Development of a Microextraction Method Based on Dissolved Carbon Dioxide Flotation after Emulsification for the Determination of Triazole Pesticides Residues in Water Samples by Gas Chromatography–Mass Spectrometry

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A novel dissolved carbon dioxide flotation after emulsification microextraction (DCF-EME) method was proposed for the determination of four triazole pesticides in water samples coupled with gas chromatography–mass spectrometry (GC-MS) in a home-made glass round flask. The DCF-EME method is based on a rapid and simple phase separation of low-density organic solvent (toluene) from the aqueous phase via introducing a saturated NaHCO$_3$ solution into the acidified sample (0.1 mol L$^{-1}$ HCl); then analytes were extracted in toluene. Various parameters affecting the extraction process were optimized. Under the optimal conditions, the recoveries for four pesticides ranged from 82.8 to 121.2%. Meanwhile the limits of detection were at the range of 0.14 – 1.04 μg L$^{-1}$, and the preconcentration factors were varied between 342 and 473 for different triazoles. The method is simple, fast and environmentally friendly, being successfully applied for the determination of triazole pesticides in water samples.

Keywords Dissolved carbon dioxide flotation, triazole pesticides, gas chromatography–mass spectrometry

(Received February 1, 2016; Accepted July 6, 2016; Published October 10, 2016)

Introduction

Triazole pesticides are one of the most important classes of pesticides that are greatly used in a variety of vegetables, fruits and grain crops, which not only have excellent antibacterial activity, but also have an ability to regulate the growth of plants.\(^1\) However, the widespread use of triazoles has increased concern about detrimental effects in ecosystems and human health, which can cause endocrine disorder and even carcinogenic effects.\(^2\) Since triazole pesticides have been found in food and water, there is a need to develop better, less labor-consuming, faster, greener and more accurate analytical procedures capable of detecting low concentrations of pesticides in environmental water samples and a variety of food matrices.

As mentioned in the literature, several methods have been developed for determination of triazole pesticides residues. For example, solid-phase extraction (SPE) has been successfully applied to the preconcentration of triazole pesticides in honeybees, vegetables, fruits and cereals, which enriches and purifies the sample at the same time.\(^3\) However, SPE can still be time-consuming, relatively expensive, and sometimes limited by the volume of the sample. Solid-phase microextraction (SPME), a solvent-free process that includes simultaneous extraction and preconcentration of analytes from the headspace of the samples, has also been used in the extraction of triazoles in fruit samples.\(^6\) However, SPME suffers from some drawbacks, such as sample carry-over, relatively high cost and fiber fragility. Dispersive liquid-liquid microextraction (DLLME) is a fast, accurate, simple and low-cost method, which only requires trace amounts of organic solvent, while providing high enrichment factors; it also has been proposed for the extraction of triazoles.\(^8\) Recently, DLLME has shown a rapid growth, such as air-assisted liquid-liquid microextraction (AALLME),\(^9\) dispersive liquid-liquid microextraction based on solidification of floating organic droplet (DLLME-SFO),\(^11\)–\(^14\) stir bar sorptive extraction combined with dispersive liquid-liquid microextraction (SBSE-DLLME),\(^15\) ultrasound-enhanced temperature-controlled ionic liquid dispersive liquid-liquid microextraction (UETC-IL-DLLME),\(^16\) homogeneous liquid-liquid extraction based on a narrow-bore tube (HLLE),\(^17\)–\(^18\) reversed-phase dispersive liquid-liquid microextraction (RP-DLLME),\(^19\) vortex-assisted ionic liquid dispersive liquid-liquid microextraction (VA-IL-DLLME),\(^20\) ultrasound-assisted salting-out homogeneous liquid-liquid microextraction (USASO-HLLME)\(^22\) and so on. In conventional DLLME, extraction solvents, which are usually water insoluble and have a density greater than that of water, were used for a simple separation of the extraction phase after centrifugation. However, few organic solvents can meet these requirements, and most of them are halogenated hydrocarbons, which are hazardous. These methods mentioned above need centrifugation to break down the emulsion, except for the home-made narrow-bore tube, which is subjected to the centrifuge specifications, and the volume of a tested sample can not be enlarged.
Recently, a novel effervescence-assisted dispersive liquid–liquid microextraction (EA-DLLME) method was proposed for the determination of different pesticides.\textsuperscript{26-30} There were two patterns in the EA-DLLME procedures, for dispersion and for deemulsification. Bubbles were generated, making possible easy dispersion of the extraction solvent, and were used to break up the organic solvent in water emulsion so as to finish the extraction process. These EA-DLLME methods did not need any centrifugation or dispersive solvent, thus bringing many advantages. However the narrow-bore tube used in the above methods is inconvenient for cleaning, and thus cross-contamination will be caused.

