

Analytical Methods

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4 **Mesoporous alumina as solid phase extraction adsorbent for the**
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7 **determination of abamectin and ivermectin in vegetables by liquid**
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9 **chromatography-tandem mass spectrometry**
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Abstract

Here we report the synthesis of mesoporous alumina by template method and its applications as a new solid phase extraction adsorbent with abamectin and ivermectin as the target analytes by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The chromatographic separation was conducted on a C₁₈ column using a gradient mobile phase consisting of methanol (solvent A) and 0.2 % formic acid in 5 mmol L⁻¹ ammonium acetate (solvent B) buffer at a flow rate of 0.3 mL min⁻¹. The linearity of the calibration curves was excellent and yielded the correlation coefficients ($r^2 = 0.9990-0.9999$) at a range of 0.02-0.50 mg L⁻¹. Samples were spiked with known quantities of the analytes at three different concentration levels (0.05, 0.10, and 0.20 mg L⁻¹) and the recoveries were in the range of 87.3 %-104.5 % with relative standard deviation (RSD) values ranging between 0.53 % and 5.8 %. The limits of detections and limits of quantization were 0.16 ng g⁻¹ and 0.53 ng g⁻¹, respectively. These results indicated that mesoporous alumina had enormous potential in food security field as a novel solid phase extraction adsorbent material.

Keywords mesoporous alumina; LC-MS/MS; solid phase extraction; abamectin; ivermectin

1. Introduction

Pesticides are widely used for the protection of a variety of crops, fruit and vegetables due to their properties of being stable and resistant to a wide range of pH, temperature and light. Abamectin (ABA) and ivermectin (IVR) are widely used for pesticides purposes due to their antiparasitic properties at low doses. The structures of abamectin and ivermectin are shown in Fig. 1(a) and Fig. 1(b) respectively. They belong to the family of avermectins, a class of macrocyclic lactones produced by a soil actinomycete and streptomycetes avermitilis. These chemicals can be slowly eliminated from food and their extensive or extra-label use may result in the presence of their residues in milk, liver, and muscle tissue [1]. The widespread use of these antiparasitic drugs may present a potential risk to the consumer if residues enter the food chain. Therefore, it is important to control their residues in food.

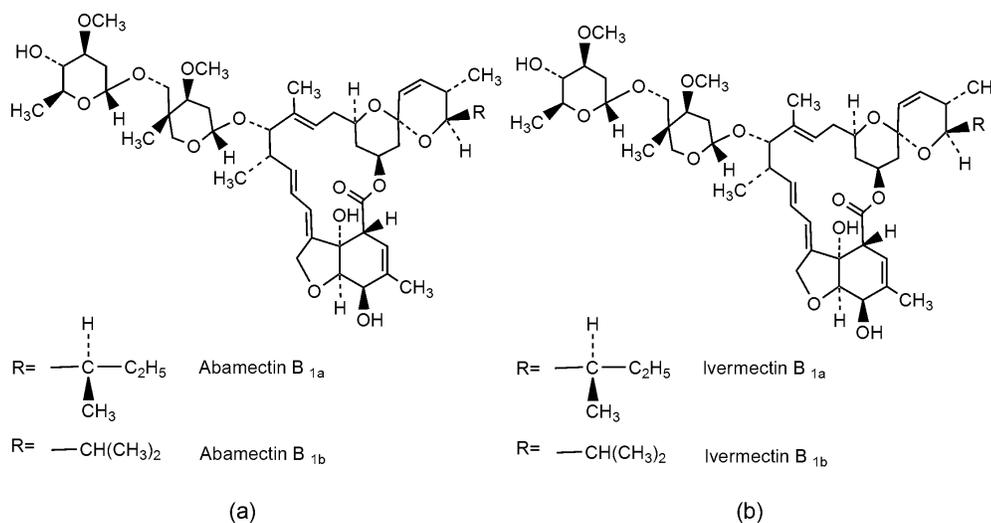


Fig. 1 Chemical structures of abamectin (a) and ivermectin (b)

So far, numerous methods have been developed for the determination of avermectins in animal tissues, liver, milk and urine, including Liquid chromatographic separation followed by

