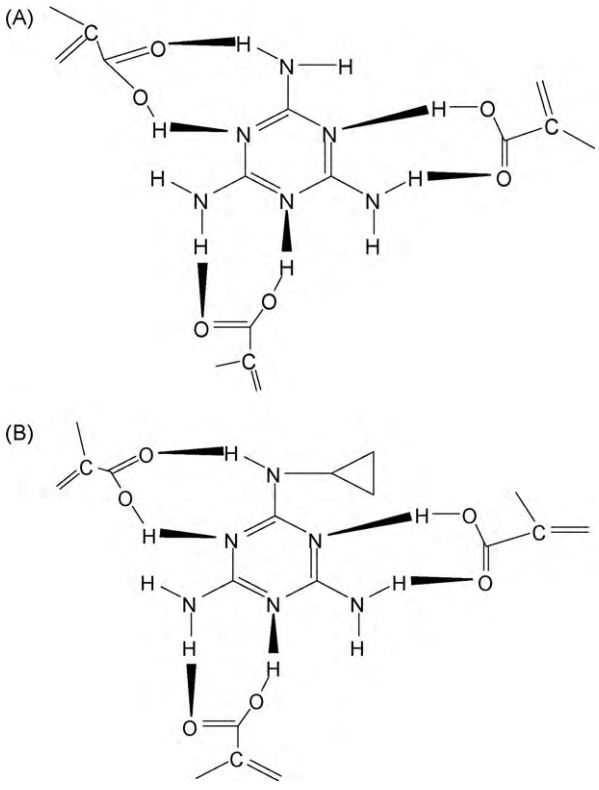

Fig. 2. Schematic representation of intermolecular interaction with methacrylic acid: (A) melamine and (B) cyromazine.

Due to having two or more dual-hydrogen bonds, four molar ratios between template and monomer of 1:4, 1:6, 1:8 and 1:10 were tested in this experiment, while the molar ratio of monomer to cross-linker was selected at 1:3 and 1:5 to ensure the formation of recognition sites within polymer [28]. The ratio of adsorption between MIPs and NIPs under the same concentration was showed in Table 1. As described above, dual-hydrogen bonds were formed between the hydrogen atom of the amino group and carboxylic group of MAA. However, the other hydrogen atom of the amino group could form hydrogen bond with carboxylic group of MAA. Thus, one melamine could form hydrogen bonds with six MAA. An excess of monomer increases the non-specific binding which lowers the overall selectivity of the MIPs [29]. From Table 1, with the ratio of monomer to cross-linker decreasing, the ratio of adsorption between MIPs and NIPs increased if the ratio of the template to monomer did not change. The results revealed that the MIPs with the molar ratios of 1:6:30 within template, monomer and cross-linker showed best specific affinity.

Traditional bulk polymerization was the most widely used approach for preparation of MIPs. In this method, the apparatus acquired for synthesis is relatively simple, and the reaction conditions could be easily controlled. Unfortunately, the chromatographic performance of these particles was usually unsatisfactory due to their irregular size and shape. Furthermore, the tedious and time-consuming process and low yield of MIPs prevented their industrial production and acceptance in analytical laboratories [30]. To overcome these drawbacks MIPs in the form of microspheres were prepared by dispersion polymerization. The dispersion polymerization was applied in the presence of a larger amount of porogen than that typically used in the bulk method. Solvent plays an important role not only in the formation of the porous structure, but also in the effect on complexation of monomers with the template [29]. Thus, MeCN was employed in the polymerization and different porogen volumes of 10, 12, 15, 18 and 20 mL were investigated. The results of the ratio of adsorption between MIPs and NIPs with different volumes were 1.98, 2.63, 3.24, 3.67, and 2.74, respectively. The optimum was confirmed at 18 mL.

Micro- and nanospheres was generated when accurate control of the parameters governing the polymerization is achieved [31]. The MIPs particles obtained by the method are microspheres of about 800 nm diameter with narrow particle size distribution (Fig. 3), unlike the typical amorphous MIPs particles obtained from the grinding of bulk polymers [19,20]. Moreover, the MIPs microspheres in the method could be directly packed to SPE without further processing. Since specific surface area and pore size influenced the efficiency of adsorption, the parameters were obtained using a Brunnauer–Emmet–Teller (BET) analysis routine. The specific surface areas, pore volumes and pore size from nitrogen adsorption experiments were 22.07 $m^2\ g^{-1}$, 0.029 $cm^3\ g^{-1}$ and 3.409 nm for the MIPs, 30.35 $m^2\ g^{-1}$, 0.058 $cm^3\ g^{-1}$ and 5.911 nm for the NIPs, respectively. Total pore volume and BET surface area

