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Analytical Method for the Determination of Trace Toxic Elements in Milk Based on Combining Fe₃O₄ Nanoparticles Accelerated UV Fenton-like Digestion and Solid Phase Extraction

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S Supporting Information

ABSTRACT: A UV Fenton-like digestion method was developed first time for a complete digestion of milk samples by using 1.6 g L⁻¹ Fe₃O₄ magnetic nanoparticles, 0.2% (v/v) nitric acid, and 6% (w/w) H₂O₂. During the digestion, the liberated As-, Sb-, and Bi-containing species were preconcentrated onto the surface of Fe₃O₄ magnetic nanoparticles, which were conveniently separated with a hand-held magnet and subsequently dissolved in hydrochloric acid prior to hydride generation atomic fluorescence spectrometric detection. Owing to the integration of UV Fenton-like digestion, solid phase extraction, and magnetic separation into a single step, the developed method significantly simplifies sample preparation steps and reduces chemical consumption and hazardous waste. Limits of detection of 0.0015, 0.0022, and 0.0025 μg L⁻¹ were obtained for As, Sb, and Bi, respectively, using a 50 mL milk sample. The method was applied to the determination of these elements in a Certified Reference Material and milk samples.

KEYWORDS: Fe₃O₄ MNPs, Fenton reaction, sample pretreatment, hydride generation, solid phase extraction

INTRODUCTION

Generally, the accurate and precise determination of toxic or essential elements in food samples involves a sample pretreatment step (e.g., digestion, separation, and concentration) and a signal acquisition step. The performance of the latter has been significantly improved in recent years; however, the sample pretreatment techniques have changed little. Unfortunately, sample pretreatment not only seriously affects the accuracy and reproducibility of final results but also generates wastes which are toxic and have adverse effects on the environment. Consequently, there is a growing interest in developing green sample pretreatment procedures that produce less hazardous wastes to the environment.^{1–4} For the determination of trace metals in solid or organic matrix food samples by atomic spectrometry, samples need to be digested into homogeneous aqueous solutions prior to their analyses. Typically, digestion is carried out by either open-vessel or closed-vessel techniques with relative large amounts of concentrated acids (e.g., HCl, HNO₃, H₂SO₄) at high temperature conditions.^{5–10} Major drawbacks of open-vessel digestion techniques include the potential analyte loss and generation of toxic acid mist through volatilization. Closed-vessel digestion techniques, especially microwave assisted-digestion, can reduce digestion time, minimize analyte loss, and alleviate toxic wastes. However, the concentrated mineral acids in the digested solutions are not favorable to the subsequent measurements, thus, a tedious and time-consuming procedure of separation and concentration is needed, such as extraction and evaporation.

UV photo-oxidation is considered as a green alternative for the digestion of organic matrix sample.¹¹ Compared to the

conventional acid digestion, only small amounts of reagents are required. Thus, the potential pollution is significantly minimized, which benefits the operator and the environment. In general, a few hours are needed for a complete digestion of samples in typical UV digestion (UVD).¹¹ As a result, microwave assistance^{12–14} was frequently applied in this system to improve the digestion efficiency. The Fenton reaction may be considered as one of the most efficient and advanced oxidation techniques because of its long historical association with degradation of organic compounds and other pollutants.^{15–17} Similar to UVD, the active species (for example, •OH) are produced by the reduction of H₂O₂ with Fe(II) or other transition metal ions at a suitable pH range. As a result, Fenton reaction has been frequently combined with UVD to significantly improve the degradation efficiencies of pollutants and reduce the degradation time.^{18,19} Although homogeneous Fenton reaction using Fe(II) or other transition metal ions is of high efficiency, it remains several disadvantages including narrow working pH range (2.5–3.5) and the accumulation of large excess of unrecyclable transition metals containing sludge to environment.²⁰ To overcome these drawbacks, the development of heterogeneous Fenton reaction such as using solid iron oxides is gaining popularity as an alternative to homogeneous Fenton reaction.^{21,22} Unfortunately, additional energies are required due to their weak catalytic activity of bulk materials.²³

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Despite its 120-year history, Fenton reaction technique has been continuously improved over the last of a few decades. Recently, an intriguing oxidase-mimic property of Fe_3O_4 magnetic nanoparticles (MNPs) which can convert H_2O_2 into $\cdot\text{OH}$ radicals to efficiently break down organic substances, was discovered by Yan et al.²⁴ Other nanomaterials have also attracted attention on their use for the degradation of various organic compounds.^{25–29} Despite an increased interest in the Fenton reaction over the past 50 years, applications in sample pretreatment have remained limited. Recently, Shelor et al.³⁰ reported an interesting study on the use of homogeneous Fenton reaction for the digestion of milk prior to the determination of iodine by isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS), but the whole digestion had to be held in an oven overnight at 60 °C. On the basis of previous studies,^{24–30} it is suggested that the performance of UV Fenton digestion (FD) may be significantly improved by the oxidase activity of Fe_3O_4 MNPs. However, no such attempt has emerged.