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4 fluorescence detection (LC-FLD) and LC-MS/MS [1]. LC-FLD has become the most frequently
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6 used technique for determination of avermectins [2-5], however, fluorescence detection often
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8 requires derivatization of analytes. The low stability of the derivatized samples led to a decrease
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10 in the reproducibility of the results, and derivatization reagents produced by-products can reduce
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12 the lifetime of the chromatographic column. LC-MS/MS commonly enables the determination of
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14 drugs in environmental and food samples with high sensitivity, high speed and high molecular
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16 masses as well as polar, non-volatile and thermally labile analytes without derivatization. A
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18 number of LC-MS/MS methods have been described for the analysis of avermectin residues in
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20 different matrices, such as milk [6], bovine tissue [7-8], food [9], water [10-12], sediment [13],
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22 soil [14] and meat [15].
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31 In general, the method used to determine the content of pesticide residue in fruit or
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33 vegetable requires an extraction of the analytes from the homogenized sample by a clean-up
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35 procedure. Efforts have been directed to extract efficiently the target compounds from the sample
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37 matrices before analysis. The preconcentration and clean-up procedures include disperse
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39 liquid-liquid microextraction (DLLME) [16-17], solid phase extraction (SPE) [18-24],
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41 cloud-point extraction (CPE) [25], and solid-phase microextraction (SPME) [26], etc. Compared
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43 with other extraction techniques, solid phase extraction is one of the most effective methods,
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45 which generally provides lots of advantages such as lower cost, high extraction recoveries with
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47 acceptable RSD values, short extraction time and less consumption of organic solvents. However,
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49 the sorbent is the key part which determines whether the purification performance is good or not.
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51 Hence screening new sorbents for cleanup is become more important.
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4 Recently, nanometer-sized material, such as TiO₂ nanotubes [18-20], carbon nanotubes
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6 [21-22] and nano-Al₂O₃ [27-28] have attracted more and more attention as SPE adsorbents.
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8 Among these materials, γ -Al₂O₃ presented good properties as adsorbents because of its unique
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10 thermal, chemical and mechanical stability. Its application performance can be adjusted via
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12 controlling its crystallinity, morphology, textural properties, and other physicochemical
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14 properties. Moreover, mesoporous γ -Al₂O₃ offers several advantages over the traditional SPE
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16 sorbents such as larger surface area, higher adsorption capacity, easily to be prepared and
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18 recycled. Some papers proved that nano-Al₂O₃ sorbents can be used for determine of ultra-trace
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20 inorganic selenium species [27], food dyes [29], fluoride and arsenic [30-31].
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28 Although activated alumina [6, 9] was used as a good sorbent material in SPE technique, to
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30 the best of our knowledge, few researches worked on the use of nanometer-sized Al₂O₃ for
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32 adsorbing abamectin and ivermectin as the target analytes in vegetables by LC-MS/MS. As one
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34 of the most important metal oxides, in this paper, we synthesized mesoporous γ -Al₂O₃ using
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36 F127 as a structure directing agent and characterized the product by BET, XRD and SEM. The
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38 main purpose of this work is to investigate the feasibility of mesoporous alumina as SPE
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40 adsorbents for the extraction of abamectin and ivermectin as the model analytes by LC-MS/MS
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42 for the sake of enlarging its application in the food security field.
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49 **2. Experimental Section**

50 **2.1 Reagents and chemicals**

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52 Pluronic F127 (M_{av}=12600, EO₁₀₆PO₇₀EO₁₀₆) were purchased from Sigma -Aldrich.
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55 Aluminum iso-propoxide and HNO₃ were purchased from Beijing Chemical Reagents. The
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standards of abamectin and ivermectin with 97 % purity were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Methanol and acetonitrile HPLC grade were purchased from Tedia (USA). Formic acid (HPLC grade) was purchased from ROE Scientific Inc (Newark, USA). Ammonium acetate was obtained from Sigma (Netherlands, USA). Ultrapure water (>18.2 MΩ) used throughout the preparation of mobile phase and other reagents was generated by Milli-Q system (Millipore, Bedford, MA, USA).

2.2 Equipment and standard solutions

The UPLC-MS/MS analyses were performed using an Acquity™ UPLC system (Waters Corp., Milford, MA, USA) linked to a Quattro Premier XE with an electrospray ionization (ESI) source (Waters Corp., Milford, MA, USA). The analytical column was a waters BEH C₁₈ (100 mm×2.1 mm, I.D, 1.7 μm), which obtained from USA; centrifuge (SIGMA 3-30K) was from Sigma (USA); nitrogen evaporator (N-EVAP 116) obtained from Organomation Associates (USA); the vortex mixer was from Jiangsu Kangjian Medical Apparatus Co., Ltd. (Jiangsu, China). Stock standard solutions of individual abamectin and ivermectin were prepared at a concentration of 2 mg L⁻¹ and were stored at -20 °C in the dark before use for the preparation of standard working solutions. The mixed standard working solutions were prepared by diluting and mixing each stock solution with acetonitrile, stored at 4 °C in the dark. Matrix standards containing the abamectin and ivermectin at concentrations of 0.02, 0.05, 0.10, 0.20 and 0.50 mg L⁻¹ were obtained by adding the mixed working solutions to the blank sample.

2.3 Synthesis of mesoporous alumina

Mesoporous alumina was synthesized using a similar procedure reported by Yuan and

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4 co-workers [32]. In a typical synthesis process, 6.0 g of Pluronic F127 was dissolved in 120 mL
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6 absolute ethanol at room temperature. Then 9 mL nitric acid (67 wt%) and 12.24 g aluminum
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8 iso-propoxide were added into the above solution with vigorous stirring at room temperature for
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10 about 5 h, then the final solution was transferred to a 100 °C drying oven to undergo the solvent
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12 evaporation process in static air. After 2 days of aging, the solution became a light-yellow solid.
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15 The precipitates were collected by filtration, washed by absolute ethanol and deionized water for
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17 several times, and dried in air at 90 °C overnight. Calcination was carried out by slowly
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19 increasing temperature from room temperature to 400 °C with a heating rate of 1 °C min⁻¹ and
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21 by heating at 400 °C for 4 h in flowing air. Different high-temperature treatment was carried out
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23 in air for 3 h with a temperature ramp of 10 °C min⁻¹ to remove the template.
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31 **2.4 Characterization of mesoporous alumina**

