# Analytical Methods

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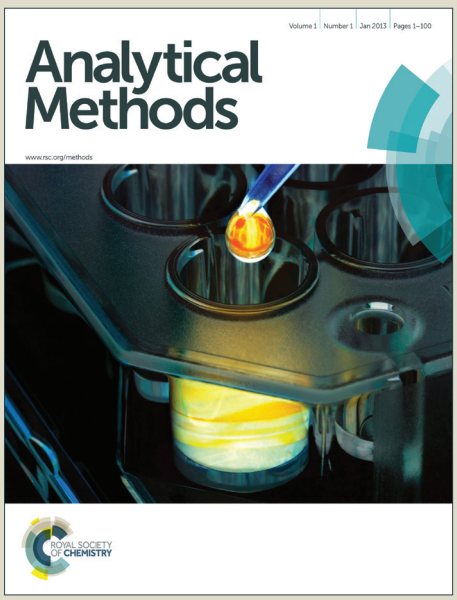





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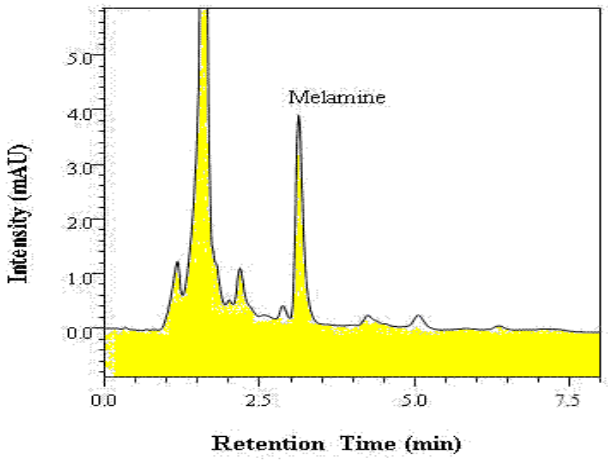
Graphic Abstract

Ultrasonic-assisted extraction with high performance liquid chromatography was used for rapid and low-cost determination of melamine in soil and sediment.



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ARTICLE TYPE

# Fast and low-cost method for determination of melamine in soil and sediment using high performance liquid chromatography

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This paper describes a fast and low-cost method for determination of melamine, an emerging environmental contaminant, in soil and sediment using ultrasonic-assisted extraction coupled with high performance liquid chromatography. The parameters for sample pretreatment and chromatographic analysis were tested and optimized. The samples were extracted by 5% (v/v) ammonia in methanol with ultrasonic assistance for 5 min, followed by centrifugation and nitrogen blowing, without cleaning procedure such as solid phase extraction. Chromatographic separation was achieved by a cyano column using acetonitrile/water (5/95, v/v) as mobile phase containing no ion-pair reagent, and the quantification was carried out by external standard method. An excellent linearity for melamine was obtained over the concentration range of 0.05-5 mg $kg^{-1}$ with a correlation coefficient of 0.9999. The limit of detection (S/N=3) and the limit of quantification (S/N=10) for melamine were 0.01 mg $kg^{-1}$ and 0.04 mg $kg^{-1}$, respectively. The recovery for melamine ranged from 95.0% to 105.9%, with relative standard deviations (RSDs) (n=3) of 1.6-5.3%. The proposed method was shown to be simple, rapid, cheap, sensitive and accurate, and was applied to the analysis of melamine in soil and sediment samples with satisfactory results.

## Introduction

Melamine (1, 3, 5-triazine-2, 4, 6-triamine, $C_3H_6N_6$) is an organic compound produced in large amounts and used in the manufacture of plastics, flame retardants, coatings and other products. Melamine-contaminated infant formulas have resulted in serious health problems such as kidney stones and renal failure in China, and caused a worldwide food security concern. [1] Besides its presence in foods and feeds due to the intentional illegal addition, melamine has also been present in the environment as a result of its widespread uses, such as in the manufacturing of fertilizer-urea mixtures as a slow release source of nitrogen. [2] Melamine can also be introduced to the environment via various industrial effluents. [3, 4] In addition, cyromazine, an insect growth regulator, can metabolize via dealkylation reactions and undergo environmental degradation to form melamine. Consequently, the presences of melamine in soil and water sources from the industrial uses/ manufacturing of melamine have been detected. [4-6] Nowadays, melamine has been regarded as one of the emerging contaminants. [7] Therefore, it is necessary to establish suitable analytical methods for determination of melamine content in environmental samples.

