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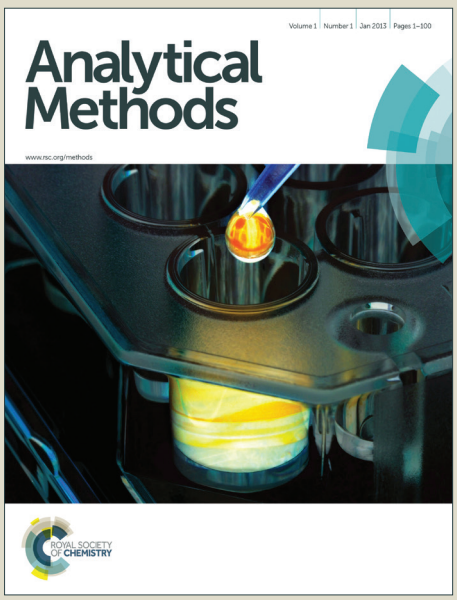

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A new liquid-liquid microextraction method by ultrasound assisted salting out for determination of triazole pesticides in water samples coupled by gas chromatography-mass spectrometry

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A simple, fast and environment friendly method termed "ultrasound assisted salting-out homogenous liquid-liquid microextraction (UASO-HLLME)" has been developed and validated for the quantitative determination of triazole pesticides in water samples coupled with gas chromatography-mass spectrometry (GC-MS). In this work, water-miscible solvent of acetonitrile was used as extractant instead of high toxic extractant in traditional dispersive liquid-liquid microextraction (DLLME) to form a homogeneous water solution, after that, salting out a small amount of acetonitrile to the purpose of extraction and enrichment. Various parameters affecting the extraction process including kind and volume of extractant, ultrasound assisted promoting solvent collection and solvent blending were optimized. Under optimum conditions, matrix matched calibration curves were established using standard solution spiked water sample. Good linear relationships as well as low limits of detection, LODs (0.4-14.4 μg L^{-1}) and quantification, LOQs (1.3-48.1 μg L^{-1}) were obtained. The relative standard deviations (RSD%) of spiked water sample and real environment water sample were 4.5-8.1% and 0.4-8.1%, respectively, with enrichment factor (EF) of 120-185. Recoveries obtained from spiked environmental water samples at three concentration levels ranging from 89.6 to 119%. The results of the analysis revealed that this method is simple, fast and environment friendly, being successfully applicable for the determination of triazole pesticides in water sample.

Instruction

Pesticides play an important role in improving the unite outputs since they can efficiently control the crop diseases, insects and weeds. Triazole pesticide is one of the most important classes of pesticides, which not only has great bacteriostatic action such as control powdery mildew, rust, sheath blight and other diseases, but also has effect on plant physiological regulation to achieve insecticidal and herbicidal activities. With widespread use of triazole, people give more concern on its detrimental effects in ecosystems and human health, which has been proved it would lead to tumorigenic[1-3] and endocrine disrupting.[4, 5] Since triazole has been found in food and water, a rapid, simple, accurate determination method of triazole levels is necessary for food safety monitoring and regulatory purposes.

There are several methods were established to determination of triazole. Such as, solid-phase extraction (SPE) is a method for enrichment and purification the sample at the same time, it has been used for preconcentration triazole fungicides in honeybees, vegetables, fruits and cereals.[6-8] Solid-phase microextraction (SPME) doesn't require solvents, but can carried out the analytical compounds directly from the liquid

phase or from the headspace over the samples. This technology has been used in the extraction of triazole fungicides in juice, wine and fruit samples and so on.[9-12] Dispersive liquid-liquid microextraction (DLLME) is a fast, cheap, simple method, which only requires minute amounts of organic solvent as well as provides high enrichment factors. It has been reported on the application of triazloe fungicides.[13, 14] Based on the DLLME technology, several improved methods were proposed to enrich triazole fungicides, like ultrasound-assisted emulsification microextraction (USAEME),[15] air-assisted liquid-liquid microextraction (AADLLME),[16,17] elevated temperature dispersive liquid-liquid microextraction (ETDLLME),[18] homogeneous liquid-liquid extraction (HLLE),[19] silylated extraction vessel-dispersive liquid-liquid microextraction (SEV-DLLME)[20] and so on. Apart from those methods, Li established a method named "ultrasound-enhanced temperature-controlled (UETC) ionic liquid dispersive liquid-liquid microextraction (IL-DLLME)" to determinate the triazole pesticides in rat blood.[21] Wang applied Magnetic solid-phase extraction (MSPE) with graphene-coated Magnetic nanocomposite as adsorbent followed to analysis the triazole fungicides in vegetable samples.[22] Besides, biological detection method can also be used for the detection of triazole fungicides, for example, enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies for the detection of triazole fungicides in fruit juices.[23]