In general, the accurate determination of trace elements in many environmental and biological samples is a challenge even when complete digestion of sample can be realized, because of their very low contents. Thus, numerous preconcentration protocols have been developed to improve the detection limits.^{31–33} Among those, solid phase extraction (SPE) using Fe_3O_4 MNPs has been proven to be a simple, organic solvent free and environmentally benign technique for the preconcentration of analytes, wherein analytes can be conveniently separated from matrix by using a permanent hand-held magnet.^{34–36} In this study, a novel UV Fenton-like digestion (FLD) method using Fe_3O_4 MNPs and UV-FD under simple and mild conditions was developed for the determination of trace metals in food samples. The determination of As, Sb, and Bi in milk was selected to evaluate the proposed methodology since these elements are very toxic to humans and animals.^{35,36} To the best of our knowledge, this is the first study utilizing Fe_3O_4 MNPs as an effective catalyst and adsorbent to accomplish complete digestion of milk, and to integrate UV-FLD, SPE and magnetic separation into a single step. The separated Fe_3O_4 MNPs were dissolved in hydrochloric acid prior to hydride generation (HG) AFS detection. The proposed method not only significantly alleviates the disadvantages of conventional acid digestion but also provides unique advantages of excellent digestion efficiency, high sample throughput, green analytical chemistry, and the elimination of tedious separation steps during SPE.

MATERIALS AND METHODS

Chemicals. All reagents used were analytical grade or better. 18.2 M Ω -cm deionized water (DIW) produced by a water purification system (Chengdu Ultrapure Technology Co., LTD, China) was used to prepare solutions throughout the study. Standard solutions were prepared daily by diluting stock solutions (1000 mg L⁻¹) of As(III), Sb(III), and Bi(III) that were purchased from National Research Center of China (NRCC, Beijing, China). Thiourea was purchased from Kelong Chemical Reagents Co. (Chengdu, China) and used for prereduction of high oxidation state of As, Sb, and Bi prior to hydride generation. H_2O_2 (30%, w/w), HCl, HNO_3 , KOH, KBH_4 , FeCl_2 , FeCl_3 , FeSO_4 , and $\text{NH}_3\cdot\text{H}_2\text{O}$ were also purchased from Kelong Chemical Reagents Co. KBH_4 solution was prepared daily by dissolving a proper amount of solid reagent in 0.5% (w/v) KOH. High-purity argon (Ar) and nitrogen (N_2) were obtained from Qiaoyuan Gas Co. (Chengdu, China).

A commercial milk sample (HX) spiked at 20 $\mu\text{g L}^{-1}$ of Sb(III) and 10 $\mu\text{g L}^{-1}$ of As(III) and Bi(III) was used to investigate the optimum experimental conditions. A Certified Reference Material (CRM, milk powder, GBW10017-GSB-8) from Beijing Century Oak Biotechnology Co., Ltd. and four commercial milk products (HX, YL, GM, and MN) bought at the local market were analyzed to evaluate the accuracy of the proposed method.

Instrumentation. A homemade UV digestion device consisting of a 15 W low pressure UV mercury lamp (Philips Co., Holland), a cylindrical glass container, a black rubber stopper, and six quartz digestion tubes (11 cm length \times 8 mm i.d. \times 10 mm o.d.; about 7 mL internal volume) or four bigger quartz digestion tubes (11 cm length \times 28 mm \times 30 mm o.d.; about 60 mL internal volume) was used to digest milk samples. The lamp and the quartz tubes were tightly bound together and further fastened through the holes made on the stopper, and then jam-packed and housed in the container over which a flow of Ar was passed to eliminate oxygen to avoid ozone generation. The container was wrapped with aluminum foil to reflect UV radiation from the lamp, enhancing digestion efficiency, and protecting the operator.

A commercial double-channel HG nondispersive atomic fluorescence spectrometer (AFS, Model AFS-2202, Beijing Haiguang Instrument Co., Beijing, China) was used for all measurements. This instrument as described in details in previous works^{37,38} was equipped with coded high intensity hollow cathode lamps (HCL) of As, Sb, and Bi, a quartz argon–hydrogen flame atomizer and a hydride generator consisting of a four-channel peristaltic pump and a quartz gas liquid separator (GLS). An Agilent 7700x inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, U.S.A.) was also used to evaluate the proposed method. An inductively coupled plasma optical emission spectrometer (ICP-OES) (ARCOS FHS12, SPECTRO Analytical Instruments GmbH, Germany) was used to determine the concentration of $\text{Fe}^{2+}/\text{Fe}^{3+}$ leached from Fe_3O_4 MNPs. Typical operating conditions for AFS and ICP-MS are summarized in Table S1 (see Section 1 of the Supporting Information, SI).

The size and surface morphology of Fe_3O_4 MNPs were characterized by scanning electron microscopy (SEM, JSM-7500F, JEOL, Japan). Wide-angle (10–70°, 40 kV/35 mA) powder X-ray diffraction (XRD) measurements were carried out using a X'Pert Pro X-ray diffractometer (Philips, Holland) with Cu K α radiation ($K = 1.5406 \text{ \AA}$).