Many analytical techniques have been developed to detect melamine in foods and animal feeds, including liquid chromatography–ultraviolet detection (LC–UV), [8, 9] gas chromatography–mass spectrometry GC–MS, [10] liquid chromatography–tandem mass spectrometry (LC–MS/MS), [11, 12] and gas chromatography–tandem mass spectrometry (GC–MS/MS). [13, 14] However, so far only a few methods for determination of melamine in soil and sediment haves been reported. [15] Although GC/LC–MS or GC/LC–MS/MS systems can provide great sensitivity and selectivity for melamine determination, the high cost of operation and maintenance of them as well as the time-consuming derivatization procedure required for GC analysis limits their practical use. Therefore, the HPLC–UV method is nowadays the popular choice for most analyses. In order to improve the liquid chromatographic behavior of melamine on commonly used C18 column, expensive ion-pair reagent such as sodium heptanesulfonate is often needed for mobile phase, [16, 17] which will certainly yield a rise in analysis cost. In sample preparation, for solid samples or liquid samples with complex matrixes, solvent extraction is generally utilized to separate melamine from samples at first and solid phase extraction (SPE) is then used for cleanup and preconcentration of the extract. SPE is a widely used sample-preparation technique, [18, 19] which generally includes four procedures, i.e., conditioning, loading, rinsing and elution. Therefore, SPE is always time-consuming.



 

Soil and sediment are well known important environmental fates for various pollutants. Considering that the chance of water, soil and sediment being contaminated by melamine has become more and more likely, it is of great importance to establish adequate analytical techniques to monitor the contents of melamine in environmental mediums, so as to prevent this hazardous compound from entering the human food supply. The aim of this work was to develop a fast, low-cost and reliable analytical approach for the routine determination of low levels of melamine in soil and sediment. Melamine was extracted from these environmental samples using ammonia-methanol solution with ultrasonic assistance, and LC–DAD was employed in melamine analysis with a cyano column for separation. The effects of extraction parameters and chromatographic conditions were investigated, and the analytical performance of the proposed approach was evaluated. Finally, the developed method was utilized in the determination of melamine in soil and sediment samples to examine its feasibility for practical application.

## Experimental

### Chemicals

Deionized water (18.6 MΩ cm) was prepared by a water purification system (Milli-Q Gradient). Acetonitrile and methanol were HPLC-grade, and 28% ammonia solution was analytical reagent grade.

Melamine (>99.9%) was purchased from Aladdin reagent (Shanghai) Co., Ltd (Shanghai, China). Stock solution of melamine (1000 mg $L^{-1}$) was prepared in methanol and stored in the dark at 4 ℃, and the working standard solutions were prepared by appropriate dilutions of the stock standard solution with methanol just before use. (5%, v/v) ammonia in methanol was prepared by the mixture of 5 mL ammonia solution and 95 mL methanol.

### Apparatus

The separation and analysis were performed on a HPLC system (SHIMADZU, Kyoto, Japan) equipped with a diode-array detector (SPD-M20A), two pumps (LC-20AT), autosampler (SIL-20AC) and in-line degasser (DGU-$20A_5$). A GL Inertsil CN-3 cyano column (150×4.6 mm I.D.) and a six-port valve with a 20 μL sample loop injector was used.

The samples were vortex mixed with a XW 80A vortex agitator (Kylin-Bell, Jiangsu, China), extracted with a KQ-100DB ultrasonic cleaner (Hechuang, Kunshan, China) and centrifuged by a KDC-80 low-speed centrifuge (Keda, Hehui, China). Ultraviolet data were measured with a TU-1901 spectrophotometer (Puxi, Beijing, China).

