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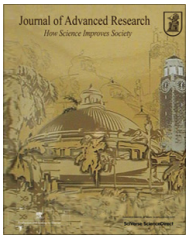

ORIGINAL ARTICLE

Determination of kresoxim-methyl and its thermolabile metabolites in pear utilizing pepper leaf matrix as a protectant using gas chromatography

Md. Musfiqur Rahman [a], Jin Jang [a], Jong-Hyouk Park [a], A.M. Abd El-Aty [b,*], Ah-Young Ko [a], Jeong-Heui Choi [c], Angel Yang [a], Ki Hun Park [a], Jae-Han Shim [a,*]

[a] *Biotechnology Research Institute, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Republic of Korea*
[b] *Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt*
[c] *Institute of Environmental Research, Faculty of Chemistry, Dortmund University of Technology, 44227 Dortmund, Germany*

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ABSTRACT

Kresoxim-methyl and its two thermolabile metabolites, BF 490-2 and BF 490-9, were analyzed in pear using a pepper leaf matrix protection to maintain the metabolites inside the gas chromatography system. Samples were extracted with a mixture of ethyl acetate and *n*-hexane (1:1, v/v) and purified and/or separated using a solid phase extraction procedure. The pepper leaf matrix was added and optimized with cleaned pear extract to enhance metabolite sensitivity. Matrix matched calibration was used for kresoxim-methyl in the pear matrix and for metabolites in the pear mixed with pepper leaf matrix. Good linearity was obtained for all analytes with a coefficient of determination, $r^2 \geqslant 0.992$. Limits of detection (LOD) and quantification (LOQ) were 0.006 and 0.02 mg kg^{-1} and 0.02 and 0.065 mg kg^{-1} for kresoxim-methyl and the metabolites, respectively. Recoveries were carried out at two concentration levels and were 85.6–97.9% with a relative standard deviation $<2.5\%$. The method was successfully applied to field incurred pear samples, and only kresoxim-methyl was detected at a concentration of 0.03 mg kg^{-1}.

Introduction

Kresoxim-methyl(methyl(E)-2-(methoxyimino)-2-[2-(*o*-tolyloxymethyl)phenyl]acetate), a strobilurin fungicide, is used to control powdery mildew and scab in apples, pears, grapes, cucumbers, strawberries, and vegetables [1]. The mode of action of strobilurins is to inhibit mitochondrial respiration by binding to the ubihydroquinone oxidation center of the mitochondrial bc1 complex and thereby blocking electron transfer [2,3]. The major reasons for the success of strobilurins

* Corresponding authors. Tel.: +20 2 27548926; fax: +20 2 35725240 (A.M. Abd El-Aty), Tel.: +82 62 530 2135; fax: +82 62 530 0219 (J.H. Shim).
E-mail addresses: abdelaty44@hotmail.com (A.M. Abd El-Aty), jhshim@chonnam.ac.kr (J.H. Shim).

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vary between individual active ingredients, but consist of one or more of the following: broad spectrum activity, control of fungal isolates resistant to other fungicide modes of action, low use rate, and excellent yield and quality benefits [4]. However, residues may remain in the crops and environment and might constitute a public health hazard to consumers. Thus, residues are regulated in different countries in terms of maximum residue limits (MRLs) to maintain food quality and prevent consumer health problems.

Kresoxim-methyl is registered in the Republic of Korea for application on pear with maximum residue limits of 1.0 mg kg^{-1} [5]. The European Commission has revised the residue definition of kresoxim-methyl and proposed the sum of total kresoxim-methyl and its metabolites, α-[(*o*-hydroxymethyl)phenoxy]-*o*-tolyl(methoxyimino) acetic acid (BF 490-2) and α-(*p*-hydroxy-*o*-tolyloxy)-*o*-tolyl(methoxyimino) acetic acid (BF 490-9) for risk assessment [6]. The chemical structures of kresoxim-methyl and its two metabolites are shown in Fig. 1.