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33 X-ray diffraction (XRD) was performed on a diffractometer (BDX3200) with Cu K α
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35 radiation ($\lambda=1.5418 \text{ \AA}$) for understanding the crystallization and composition of the sample.
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37 Scans were made in the 2θ range 10 - 80°, with a scan rate of 2° min⁻¹.
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41 Pore size distributions, BET surface areas, and pore volumes of the powders were examined
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43 via nitrogen adsorption/desorption experiments using a NOVA 2000e gas sorption analyzer
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45 (Quantachrome Corp.).
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49 The morphologies of the as-prepared samples were characterized by field emission scanning
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51 electron microscopy (FESEM, Nova 400 Nano-SEM).
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55 **2.5 Sample preparation, extraction and clean-up**

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57 All vegetable samples were purchased from the local market. Typically, homogenized
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4 sample of vegetables (5.0 g) was prepared into a 50 mL polypropylene tube, 10 mL of
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6 acetonitrile were added and the sample tube was then vortexed for 2 min and shaken for 30 min.
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9 After being centrifuged at 6000 rpm for 5 min, the supernatant was transferred to another 50 mL
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11 polypropylene centrifuge tube. The residue was extracted with 10 mL acetonitrile once more.
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15 Afterwards, a solid phase extraction was performed using the mesoporous alumina. The
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17 SPE column was washed prior to sample extraction with 5 mL of acetonitrile and eluted with 5
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19 mL acetonitrile after all the supernatant clean-up. The collected eluting solution was evaporated
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21 to dryness at 40 °C under a gentle stream of nitrogen, then the residues were reconstituted in 1
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23 mL acetonitrile with a vortex mixer for 1 min. After filtration through a 0.22 µm nylon
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25 membrane filter, aliquots of 10 µL were subjected to LC-MS/MS analysis.
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31 **3. Results and Discussion**

32 **3.1 Characterization of mesoporous alumina**

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36 Fig. 2(a) shows the wide-angle XRD patterns of the mesostructured aluminum calcined at
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38 800, 900, and 1000 °C, respectively. The characteristic peaks of (111), (220), (311), (222), (400),
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40 (511) and (440) of γ -Al₂O₃ phase (JCPDS Card No. 10-0425) initially appeared. However, the
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42 broad peak means poor crystallization of the sample at 800 °C. The sharp XRD line can be
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44 observed for the samples after calcination at 900 and 1000 °C, respectively, indicating a
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46 well-crystallized samples and the sintering effects as well as relatively bigger crystalline size.
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48 The synthesized time effect on the products was also studied by XRD. The results of samples
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50 synthesized for 16 h, 24 h, 36 h and 48 h are shown in Fig. 2(b), all samples were calcined at
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52 900 °C. After 16 h hydrothermal treatment, the product could be completely changed to
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boehmite and after calcination at 900 °C, all changed to γ -Al₂O₃. With the hydrothermal treatment time increasing, the diffraction peak intensity increased, which indicates the crystallization increased.

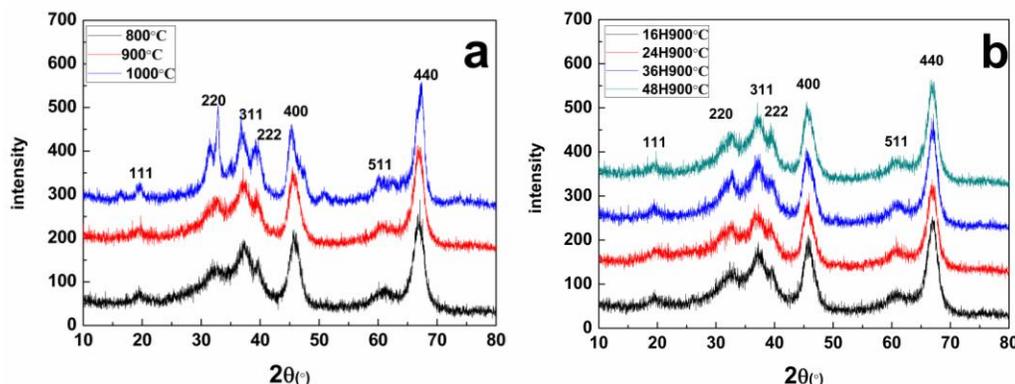


Fig. 2 XRD patterns of the mesoporous alumina calcined at different temperatures (a) and synthesis in different times (b)

The BET surface areas, pore sizes and volumes of the mesoporous alumina synthesized in different conditions are summarized in Table 1. These samples displayed high specific surface areas from 115 to 243 m² g⁻¹, pore volumes from 0.39 to 0.56 cm³ g⁻¹ and average mesopore sizes from 9.17 to 16 nm, which makes them suitable as solid phase extraction clean-up column. These results of the mesoporous alumina synthesized in this work were similar to those of previous published paper. The BET surface areas are larger and the pore sizes are smaller compared with the data reported by the reference [32].

