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Rapid and sensitive analysis of nine fungicide residues in chrysanthemum by matrix extraction-vortex-assisted dispersive liquid–liquid microextraction

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ABSTRACT

A simple sample pretreatment for simultaneous determination of nine fungicides (triadimefon, picoxystrobin, kresoxim-methyl, diniconazole, epoxiconazole, trifloxystrobin, triticonazole, difenoconazole, and azoxystrobin) in chrysanthemum was developed using matrix extraction-vortex-assisted dispersive liquid–liquid microextraction (ME-VADLLME) prior to gas chromatography with electron capture detection. The target fungicides were firstly extracted with acetonitrile and cleaned with the mixture of primary secondary amine and graphite carbon black. The VADLLME procedure was performed by using toluene with lower density than water as the extraction solvent and the acetonitrile extract as the dispersive solvent, respectively. After vortexing and centrifugation, the fine droplet of toluene was collected on the upper of the mixed toluene/acetonitrile/water system using a 0.1-mL pipettor. Under the optimum conditions, the relative recoveries ranged from 73.9 to 95.1% with relative standard deviations of 3.5–9.7% for all of the analytes. The limits of detection were in the range of $(0.005–0.05)\times10^{-3}$ mg kg^{-1}. In the proposed method, the ME step provides more effective cleanup for the chrysanthemum matrix, and VADLLME introduces higher sensitivity with the remarkable enrichment factors up to 88-fold compared with the conventional QuEChERS or SPE. The good performance has demonstrated that ME-VADLLME has a strong potential for application in the multi-residue analysis of complex matrices.

1. Introduction

The use of herbal plants for medicines or food ingredients in China has been a tradition for thousands of years. Chinese herbal medicines (CHMs) have been considered to be gentle, and non-toxic because of their natural origins and no industrial processing. The international popularity of CHMs has increased its production by applying pesticides to CHMs, which can reduce loss from diseases. However, the pesticide residues in CHMs may lead to potential health risk to consumers or force unnecessary pressure on the environment. Contamination of crude medicinal plants as well as their products has been increasingly reported. In these studies, most concerns focused on organochlorine and pyrethroid pesticides [1–4]. Additionally, the analytical methods for determining pesticide residues in CHM matrices are limited, including modified QuEChERS methods [5,6], treatment with sulfuric acid [3], and matrix solid-phase dispersive extraction [7]. Monitoring of a wide range of pesticide residues in CHMs can be an important task in public health safety and international trade.

Chrysanthemum, *Dendranthema grandiflora*, is a kind of the most important cut flowers and pot plants all over the world. The flower is commonly processed into beverages and CHMs due to its special flavor and effectiveness against colds and high blood pressure. Chrysanthemum is known as a complex matrix comprising of pigments, alkaloids, flavonoids, sterols, and essential oils, etc. To remove the potential co-extractives that may interfere with the determination of the analytes of interest, isolation of pesticides from chrysanthemum needs complicated cleanup procedures, involving gel permeation chromatography (GPC) and solid phase extraction (SPE) [8]. These procedures usually require large amounts of solvents and are time-consuming, and also presented relatively high limits of detection (LODs). Therefore, the

Abbreviations: ME-VADLLME, matrix extraction-vortex-assisted dispersive liquid–liquid microextraction; SPE, solid phase extraction; DSPE, solid phase extraction; GC-ECD, gas chromatography with electron capture detection; PSA, primary secondary amine; GCB, graphite carbon black; EF, enrichment factor; ER, extraction recovery; RR, relative recovery; RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantification.

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development of a rapid and simple multi-residue analytical method with sufficient cleanup and high sensitivity for chrysanthemum is clear needed.

Dispersive liquid–liquid microextraction (DLLME) is a recently developed microextraction technique introduced by Assadi [9]. This technique makes use of microliter volume of a mixture of extraction solvent and dispersive solvent with high miscibility in acceptor solutions [10]. Due to the simplicity of operation, rapidity, high recovery and sensitivity, DLLME has been used for separation and preconcentration of different organic compounds in aqueous samples [9,11–16], environmental soils [17] and sediments [18,19]. With the development of DLLME, it has been extended to the analysis of targets in vegetable and fruit samples, including bananas [20,21], apples [22–24], grapes [25,26], tomatoes [27,28], watermelons and cucumbers [29–32]. As always, limitations still exist in the analysis of complex matrices, such as CHM samples, because unique chemical or nutritional compositions contained in CHMs usually make the extraction and cleanup steps difficult. The rich flavonoids and essential oil in chrysanthemum contribute some special restrictions on the capacity of the conventional DLLME.