**Sample preparation**

Samples of soil and sediment were collected from farmland and fishpond located in Guangzhou, China, respectively. Extraction of these samples was performed according to the following procedures: a portion of soil or sediment sample (2.00 g) was transferred to a 50-mL plastic centrifugal tube containing 10.0 mL of 5% (v/v) ammonia in methanol. Each sample was vortex mixed for 1 min, and then subjected to ultra-sonication for 5 min, followed by centrifugation for 5 min at 4000 rpm. The supernate was carefully transferred into a 50-mL flask. Thereafter, a second 10-mL portion of extraction solvent was added and the sample was extracted again. After centrifugation the supernate was transferred into the same flask containing the first extract. A portion of 5 mL of the supernate was evaporated to dryness in a water bath at 50 °C under blowing nitrogen. Upon reaching dryness, the residue was reconstituted in 1 mL of the LC mobile phase and then filtered through a membrane of 0.22 μm pore size for determination.

In spiking experiments, appropriate amount of melamine standard solution was added into 2.00 g soil or sediment sample, resulting in spiking concentration of melamine at 0.20 and 2.0 mg $kg^{-1}$, respectively. After vortex mixing for 1 min, standing at room temperature for 12 h to remove methanol in the standard solution, each sample was extracted according the procedure described above.

**HPLC analysis**

Chromatographic separation was achieved by a GL Inertsil CN-3 column using acetonitrile/water (5/95, v/v) as mobile phase. The flow rate of mobile phase was set at 1.0 mL $min^{-1}$. A 20-μL volume of sample solution was injected into the column. Peak identification and purity of melamine were assessed by comparison of its retention times and UV spectra with those previously recorded for melamine standard. Quantification was carried out by using matrix-matched standards calibration. The linear equation for the relationship between peak area of melamine and its concentration was determined by least-squares method.

## Results and discussion

**Optimization of ultrasonic-assisted extraction conditions**

***Selection of extraction solvent***

Since melamine is a polar compound, initial extraction is therefore performed with polar solvents such as a dilute acidic solution, a polar organic solvent, or a mixture of organic agent and water. In this work, 5% trichloroacetic acid solution, methanol and acetonitrile were tested for extraction of melamine from the samples. It was found that when 5% trichloroacetic acid solution was used, the extract should be further cleaned by cationic exchange solid phase extraction before LC



 

analysis. Using acetonitrile as the extraction solvent, it would take longer time to evaporate the supernatant to dryness, because the boiling point of acetonitrile is relatively high (81.1 °C). In addition, acetonitrile had a high price. The extraction recovery for melamine was about 70% when methanol was utilized. However, it was observed that the addition of ammonia to methanol could significantly improve the extraction efficiency. The effect of ammonia concentration in methanol solution was examined in the range of 1- 8% (v/v). The recovery of melamine as a function of ammonia concentration is presented in Fig. 1. It is evident that the recovery enhanced with the increase of ammonia concentration from 1% to 5%, then kept practically unchanged up to 6%, and thereafter dropped slightly. Accordingly, 5% ammonia-methanol solution was selected as the extraction solvent. Tang et al found that adsorption of melamine was significantly negatively correlated with soil pH, since cation adsorption was the predominant sorption mechanism of melamine on soil. [20] In this work, the addition of ammonia in methanol increased the extraction solvent pH, thus lessened the adsorption of positively charged-melamine on the soil, and enhanced the extraction efficiency.

***Selection of extraction solvent volume***

To investigate the effect of extraction solvent volume on analytical performance, experiments were carried out by changing the amount of 5% ammonia- methanol solution from 10 to 30 mL by an increment of 5 mL. As shown in Fig.2, the extraction recovery was increased with the elevation of extraction solvent volume. When the volume exceeded 20 mL, extraction recoveries higher than 96% could be obtained and changed insignificantly. Thus, in our experiment, 20 mL of the extraction solvent was used throughout.

