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# Simultaneous determination of sixteen amide fungicides in vegetables and fruits by dispersive solid phase extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

A modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method using multi-walled carbon nanotubes (MWCNTs) as a reversed-dispersive solid phase extraction (r-dSPE) material combined with ultra-high liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) was developed for the simultaneous determination of 16 novel amide fungicides in vegetables and fruits. After extraction with acetonitrile, a dSPE cleanup procedure, which was developed after the optimization of the type and amount of MWCNTs, the pH value of the extract, the extraction time for MWCNTs, and the type of eluent with MWCNTs material, was conducted. The determination of the target compounds was conducted in less than 7.0 min while the specificity is ensured through the MRM acquisition mode. The linearity of the analytical response across the studied range of concentrations (0.25–500 μg/L) was excellent, obtaining correlation coefficients higher than 0.997. The samples were quantified with the matrix matched standard solutions. The average recoveries in cabbage, celery, strawberry, and grape at three spiked levels (0.01, 0.5, and 5.0 mg/kg) were ranged from 72.4 to 98.5% with all RSDs lower than 10%. The limits of detection were below 0.003 mg/kg and the limits of quantification did not exceed 0.01 mg/kg in all matrices. The method demonstrated to be suitable for the simultaneous determination of 16 novel amide fungicides in vegetables and fruits.

## 1. Introduction

Since the first synthesis of carboxin by Schmeling and Kulkain in 1966, amide fungicides have been used for controlling plant diseases for more than 40 years [1]. Recently, many novel amide derivatives (Fig. 1) have been developed and commercialized as fungicides because of their high antifungal activities. In China, seven novel amide fungicides (boscalid, fenoxanil, mandipropamid, zoxamide, fluopicolide, fluopyram, and thifluzamide) have already been registered and widely used in vegetables and fruits [2]. Although these amide fungicides are moderately or low toxicity pesticides for mammals, several authorities around the world have established maximum residue limits (MRLs) in vegetables and fruits to protect consumers [2–4]. Therefore, there is a need for the development of a simple, rapid, specific, inexpensive, and sensitive method to detect the presence of these amide fungicides in vegetables and fruits.

To determine these novel amide fungicides in agricultural products, some enzyme-linked immunosorbent assay (ELISA) [5,6], gas chromatography (GC) [7–9], liquid chromatography (LC) [10], gas chromatography–mass spectrometry (GC–MS) [11–14] and liquid chromatography–mass spectrometry (LC–MS and LC–MS/MS) methods [15–17] have been developed. However, each of ELISA methods can only determine one kind of the novel amide fungicides [5,6]. Moreover, the varieties of the novel amide fungicides are too few (the largest numbers of the novel amide fungicides are usually less than six) for many instrumental methods [11–14,16], which can simultaneous determine multiclass fungicides involved the novel amide fungicides in agricultural products with good sensitivity and accuracy. However, no development of the simultaneous determination method has been reported for those novel amide fungicides (boscalid, fenoxanil, mandipropamid, zoxamide, fluopicolide, fluopyram, and thifluzamide) with MRLs in fruits and vegetables. Recently, although Chen et al. have developed a LC–MS/MS method for determination of six novel amide fungicides