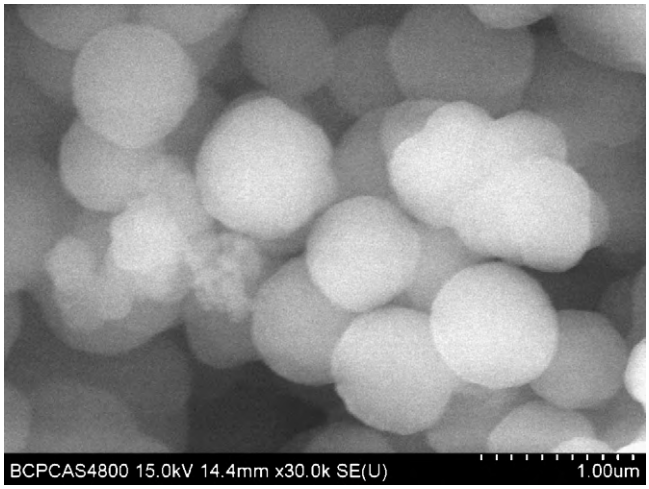

Fig. 3. Scanning electron microscopy of the MIPs.

of MIPs could be smaller than NIPs as the micropore volume and specific surface area is not detectable by gas adsorption measurements at pore diameters of 0.7–2 nm in the nanospheres [28]. It is assumed that the difference of the surface area between MIPs and NIPs could be because most of the pore diameter in MIPs is in the range of 0.7–2 nm.

3.2. Binding isotherm for melamine

With the usage of the optimized conditions established, the imprinting effect was initially evaluated by binding assay. In our preliminary binding assay, we found the MIPs obtained had better binding capability for melamine in MeCN than in water and methanol. It can be explained that H_2O and methanol affected affinity between the MIPs and melamine via hydrogen binding. Therefore, MeCN was selected as the solvent for the binding experiment. In MeCN, the MIPs adsorbed more than 85% of the melamine within 50 min (Fig. 4A). In comparison, NIPs adsorbed less than 16% of melamine during the same time period. B_{max} of 53.20 and 11.56 nmol mg^{-1} for melamine was obtained for the MIPs and NIPs, respectively (Fig. 4B), and the overall imprinting effect B_{max}(MIPs)/B_{max}(NIPs) of 4.6 (B_{max} is the binding site capacity in nmoles mg^{-1} of polymers). In addition to the good binding capability, a dissociation constant K_d of 90.45 μM demonstrated the high binding capacity between MIPs and analyte. When compared to MIPs for melamine reported in the literature, the MIPs in this study have higher binding capacity [18,19]. All these characteristics indicated that the MIPs were potential sorbents for selective enrichment and separation, detection of melamine from sample matrix.

3.3. Method optimization

In order to evaluate application of the MIPs for separation and determination of trace melamine by GC–MS, a general procedure for a generic SPE (conditioning, loading, washing, eluting) was optimized to achieve good sensitivity and precision of this method.

As melamine could be dissolved in H_2O, methanol and MeCN, five loading solvents were investigated. Recoveries of melamine in solvents were 97.02% (H_2O), 101.07% (methanol), 80.42% (H_2O/methanol), 74.73% (H_2O/MeCN), and 68.20% (methanol/MeCN), respectively. The results showed that the recovery in methanol is optimum, but the recovery of melamine in H_2O was also over 97%. Then the effect of pH value of H_2O was compared, as an optimum pH is crucial for trapping [32]. When 0.5