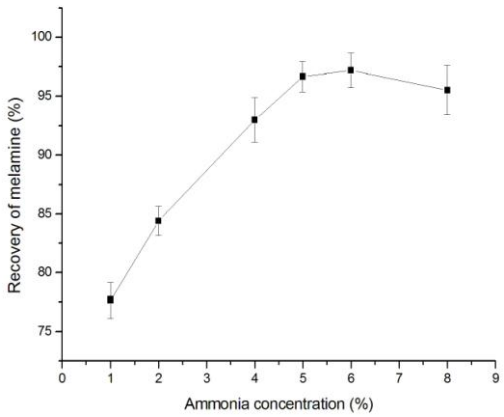


**Fig. 1.** Effect of ammonia concentration in extraction solvent on recovery of melamine.

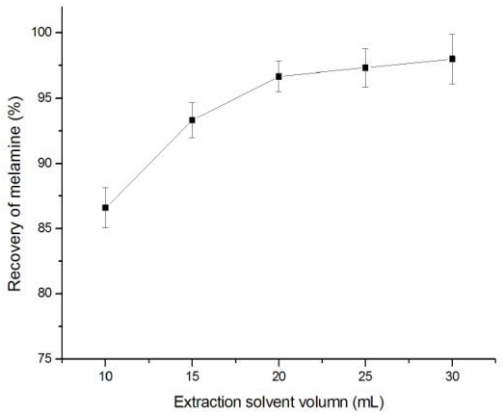


**Fig. 2.** Effect of extraction solvent volume on recovery of melamine.

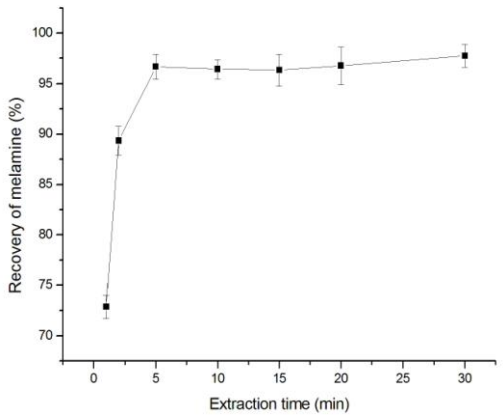


**Fig. 3.** Effect of extraction time on recovery of melamine.

***Selection of extraction time***

Extraction time usually plays an important role in extraction of analytes from matrixes. In this work, the power of ultrasound used for extraction was 100 w, and the extraction time was spanned from 1 to 30 min. Fig.3 shows the dependence of recovery for melamine on the extraction time. As can be seen, the recovery increased sharply when extraction time increased from 1 to 5 min, and showed little enhancement when up to 30 min. Consequently, the extraction time was maintained at 5 min throughout the work to obtain a satisfactory recovery.

Vortex extraction of melamine from soil and sediment was also investigated. The mixture (sample and extraction solvent) was vigorously shaken for 5 min (the same time as for ultrasonic extraction) at 2800 rpm (maximum setting). It was found that using vortex extraction, the recovery for melamine was lower (<85%) than that obtained by ultrasonic extraction (>95%).

***Selection of centrifugation speed and time***

The influences of centrifugation speed and time on the extraction recovery of melamine were also studied in the range of 2000-5000 rpm and 1-10 min, respectively, and the results revealed that a recovery higher than 95% could be achieved when centrifugation speed was larger than 4000 rpm and centrifugation time larger than 3 min. Considering that a longer centrifugation time is beneficial for the complete matrix sedimentation, 4000 rpm and 5 min were chosen as the optimum centrifugation speed and time respectively.

After centrifugation, evaporation and filtration, the sample solution could be directly injected into the chromatographic system for separation and determination, without subjection to purification using a strong cation-exchange resin for solid phase extraction.

**Optimization of chromatographic conditions**

***Selection of analytical wavelength***

In order to choose a suitable detection wavelength, a standard solution containing 100 mg $L^{-1}$ of melamine was scanned with a TU-1901 spectrophotometer from 200 to 400 nm. It was found that the maximum absorption wavelength for melamine was 210 nm. So,



 

the following UV absorbance detection was performed at the wavelength of 210 nm.

***Selection of separation column***

The key factor affecting separation is chromatographic column. Because melamine is small and highly polar molecule, it is difficult to achieve sufficient retention of melamine by using traditional C18 columns. To improve its retention and separation, some ion-pair reagents such as sodium heptanesulfonate were used in the mobile phases. [13, 16]

GL Inertsil CN-3 column utilized in this work is a cyano column which has unique polar selectivity. The experimental results indicated that using this column, effective separation of chromatographic peak of melamine from the matrix peaks could be achieved. The retention time for melamine was 3.1 min. Thus, a GL Inertsil CN-3 column was selected for separation. In order to protect the analytical column, a guard column was needed.