Table 1 BET surface areas, pore sizes and volumes of mesoporous alumina

Sample	Synthesis time	Calcined temperature	BET surface area (m ² g ⁻¹)	Pore Volume (cm ³ g ⁻¹)	Pore Size (nm)
Al-1	48h	800°C	186.57	0.51	10.95
Al-2	48h	900°C	154.54	0.42	10.87
Al-3	48h	1000°C	115.06	0.46	16.00
Al-4	16h	900°C	243.99	0.56	9.17

Al-5	24h	900 °C	148.53	0.39	10.58
Ref.[32]	48h	900 °C	225	0.42	4.4

In order to optimize the preparing conditions, the nitrogen adsorption and desorption isotherms and the corresponding pore size distribution of the mesoporous alumina synthesized in 48 h and calcined at different temperatures are respectively shown in Fig.3 (a-b). As observed in Fig. 3(a), all the isotherms are type IV isotherm (definition by IUPAC), which is a characteristic of mesoporous material. The samples show H2 shape hysteresis loops, indicate the presence of “ink-bottle” shaped pores existed in these materials. The pore size distributions of these materials are displayed in Fig. 3(b). Apart from the materials calcined at 800 °C, other materials revealed relative wide pore size distributions. They still show nanocrystalline nature, with a growth of the particle size from 10 to 16 nm.

The nitrogen adsorption/desorption isotherms and corresponding pore-size distribution for samples synthesized in different times and calcined at 900 °C are presented in Fig.3 (c-d) respectively. All isotherms are of type IV, characteristic of mesoporous materials. Nitrogen adsorption isotherms for these samples are very similar to one another and exhibit distinct capillary condensation steps, indicating uniform mesoporosities. Samples calcined at 900 °C exhibited narrow distribution of pore size and high surface area, implying that γ -alumina nanoparticles synthesized in this optimal condition makes it suitable for solid phase extraction column.

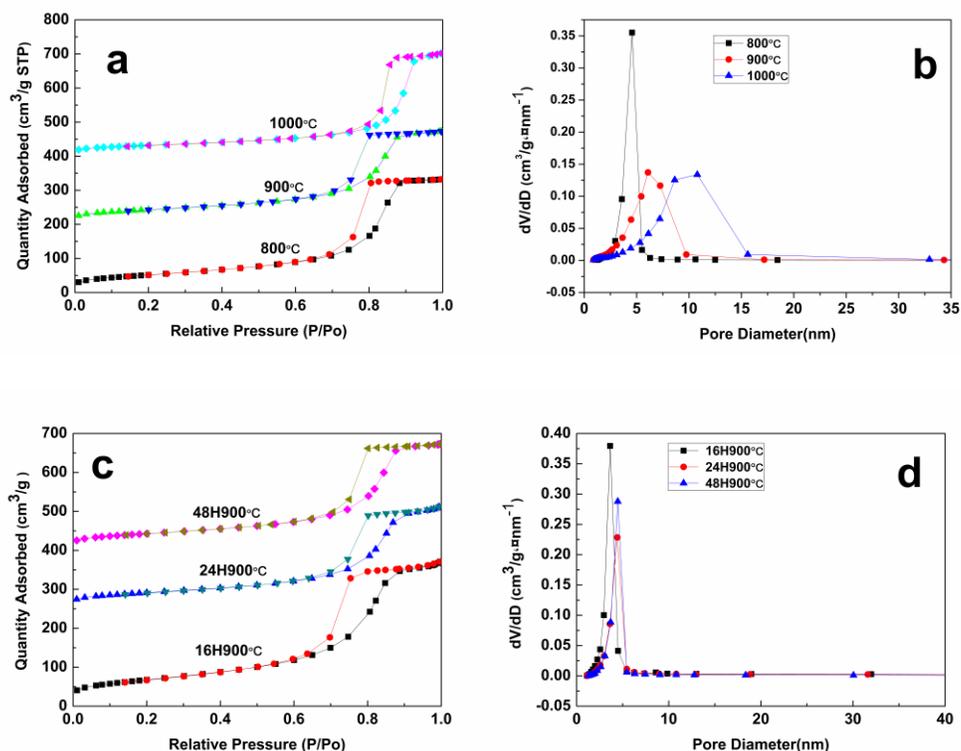


Fig. 3 Nitrogen adsorption-desorption isotherms (a) and pore-size distribution curves (b) of mesoporous alumina synthesized in 48 h and calcined at different temperatures, Nitrogen adsorption-desorption isotherms (c) and pore-size distribution curves (d) of mesoporous alumina synthesized in different times and calcined at 900 °C.

The morphology of the product synthesized at the optimal condition were studied using Field Emission Scanning Electron Microscopy and is shown in Fig. 4. It shows that the sample displays mesoporous structure with the size of 20 to 30 nm.

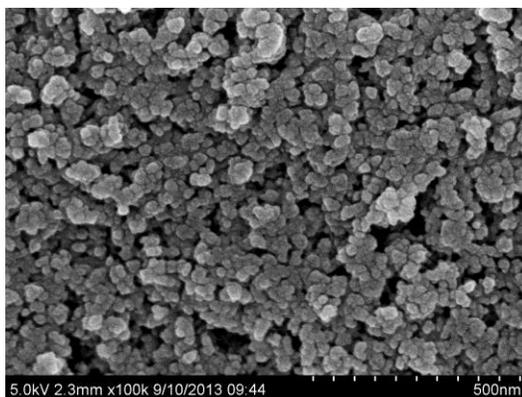


Fig. 4 The SEM of mesoporous alumina synthesized in 48h and calcined at 900 °C.