Preparation and Characterization of Fe_3O_4 MNPs. Fe_3O_4 MNPs were prepared following a previous coprecipitation method³⁹ (See section 2 of the SI). The representative SEM images of the prepared Fe_3O_4 MNPs were reported in our previous study,³⁹ and showed that these MNPs are spherical and homogeneous. The crystalline structure and the purity of the prepared MNPs were further characterized by powder X-ray diffraction. The positions and relative intensities of diffraction peaks agree well with the standard pattern of Fe_3O_4 indexed from the JCPDS 75-1609 card.

Fe_3O_4 MNPs Accelerated UV Fenton-like Digestion. Digestion of milk samples using the proposed method was performed for five samples and one blank simultaneously. One milliliter of each tested milk sample was transferred to individual quartz digestion tube, and then 8.0 mg of Fe_3O_4 MNPs with diameter of 30 nm, 1.0 mL of 30% (w/w) H_2O_2 and 2.0 mL of DIW were added. The contents in each tube were mixed on a vortex mixer (XH-B, Jiangsu Kangjian Medical Apparatus Co., Ltd., China). The mixture was acidified with 10 μL of HNO_3 and subsequently diluted to 5 mL with DIW. The digestion tubes were capped loosely and inserted into the UVD device, and allowed to expose to UV irradiation for 60 min. After the UV irradiation, Fe_3O_4 MNPs were deposited onto the bottom of the digestion tube with the aid of a permanent hand-held magnet. The supernatant was discarded, and the residual MNPs were directly dissolved in 0.3 mL of HCl and diluted to 5 mL solution with thiourea solution prior to analysis by HG-AFS.

In order to demonstrate the feasibility of both digestion and solid phase extraction, 10 mL of milk and proportional amounts of MNPs, H_2O_2 and HNO_3 were added into a 60 mL quartz digestion tube and operated using the above-described procedure except that the residual

MNPs were dissolved and diluted to 2.5 mL solution with thiourea prior to analysis.

Microwave Assisted Digestion. In order to evaluate the accuracy of the proposed method, a microwave assisted digestion method was employed to digest milk samples prior to their determination by ICP-MS. Briefly, each 5 mL of the tested milk samples, 10 mL of concentrate nitric acid and 5 mL of 30% (m/v) hydrogen peroxide were added into precleaned Teflon vessels. Sample blanks were processed along with the samples. The vessels were closed, fastened, and then placed in a microwave oven (Master 40, Shanghai Sineo Microwave Chemistry Technology Co., China) for microwave-assisted digestion. The digestion program was performed as follows: 5 min at 130 °C and 2200 W; 10 min at 150 °C and 2200 W; and 15 min at 180 °C and 2200 W. After cooling, the caps were removed and the digests were transferred to precleaned 50 mL volume Teflon tubes. The digests were then diluted to 50 mL with DIW prior to analysis by ICP-MS.

Analytical Procedure and Data Analysis. Quantification of As, Sb, and Bi was accomplished by flow injection HG-AFS, which is similar to our previous works.^{37,38} Briefly, 2 mL of tested solution was initially directed to the sample loop through a six-port valve by a peristaltic pump. The valve was activated to pass the carrier solution (3% (v/v) HCl solution) to flush the digested solution to mix with 2% (m/v) KBH_4 solution containing 0.5% (m/v) KOH for the generation of As, Sb, and Bi hydrides. Finally, the sample was flushed into a GLS by the carrier solution and Ar carrier gas, wherein the resultant hydrides were separated from liquid phase and then introduced into the AFS for detection. Peak area signal of the atomic fluorescence intensity was recorded.

For comparison purpose, samples were also digested using a conventional microwave oven and analyzed by ICP-MS. Statistical analysis of *t* test was applied to the results obtained using the proposed method in the Certified Reference Material in order to validate the method.

RESULTS AND DISCUSSION

Experiment Design and Possible Reaction Mechanism. An initial attempt was made to integrate conventional UVD and FD for the digestion of milk sample aiming to achieve high sample throughput, minimize chemical consumption, and alleviate interference on the subsequent hydride generation. Unfortunately, complete digestion of a milk sample was not possible under conditions of 5 mL mixture containing 1 mL of milk, 100 μL of 0.1 M Fe^{2+} , 1 mL of 30% (w/w) H_2O_2 , and 20 μL of HNO_3 even with a long digestion time of 6 h. Since Fe_3O_4 MNPs can catalytically generate $\bullet\text{OH}$ radicals from H_2O_2 for a rapid degradation of complex organic substances, 20 mg of Fe_3O_4 MNP with diameter of 80 nm was thus added into 5 mL mixture containing 1 mL of milk, 1 mL of 30% (w/w) H_2O_2 and 20 μL of HNO_3 . This mixture was subsequently exposed to UV irradiation. The milk solution turned clear and transparent within 90 min.