The aim of this work is to use dissolved carbon dioxide flotation after employing the emulsification microextraction (DCF-EME) method in a new mode performed in a glass round flask for the determination of triazole pesticides in water samples by gas chromatography–mass spectrometry. The proposed method is an improvement on the traditional method of DLLME. It is very simple and fast; also, the whole procedure can be completed in 3 min. The effects of various experimental parameters were studied, and the performance of the presented method for the analysis of real samples was tested. This method breaks through the limit of volume with centrifugation; the device is easy to clean and this method exhibits many merits, such as excellent EFs, very low LODs, excellent sensitivity, and short extraction time.

**Experimental**

**Reagents and materials**

Standards of myclobutanil (≥96.5%), tebuconazole (≥97%), difenoconazole (≥97.2%) and epoxiconazole (≥96%) were purchased from Wellington Laboratories Inc. (Canada). All standard solutions were prepared in methanol and were stored at 4°C. Analytical-grade acetonitrile, methanol, toluene, n-hexane, cyclohexane, 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). Distilled water was obtained from a Purite RO200-Stillplus HP System (Purite Oxon, UK).

**Instrumentation and chromatographic conditions**

GC-MS analysis was performed on a GC 2000-Mars 6100 (Focused Photonics Inc., Hangzhou, China). Chromatographic separation was achieved on a DB-5 MS capillary column (30 m × 0.25 mm × 0.25 μm). The column oven was initially kept at 180°C for 1 min; then, the temperature was increased to 190°C at 5°C min\textsuperscript{-1}, which remained for 1 min; after that it was increased to 220°C at 2°C min\textsuperscript{-1}. Finally, the temperature was ramped up at 10°C min\textsuperscript{-1} to 290°C and held for 6 min. High-purity helium (≥99.999%) was used as the carrier gas at a constant flow rate of 1 mL min\textsuperscript{-1}. The temperature of the injector was held at 280°C with a 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 4 ranges: the first range was 3.0 – 15.5 min for myclobutanil with specific ions of 179 and 152; the second was 15.5 – 18.43 min for tebuconazole with specific ions of 125 and 250; the third was 18.43 – 20.0 min for epoxiconazole with specific ions of 192 and 138; the last one was 20.0 – 29.0 min for difenoconazole with ion fragments of 265 and 323. A SY-360 ultrasonic bath from Ultrasonic Instrument Company (Ningshang, Shanghai, China) was used to facilitate extraction. The extraction procedure was performed in a home-made glass round flask with a manifold and an adapter.

**DCF-EME procedure**

The DCF-EME procedures mainly consist of the following steps: firstly, 2 mL acetonitrile and 60 μL toluene were added into the home-made glass round flask with a manifold to form the homogeneous mixture with ultrasound assistance; then, 20 mL of acidified water sample (0.1 mol L\textsuperscript{-1} HCl) was injected into the mixture. Secondly, the flask was gently shaken by hand for several seconds (about 15 s), and then put into an ultrasonic bath for 30 s to accelerate forming the emulsion. In this step, the triazoles in the water sample were extracted into fine droplets of toluene. Thirdly, 2 mL of a saturated NaHCO\textsubscript{3} solution was injected into the emulsion from the manifold, and the flask was immediately transferred into the ultrasonic bath again for 90 s. In this way, the effervescence occurred homogeneously from the solution up to the top of the liquid surface, and the emulsion was broken down. After that, a saturated NaCl solution was added into the flask by the side tube to raise the liquid level, and the toluene organic phase (7 ± 1 μL) was taken out by a syringe into a 0.5-mL cone-bottom plastic PCR pipe with a small amount of anhydrous sodium sulfate added to remove any trace moisture. Finally, 1 μL of toluene was injected into the GC-MS system for analysis. The whole flow chart is shown in Fig. 1.
**Results and Discussion**

A systematic study was conducted to optimize the extraction conditions influenced by many factors. To select the optimal extraction conditions in the DCF-EME method, single-factor experiments were used. Every experiment was repeated three times at the concentration levels of four triazole pesticides at 100 μg L⁻¹ spiked in the water sample.

**Selection of the way of dispersion and the solution to raise level**

A set of control experiments were designed to verify the effect of ways of dispersion; one was operated with ultrasound assistance, while the other was with manual shaking. The results (Fig. S1) demonstrated that the peak area obtained with ultrasound assistance was higher than that with manual shaking; so, ultrasound assistance was chosen.

Solutions used to raise the level were also investigated. There were two solutions: distilled water and saturated NaCl solution; the results (Fig. S2) indicated that a saturated NaCl solution was better. That was because the saturated NaCl solution decreases the solubility of analytes in the aqueous phase, and thus enhances their extraction into the organic phase.