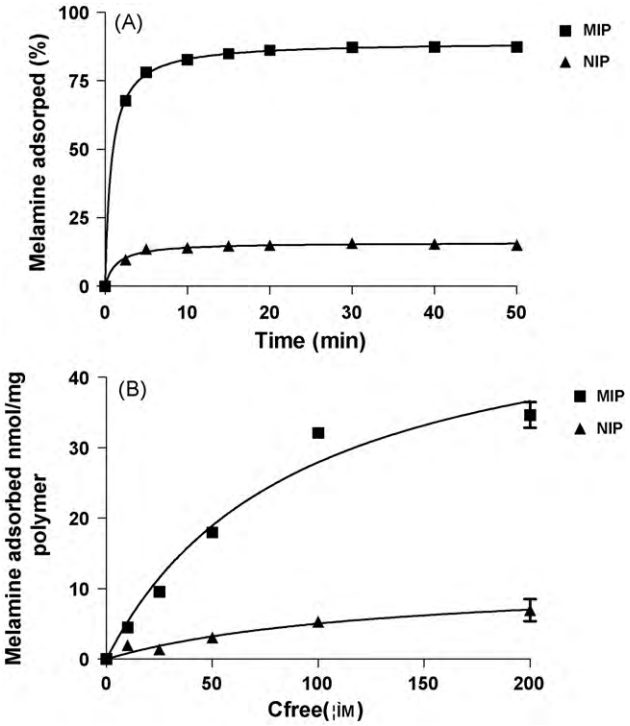

Fig. 4. (A) Time profile of melamine (0.1 mM) binding by 1 mg mL^{-1} MIPs and NIPs from MeCN. (B) Binding isotherm of MIPs and NIPs for melamine from MeCN.

and 1% trichloroacetic acid solutions were used, losses of melamine were over 50%. Then the effect of pH at 6, 7, 8, 9, 10, and 11 were tested. The results showed approximately 3% loss of melamine was inevitable, although the loss at pH 8 was the lowest (Fig. 5). So methanol was chosed as extract solvent finally in this study.

The washing step was a crucial procedure to maximize the specific interactions between the analytes and binding sites, and to simultaneously decrease non-specific interactions to discard matrix components in the polymers [33]. In this study, acetone, tolune, hexane, methanol, MeCN and dichloromethane were investigated. The results showed that loss of melamine was the smallest when MeCN was used as washing solvent (Fig. 6). Methanol–acetic acid solution (80:20, v/v) was used as of eluting solvent which could absolutely elute the melamine from MIPs and NIPs cartridges.

3.4. Validity

The optimized condition described above was validated before application to the detection of real samples. Linear calibration curves of melamine in milk and feed were obtained from the

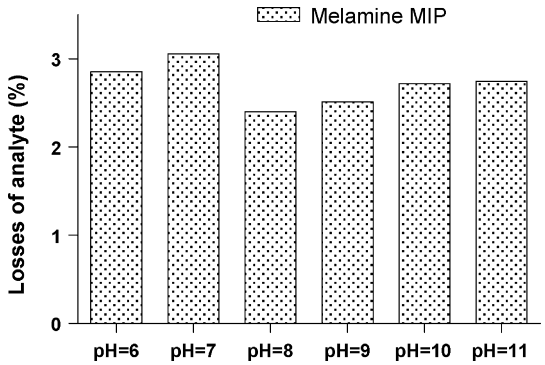

Fig. 5. Losses of melamine with different pH loading conditions, $n = 4$.

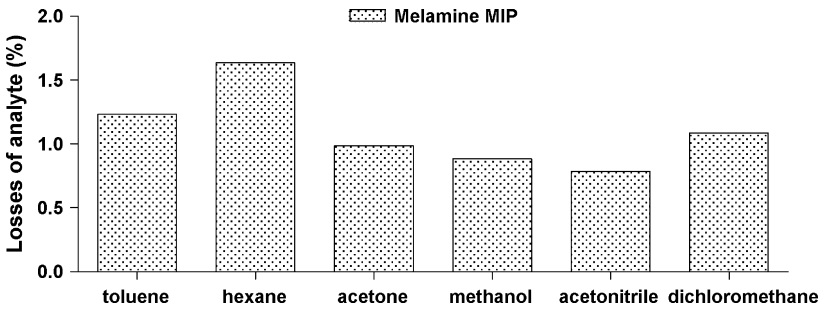

Fig. 6. Losses of melamine with different washing solvents, $n = 3$.

Table 2
Recoveries of melamine from spiked feed and milk samples.

Matrices	Content	Recoveries (%)			Relative standard deviation (%)	
		Means	Max	Min	Inter-day repeatability ($n = 6$)	Intra-day repeatability ($n = 3$)
Milk ($\mu g\,mL^{-1}$)	0.05	95.0	99.8	91.0	2.88	3.51
	1.5	99.7	105.1	93.9	3.11	4.44
	10	99.8	107.3	94.2	3.96	4.05
Feed ($\mu g\,g^{-1}$)	0.05	93.1	96.2	87.6	4.45	5.13
	2	99.8	104.8	94.1	3.28	2.97
	10	101.3	107.2	95.4	3.66	3.19
	50	100.4	109.7	93.3	4.06	5.34