In this work, a simple, fast and environment friendly method termed "ultrasound assisted salting-out homogenous liquid-liquid microextraction (UASO-HLLME)" has been developed and validated for the quantitative determination of triazole pesticides in water samples coupled with gas chromatography-mass spectrometry (GC-MS). The proposed method is the improvement of traditional method of DLLME. This method doesn't adopt the high toxic extractant, and water-miscible low density dispersant of acetonitrile is taken instead of the extractant in traditional DLLME. After acetonitrile dissolved with water to form a homogenous mixture solution, add some sodium chloride, a small amount of acetonitrile is salted out to achieve the purpose of extraction and enrichment. This method is very simple and fast with the whole procedure can be completed in 2 min. Moreover, the experiment material is very easy to buy from the market, without the need for custom processing. In brief, this method is simple and efficient, being successfully applicable for the determination of triazole pesticides in water sample.

Experimental

Chemicals and solutions

Tricyclazole (≥95%), myclobutanil (≥96.5%), tebuconazole (≥ 97%), difenoconazole (≥97.2%), epoxiconazole (≥96%) were purchased from wellington laboratories Inc. (Canada). Acetonitrile, ethyl acetate and acetone were obtained from Huadong Medicine Company (Hangzhou, China). Sodium chloride and sodium sulfate were from Sihewei Chemical Co., Ltd. (Shanghai, China). Double distilled water was obtained from a Purite RO200-Stillplus HP System, (Purite Oxon, UK).

A stock solution of the studied compounds was prepared by dissolving five triazole pesticides in acetone with the concentration of myclobutanil, tebuconazole, epoxiconazole were 10 X 10^3 μg L^{-1} and tricyclazole, difenoconazole were 50 X 10^3 μg L^{-1}, and stored in a refrigerator at 4°C. This solution was directly injected into the chromatographic system twice a day for quality control and areas of the obtained peaks were used in calculation of enrichment factors (EF) and recoveries.

Working solutions on six concentration levels (the concentration of myclobutanil, tebuconazole and epoxiconazole were 10 μg L^{-1}, 20 μg L^{-1}, 50 μg L^{-1}, 100 μg L^{-1}, 150 μg L^{-1}, 200 μg L^{-1}, while the concentration of tricyclazole and difenoconazole were 50 μg L^{-1}, 100 μg L^{-1}, 250 μg L^{-1}, 500 μg L^{-1}, 750 μg L^{-1}, 1000 μg L^{-1}) were prepared daily by appropriate dilutions of the stock solution with double distilled water.

The real environment water sample was got from the southern end of the Beijing-Hangzhou Grande Canale. Then, the environment sample was spiked with pesticides standard solution to obtain three different concentrations levels with myclobutanil, tebuconazole and epoxiconazole on concentrations of 10 μg L^{-1}, 50 μg L^{-1} and 100 μg L^{-1}, while tricyclazole and difenoconazole on their concentrations with 50 μg L^{-1}, 250 μg L^{-1} and 500 μg L^{-1}.