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4 In summary, well structured mesoporous alumina with high surface area and narrow pore size
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6 distribution was synthesized by an one-pot evaporation induced self-assembly method. The
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8 optimal reaction time and calcined temperature are 48 h and 900 °C, respectively. This sample
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10 which synthesized in the optimal conditions was used to fill the SPE.
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14 15 **3.2 Optimization of chromatographic condition** 16

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18 Parameters such as mobile phase composition, flow rate, and injection volumes were
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20 studied in order to achieve good peak shape and improve the separation. The optimal
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22 chromatographic separation condition was carried out as following. The separation was
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24 performed on an Acquity UPLC BEH™ C₁₈ column (2.1×100mm×1.7 μm, Waters Corp.,
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26 Milford, MA, USA) with a column temperature of 35 °C. The flow rate and injection volume
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28 were 0.3 mL min⁻¹ and 10 μL, respectively. Avermectins are weak polarity substances, which can
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30 retain on the reversed-phase chromatography for a long time. A gradient profile was used with
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32 the mobile phase consisting of methanol (solvent A) and 0.2% formic acid in 5 mmol L⁻¹
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34 ammonium acetate (solvent B) as follows: 20.0 %A (0-2 min), 20.0-70.0 %A (2-4 min),
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36 70.0-90.0 %A (4-6 min), 90.0 %A (6-9 min), 90.0-20.0 %A (9-10 min). All the abamectin and
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38 ivermectin could be eluted within 10 min. The experiment proves that the gradient profile ensure
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40 the sensitivity and good peak shape of abamectin and ivermectin. Total ion chromatograms of
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42 abamectin and ivermectin are shown in Fig. 5.
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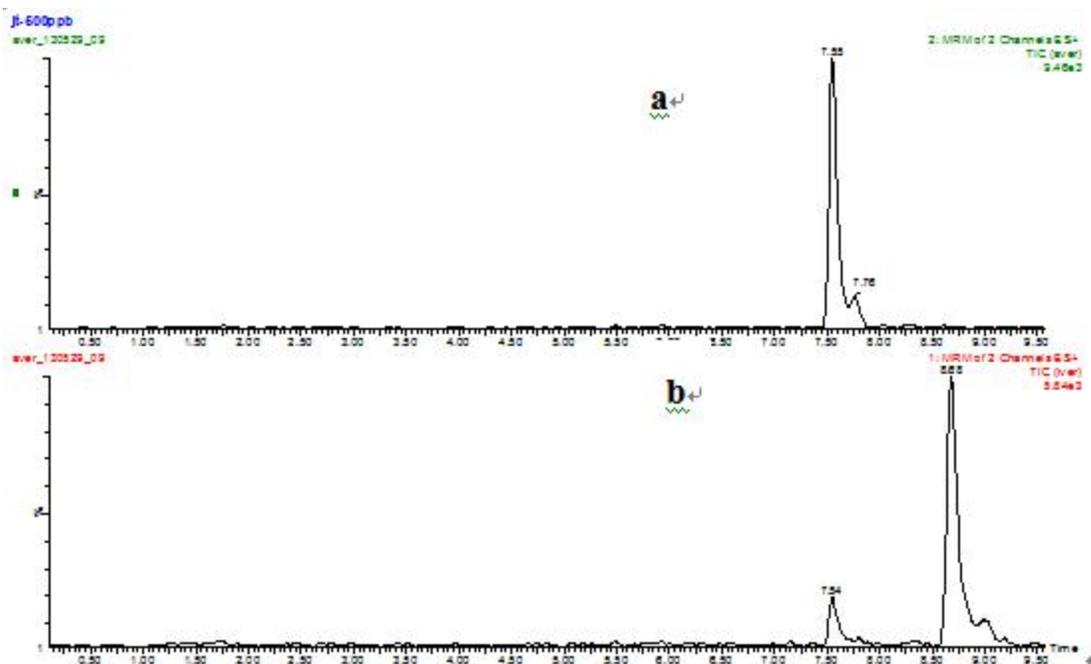


Fig. 5 Total ion chromatograms of abamectin (a) and ivermectin (b)

3.3 Optimization of MS/MS condition

The chromatography conditions were adjusted in order to develop a quick method and to improve the analytes separation. Mass spectrometric detection was performed on a quattro/premier tandem quadrupole mass spectrometer (Quattro Premier XE LC/MS/MS System, waters, USA) equipped with electrospray ion source (ESI) and operated in positive mode. For the MS/MS detection, electrospray was operated in positive ion mode and the ionization source parameters were: capillary voltage, 2.0 kV; ion source temperature, 110 °C; desolvation temperature, 350 °C; desolvation gas flow, 650 L h⁻¹; cone gas flow, 50 L h⁻¹; collision gas flow, 0.28 mL min⁻¹. Adding 5 mmol L⁻¹ ammonium acetate to the mobile phase was able to promote the ion formation, in the mean while, it enhanced the ionization of avermectins in the addition of 0.1% acetic acid. The multiple reaction monitoring transitions as well as the cone and collision energy voltages applied are summarized in Table 2.