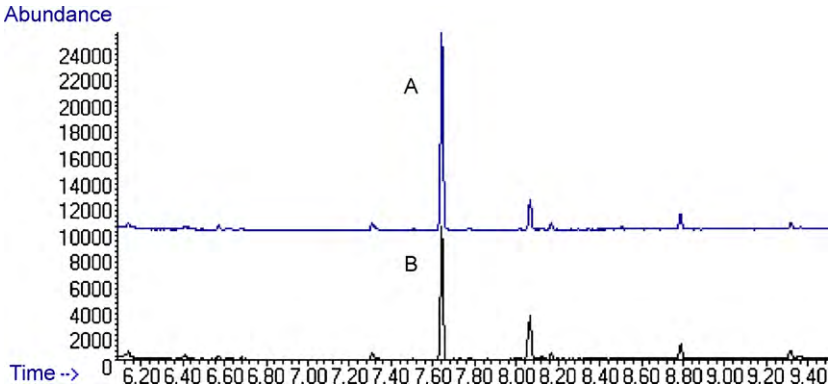

Fig. 7. Chromatograms of feed and milk samples using MISPE-GC–MS: (A) milk spiked at 1.5 $\mu g\,mL^{-1}$ and (B) feed spiked at 2 $\mu g\,g^{-1}$.

concentration 0.05 to 20 $\mu g\,mL^{-1}$, and 0.05 to 100 $\mu g\,g^{-1}$ in real sample, respectively. The correlation coefficients were 0.9914 and 0.9906, respectively.

Different spiked concentrations in the milk and feed (0.05, 1.5, 10 $\mu g\,mL^{-1}$; 0.05, 2.0, 10, 50 $\mu g\,g^{-1}$, respectively) were adopted to examine the recovery and precision of the method (Table 2). The recoveries of different concentrations in milk were from 95.0 to 99.8%. The intra-day and the inter-day relative standard deviation (RSD) values all ranged from 2.88 to 4.44 for all spiked levels in milk. The recoveries of different concentrations in feed were from

Table 3
Comparative results between MISPE-GC–MS method and available standard methods.

Sample	Melamine	
	Available standard method ($n = 3$)[a,b]	MISPE-GC–MS method ($n = 3$)
Milk ($\mu g\,mL^{-1}$)	Mean (%RSD)	
A	0.12 (8.80)	0.14 (5.96)
B	ND[c]	0.06 (4.59)
C	ND	ND
D	0.27 (6.40)	0.31 (3.68)
Feed ($\mu g\,g^{-1}$)		
A	ND	ND
B	ND	0.07 (7.30)
C	1.21 (5.72)	1.35 (3.73)
D	2.31 (4.21)	2.55 (3.42)
E	ND	ND

[a] NY/T 1372-2007, Determination of melamine in feedstuffs, Chinese Ministry of Agriculture Standard.
[b] GB/22388-2008, Determination of melamine in raw milk and dairy products, Chinese National Standards.
[c] Not detected.

93.1 to 101.3%. The intra-day and the inter-day RSD values ranged from 2.97 to 5.34 for all spiked levels in feed. The results showed the method had good precision even at the low concentrations. The limit of detection (LOD, based on signal-to-noise ratio of 3, S/N = 3) and limit of quantitation (LOQ, S/N = 10) of the milk and feed were 0.01 $\mu g\,mL^{-1}$ ($\mu g\,g^{-1}$) and 0.05 $\mu g\,mL^{-1}$ ($\mu g\,g^{-1}$), respectively. An overlaid chromatogram of the spiked milk and feed was shown in Fig. 7.

3.5. Analysis of real samples

To determine the applicability of the developed method and make comparisons to the available standard methods used in China [3,4], the new method was applied to the analysis of melamine in real samples. Four milk and five feed samples were chosed again and analyzed with the improved method. The results were showed in Table 3. It looks better than the available standard methods now used in China.

4. Conclusions

An accurate and selective analytical method has been developed for the determination of melamine in milk and feed. The method employs molecularly-imprinted microspheres designed for the separation and extraction of melamine as a MISPE clean-up step to avoid major traditional problems associated with MIPs with irregular size and shape. The method was also shown to be applicable for various samples. Finally MISPE-GC–MS has offered accurate results for the separation and analysis of melamine in the milk and feed.

Acknowledgement

Financial support from National Natural Science Foundation of China (Nos. 30771577 and 20775007) is appreciated.