Apparatus

GC-MS analysis was performed on a GC 2000-Mars 6100 (Juguang Technology co., ltd., Hangzhou, China). The chromatographic separation was achieved on a DB-5 MS capillary column. The column oven was initially held at 120°C for 1 min, then the temperature was subsequently increased to 190°C at 35°C min^{-1} and remained for 5 min, it was then increased to 200°C at 2°C min^{-1} and remained for 9 min. After that, the temperature was ramped at 20°C min^{-1} to 220°C and held for 5 min. At last, it was increased to 290°C at 20°C min^{-1} and maintained for 5 min. Electronic flow control (EFC) was used to maintain a constant helium carrier gas flow of 1.0 mL min^{-1}. The temperature of the injector was held at 280°C with splitless mode. The mass detector conditions were: transfer line temperature: 250°C; ion source temperature: 180°C; ionization mode-electron impact at 70 eV. SIM (Selected ion monitoring) scan spectra were acquired in 5 ranges: the first range was 5-18.2 min for tricyclazole with specific ion of 189 and 162; the second was 18.2-21 min for myclobutanil with specific ion of 179 and 152; the third was 21-26.8 min for tebuconazole with the specific ion of 125 and 250; the forth was 26.8-30 min for epoxiconazole with specific ion of 192 and 138; the last one was 30-39.0 for difenoconazole with ion fragmentation of 265 and 323.

A KQ-50E ultrasonic bath from Ultrasonic Instrument Company (Kunshan, China) was used to facilitate extraction.

Ultrasound assisted salting-out homogenous liquid-liquid extraction procedure

The polyethylene Pasteur pipette sold on the market is divided into three sections with diameters reducing from the top to bottom. The diameter of second section is too narrow (5 cm) to add into salt. In order to conquer that problem, we modified two Pasteur pipette slightly which were shown in Fig. 1. The maximum section (top) of upper Pasteur pipette was cut off partly, and the second Pasteur pipette was cut off from the middle of the second part, then combination both of them to form a simple funnel device. 1.30 g of salt was added into the Pasteur pipette easily from the maximum diameter part. After that, the homogenous mixture solution with ultrasound assisted obtained (3 mL of water sample and 650 μL of acetonitrile) was immediately injected into the Pasteur pipette using 5 mL syringe. Gently shaking the Pasteur pipette by hand for seconds until the salt can't be dissolved any more. Solution was

saturated at that moment. Then, Pasteur pipette was put into to ultrasonic bath for 30 s to accelerate acetonitrile floating to the upper surface. Afterwards, inject into saturated salt water to raise the level of acetonitrile to the upper narrow neck of Pasteur pipette. Next acetonitrile was transferred to 0.5 mL cone bottom plastic PCR pipe with a little anhydrous sodium sulfate to remove trace moisture. Finally, 1 μL of acetonitrile was injected into the GC-MS system for analysis.

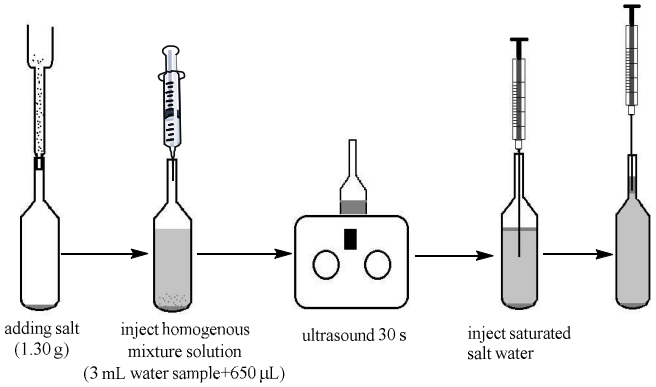

Fig. 1 Schematic of ultrasound assisted acetonitrile salting-out homogenous liquid-liquid extraction (UASO-HLLME)

Results and discussion

Influence of ultrasound assisted to promote solvent collection

Water-miscible solvent was selected for extraction of triazole pesticides in water samples. Solvent can be collected or not is the critical to the success of this experiment. Ultrasonic device was introduced into the experiment procedures, with the aidded of ultrasonic energy to make triazole pesticide dissolving in solvent rather than in water as well as to accelerate solvent salting-out. A set of control experiments were designed to verify the effect of ultrasonic assisted, one was joined ultrasound assisted while the other was not. The results were shown as Fig. 2. It revealed that ultrasound assisted indeed have a certain effect on accelerating extraction of triazole pesticide.

