Rapid Determination of Dichlofluanid Residues in Vegetables Using Dispersive-SPE Sample Preparation Combined with Gas Chromatography–Mass Spectrometry

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Received 24 December 2014; Revised 8 January 2016

Abstract

A method for rapid determination of dichlofluanid residue in vegetables using dispersive solid-phase extraction (dispersive-SPE) sample preparation combined with gas chromatography–mass spectrometry (GC–MS) was developed. Samples were extracted with acetone–ethyl acetate (1:1, V/V), and then detected by GC–MS with an external standard method after being purified by optimized primary secondary amine, graphitized carbon black and anhydrous magnesium sulphate (MgSO₄). It turned out that dichlofluanid showed a good linearity ($y = 2.7E + 5x - 2710.5$) over the range of 0.02–2.00 mg/L with a correlation coefficient of 0.9994. The limit of detection was 0.13 µg/kg (S/N = 3) and the limit of quantification was 0.43 µg/kg (S/N = 10). The recoveries of the dichlofluanid were in the range of 73.3–106.7, 83.3–116.7 and 83.3–106.7% with the spiked levels of 0.01, 0.02 and 0.05 mg/kg, and the relative standard deviations were in the range of 4.1–22.3%. Compared with the reported literature, the method is more simple, rapid, sensitive, reliable and can be applied to many vegetables.

Introduction

Dichlofluanid (chemically known as N-[(dichlorofluoromethyl) thio]-N,N′-dimethyl-N-phenylsulfamide; Figure 1), introduced in 1965 by Bayer AG (trademarks are “Euparen” and “Elvaron”), is a protective fungicide with a broad spectrum against grey mold, powdery mildew, downy mildew and so on, which is used to control spoilage of vegetables (1–4). Dichlofluanid has a certain toxicity. When made in spray, it can kill tetranychus cinnabarinus. The mail rats’ oral amount of LD₅₀ is 2,500 mg/kg and the humans’ daily allowed intake is 0.3 mg/kg. Dichlofluanid must be stopped using in 7–14 days before the harvest of the vegetables (5–7). Therefore, developed countries have formulated the largest residues of dichlofluanid in vegetables, for example, the formulated largest residues of dichlofluanid of China to onion, lettuce, tomato, chili, cucumber and potato were 0.1, 10, 2, 2, 5 and 0.1 mg/kg, respectively (8).

Traditional sample preparation procedures include solid-phase extraction (SPE) (5, 9, 10), solid-phase micro-extraction (SPME) (11) and gel permeation chromatography (GPC) (12), accelerated solvent extraction (ASE) (13), matrix solid-phase dispersion (MSPD) (14) and microwave-assisted extraction (MAE) (15). However, most of these techniques are rather time-consuming, labour-intensive, complicated, expensive and produce considerable quantities of waste. An alternative technique called QuEChERS (quick, easy, cheap, effective, rugged, and safe) was introduced first by Anastassiades and has
been widely used. Dispersive-SPE was transformed from QuEChERS. The dispersive-SPE method uses a single step organic solution extraction and salting out liquid–liquid partitioning from the water in the sample with MgSO₄ and NaCl. Dispersive-SPE cleanup is done to remove organic acids, excess water and other compounds with a combination of adsorbents and MgSO₄. However, the determination of dichlofuanid residues in vegetables using dispersive-SPE to purify has rarely been reported. Primary secondary amine (PSA) bonds the group of ethylenediamine–N-propyl on silica, which can remove organic acids, sugars, anthocyanins and other plant phenols. Graphitized carbon black (GCB) has regular polyhedron structure of uniform graphite surface, which is widely used to remove pigments, especially chlorophylls. Therefore, PSA and GCB were chosen as adsorbents to purify impurities in this dispersive-SPE method.

Different methods have been developed for the determination of dichlofuanid residues, which include gas chromatography–mass spectrometry (GC–MS) (16, 17), gas chromatography (GC) (18), ultra high-performance liquid chromatography–tandem mass spectrometry (UPLC–MS–MS) (19) and so on. GC has highly efficient separation and rapid analysis. MS provides accurate structure information. GC–MS has become one of the most powerful methodologies to detect the dichlofuanid residue in vegetable samples.

Many experimental conditions of dichlofuanid determination in vegetables were investigated such as extraction and purification. This method made full use of the advantages of dispersive-SPE, which resulted in excellent recoveries, much faster sample analyses, significant reductions in solvent usage and hazardous waste production (20–22). What is more, the amount of adsorbent is changeable according to the experimental needs, so ion exchange capacity is variable. Therefore, in this research, a method for the rapid determination of dichlofuanid residues in vegetables using dispersive-SPE sample preparation combined with GC–MS was developed. The method was more rapid, simple, sensitive and reliable, so it could be applied in the determination of dichlofuanid residues in vegetables.