In this work, a rapid and sensitive matrix extraction-vortex-assisted DLLME (ME-VADLLME) was developed to determine nine fungicides in chrysanthemum samples by gas chromatography with electron capture detection (GC-ECD). Several modifications have been made to extend the application of the conventional techniques to chrysanthemum samples. Under the optimum conditions, the fungicides were firstly extracted with acetonitrile and cleaned with the mixture of primary secondary amine (PSA) and graphitized carbon black (GCB). Afterwards, a comprehensive investigation of different experimental parameters was performed to develop the VADLLME procedure. Finally, a 0.1-mL pipettor for collecting toluene with lower density than water from the top of the toluene/acetonitrile extract/water system instead of special extraction devices [33], making the method simple, rapid and cheap. To our knowledge, this is the first report using ME-VADLLME for simultaneous determination of residue levels of nine fungicides in chrysanthemum, a complex CHM matrix. The optimized method was compared with some other reported methods in CHMs. The approach with the good accuracy, purification, separation and low limits of detection reveals that it is a reliable technique for the analysis of the selected targets in chrysanthemum samples. The combination of acetonitrile extraction, adsorbent mixture cleanup and VADLLME would extend the applicability of DLLME techniques to complex solid samples.

2. Experimental

2.1. Reagents and materials

All HPLC-grade solvents including isooctane, cyclohexane, n-hexane, toluene, acetonitrile, methonal, acetone and ethyl acetate were purchased from Dikma, USA. Distilled water was from a Milli-Q water purification system. Sodium chloride (analytical-reagent grade, Merck) was used to adjust the ionic strength of aqueous solutions. Primary secondary amino (PSA, 40–60 μm in size) and graphitized carbon black (GCB, 40–60 μm in size) were obtained from Agela Technologies, China.

An RJ-TDL-40B low-speed desktop centrifuge was purchased from Ruijiang Co., Jiangsu, China. The shaker (HZQ-C) was from Donglian Electron Technology Exploiter Co., Ltd., Harbin, China. The vortex meter and hand centrifuge were supplied by Qilinbeier Co., Jiangsu, China. The pipettor in a range of 0.1 mL was obtained from Guangdahengyi Co., Beijing, China.

Nine fungicides (triadimefon, picoxystrobin, kresoxim-methyl, diniconazole, epoxiconazole, trifloxystrobin, triticonazole, difenoconazole, and azoxystrobin) of analytical standard grade with the purity of >95% were purchased from Standard Technology Development, Beijing, China. The chrysanthemum samples provided by Institute of plant protection in Anhui, China, were cut into slices before being used.

2.2. Preparation of standards

Individual 1000 mg L^{-1} stock solution was prepared by dissolving 0.010 g of each pesticide in 10 mL acetonitrile. For daily preparation of working solutions, two composite stock standard solutions were prepared in acetonitrile at a concentration of 10 mg L^{-1} for each analyte. All solutions were stored at −20 °C in the dark.

2.3. Sample preparation

The development of an analytical method needs to maximize the accuracy and sensitivity of the analytes while minimize interferences from the matrix. Due to the low detection levels required by regulatory bodies and the complexity of chrysanthemum in which the target compounds are present, efficient extraction of analytes, separation from interferences and low-detection determination are significant aspects of sample preparation. Nine fungicides were chosen as model compounds to evaluate the performance of this proposed method.

2.3.1. The optimization of ME-VADLLME

With the aim of achieving the best efficiency of the proposed method, different factors were investigated, including (i) the type of the extraction solvent: methanol, acetone and acetonitrile; (ii) the type of the DSPE adsorbent: PSA and the mixture of PSA and GCB; (iii) the type of the extraction solvent in VADLLME: four chlorinated organic solvents heavier than water (C_6H_5Cl, CCl_4, $CHCl_3$, and CH_2Cl_4) and four organic solvents lighter than water (isooctane, n-hexane, cyclohexane, and toluene); (iv) the volume of the extraction solvent in VADLLME: 30, 40, 50, 60, 70, 80, 90, and 100 μL; (v) the volume of the dispersive solvent in VADLLME: 0.5, 1, 1.5, and 2 mL; (vi) the volume of distilled water in VADLLME: 3, 4, 5, 6, 7, and 8 mL; (vii) the extraction condition and time: vortexing for 1, 2, and 3 min, and ultrasonication for 2, 4, 6, 8, and 10 min; (viii) the amount of NaCl (0%, 4%, 8%, 12%, 16%, and 20%, w/v). Then, the optimum conditions were applied for the method.

2.3.2. The proposed ME-VADLLME procedure

The samples were homogenized for 10 s. For recovery determination, the portion samples (1 g, weighed to a precision of 0.01 g) were spiked by the addition of the standard stock solutions at three levels. The spiked samples were allowed to stand for a few minutes for the spiking solutions to penetrate the matrix. The homogenized sample (1 g, weighed to a precision of 0.01 g) was placed in a 15-mL centrifuge tube. A total of 5 mL of acetonitrile was added and the mixture was shaken for 10 min. After that, the solution was centrifuged at 3800 rpm for 5 min to separate the fine solid particles of chrysanthemum from the solution. Then 1.8 mL of the extract was transferred for cleanup by mixing with 50 mg of PSA and 50 mg of GCB. The mixture was vortexing for 1 min immediately. After high-speed centrifugation at 10,000 rpm, the supernatant was collected and filtered through a 0.22-mm organic system filter.

In the VADLLME step, 1.5 mL of the supernatant acetonitrile solution (as the dispersive solvent) was mixed with 50 μL of toluene (as the extraction solvent). The acetonitrile-toluene was transferred rapidly into a 15-mL screw cap plastic tube with conical bottom containing 7 mL of distilled water. Next the tube was vigorously shaken on a vortex meter immediately for 2 min, and a cloudy