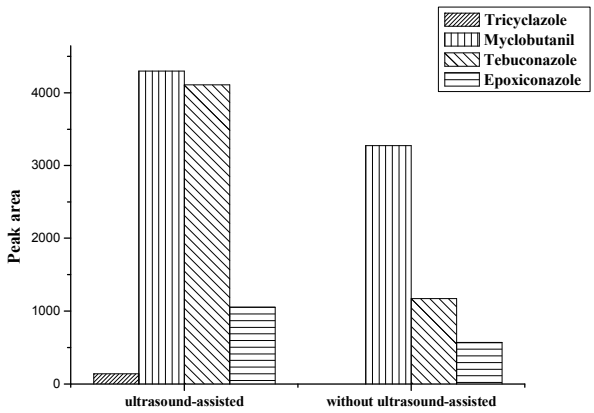

Fig. 2 Effect of ultrasound assisted to promote solvent precipitation. Extraction conditions: salt 1.30 g, mixture solvent with water sample 3.0 mL and acetonitrile 800 μL injected into the Pasteur pipette immediately, slightly shaking by hand.

Kind and volume of water-miscible organic solvent

The selection of an appropriate extraction solvent is very critical to UASO-HLLME procedure. In this method, organic solvent was selected based on its miscibility with aqueous solution, the ability of two-phase system formation after adding salt, density lower than water, extraction capability of the interested compounds, and less toxicity. On the basis of those considerations, acetonitrile (density, 0.790 g mL^{-1}), acetone (density, 0.788 g mL^{-1}) and ethyl acetate (density, 0.902 g mL^{-1}) were chosen and tested as the extraction solvent. But different types of extraction solvent were injected into different volumes, because they are special water-miscible and salting-out effect. Acetonitrile and acetone infinite miscible with water, while acetonitrile could be collected when its volume above 640 μL and acetone needed more than 1.7 mL. The water-solubility of ethyl acetate is 8.3% ($V_{ethyl\ acetate}/V_{water}$=8.3%, 20°C), which means the maximum volume of ethyl acetate dissolved in 3 mL water is 249 μL. Therefore, we optimized the type and volume of extraction solvent individually.

The tested volumes of acetonitrile were 800 μL, 700 μL, 670 μL, 650 μL, 640 μL, respectively. And the volumes of ethyl acetate were 200 μL, 160 μL, 140 μL, 120 μL, 110 μL, respectively. The results of both sets of experiments were listed in Fig. 3. However, the volume of acetone only tested once of 1.8 mL, because the limitation of Pasteur pipette capacity (5 mL) and acetone could be collected only when the volume was more than 1.8 mL. It was difficult to shake Pasteur pipette to accelerate salt dissolving without solution spread out when it reached 4.8 mL. So acetone was abandoned. From the Fig. 3, we know that the extraction efficiency of acetonitrile was better than ethyl acetate. Thus, acetonitrile was selected as extraction solvent. When it came to the volume of extraction solvent, we finally chose to inject 650 μL of acetonitrile. That was because when the volume lowered than 640 μL, acetonitrile couldn't be collected. Besides, it was hardly to obtain acetonitrile when the injected volume was 640 μL. As we could see, 650 μL of acetonitrile achieved the highest extraction efficiency. What's more, at that condition there was nearly 10 μL of acetonitrile, which can be easily removed by 50 μL syringe. Above all, we chose acetonitrile as extraction solvent and its volume was 650 μL.