To investigate the possible mechanism of the above reaction, free iron ion concentration in the leaching solution was determined by ICP-AES. Fe_3O_4 MNPs with diameter of 30 nm were incubated under typical experimental conditions of 0.2% (v/v) HNO_3 and 6% (w/w) H_2O_2 for 60 min, and then removed by a hand-held magnet. The iron content of the leaching solution was found to be 0.45 mM, which agrees well with the amount required in the conventional Fenton reaction.^{15,16,33} A comparison study to evaluate the digestion efficiencies induced by the leaching solution of Fe_3O_4 MNPs, 0.45 mM FeSO_4 , or 10 mg of Fe_3O_4 with diameter of 30 nm, was performed by using each 5 mL mixture containing 1 mL milk sample, 6% (w/w) H_2O_2 and 0.2% (v/v) HNO_3 . As

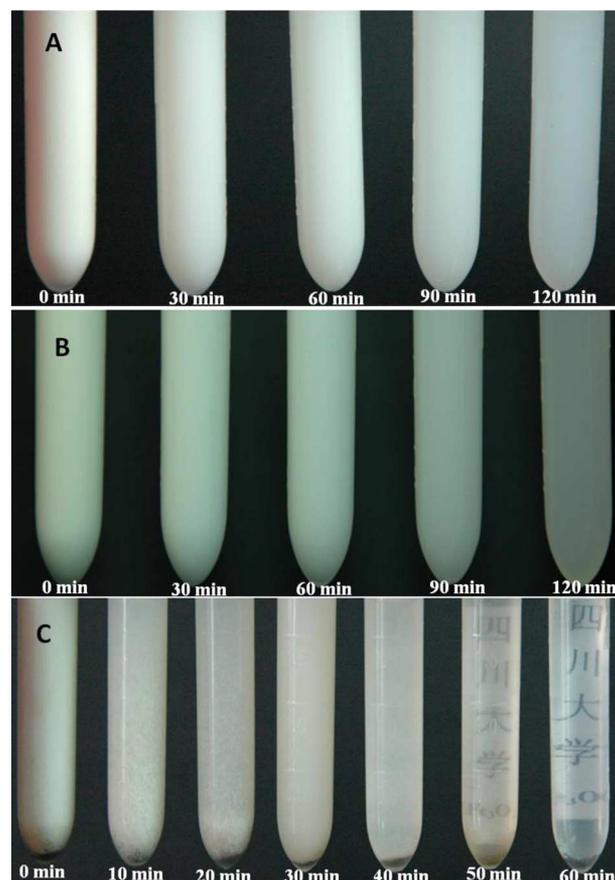


Figure 1. UV digestion of 5 mL mixture containing 1 mL milk sample, 6% H_2O_2 and 0.2% (v/v) HNO_3 using different catalyst. (A) the leaching solution of Fe_3O_4 MNPs; (B) 0.45 mM FeSO_4 solution; and (C) 10 mg of Fe_3O_4 with diameter of 30 nm.

shown in Figure 1, the milk sample was still opaque in the case of using either the leaching solution (Figure 1A) or 0.45 mM FeSO_4 (Figure 1B) even with the digestion time longer than 120 min, whereas it became clear and transparent within 60 min with use of Fe_3O_4 MNPs (Figure 1C). This observation indicates that the digestion activity provided by the MNPs is not only generated from the iron ions leaching into solution, but also attributed to the MNPs. The role of UV irradiation in the digestion was investigated by comparing the digestion efficiencies that carried out with and without UV irradiation, respectively. In the absence of UV, the tested milk samples could not be completely digested within 8 h with use of 30 nm of Fe_3O_4 MNPs as catalyst, confirming that the generation efficiency of hydroxyl radical was improved in the presence of UV irradiation.

According to the previous studies by Yan,²⁴ Xu,²⁵ Luo,²⁶ and Ai,³⁹ and their colleagues, this rapid digestion may be mainly attributed to the iron species of $\equiv\text{Fe}^{2+}/\equiv\text{Fe}^{3+}$, rather than just the free Fe^{2+} ions leached from the MNPs as previously presumed in the conventional Fenton reaction, where $\equiv\text{Fe}^{2+}$ and $\equiv\text{Fe}^{3+}$ represent Fe(II) and Fe(III) sites on the MNPs surface, respectively. On the basis of the observations from previous studies^{24–26,39} and the above study, the reaction mechanism may consist of a dominant digestion and a subsidiary digestion, which have occurred on the surface of MNPs and in the leaching solution, respectively. Figure 1A,B shows that the mixture can be digested to some extent in the