Hydrophilic interaction liquid chromatography (HILIC) column and strong cation exchange chromatography (SCX) column can also be applied in separation of melamine. However, compared with the used CN-column, in order to achieve satisfactory chromatographic separation, using of these two types of columns usually require a higher proportion of costly acetonitrile in mobile phase and take a longer run time. [21, 22]

***Selection of mobile phase***

Acetonitrile/water and methanol/water were tested as the mobile phase. The results showed that good separation was attainable by using each of them. However, it was also observed that acetonitrile/water could elute melamine off from the column within a shorter time and yielded a sharper peak compared with methanol/water. So, acetonitrile/water instead of methanol/water was utilized in the experiments.

The proportion of acetonitrile in mobile phase had a significant influence on the retention time of melamine. It was found that melamine would be eluted from the column very fast when acetonitrile concentration was larger than 10%, resulting a overlapping of peaks of melamine and a matrix component. When 5% acetonitrile was used, satisfactory separation degree, peak shape and retention time were obtained. Thus, acetonitrile/water (5/95, v/v) was adopted as the mobile phase. It is obvious that the method is advantageous as it avoids the use of expensive ion-pair reagents and high proportion of costly acetonitrile in mobile phase.

***Evaluation of the method performance***

To evaluate the performance of the developed method, limits of detection (LOD) and quantification (LOQ), precision (RSD), linearity and correlation coefficient (r) were investigated under the optimum conditions. The

results are summarized in Table 1. A calibration curve was obtained by plotting the peak area vs. melamine concentration over the range of 0.05-5 mg $Kg^{-1}$, which resulted in an r value of 0.9999. The LOD defined as 3 S/N and LOQ as 10 S/N were 0.01 and 0.04 mg $kg^{-1}$ for melamine, respectively. The precision of the method was studied by six replicate experiments at the same spiked concentration (0.2 mg $kg^{-1}$), which yielded an intra-day RSD of 1.3% and an inter-day RSD of 3.4%, respectively. The robustness of the developed method was determined by analysis of samples under a variety of conditions such as small changes in the ammonia concentration in extraction solvent (from 5.0% to 5.5%), in the extraction solvent volume (from 20 mL to 18 mL) and in the extraction time (from 5.0 min to 4.5 min). The effects on sample (spiked with melamine at 0.2 mg $kg^{-1}$) recovery were studied. The method was found to be robust when the extraction conditions were varied. During these investigations, the recoveries for melamine were 95.6–103.7%, and the RSDs were 1.8–4.2%. All these data indicate that the proposed method can provide good sensitivity and linearity with excellent accuracy and precision for the quantitation of melamine.

Both solid phase extraction (SPE) and solvent evaporation to dryness are time consuming procedures. Because the solvent used for elution of melamine from SPE adsorbent is different from LC mobile phase, it also needs evaporation to dryness before LC analysis. [6] Since no SPE procedure is

**Table 1** Figures of merit of the proposed method.

| Parameters (units) | Analytical feature |
|---|---|
| Linear dynamic range (mg $Kg^{-1}$) | 0.05—5 |
| r | 0.9999 |
| LOD (mg $Kg^{-1}$) | 0.01 |
| LOQ (mg $Kg^{-1}$) | 0.04 |
| Intra-day RSD (%, n=6) | 1.3 |
| Inter-day RSD (%, n=6) | 3.4 |

**Table 2** Analytical results of melamine in soil and sediment samples.

| Sample | Add (mg $Kg^{-1}$) | Found (mg $Kg^{-1}$)[a] | Recovery (%) |
|---|---|---|---|
| Soil | 0 | 0.057±0.003 | |
| sample 1 | 0.20 | 0.251±0.006 | 97.0 |
| | 2.00 | 1.987±0.032 | 96.5 |
| Soil | 0 | nd[b] | |
| sample 2 | 0.20 | 0.201±0.004 | 100.5 |
| | 2.00 | 2.043±0.059 | 102.5 |
| Sediment | 0 | nd[b] | |
| sample 1 | 0.20 | 0.205±0.011 | 102.5 |
| | 2.00 | 2.117±0.069 | 105.9 |
| Sediment | 0 | 0.256±0.011 | |
| sample 2 | 0.20 | 0.446±0.018 | 95.0 |
| | 2.00 | 2.293±0.049 | 101.9 |

[a] Mean of three determinations ± standard deviation.

[b] Not detected (i.e., below the limit of quantification).