**Selection of extraction solvent and disperser solvent**

The selection of an appropriate extraction solvent and a disperser solvent is of great importance in the DCF-EME procedure. The primary requirements of an adequate extraction solvent for the proposed method are: low-solubility in water, low density solvents, and high extraction capability for the analytes of interest. On the basis of these considerations, toluene (ρ = 0.87 g mL⁻¹), cyclohexane (ρ = 0.78 g mL⁻¹), n-hexane (ρ = 0.66 g mL⁻¹) and ethyl acetate (ρ = 0.90 g mL⁻¹) were tested using acetonitrile (2 mL) as the disperser solvent. As a result, emulsion could not be formed using ethyl acetate as the extraction solvent when the volume was up to 200 μL; the extraction efficiency of three other organic solvents using different amounts to get the same volume of the collected phase for four target analytes indicated that toluene was the best among these solvents (Fig. S3). Besides, the lower viscosity of toluene leads to a rapid and highly efficient emulsification process. Thus, toluene was chosen as the extraction solvent for subsequent experiments.

The miscibility of the disperser solvent with the organic extraction solvent and the aqueous phase is the main criterion for selecting the disperser solvent in DLLME. It is necessary that the trace extraction solvent is dispersed well into the aqueous sample as very fine droplets, in order to obtain a very high amount of contact area, and to achieve fast migration of the analytes from the aqueous sample into the extraction phase. Acetonitrile, methanol and acetone were tested for concerning their efficiencies (using 2 mL of each disperser solvent). The volume of the collected phase was different: 8 μL for acetonitrile, 4.5 μL for methanol and 4 μL for acetone, respectively. The extraction efficiency of acetonitrile for four target analytes was found to be the best by considering the volume of the collected phase (Fig. S4). Thus, acetonitrile was chosen as the disperser solvent for subsequent experiments.

**Optimization of extraction time and deemulsification time**

In this paper, the extraction time was calculated from ultrasound beginning to the end forming the emulsion. To investigate the effect of the extraction time, 0, 15, 30, and 45 s were considered. The peak areas for four target analytes (shown in Fig. 2) were increased as the extraction time increased from 0 to 30 s; and when the extraction time was up to 45 s, the areas were decreased. That was because the extraction was insufficiently within a short time; and with the extraction time going by, the emulsion will become unstable. Above all, 30 s was chosen as the optimal extraction time.

Due to the slow rate of CO₂ generation after the addition of the NaHCO₃ solution, ultrasound was used to accelerate breaking down the emulsion. The ultrasound time for deemulsification was an important factor that influenced the extraction efficiency. Thus, different times ranging from 60 to 120 s were tested; the results (Fig. S5) demonstrated that 90 s was the best deemulsification time.

**Effect of the amount of the generated CO₂ gas**

In this work, the intensity of generated CO₂ gas was responsible for breaking down the emulsion and accelerating the phase-separation process. The amount of the generated gas was tested using the injection of various amounts of a sodium bicarbonate solution into a 20-mL acidified sample solution of corresponding concentration (0.02 mol L⁻¹ HCl, 0.05 mol L⁻¹ HCl, 0.1 mol L⁻¹ HCl and 0.2 mol L⁻¹ HCl) to assure that the final solution was neutral. The results (Fig. 3) showed that the amount of CO₂ gas generated by 2 mL of saturated NaHCO₃ solution and 20 mL 0.1 mol L⁻¹ HCl was the optimum value. That was because as the amount of CO₂ gas increased at the beginning, the deemulsification was more sufficient; and at higher CO₂ amount because of the evaporation, the extraction efficiency decreased.
The triazole pesticides were extracted with different volumes of toluene. At volumes of less than 2 μL, the DLLME procedure could not be performed well, which may be attributed to the formation of larger droplets of the organic phase; at volumes larger than 2.5 μL, it is not easy to obtain the toluene organic phase (Fig. S6). Therefore, 2 μL of toluene was the optimum value.

Validation of the DCF-EME method

To assess the analytical characteristics of the method, some quantitative parameters, including the linear range (LR), limits of detection (LODs), limits of quantification (LOQs), relative standard deviation percentages (RSD, %), and enrichment factors (EFs), were investigated under the optimum conditions. The results are listed in Table 1.