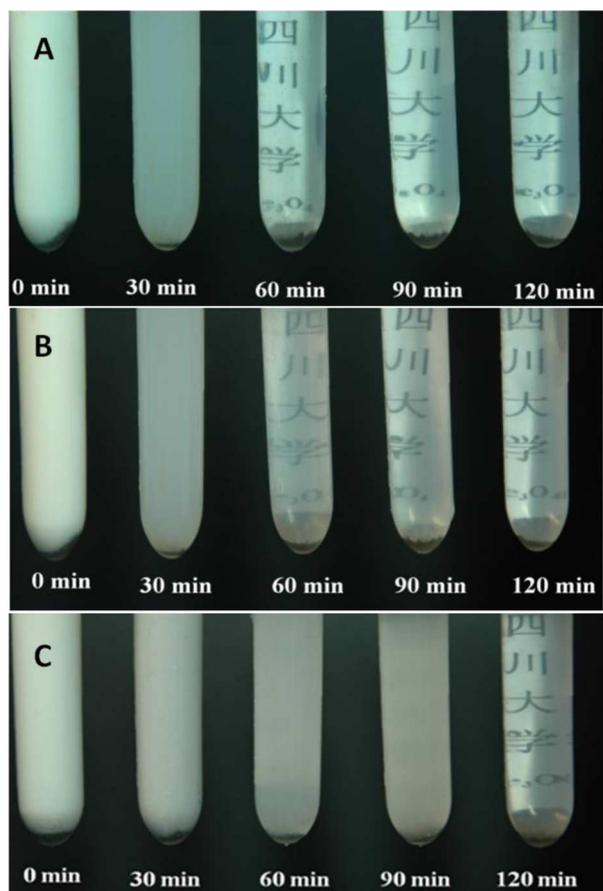


Figure 2. Effect of the diameter of Fe_3O_4 MNPs on the UV Fenton-like digestion: (A) 30 nm; (B) 80 nm, and (C) 120 nm. Experimental condition: 5 mL of mixture containing 1 mL of spiked milk, 10 mg Fe_3O_4 MNPs, 9% (w/w) H_2O_2 , and 0.3% (v/v) HNO_3 .

cases using leaching solution or 0.45 mM FeSO_4 solution. This supports the generally accepted mechanism of conventional FD^{18,19,30} that part of organic compounds (OC) in milk were degraded by free hydroxyl radicals ($\bullet\text{OH}_{\text{free}}$ and $\bullet\text{OOH}_{\text{free}}$) produced from free Fe ions. The dominant digestion of the sample has occurred on the surface of MNPs because the milk sample can only be completely digested in the presence of Fe_3O_4 MNPs (Figure 1C). $\equiv\text{Fe}^{2+}$ react with H_2O_2 to generate surface-bound hydroxyl radicals ($\bullet\text{OH}_{\text{ads}}$) and $\equiv\text{Fe}^{3+}$. Then the original $\equiv\text{Fe}^{3+}$ together with the generated $\equiv\text{Fe}^{3+}$ interact with H_2O_2 or $\bullet\text{OOH}$ to produce more $\equiv\text{Fe}^{2+}$, which further improve the generation of $\bullet\text{OH}_{\text{ads}}$. Moreover, the $\bullet\text{OH}_{\text{ads}}$ can react with H_2O_2 to produce $\bullet\text{OOH}_{\text{ads}}$. Finally, the OC of milk were adsorbed onto the surface of MNPs and then efficiently decomposed to CO_2 and H_2O by $\bullet\text{OH}_{\text{ads}}/\bullet\text{OOH}_{\text{ads}}$ on the surface of MNPs.

Experimental Conditions for MNP Based Digestion. In order to investigate the effect of diameter of MNPs on the milk digestion and to gain further insight into the role played by the MNPs in the proposed method. Fe_3O_4 MNPs with diameter of 30, 80, and 120 nm were tested to digest the milk mixture under the same experimental conditions, as shown in Figure 2. Interestingly, catalytic activity of Fe_3O_4 MNPs was clearly decreased with increasing of its particle diameter, and the milk solution can be completely digested within 60 min using MNPs with 30 nm diameters, while a much longer time was required in the case of MNPs with 80 or 120 nm diameters. This much

more efficient digestion may be attributed to the fact that smaller MNPs provide higher adsorption capability and more $\equiv\text{Fe}^{2+}/\equiv\text{Fe}^{3+}$ because of its greater surface-to-volume ratio. Fe_3O_4 MNPs with a diameter of 30 nm were thus chosen in all subsequent experiments.

As previously reported, the catalytic activity of Fe_3O_4 MNPs is also strongly dependent on the concentrations of Fe_3O_4 MNPs and H_2O_2 as well as acidity and reaction temperature. Thus, further investigations of these parameters are necessary. The effect of the concentration of MNPs on response is summarized in Figure 3A. The results show that the responses of As, Sb, and Bi increased in a range from 0.4 to 1.6 g L^{-1} Fe_3O_4 MNPs, and followed by a plateau at higher concentrations. This is probably due to inefficient digestion at lower concentrations of Fe_3O_4 MNPs. A concentration of 1.6 g L^{-1} Fe_3O_4 MNPs was chosen for the subsequent experiments. The effects of concentrations of H_2O_2 and HNO_3 on the digestion efficiencies of milk samples are presented in Figure 3B,C, respectively. The AFS responses increased as H_2O_2 concentration increased from 0 to 3% (w/w) and reached a plateau at higher concentrations. Consequently, the concentration of 6% (w/w) H_2O_2 was selected for all subsequent experiments in a consideration of achieving efficient digestion and the minimization of H_2O_2 consumption. The responses for As(III), Sb(III), and Bi(III) increased as HNO_3 concentration increased from 0 to 0.2% (v/v), remained relatively stable in the range of 0.2–0.5% (v/v), and then decreased at higher concentrations. Low acidity results in low catalytic activity of the Fe_3O_4 MNPs and insufficient digestion of milk, while high acidity leads to dissolution of MNPs and thus decreases its catalytic activity. Consequently, a concentration of 0.2% (v/v) of HNO_3 was chosen for further studies, which is much lower than that needed in the conventional acid digestion and benefits the reduction of chemical consumption and subsequent hydride generation.