Table 2 Acquisition MRM parameters of abamectin and ivermectin

Analytes	Parent ion (m/z)	Daughter ion (m/z)	Dell times (s)	Cone voltage (V)	Collision energy (eV)
AVER	895.7	449.3	0.100	60.00	48
	895.7*	751.6*	0.100	60.00	46
IVER	897.6	609.1	0.100	60.00	48
	897.6*	752.9*	0.100	60.00	46

“*” Quantitative ion pair

3.4 Optimization of sample pretreatment procedure

Several experiment parameters produce an effect on the extraction efficiency. The extraction solvent, elution conditions and purification condition were optimized.

Avermectins are weak polarity substances, which are soluble in toluene, acetone, ethyl acetate, acetonitrile and methanol, slightly soluble in hexane and petroleum ether, and insoluble in water. In our studies, the effects of acetone and acetonitrile on fortified recovery were investigated. Acetone will dissolve a large amount of impurities which may interfere with the detection, resulting in recoveries only 60%. On the other hand, acetonitrile showed to be more adequate to extract all the target compounds, the extraction of acetonitrile was highly efficient, small impurity interferential and the recoveries can reach higher than 80%. Acetonitrile is preferred option as extraction solvent, so we chose acetonitrile as the extraction solvent in the following work. The elution conditions were optimized in the same time, and the results indicated that the recoveries were improved with the volume of the eluent increasing, and reached higher than 95% when acetonitrile volume was more than 5 mL. Therefore, the elution volume was selected to be 5 mL.

Extraction solvent contains a lot of impurities, and hence it is necessary to select an

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4 appropriate cleaning method to eliminate possible interferences from the crude sample extracts.
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7 In this work, we compared two different ways of passing through solid phase extraction columns.
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10 The first one is passing through alumina SPE with extraction solution which evaporated to about
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12 2-3 mL in a water bath at 40 °C. The second one is passing through alumina SPE with all
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14 extraction solution. The mesoporous alumina SPE was washed with 5 mL acetonitrile prior to
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16 sample extraction. Then the collected extraction solutions were passed through alumina SPE,
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18 which remove the main interferent and allow the analytes to pass through in slowly flow rate,
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20 appeared to give satisfactory results. Finally, the target compounds were eluted with 5 ml of
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22 acetonitrile and the eluate was evaporated to dryness with stream of nitrogen. The flow rate of
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24 the sample solution can not only affects the recoveries of the analytes, but also controls the time
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26 of analysis. However, the extraction solution which was evaporated pass through in a fast rate
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28 resulting unsatisfactory recoveries. So we choose the second method which all the extraction
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30 solution was subjected to solid-phase extraction clean-up.
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39 **3.5 Matrix Effect**

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41 The matrix effect of the insecticides was investigated by comparing the responses obtained
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43 from standard mixture of avermectins injected in solvent and standards spiked into the vegetable
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45 homogenate before extraction. The slope of matrix matched standard calibration curves and the
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47 standard curves obtained are shown in Fig. 6. From the results, we can conclude that the matrix
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49 effect of abamectin and ivermectin isn't distinct for cabbage, cucumber and onion, but obvious
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51 for ternip. Therefore matrix matched standard calibration curves were established to compensate
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53 for interference of the matrix and to ensure the accuracy of qualitative and quantitative analysis.
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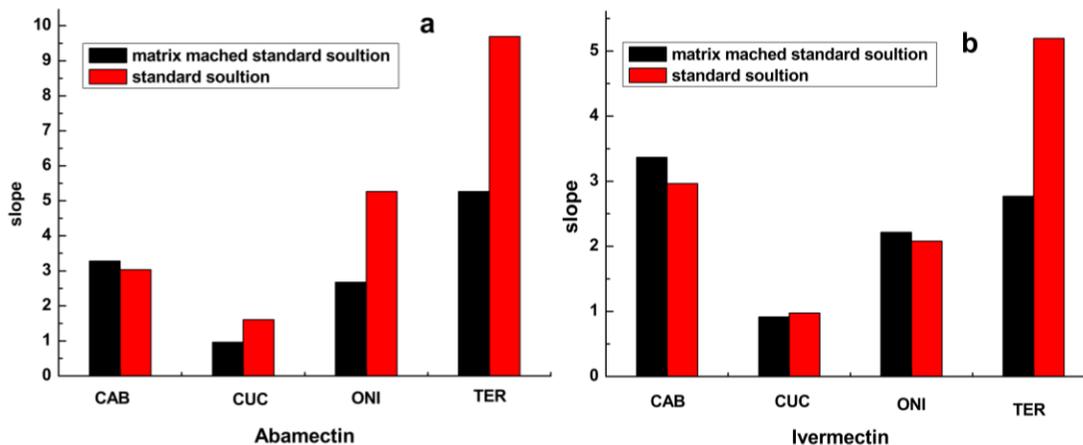


Fig. 6 Matrix effect for abamectin (a) and ivermectin (b) in cabbage (CAB), cucumber (CUC), onion (ONI), and ternip (TER) samples