**Experimental process**

**Chemicals and reagents**

Dichlofuanid (99.6%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetone and ethyl acetate (HPLC grade) were obtained from Tedia (Fairfield, OH, USA). PSA and GCB were purchased from Agilent Technologies (Santa Clara, CA, USA) and their particle size was 60 μm. Sodium chloride and magnesium sulphate were of analytical grade. Ultra-pure water was prepared by Milli-Q-plus ultrapure water system (Milford, MA, USA) throughout the study. All of the vegetables were purchased from various local supermarkets (Chongqing, China).

**Instrumentation**

The instruments used are as follows: 7890 gas chromatography–5975 mass spectrometry (Agilent, USA); a XH-B vortex mixer (Jiangsu Healthcare Medical Supplies Company, Ltd); a 3–30 K refrigerated centrifuge (Sigma Company, Germany); rotary vacuum evaporators (4011 Digital) (Heidolph Company, Germany); pipettes (adjustable range: 10–100, 20–200, 10–1,000, 1,000–5,000, 1–10 mL) (Eppendorf Company, Germany) and a SR-2DS powerful oscillator (TAITEC, Japan).

**Standard solutions**

Stock solution of the dichlofuanid was prepared by dissolving the weighed compound in acetone at a final concentration of 1,000 mg/L in a glass vial, which was stored at −18°C. The stock solution was further diluted by acetone to make the working standard solutions, which were stored at 4°C. The concentrations of working standard solutions were 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 and 2.00 mg/L.

**GC–MS analysis**

All analyses were carried out on a 7890 GC equipped with a 5975 MS. Separation was done by using a DB-5 MS capillary column (30 m × 0.25 mm × 0.25 μm). Splitless injections of 1 μL of the sample extracts were carried with an injector temperature of 250°C. Helium (99.999% pure) was used as the carrier gas, at a flow rate of 1.2 mL/min. The oven temperature was programmed (Table I) as follows: start at 100°C (hold for 2 min), increase to 200°C at a rate of 30°C/min, then increase to 280°C at a rate of 10°C/min (hold for 2 min), with a final phase of heating at 280°C. The total run time was 15.333 min. The MS was operated with an electron impact ionization source in the selected ion scanning mode. The electron energy was 70 eV, and the temperature of the ion source, quadrupole and interface were 230, 150 and 280°C, respectively. The analysis was performed with a solvent delay of 5 min to prevent instrument damage (Table II).

**Sample preparation**

A dispersive-SPE sample preparation method was used to extract and clean up the target compound. For the extraction procedure, 10.0 g of...
the homogenized sample was weighed in a 50-mL teflon centrifuge tube and then 20 mL acetone-ethyl acetate (1:1, v/v) was added. The sample was then extracted for 10 min by a powerful oscillator. After the addition of 3.5 g MgSO4 and 3.5 g NaCl, the mixture was vortexed for 1 min, and then centrifuged for 3 min at 5,000 rpm. During the clean-up step, a 10-mL upper organic layer was transferred into a 15-mL teflon centrifuge tube and then cleaned up using dispersive-SPE with 350 mg PSA, 50 mg GCB and 500 mg MgSO4. The tube was vortexed for 90 s and centrifuged for 3 min at 5,000 rpm. After dispersive-SPE procedures, a 10 mL of the supernatant was transferred to a 25-mL round-bottomed flask and was then concentrated to almost dryness on a rotary vacuum evaporator below 40°C. The residues were dissolved with 2 mL acetone. The content was filtered through a 0.22-µm nylon membrane filter and was then injected into the GC–MS system for analysis.

**Results**

There are various factors that affect the extraction process. They are selection of a suitable extraction solvent, selection of suitable adsorbents, selection of the amount of adsorbents, the salt effect and so on. It is very important to optimize them in order to obtain the good recovery strategy forms.