The digestion time is usually reduced by increasing the digestion temperature during UVD.¹² To gain insight into the effect of temperature on digestion, a mercury thermometer was inserted into the quartz digestion tube to measure the temperature. The temperature was found to increase to about 65 °C after 20 min of UV irradiation and remained relatively stable at longer irradiation times. Therefore, the effect of temperature on the digestion time was investigated by putting the UVD device in a water bath to conveniently control the digestion temperature at 30, 40, 60, 70, and 90 °C, respectively. The results show that the digestion time was reduced with increased temperature from 30 to 70 °C. However, dark brown floccules were formed and the mixture could not be completely digested at 90 °C. This phenomenon may be resulted from the fact that the proteins in milk solution were denaturated and deposited onto the bottom of digestion tube at this temperature. Fortunately, on the basis of the above results, the optimal digestion temperature can be achieved with use of UV lamp only, and thus no additional heating source is required, which significantly simplified the operational process and experimental setup.

The effect of digestion time on response was investigated under the previously optimized conditions of 6% (w/w) H_2O_2 , 0.2% (v/v) of HNO_3 , and 1.6 g L^{-1} of 30 nm Fe_3O_4 MNPs. The effect of digestion time on response is summarized in Figure 3D. It is evident that maximum responses can be achieved with a 40 min digestion or longer. Sixty min of

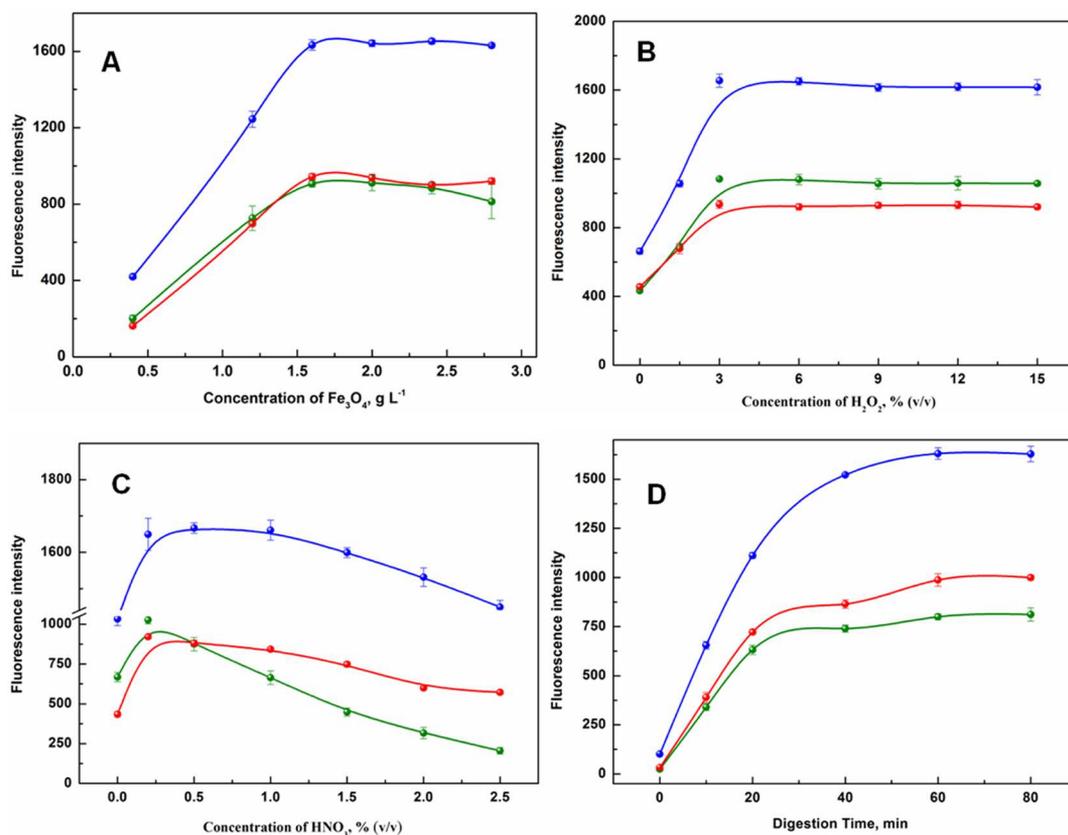


Figure 3. Effects of experimental conditions on the Fe₃O₄ MNPs accelerated, and UV-induced Fenton-like digestion (blue, As; red, Sb; green, Bi). (A) effect of the concentration of Fe₃O₄; (B) effect of the concentration of H₂O₂; (C) effect of the concentration of HNO₃; and (D) effect of the digestion time. Error bars indicate standard error of the mean ($n = 5$).