References

[1] B. Pushner, R.H. Poppenga, L.J. Lowenstine, M.S. Filigenzi, P.A. Pesavento, J. Vet. Diagn. Invest. 19 (2007) 616.
[2] R.A. Yokley, L.C. Mayer, R. Rezaaiyan, M.E. Manuli, J. Cheung, J. Agric. Food Chem. 48 (2000) 3352.
[3] NY/T 1372-2007, Determination of melamine in feedstuffs, Chinese Ministry of Agriculture Standard.
[4] GB/22388-2008, Determination of melamine in raw milk and dairy products, Chinese National Standards.
[5] X.M. Xu, Y.P. Ren, Y. Zhu, Z.X. Cai, J.L. Han, B.F. Huang, Y. Zhu, Anal. Chim. Acta 650 (2009) 29.
[6] M.S. Filigenzi, B. Puschner, L.S. Aston, R.H. Poppenga, J. Agric. Food Chem. 56 (2008) 7593.
[7] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[8] K. Haupt, Chem. Commun. (2003) 171.
[9] S.A. Piletsky, S. Alcock, A.P.F. Turner, Trends Biotechnol. 19 (2001) 9.
[10] N. Lavignac, C.J. Allender, K.R. Brain, Anal. Chim. Acta 510 (2004) 139.
[11] L. Andersson, Bioseparation 10 (2001) 353.
[12] K. Haupt, K. Mosbach, Chem. Rev. 100 (2000) 2495.
[13] G. Wulff, Chem. Rev. 102 (2002) 1.
[14] R.J. Krupadam, B. Bhagat, S.R. Wate, G.L. Bodhe, B. Sellergren, Y. Anjaneyulu, Environ. Sci. Technol. 43 (2009) 2871.
[15] M. Gros, T.M. Pizzolato, M. Petrović, M.J.L. de Alda, D. Barceló, J. Chromatogr. A 1189 (2008) 374.
[16] R. Mohamed, J.R. Payot, E. Gremaud, P. Mottier, E. Yilmaz, J.C. Tabet, P.A. Guy, Anal. Chem. 79 (2007) 9557.
[17] R.N. Liang, R.M. Zhang, W. Qin, Sens. Actuators B: Chem. 141 (2009) 544.
[18] H.H. Yang, W.H. Zhou, X.C. Guo, F.R. Chen, H.Q. Zhao, L.M. Lin, X.R. Wang, Talata 80 (2009) 821.
[19] L.M. He, Y.J. Su, Y.Q. Zheng, X.H. Huang, L. Wu, Y.H. Liu, Zh.L. Zeng, Zh.L. Chen, J. Chromatogr. A 1216 (2009) 6196.
[20] L.M. He, Y.J. Su, X.G. Shen, Y.Q. Zheng, H.B. Guo, Zh.Z. Zeng, J. Sep. Sci. 32 (2009) 3310.
[21] F.G. Tamayo, E. Turiel, A. Martín-esteban, J. Chromatogr. A 1152 (2007) 32.
[22] L. Ye, I. Surugiu, K. Haupt, Anal. Chem. 74 (2002) 959.
[23] A. Ellwanger, C. Berggren, S. Bayoudh, C. Crecenzi, L. Karlsson, P.K. Owens, K. Ensing, P. Cormack, D. Sherrington, B. Sellergren, Analyst 126 (2001) 784.
[24] M.T. Muldoon, L.H. Stanker, J. Agric. Food Chem. 43 (1995) 1424.
[25] J. Matsui, Y. Miyoshi, O. Doblhoff-Dier, T. Takeuchi, Anal. Chem. 67 (1995) 4404.
[26] M. Siemann, L.I. Andersson, K. Mosbach, J. Agric. Food Chem. 44 (1996) 141.
[27] G.J. Welhouse, W.F. Bleam, Environ. Sci. Technol. 27 (1993) 494.
[28] S. Wei, A. Molinelli, B. Mizaikoff, Biosens. Bioelectron. 21 (2006) 1943.
[29] D.A. Spivak, Adv. Drug. Deliver. Rev. 57 (2005) 1779.
[30] A. Martín-Esteban, Fresen. J. Anal. Chem. 370 (2001) 795.
[31] R. Say, E. Birlik, A. Ersöz, F. Yilmaz, T. Gedikbey, A. Denizli, Anal. Chim. Acta 480 (2003) 251.
[32] S. Zorita, B. Boyd, S. Jönsson, E. Yilmaz, C. Svensson, L. Mathiasson, S. Bergström, Anal. Chim. Acta 626 (2008) 147.
[33] C.Y. He, Y.Y. Long, J.L. Pan, K. Li, F. Liu, J. Biochem. Biophys. Methods 70 (2007) 133.