3.6 Method of Validation

3.6.1 Matrix matched standard calibration curves

As the optimum experimental conditions were determined, the parameters such as the linear range, correlation coefficients, limits of detection (LOD) and limits of quantification (LOQ) were evaluated and the results were listed in Table 3. The limits of detection were three times of the value of signal/noise calculated from the signal/noise value at 0.02 mg L^{-1} , while the limits of quantification were ten times of the value of signal/noise. Matrix matched standard calibration curves were built by extracting and purifying blank vegetable samples as described before, then mixed standard solutions containing abamectin and ivermectin with the concentration sequence of $0.02, 0.05, 0.10, 0.20$ and 0.50 mg L^{-1} were used for dissolving residues instead of acetonitrile. The calibration graphs were obtained following linear regression analysis by plotting concentration (X) against peak area (Y). The results showed that good linear relationships and good coefficients of determination ($r^2=0.9990-0.9999$) were achieved over the concentration range of $0.02-0.50 \text{ mg L}^{-1}$.

Table 3 Linear equations, correlation coefficients, detection limits (S/N=3) and quantification limits (S/N=10) for the determination of avermectin (AVER) and ivermectin(IVER)

Compound	Bulk material	Fortified concentration (mg L ⁻¹)	Correlation coefficient (R ²)	Linear equation	LOD (ng/mL)	LOQ (ng/mL)
AVER	cabbage	0.02-0.50	0.9997	Y=3.2822X+0.8069	0.16	0.53
	cucumber	0.02-0.50	0.9990	Y=0.96116X+10.36		
	onion	0.02-0.50	0.9992	Y=2.6748X+15.98		
	ternip	0.02-0.50	0.9991	Y=5.2641X-11.12		
IVER	cabbage	0.02-0.50	0.9999	Y=8.4576X+5.81	0.16	0.53
	cucumber	0.02-0.50	0.9992	Y=0.91258X+6.33		
	onion	0.02-0.50	0.9998	Y=2.2156X+18.28		
	ternip	0.02-0.50	0.9993	Y=2.7686X+13.70		

3.6.2 Accuracy and precision

To assess matrix effects, the avermectin and ivermectin from the vegetables considered was evaluated at three spiked levels: 0.05, 0.10 and 0.20 mg kg⁻¹ (n=10 replicates per level). Recoveries were calculated by comparing the concentrations of the extracted avermectin and ivermectin with those from the matrix-matched standard calibration curves. These data were also used to determine the precision of the method via the evaluation of RSD. The results are reported in Table 4. Satisfactory recoveries and RSD of mesoporous alumina as solid phase extraction for clean-up were 87.3 %-104.5 % and 0.53 %-5.8 %. In order to make a comparison, conventional SPE method was also used for the determination of avermectin and ivermectin and the results were listed in Table 4. All of these results indicate the good performance of the proposed analytical method and mesoporous alumina had enormous potential in food security field as a novel SPE adsorbent material.

Table 4 Mean recovery and precision (RSD) in the three fortified levels (n = 10) for purification using mesoporous alumina as SPE adsorbent material and conventional SPE

	compounds	Bulk materials	Spiked level (mg kg ⁻¹)					
			0.05		0.10		0.20	
			mean recovery/%	RSD%	mean recovery/%	RSD%	mean recovery/%	RSD%
clean-up by mesoporous alumina SPE	AVER	cabbage	99.4	3.8	87.3	5.3	89.5	3.5
		cucumber	104.5	5.5	103.9	4.7	104.2	0.85
		onion	100.7	1.2	95.5	4.6	99.1	1.5
		ternip	102.4	1.2	101.9	1.7	100.3	0.91
	IVER	cabbage	99.3	4.1	92.3	5.8	89.5	4.8
		cucumber	97.7	1.5	102.1	4.9	100.7	1.3
		onion	98.4	1.4	97.6	2.2	94.3	0.53
		ternip	97.2	0.99	98.4	0.79	100.6	1.7
clean-up by conventional SPE	AVER	cabbage	85.8	9.1	102.3	6.0	104.1	3.3
		cucumber	84.3	7.1	102.3	2.9	95.0	2.3
		onion	85.6	2.7	94.7	6.5	86.7	4.7
		ternip	83.1	2.3	82.8	4.2	97.2	3.3
	IVER	cabbage	100.4	9.3	98.8	5.5	88.0	4.8
		cucumber	89.1	9.6	93.4	2.1	93.8	1.5
		onion	81.6	7.0	82.3	5.6	83.5	2.8
		ternip	88.1	3.0	98.7	1.2	96.9	2.3

3.7 Analysis of real vegetable samples

Four vegetable samples, including cabbage, cucumber, onion, and ternip, which available from a local market were extracted and purification using mesoporous alumina as SPE adsorbent material prior to the LC-MS/MS determination. No target analytes were detected.

4. Conclusions

In this work, mesoporous alumina was synthesized, characterized and used for the solid phase extraction of the determination of abamectin and ivermectin in vegetables samples. The accuracy, precision, linearity, LOD and LOQ values are satisfactory for this method. The experimental results showed that mesoporous alumina had good purification capacity for abamectin and ivermectin, and the developed SPE with mesoporous alumina was an important

and valuable alternative to the routine analytical methods for such target fungicides.