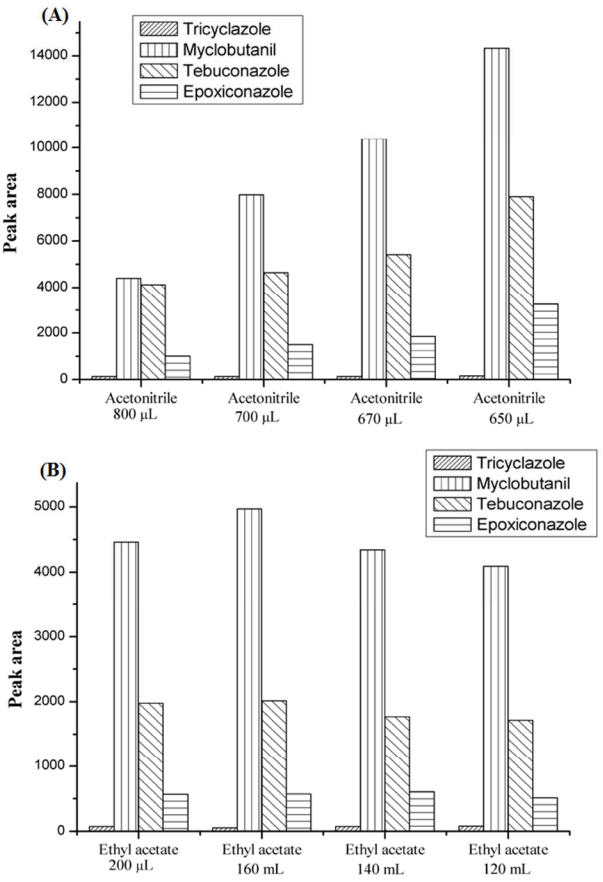

Fig. 3 (A) Different volume of acetonitrile on extraction efficiency. (B) Different volume of ethyl acetate on extraction efficiency. Extraction conditions: salt 1.30 g, mixture solvent with water sample 3.0 mL and different types and volumes injected into the Pasteur pipette immediately, slightly shaking by hand, ultrasound assisted to promote solvent precipitation.

Influence of ultrasound assisted to promote solvent blending

At the beginning of experiments procedure, 650 µL of acetonitrile was injected into the water sample, in order to get a homogenous mixture solution, ultrasound assisted was taken for homogeneity and shorten the time. As we know, ultrasonic energy can accelerate the mass transfer, so acetonitrile can reach dissolution equilibrium within short time in water when it coupled with ultrasound assisted. The dissolution balance time only needed for 30 s. Fig. 4 illustrated the results of setting aside for 30 s and ultrasound assisted blending for 30 s. It revealed that ultrasound assisted blending in the short time was conductive to extraction efficiency.

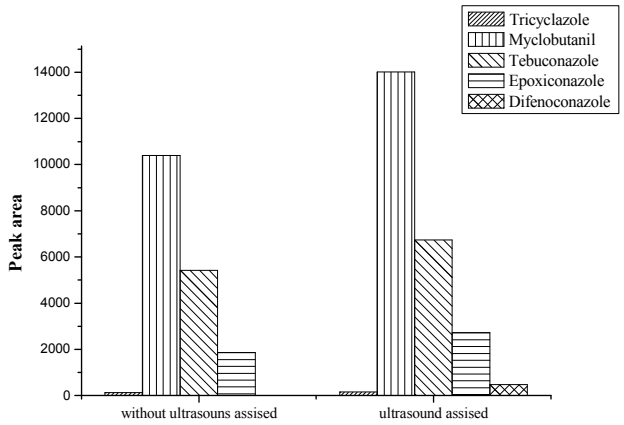

Fig. 4 Effect of ultrasound assisted to promote solvent blending. Extraction conditions: salt 1.30 g, mixture solvent with water sample 3.0 mL and acetonitrile 650 µL injected into the Pasteur pipette immediately, slightly shaking by hand, ultrasound assisted to promote solvent precipitation.

Evaluation of the Method Performance

To assess the analytical characteristics of the method, some quantitative parameters including linear range of calibration graphs (LR), correlation coefficients (R^2), limits of detection (LOD), limits of quantification (LOQ), relative standard deviations (RSD%), and EF were investigated. The results were listed in Table 1. As given in the table, the LRs were in ranges of 10-200 µg L^{-1} for myclobutanil, tebuconazole and epoxiconazole, 50-1000 µg L^{-1} for tricyclazole and difenoconazole with correlation coefficients in the range of 0.9913-0.9992. LODs and LOQs for the five tested triazole pesticides were in the ranges of 0.4-14.4 and 1.9-16 µg L^{-1}, respectively. Moreover, the repeatability study was performed on the concentration level of myclobutanil, tebuconazole and epoxiconazole at 100 µg L^{-1}, tricyclazole and difenoconazole at 500 µg L^{-1} and the RSD percentages were obtained in the range of 4.0-6.1% for six repeated determinations. The EFs ranging from 120 to 185 were obtained. Wide linear ranges, low LODs and LOQs, and high EFs are the main advantages of the proposed method.