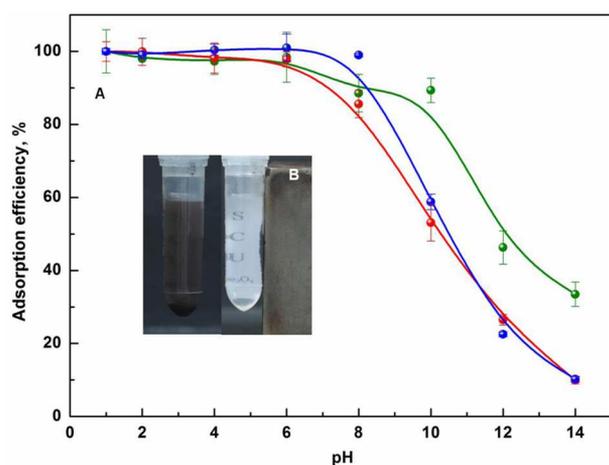


Figure 4. (A) Effect of pH of sample solution on the adsorption efficiencies of As(III), Sb(III), and Bi(III) (blue, As; red, Sb; green, Bi; $n = 5$, error bars indicate standard error of the mean); and (B) visual demonstration of the feasibility of magnetic separation of the prepared Fe₃O₄ MNPs.

digestion time was thus chosen for all subsequent experiments to ensure a complete digestion.

Experimental Conditions for Fe₃O₄ MNPs Based Solid Phase Extraction. In order to improve limits of detection of the proposed method, Fe₃O₄ MNPs were used not only as a catalyst to improve digestion, but also as an adsorbent to preconcentrate analytes. SPE efficiency for metals is normally dependent on the pH of the sample solution and the

Table 1. Figures of Merit

	without preconcentration	preconcentration
As/LOD, $\mu\text{g L}^{-1}$	0.027	0.0015
Sb/LOD, $\mu\text{g L}^{-1}$	0.038	0.0022
Bi/LOD, $\mu\text{g L}^{-1}$	0.040	0.0025
As ^a /precision ($n = 9$), % RSD	2.4	5.2
Sb ^a : precision ($n = 9$), % RSD	3.6	4.4
Bi ^a : precision ($n = 9$), % RSD	4.0	5.1

^a5 $\mu\text{g L}^{-1}$ (without preconcentration); 0.5 $\mu\text{g L}^{-1}$ (preconcentration)

adsorption time. Therefore, 50 mL mixture containing 10 mL spiked milk was used to investigate effects of these parameters on the adsorption efficiencies of As(III), Sb(III), and Bi(III). The adsorption efficiency was estimated from a comparison of the relative concentrations of analytes in the tested solution and in its supernatant after SPE. In order to avoid the dissolution of MNPs, the effect of pH on the adsorption efficiencies of analyte ions was studied in a range of pH 1.0–14.0. The adsorption efficiency was estimated from a comparison of the relative concentrations of analytes in the tested standard solution and in its supernatant after the adsorption by Fe₃O₄ MNPs. As shown in Figure 4A, all these tested analytes can be efficiently adsorbed in the pH range of 1.0–6.0, which agree well with those reported earlier.^{40,41} This optimum pH range is particularly beneficial, since tedious pH adjustment is eliminated prior to analysis because the pH value of the digested sample solution is about 2, within this optimum pH range.

Table 2. Analytical Results of As, Sb, and Bi in Milk Samples

sample	added, $\mu\text{g L}^{-1}$			detected ^{a,b} $\mu\text{g L}^{-1}$			recovery, %		
	As	Sb	Bi	As	Sb	Bi	As	Sb	Bi
HX	10	20	10	10.5 \pm 0.2 (10.0 \pm 0.1)	17.7 \pm 0.8 (18.8 \pm 0.6)	8.9 \pm 0.5 (10.2 \pm 0.3)	105	88	89
YL	10	20	10	10.6 \pm 0.1 (9.8 \pm 0.3)	18.0 \pm 0.6 (19.5 \pm 0.4)	9.2 \pm 0.5 (10.1 \pm 0.2)	106	90	92
GM	10	20	10	10.6 \pm 0.4 (9.6 \pm 0.3)	18.2 \pm 0.5 (20.2 \pm 0.5)	8.7 \pm 0.2 (9.7 \pm 0.2)	106	91	87
MN	10	20	10	10.3 \pm 0.2 (10.5 \pm 0.4)	18.0 \pm 0.8 (19.5 \pm 0.3)	9.3 \pm 0.4 (10.3 \pm 0.1)	103	90	93
GBW10017-GSB-8 ^c	0.031 \pm 0.007 ^d			0.030 \pm 0.002					

^aMean and standard deviation ($n = 3$). ^bDetected by ICP-MS after microwave assisted digestion. ^cmg kg⁻¹. ^dCertified value.

The adsorption time counted after the digestion procedure, and its effect on adsorption efficiencies of As(III), Sb(III), and Bi(III) was investigated. The results show that adsorption equilibria were obtained within a short time of less than 1 min. This remarkably fast adsorption actually can be attributed to the fact that the adsorption must be synchronized with the digestion. Therefore, no additional adsorption time was needed for subsequent experiments, and the sample throughput is increased.