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References

- [1] Danaher M, Howells LC, Crooks SRH, Cerkvenik-Flajs V, O’Keeffe M (2006) *J Chromatogr B* 844:175-203
- [2] Hernández-Borges J, Ravelo-Pérez LM, Hernández-Suárez EM, Carnero A, Rodríguez-Delgado MÁ (2007) *J Chromatogr A* 1165:52-57
- [3] Borges JH, Ravelo-Pérez LM, Hernández-Suárez EM, Carnero A, Rodríguez-Delgado MÁ (2008) *Chromatographia* 67:69-75
- [4] Xia X, Xiao ZM, Huang QS, Xia LJ, Zhu K, Wang XL, Shen JZ, Ding SY (2010) *Chromatographia* 72:1089-1095
- [5] Xie XC, Wang XR, Zhao L (2011) *Food Anal Methods* 4:203-211
- [6] Durden DA (2007) *J Chromatogr B* 850:134-146
- [7] Inoue K, Yoshimi Y, Hino T, Oka H (2009) *J Sep Sci* 32:3596-3602
- [8] Ribensam G, Barreto F, Hoff RB, Pizzolato TM (2013) *Food Control* 29:55-60
- [9] Hernando MD, Suárez-Barcena JM, Bueno MJM, Garcia-Reyes JF, Fernández-Alba AR (2007) *J Chromatogr A* 1155:62-73
- [10] Krogh KA, Björklund E, Loeffler D, Fink G, Halling-Sørensen B, Ternes TA (2008) *J Chromatogr A* 1211:60-69

- 1
2
3
4 [11] Raich-Montiu J, Krogh KA, Granados M, Jönsson JÅ, Halling-Sørensen B (2008) J
5
6 Chromatogr A 1187:275-280
7
8
9 [12] Thompson TS, Noot DK, Forrest F, van den Heever JP, Kendall J, Keenlside J (2009) Anal
10
11 Chim Acta 633:127-135
12
13 [13] Löffler D, Ternes TA (2003) J Chromatogr A 1021:133-144
14
15 [14] Park JH, Choi JH, El-Aty AMA, Park JS, Kim BM, Na TW, Park KH, Yang A, Rahman MM,
16
17 Shim JH (2013) J Sep Sci 36:148-155
18
19 [15] Tao YF, Yu G, Chen DM, Pan YH, Liu ZL, Wei HM, Peng DP, Huang LL, Wang YL, Yuan
20
21 ZH (2012) J Chromatogr B 897:64-71
22
23 [16] Viñas P, Martínez-Castillo N, Campillo N, Hernández-Córdoba M (2010) J Chromatogr A
24
25 1217:6569-6577
26
27 [17] Pizarro C, Sáenz-González C, Pérez-del-Notario N, González-Sáiz JM (2011) J Chromatogr
28
29 A 1218:1576-1584
30
31 [18] Zhou QX, Zhao XN, Xiao JP (2009) Talanta 77:1774-1777
32
33 [19] Kefi BB, Atrache LLE, Kochkar H, Ghorbel A (2011) J Environ Sci-China 23:860-867
34
35 [20] Huang YR, Zhou QX, Xie GH, Liu HJ, Lin HY (2011) Microchim Acta 172:109-115
36
37 [21] Dong MF, Ma YQ, Zhao EC, Qian CF, Han LJ, Jiang SR (2009) Microchim Acta
38
39 165:123-128
40
41 [22] Zhu SY, Niu WX, Li HJ, Han S, Xu GB (2009) Talanta 79:1441-1445
42
43 [23] Fontana AR, Rodríguez I, Ramil M, Altamirano JC, Cela R (2011) J Chromatogr A
44
45 1218:2165-2175
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 [24] Zhou QX, Gao YY, Xiao JP, Xie GH (2010) *Chromatographia* 72:1067-1071
5

6 [25] Tang T, Qian K, Shi TY, Wang F, Li JQ, Cao YS (2010) *Anal Chim Acta* 680:26-31
7

8 [26] Ji YS, Liu XY, Jiang XM, Huang HY, Xu ZG, Zhang HX, Wang CM (2009)
9
10 *Chromatographia* 70:753-759
11

12 [27] Liu QY (2010) *Cent Eur J Chem* 8:326-330
13

14 [28] Duan JK, Hu B (2009) *Talanta* 79:734-738
15

16 [29] Li WJ, Zhou X, Tong SS, Jia Q (2013) *Talanta* 105:386-392
17

18 [30] Lee G, Chen C, Yang ST, Ahn WS (2010) *Micropor Mesopor Mat* 127:152-156
19

20 [31] Jagtap S, Yenkie MKN, Labhsetwar N, Rayalu S (2011) *Micropor Mesopor Mat*
21
22 142:454-463
23

24 [32] Yuan Q, Yin AX, Luo C, Sun LD, Zhang YW, Duan WT, Liu HC, Yan CH (2008) *J. AM.*
25
26 *CHEM. SOC.* 130:3465 -3472
27
28
29
30
31
32
33
34
35
36
37
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