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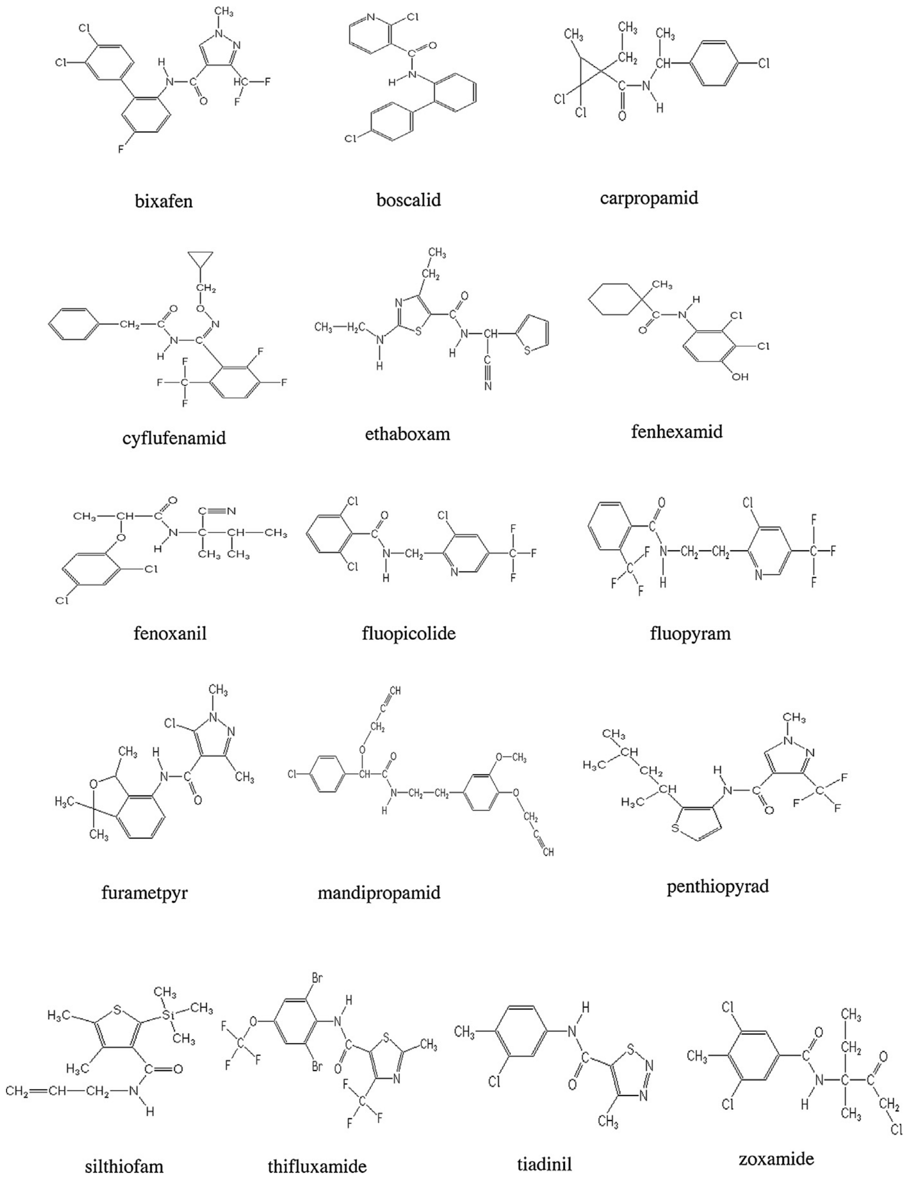

bixafen boscalid carpropamid

cyflufenamid ethaboxam fenhexamid

fenoxanil fluopicolide fluopyram

furametpyr mandipropamid penthiopyrad

silthiofam thifluxamide tiadinil zoxamide

**Fig. 1.** The chemical structures of 16 novel amide fungicides.

(silthiofam, boscalid, fluopicolide, mandipropamid, cyflufenamid, and mepanipyrim) in fruits and vegetables [19], it still cannot simultaneous determination of those amide fungicides with MRLs.

Here, we develop and validate a simple and reliable confirmatory LC–MS/MS analytical method for the analysis of 16 novel amide fungicides including those amide fungicides with MRLs. Moreover, multi-walled carbon nanotubes (MWCNTs) as a dispersive solid phase extraction (dSPE) material has been firstly used for the simultaneous determination of these novel amide fungicides. The type and amount of MWCNTs, the pH value of the extract, the extraction time with the MWCNTs, and the type of eluent have been optimized in this study. After validation studies, the method is suitable for the routine determination of the 16 novel amide fungicides in fruits and vegetables.

## 2. Materials and methods

### 2.1. Materials and reagents

Methanol (LC grade) and acetonitrile (ACN, LC grade) were obtained from Fisher Scientific (Fairlawn, USA). Formic acid (LC grade) was obtained from Tedia Company Inc. (Fairfield, USA). Sodium hydroxide was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Fenoxanil, boscalid, carpropamid, bixafen, silthiofam, thifluzamide (10 µg/L in cyclohexane), fluopyram, tiadinil, ethaboxam (10 µg/L in ACN), mandipropamid, fluopicolide, fenhexamid, cyflufenamid, zoxamide, furametpyr, and penthiopyrad were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Five types of MWCNTs with similar lengths (10–30 µm or 10–20 µm) and different outer diameters (MWCNT01 <8 nm, MWCNT02 = 10–20 nm, MWCNT03 = 20–30 nm, MWCNT04 = 30–50 nm, and MWCNT05 >50 nm) were purchased from Nanjing XF NANO Materials Tech Co. Ltd. (Nanjing, China). The specific surface areas (SSAs) of the MWCNTs were 500, 200, 110, 60, and 40 $m^2$/g for MWCNT01, MWCNT02, MWCNT03, MWCNT04, and MWCNT05, respectively. The water was purified with a Milli-Q reverse osmosis system (Millipore, Milford, Massachusetts, USA).

### 2.2. Standard solutions

Individual stock solutions of 14 compounds (excluding ethaboxam and thifluzamide) were prepared in ACN at a concentration of 1000 µg/ml. One mixed standard solution (10 µg/ml) was