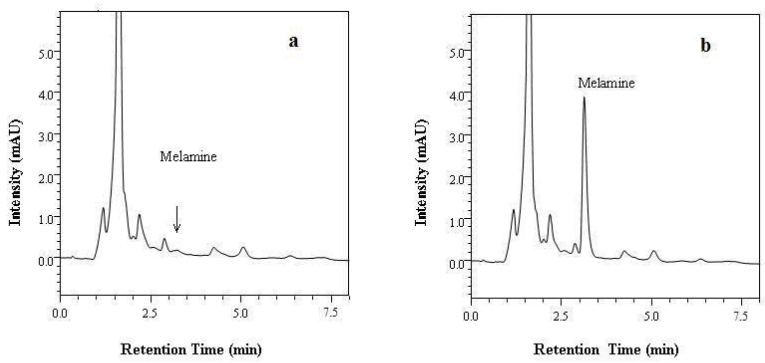


**Fig. 4.** Chromatograms of (a) soil sample and (b) soil sample spiked with 2.0 mg $kg^{-1}$ of melamine.

**Table 3** Comparison of the proposed method with other analytical techniques.

| Methods | Extraction solvent | LOD (mg $Kg^{-1}$) | Recovery (%) | RSD (%) | Time (min) | Reference |
|---|---|---|---|---|---|---|
| SE-SPE- ELISA | $H_2O$ | 0.15 | 104-107 | -- | >70 | [15] |
| SE-SPE-GC-MS | 70% $CH_3CN$/ 30% 0.050 M $(NH_4)_2CO_3$ | 0.003 | 93.5-96.8 | 9.4-17.5 | >110 | [6] |
| SE-SPE -HPLC-UV | $H_2O$+4% $CH_3COOH$ | 0.05 | 100 | 3.6-6.7 | >120 | [15] |
| SE-SPE -UPLC- MS/MS | 1%$Cl_3CCOOH$ | 0.0005 | >70 | <12 | >90 | [23] |
| SE-SPE-GC-MS | 1% $CH_3COOH$ | 0.003 | 73.5-90.7 | 3.8-6.6 | >80 | [24] |
| SE-HPLC-DAD | 5% $NH_3H_2O$ ($CH_3OH$) | 0.01 | 95.0-105.9 | 1.6-5.3 | 40 | This work |

required for further clean-up in our work, the time for overall treatment was significantly reduced.

**Sample analysis**

In order to validate the proposed method, it was applied to the determination of melamine in soil and sediment samples. The water contents of these samples were below 5%. All the measurements were made in triplicate. Samples spiked with 0.2–2.0 mg $kg^{-1}$ of melamine showed recoveries ranging from 95.0% to 105.9% with RSD of 1.6–5.3%, indicating that the method is accurate and precise over this concentration range (Table 2). Typical chromatograms obtained are shown in Fig. 4. There was no matrix peak showing noticeable interference with the chromatographic peak of melamine.

**Comparison with other techniques**

A comparison of the developed method with other reported techniques was made. Table 3 presents the LOD, recovery, RSD and required time for the solvent extraction–solid phase extraction–enzyme linked immunosorbent assay (SE–SPE–ELISA),[15] solvent extraction-solid phase extraction–gas chromatography–mass spectrometry (SE–SPE–GC–MS), [6, 24] solvent extraction–solid phase extraction– high performance liquid chromatography with UV detection (SE–SPE–HPLC–UV),[15] solvent extraction–solid phase extraction–ultra performance liquid chromatography – tandem mass spectroscopy (SE–SPE–UPLC–MS/MS) [23] and the represented method (solvent extraction– high performance liquid chromatography with diode-array detection, SE–HPLC–DAD). As can be seen from Table 3, the proposed method took the shortest time to fulfill a complete sample analysis, and achieved satisfactory accuracy, precision, and a LOD near that obtained by SE-SPE-GC-MS. Furthermore, the developed technique has the advantages of low-cost and simple operation, because it didn't need expensive instrumentation, SPE for sample purification, derivatization for increasing volatility or ion-pair reagent for improving chromatographic behavior of melamine.