Kresoxim-methyl has been analyzed by gas chromatography/nitrogen phosphorus detector (NPD)/mass spectrometry (MS) or a liquid chromatography/electrospray tandem mass spectrometry in different matrices [7–9]. No analytical method has been reported to analyze the metabolites by gas chromatography until a method was developed for total residue analysis in Korean plum in our laboratory [10]. An unpublished complex analytical method was found to analyze – kresoxim-methyl and its metabolites using liquid chromatography [11]. However, we showed in our previous study that the two metabolites of kresoxim-methyl, BF 490-2 and BF 490-9, had poor responses or peak broadening when injected into GC-μECD in pure solvent. It was really tough to integrate and analyze these types of peak due to higher detection limit and also for overestimation when compared with solvent calibration. Erney and his co-workers explained this overestimation and named it the "matrix-induced response enhancement effect." Finally, they tried to remove this effect using single additive (as a protectant) aiming to protect the analyte in solvent and subsequently equalize the response between solvent and in matrix. However, their efforts were not successful, and they suggested to use matrix matched calibration [12,13]. Anastassiades et al. in 2003 [14] re-introduce the concept of additives as analyte protectant and evaluated 93 compounds with strong hydrogen bonding capability, whereas Mastovska et al. in 2005 [15] determined that a combination of three compounds (ethylglycerol, gulonolactone, and sorbitol) among the 93 compounds provided perfect protection for the thermally affected compounds in gas chromatography-mass spectrometry. However, their application range was limited due to the solubility of the protectant was polar-dependent, here up to 20% water was needed to be mixed with acetonitrile to dissolve them. Furthermore, the applicability of the combined analyte protectant was not examined for other detectors, including ECD (electron-capture detector), FPD (flame photometric detector), or NPD (nitrogen–phosphorus detector). On the other hand, in our early studies, pepper leaf matrix was a promising analyte protectant for thermolabile metabolites such as terbufos metabolites (terbufos sulfoxide and terbufoxon sulfoxide) and kresoxim-methyl metabolites (BF 490-2 and BF-490-9) using a FPD and a ECD, respectively. A pepper leaf matrix was incorporated with the pepper and plum matrix and provided complete protection for the metabolites inside the GC system [16,17].

Therefore, the aim of this study was to adapt and optimize our previous method for analyzing kresoxim-methyl and its metabolites to determine the total field incurred residues in pear.

Material and methods

Chemicals and reagents

Analytical standard kresoxim-methyl (purity 99.9%) and two metabolites, BF 490-2 (purity 94.6%) and BF 490-9 (purity 99.7%), were purchased from Badische Anilin-und Soda-Fabrik (BASF, Seoul, Republic of Korea). High performance liquid chromatography grade ethyl acetate (EtOAc), acetone, and *n*-hexane were supplied by Burdick and Jackson (SK Chemical, Ulsan, Republic of Korea). Anhydrous magnesium sulfate ($MgSO_4$) was of analytical grade and obtained from Junsei Chemicals Co., Ltd. (Tokyo, Japan). A C_{18}-E solid phase extraction (SPE) cartridge (500 mg, 6 mL) was provided by Phenomenex (Torrance, CA, USA).

A standard stock solution of kresoxim-methyl and two metabolites (BF 490-2 and BF 490-9) were prepared individually in EtOAc at a concentration of 100 mg L^{-1} and stored at −24 °C. An intermediate solution was prepared by diluting kresoxim-methyl to 10 mg L^{-1} and mixing metabolites together to attain 10 mg L^{-1} using the same solvent. Finally, intermediate solutions were diluted separately to 0.05 mg L^{-1} using EtOAc to make a working solution. Both intermediate and working solutions were kept in a refrigerator at 4 °C pending analysis.

Field experimental design

As Naju (Southern part of Gwangju, Republic of Korea) is famous for pear cultivation, a field study was conducted at the Naju Agricultural Farm affiliated with Chonnam National University, Gwangju, Republic of Korea. Thirteen mature trees (14 years old) in the same row were selected for applying a commercial pesticide after dividing the rows in different segments for various application times. The experimental

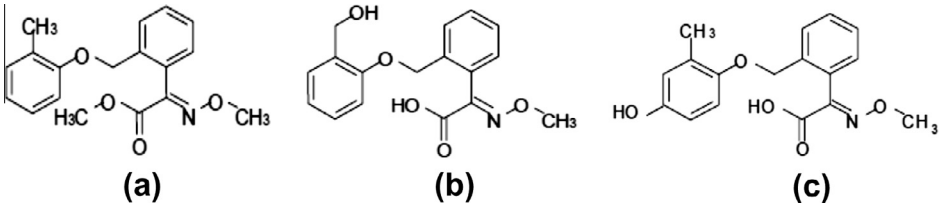

Fig. 1 Chemical structure of kresoxim-methyl (a) and its metabolites BF 490-2 (b) and BF 490-9 (c).