Real Sample Analysis

The applicability of the method was evaluated by performing recovery studies in an environmental water sample which was got from the southern end of the Beijing-Hangzhou Grande Canale. Sample was spiked with pesticides standard solutions to configure as a mixed solution of three concentration levels, the each concentration level of myclobutanil, tebuconazole and epoxiconazole were 10 µg L^{-1}, 50 µg L^{-1} and 100 µg L^{-1}, while tricyclazole and difenoconazole were 50 µg L^{-1}, 250 µg L^{-1} and 500 µg L^{-1}. Recoveries were then calculated by comparing the average peak area for the analytes in blank sample spiked before the application of UASO-HLLME procedure with the peak area of the corresponding sample spiked after the application of UASO-HLLME procedure. Under the optimized conditions, real sample was analyzed, however, none of these target analytes were detected. Typical GC-MS chromatograms of Beijing-Hangzhou Grande Canale water sample after performing the proposed method on it was shown in Fig. 5. Recoveries and the corresponding RSD% were listed in table 2.

As it can be seen, recoveries and the corresponding RSD% were pretty good with current method, they were in the range of 89.6-119% and 0.4-8.1%, respectively. Thus, the results obtained with the proposed method could be considered in agreement with the current EU legislation.[24]

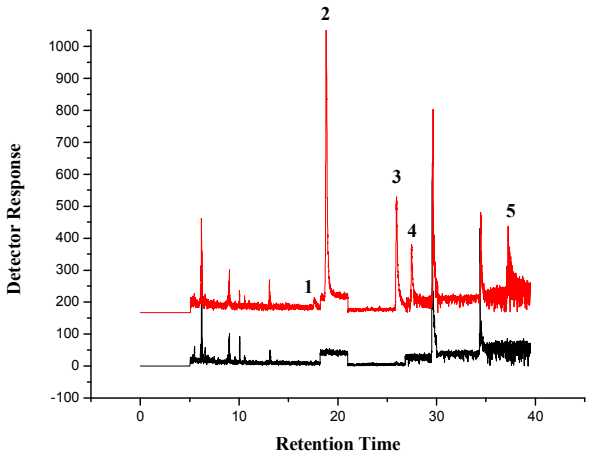

Fig. 5 GC-MS chromatography of (A) spiked Beijing-Hangzhou Grande Canale water sample with 100 μg L^{-1} of myclobutanil, tebuconazole and epoxiconazole, and 500 μg L^{-1} of tricyclazole and difenoconazole; (B) unspiked Beijing-Hangzhou Grande Canale water sample.
Identification: 1 tricyclazole, 2 myclobutanil, 3 tebuconazole, 4 epoxiconazole, 5 difenoconazole

In order to verify the feasibility of this method once again, we made a second spiked experiments at low concentration level (myclobutanil, tebuconazole and epoxiconazole were 10 μg L^{-1}, while tricyclazole and difenoconazole were 50 μg L^{-1}). The spiked concentrations of analytes were the same as real sample with myclobutanil, tebuconazole, epoxiconazole were 10 μg L^{-1} and tricyclazole, difenoconazole were 50 μg L^{-1}. The experimental results were shown in table 5. Recoveries and RSD% of real water sample at low concentration with secondary spiked were 86.6%-110%, 3.3-7.5%, respectively. These results reveal that method of UASO-HLLME can be utilized for the pesticide residues preconcentration from aqueous samples.

Conclusion

In this study, a simple, efficient and environment friendly analytical method has been proposed for sample preparation and quantitative determination of five triazole pesticides in water, using UASO-HLLME in combination with GC-MS. Water-miscible solvent of acetonitrile was used as extractant instead of high toxic extractant in traditional DLLME to form a homogeneous water solution, after that, salting out a small amount of acetonitrile to the purpose of extraction and enrichment. Under optimum conditions, matrix matched calibration curves were established using standard solution spiked water sample. Good linear relationships as well as low LODs (0.4-14.4 μg L^{-1}) and LOQs (1.3-48.1 μg L^{-1}) were obtained. The RSD% of spiked water sample and spiked real environment water sample were 4.5-8.1% and 0.4-8.1%, respectively, with EF 120-185. Recoveries obtained from spiked environmental waters with three concentration levels ranging from 89.6 to 119%. The results obtained with the proposed method could be considered in agreement with the current EU legislation, which revealed that it can be successfully applicable for the determination of triazole pesticides in water samples.

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