## Conclusions

The present study describes a fast, cheap and reliable method for analysis of melamine in soil and sediment using readily available HPLC system. The samples could be effectively extracted by 5% ammonia–methanol solution with ultrasonic assistance within a very short time (5 min), and separated using a cyano column with acetonitrile/water (5/95, v/v) as mobile phase. No procedures such as SPE were required for sample purification, and no costly ion-pair reagents or high proportion of acetonitrile was needed in LC mobile phase. Thus, the time and cost for melamine determination was significantly reduced. Moreover, high recovery (95.0-105.9%) as well as good precision (RSD < 6%) was obtained in the practical application, and the detection limit was as low as 0.01 mg $kg^{-1}$. Therefore, the developed method has the advantages of rapidity, simplicity, cheapness, sensitivity and accuracy, and can be easily applied for routine monitoring of melamine at trace levels in soil and sediment samples.

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## Notes and references


1 C. Li, Y. F. Li, B. Liang, N. Li, Z. Z. Li, Anal. Methods, 2013, **5**, 5760-5766.

2 Z. Wang, X. Ma, L. Zhang, W. Yang, L. Gong, P. He, Z. Li, Anal. Chim. Acta, 2010, **662**, 69-75.

3 L.P. Wackett, M.J. Sadowsky, B. Martinez, N. Shapir, Appl. Microbiol. Biotechnol., 2002, **58**, 39-45.

4 Y. Qin, X. Lv, J. Li, G. Qi, Q. Diao, G. Liu, M. Xue, J. Wang, J. Tong, L. Zhang, K. Zhang, Environ. Int., 2010, **36**, 446-452.

5 OECD (Organisation for Economic Co-operation and Development). Availablehttp://www.chem.unep.ch/ irptc/sids/OECDSIDS/108781.pdf1998.

6 R.A. Yokley, L.C. Mayer, R. Rezaaiyan, M.E. Manuli, M.W. Cheung, J. Agric. Food Chem., 2000, **48**, 3352-3358.

7 S. D. Richardson, Anal. Chem., 2010, **82**, 4742-4774.

8 P. Lutter, M. C. Savoy-Perround, E. Campos-Gimenenz, L. Meyer, T. Goldmann, M. C. Bertholet, P. Mottier, T. Delatour, Food Control, 2010, **22**, 903-913.

9 G. Venkatasami, J.R. Sowa, Anal. Chim. Acta, 2010, **665**, 227-230.

10 S. Squadrone, G. L. Ferro, D. Marchis, C. Mauro, P. Palmegiano, G. Amato, E. P. Genin, M. C. Abete, Food Control, 2010, **21**, 714-718.

11 S. H. Tzing, W. H. Ding, J. Chromatogr. A, 2009, **1217**, 6267-6272.

12 Y.L. Wong, C.S. Mok, Anal. Methods, 2013, **5**, 2305-2314.

13 GB/T22388, National standards of the People's Republic of China, 2008.

14 H. Miao, S. Fan, P. P. Zhou, L. Zhang, Y. F. Zhao, Y. N. Wu, Food Addit. Contam. A, 2010, **27**, 1497-1506.

15 Y. Tian, L. Chen, L. Gao, M. Wu, W. A. Dick, Sci. Total Environ., 2012, **417-418**, 255-262.

16 J.V. Sancho, M. Ibañez, S. Grimalt, O.J. Pozo, and F.Hernandez, Anal. Chim. Acta, 2005, **530**, 237-243.

17 T.Tang, S.Li, L.Fan, S.Yu, L.Tong, Chinese J. Chromatogr., 2009, **27**,513-515.

18 N. Wongsa, R. Burakham, Food Anal. Method., 2012, **5**, 849-855.

19 J.F.Cheng, X.G. Ma, Y.G. Wu, Food Anal. Methods, DOI 10.1007/s12161-013-9716-3

20 L. Tang, H. Wang, Y. Dong, D.Liu, L. Zhao, J.Zhang, L. Cheng, Q. An, Chinese J. Soil Sci., 2010, **41**,723-726.

21 Y. Li, W. Hao, Y. Wang, Q. Chen, J. Li, X. Sun,

Chinese J. Chromatogr., 2012, **30**, 716-720.

22 C. Han, Y. Wu, Y. Zhou, Y. Gong, C. Liu, L. Wang, Y. Shen, Chromatographia, 2009, **70**, 927-931.

23 Y. Xu, X.Gong, X.Zhang, X.Tian, H.Xing, J.Liu, J. Chinese Mass Spectr. Soc., 2009, **32**, 70-73.

24 Y.Wu, Chinese J. Analysis Lab., 2009, **28**, 88-90.



 