The adsorption capacities of the prepared Fe₃O₄ MNPs for As(III), Sb(III), and Bi(III) were also evaluated via spiking various amounts of these analytes into 5 mL milk sample solutions and with each 8 mg Fe₃O₄ MNPs added. Saturation of the adsorption capacities of Fe₃O₄ was found to be 10, 5, and 8 mg g⁻¹ for As(III), Sb(III), and Bi(III), respectively, which can meet the requirement of routine analysis. Magnetic separation is simpler, faster, and more convenient than centrifuge separation. In order to visually demonstrate the feasibility of magnetic separation of the prepared Fe₃O₄ MNPs, the magnetic separation of 8 mg of Fe₃O₄ after complete digestion and adsorption of analytes ions in 5 mL sample solution is shown in Figure 4B. This fast magnetic separation arising from the strong magnetic response of the prepared Fe₃O₄ MNPs significantly simplified the separation procedure, eliminated organic solvent and reduced overall analysis time.

Optimization of the HG-AFS Determination. As a sample introduction technique, HG provides unmatched sensitivity and analyte–matrix separation, elemental specificity, and high sample introduction efficiency for atomic spectrometry, and is enjoying increasing interest for inorganic trace analysis. Previous study⁴² found that HG efficiencies of As, Sb, and Bi were affected by many factors, especially KBH₄ concentration, concentration of prereducing reagent and the reaction medium. Therefore, these factors were carefully investigated, as shown in Figure S1 (see section 3 of the SI). Six percent (v/v) HCl, 2% (m/v) KBH₄, 1.0% (m/v) thiourea, and 10 min of prerduction time were finally chosen for better responses.

Since Fe MNPs were dissolved together with analytes and then directly reacted with KBH₄ for the generation of their hydrides, the effects of Fe²⁺/Fe³⁺ concentration on the HG efficiencies of 10 $\mu\text{g L}^{-1}$ of As, Sb and Bi were thus investigated. No significant interferences was observed using the proposed method, even at a concentration of Fe²⁺/Fe³⁺ as high as 2 g L⁻¹.

Analytical Performance. A series of 5 mL mixture containing 1 mL milk and various concentrations of As(III), Sb(III), and Bi(III) were analyzed to establish the calibration curves under optimal experimental conditions, as shown in Figure S2A (see Section 4 of the SI). The linear coefficient for each curve is higher than 0.999. The limits of detection (LODs) defined as the analyte concentration equivalent to three times of the standard deviation of 11 measurements of a blank

solution, were 0.027, 0.038, and 0.040 $\mu\text{g L}^{-1}$ for As, Sb, and Bi, respectively. Precisions of replicate measurements, expressed as relative standard deviations (RSDs, $n = 9$), are better than 6% at 5 $\mu\text{g L}^{-1}$ analytes concentrations. In order to evaluate the improvement of sensitivity and LODs by Fe₃O₄ based SPE, a series of 50 mL of mixture containing 10 mL milk and various concentrations of As(III), Sb(III), and Bi(III) were used. After digestion, the MNPs were dissolved and diluted to a final 2.5 mL prior to analysis. The obtained calibration curves were described in Figure S2B (see section 4 of the SI). LODs of 0.0015, 0.0022, and 0.0025 $\mu\text{g L}^{-1}$ were obtained for As, Sb, and Bi, respectively, providing 18-, 17-, and 16-fold improvements over those obtained from this method without preconcentration. The analytical figures of merit achieved are summarized in Table 1.

Preliminary Analytical Application. Four milk samples obtained from a local market were analyzed to check the accuracy of the proposed method. Analytical results are summarized in Table 2 using external standard calibration. It was necessary to spike all the original milk samples with 10 $\mu\text{g L}^{-1}$ As, 20 $\mu\text{g L}^{-1}$ Sb and 10 $\mu\text{g L}^{-1}$ Bi because their endogenous concentrations were not detectable by the proposed method. Good spike recoveries of arsenic (103–106%) for these samples were achieved, and the analytical results are in good agreement with those obtained by use of microwave assisted digestion and ICP-MS detection, confirming the accuracy of the proposed methodology for routine elemental analysis of milk. Slightly lower recoveries for Sb and Bi maybe due to sample matrix effects, and their recoveries could be increased to 92–101% when a longer digestion time (80 min) was used. The accuracy of the proposed technique was further demonstrated by the determination of As in a Certified Reference Material (milk powder, GBW10017-GSB-8), as shown in Table 2. The *t* test showed that the analytical results obtained by the proposed method were not significantly different from the certified value for As at the confidence level of 95%.

In summary, a unique and green method combining heterogeneous UV Fenton-like digestion, solid phase extraction, and magnetic separation was developed for the determination of trace toxic elements in milk. This method not only overcomes disadvantages associated with conventional acid digestion and UV digestion, but also provides several unique advantages including: simpler operation, greener analytical chemistry, higher sample throughput, cost-effectiveness, and reduction of chemical consumption. In addition, a tedious and time-consuming separation procedure was simplified in the proposed method because of the simple and convenient magnetic separation.

■ ASSOCIATED CONTENT

■ Supporting Information

Instrumental parameters, preparation of Fe₃O₄, microwave assisted digestion of milk samples, optimization of the HG-AFS determination, and calibration curves. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

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Notes

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