To assess the applicability of the proposed method, recovery studies were performed in an environmental water sample that was obtained from the southern end of the Beijing–Hangzhou Grand Canal. The sample was spiked with pesticide standard solutions to configure a mixed solution of three concentration levels; the concentration levels of myclobutanil, tebuconazole, epoxiconazole and difenoconazole were 20, 50 and 100 μg L⁻¹. Under the optimized conditions, none of these triazoles were detected in the real water sample. Typical GC-MS chromatograms of the Beijing–Hangzhou Grand Canal water sample after performing the proposed method on it are shown in Fig. 5. The recoveries and the corresponding RSD were in fairly good agreement with the current method (Table 2), in the ranges of 82.8 – 121.2 and 2.8 – 18.9%, respectively. The results showed that the proposed method is very practical and useful for the analysis of real sample.

Comparison of the proposed method with other approaches

The efficiency of the presented DCF-EME method for the selected analytes was compared with those of other methods reported in the literature, in terms of such features as the LODs, LOQs, LR and EFs; the results are summarized in Table 3. The LODs and LOQs for the proposed method are lower than those of most mentioned methods. The EFs of the proposed method are better, or comparable to other methods. By considering these results, the newly developed method can be considered to be a rapid, efficient, sensitive, reliable, and easy way to use the technique for the extraction and highly efficient preconcentration of the triazole pesticides from the water samples.

Optimization of volume of extraction solvent and disperser solvent

The volume of the extraction solvent was also investigated. The triazole pesticides were extracted with different volumes of toluene (50, 55, 60 and 70 μL). The results (Fig. 4) indicated that the peak areas of the target analytes increased as the volume of toluene increased from 50 to 60 μL, and decreased from 60 to 70 μL. This may have been caused by the dilution effect when a larger volume of extraction solvent was used. Accordingly, 60 μL of toluene was chosen for subsequent experiments.

In order to study the effect of the volume of the disperser solvent on the extraction efficiency, 60 μL of toluene was dissolved in different volumes of acetonitrile (5 – 100 μL). As given in the table, the LR was 1 – 100 µg L⁻¹ for myclobutanil, tebuconazole and epoxiconazole and 5 – 100 μg L⁻¹ for difenoconazole with correlation coefficients in the range of 0.9940 – 0.9976. The LODs and LOQs for the four tested triazole pesticides were in the ranges of 0.14 – 1.04 and 0.46 – 3.46 µg L⁻¹, respectively. Moreover, a repeatability study was performed at a concentration level of 50 μg L⁻¹ for myclobutanil, tebuconazole, epoxiconazole and difenoconazole; the RSDs obtained were in the range 8.6 – 13.2% for seven repetitions. EFs ranging from 342 to 473 were obtained. Wide linear ranges, low LODs and LOQs, and high EFs are the main advantages of the proposed method.

Application to real samples

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![Graph 1](Image 55x283 to 291x463)

**Fig. 3** Effect of the amount of the generated CO₂ gas (extraction conditions: sample solution, 20 mL; disperser solvent, 2 mL acetonitrile; extractant, 60 μL toluene; extraction time, 30 s; deemulsification time, 90 s; way of dispersion, ultrasound assistance; solution to raise level, saturated NaCl solution; spiked level, 100 μg L⁻¹ of each analyte; the error bars indicate the minimum and maximum of three independent determinations).

![Graph 2](Image 56x583 to 290x760)

**Fig. 4** Effect of the volume of toluene (extraction conditions: sample solution, 20 mL; disperser solvent, 2 mL acetonitrile; extractant, 60 μL toluene; extraction time, 30 s; deemulsification time, 90 s; way of dispersion, ultrasound assistance; solution to raise level, saturated NaCl solution; amount of CO₂ gas, generated by 20 mL 0.1 mol L⁻¹ HCl sample solution and 2 mL saturated NaHCO₃ solution; spiked level, 100 μg L⁻¹ of each analyte; the error bars indicate the minimum and maximum of three independent determinations).
Conclusions

In this paper, for the first time, a simple, efficient and environmentally friendly analytical method has been proposed for sample preparation and quantitative determination of four triazole pesticides in water, using DCF-EME in combination with GC-MS. Dissolved carbon dioxide flotation was used to break down the emulsion instead of centrifugation, reducing the time for sample preparation. Also, a larger volume of solution can be tested by the home-made experimental device. The results demonstrated that this technique exhibits many merits, such as excellent EFs, very low LODs, excellent sensitivity, and short extraction time. By considering these advantages, the developed method can be considered to be a high-performance technique for the determination of triazole pesticides in water samples.

Acknowledgements

Support of this work by the Nature Science Foundation of Zhejiang Province (LY16B050008), the Science and Technology Department of Zhejiang Province (2015C32006), Key Laboratory of Detection for Pesticide Residues of Ministry of Agriculture Project (2014PRG01), the Department of Education of Zhejiang Province (Pd2013016), Hangzhou Qianjiang Distinguished Experts Project (2014), and the Sprout Talented Project Program (2011443) are gratefully acknowledged. The authors have declared no conflict of interest.
Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References