**Optimization of the extraction solvent**

Selection of an extraction solvent is beneficial to improve the process efficiency of the analyte. In order to choose an appropriate extracting solvent, acetonitrile (AN), hexane (Hex), hexane–ethyl acetate (Hex-EAC, 1:1, v/v), acetone–ethyl acetate (AC-EAC, 1:1, v/v) were compared for solvent extraction of a blank sample of homogenized cabbage spiked with the target analyte at a concentration 0.01 mg/kg. The experiments were performed as mentioned in sample preparation, except that the type of solvent was varied and the extracts were not purified. In comparison with that obtained using acetonitrile, hexane, hexane–ethyl acetate (1:1, v/v), acetone–ethyl acetate (1:1, v/v) provided better extraction efficiency for the target analyte with recoveries of 99.18% (Figure 2), and acetone–ethyl acetate (1:1, v/v) was chosen as the extraction solvent because its good miscibility with acetone. But when acetone was used as the extracting solvent, there were so many impurities in the extract. Therefore, we chose acetone–ethyl acetate (1:1, v/v) as the extraction solvent.

**Evaluation of the salt effect**

Acetone is soluble in water, because acetone can be changed from keto to enol. So when only NaCl was used for salting out, the separation effect of the organic layer and the aqueous layer was poor. MgSO4 was added in to assist the salting out procedure. The suction effect of MgSO4 is fast and complete. MgSO4 is a neutral compound, which is unreactive to various organic compounds.

![Figure 2. Extraction recoveries of dichlofluorane in different solvents.](https://academic.oup.com/chromsci/article-abstract/54/5/858/2240569/860)

**Figure 2.** Extraction recoveries of dichlofluorane in different solvents.

![Figure 3. Purifying effect comparison of the different amount of GCB. This figure is available in black and white in print and in color at JCS online.](https://academic.oup.com/chromsci/article-abstract/54/5/858/2240569/860)

**Figure 3.** Purifying effect comparison of the different amount of GCB. This figure is available in black and white in print and in color at JCS online.
Optimization of adsorbents

Being one of weak anion exchangers, PSA can remove organic acids, sugars, anthocyanins and other plant phenols. GCB can be used to remove pigments, especially chlorophylls. C18 is often used to reduce lipids and non-polar interference. In the research, we investigated the efficiency of these three adsorbents to purify the crude extracts. The

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Figure 4. Purifying colour comparison of the different amount of GCB. This figure is available in black and white in print and in color at JCS online.

Figure 5. Purifying effect comparison of the different amount of PSA. This figure is available in black and white in print and in color at JCS online.
recovery was 120% when C_{18} was added in. The results suggested that C_{18} was not appropriate for purification of the crude extracts. So PSA and GCB were chosen to achieve good purification and recoveries.

**Optimization of the amount of adsorbents**

Different amounts of PSA (0, 50, 100, 150, 200, 250, 300, 350 and 400 mg) and GCB (0, 25, 50, 75 and 100 mg) were tested to evaluate their effects on the recoveries of dichlofluanid. As the dosage of GCB (0, 25, 50, 75 and 100 mg) increased, the recoveries of dichlofluanid were unchanged. So, different amounts of GCB (0, 25, 50, 75 and 100 mg) were used to purify the crude extracts of the blank samples of homogenized cabbage. As shown in Figure 3, when the amount of PSA kept unchanged, the purifying effects of different amounts of GCB (0, 25, 50, 75 and 100 mg) were the same. However, the purifying colours (Figure 4) of different amounts of GCB (0, 25, 50, 75 and 100 mg) showed that the purification colours of the amount of GCB (0, 25 mg) were greener than the others. In contrast, when the amount of PSA was 300 mg or higher and the amount of GCB kept unchanged, the purification effect is better than the others (Figure 5). Considering the supplies, 300 mg PSA and 50 mg GCB were the best combination for purification of the crude extracts.

**Discussion**

In order to determine the linearity of the dichlofluanid, a series of standard solutions (0.02, 0.05, 0.10, 0.20, 0.50, 1.00 and 2.00 mg/L) were tested under the optimum conditions. Good linearity (y = 2.7E + 5x−2710.5) and correlation coefficient (R² = 0.9994) were achieved for the compound studied. The detection limit of dichlofluanid was 0.13 μg/kg as the signal-to-noise (S/N) ratio was of 3. The quantification effect is better than the others (Figure 5). Considering the supplies, 300 mg PSA and 50 mg GCB were the best combination for purification of the crude extracts.

**Conclusion**

A new method, based on a dispersive-SPE extraction procedure and GC-MS, has been developed for the determination of dichlofluanid. The extraction procedure is quick, effective and cheap high through-put. The results showed the suitability of this procedure for monitoring dichlofluanid residues in vegetables in a single run and for providing data of the occurrence of the compound in a wide range of vegetables.

**Acknowledgments**

This work was supported by food and agricultural research fund project (No. csc2013yykB0165) and youth science and technology talents fund project (No. csc2014jrc-qnre00002) of Chongqing Municipal Science and Technology Commission of the People’s Republic of China.

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