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Analytical Methods

Dynamic behaviour and residual pattern of thiamethoxam and its metabolite clothianidin in Swiss chard using liquid chromatography–tandem mass spectrometry

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ABSTRACT

A simultaneous method was developed to analyse thiamethoxam and its metabolite clothianidin in Swiss chard using tandem mass spectrometry (in the positive electrospray ionisation mode using multiple reaction monitoring mode) to estimate the dissipation pattern and the pre-harvest residue limit (PHRL). Thiamethoxam (10%, WG) was sprayed on Swiss chard grown in two different areas under greenhouse conditions at the recommended dose rate of 10 g/20 L water. Samples were collected randomly up to 14 days post-application, extracted using quick, easy, cheap, effective, rugged, safe (QuEChERS) acetate-buffered method and purified via a dispersive solid phase extraction (d-SPE) procedure. Matrix matched calibration showed good linearity with determination coefficients (R^2) $\geqslant$ 0.998. The limits of detection (LOD) and quantification (LOQ) were 0.007 and 0.02 mg/kg. The method was validated in triplicate at two different spiked concentration levels. Good recoveries ($n = 3$) of 87.48–105.61% with relative standard deviations (RSDs) < 10 were obtained for both analytes. The rate of disappearance of total thiamethoxam residues in/on Swiss chard was best described by first-order kinetics with half-lives of 6.3 and 4.2 days. We predicted from the PHRL curves that if the residues were <19.21 or 26.98 mg/kg at 10 days before harvest, then total thiamethoxam concentrations would be below the maximum residue limits during harvest.

1. Introduction

Leafy vegetables represent a broad variety of plants, including lettuce, cabbage, spinach, leek, kale, and Swiss chard, which form an important component of the human diet. Typically, they are low in calories and fat; however, their protein, dietary fibre, iron, calcium, and phytochemical contents are high (Walorczyk, 2008). Dietary fibre has been recognised as a good source for preventing and treating constipation in humans. Furthermore, it is an important component to reduce the risk of certain diseases, including cancer, cardiovascular disease, and diabetes (O'Sullivan & Cho, 1998). Jenkins et al. (2001) tested the effects of a fruit and vegetable diet and concluded that very high-vegetable fibre intake reduces the risk for cardiovascular diseases and possibly colon cancer. A wide range of pesticides are used to protect vegetables from heavy infestation by pests. Vegetables are consumed raw (after washing with tap water), boiled, or steamed. Overall, leafy vegetables contribute to 2% of total human vegetable consumption but may lead to human health impacts comparable or even higher than ingesting cereals (Fantke, Juraske, Antón, Friedrich, & Jolliet, 2011). Therefore, an analytical approach to determine contaminants in complex matrices such as plant material with improved capabilities, reduced clean-up and concentration steps, few toxic solvents, and improved detection limits is needed (Barriada-Pereira, Serôdio, González-Castro, & Nogueira, 2010).

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The neonicotinoid insecticides thiamethoxam (TMX) and clothianidin (CLO) (Fig. 1), have been used widely for long-term control of a wide variety of suckling insect pests, including Hemiptera, Thysanoptera, Coleoptera, Lepidoptera, and Diptera, with excellent systemic action using a variety of application methods (Uneme, 2011). Besides their beneficial effects to control pests, neonicotinoids may promote growth and protect plants against biotic and abiotic stressors (Ford, Gulevich, Swenson, & Casida, 2011). However, they may induce neurobehavioral alterations in mammals (de Oliveira, Nunes, Barbosa, Pallares, & Faro, 2010). The insecticidal activity of neonicotinoids is primarily attributed to their action on nicotinic acetylcholine receptors (Tomizawa & Casida, 2005). CLO is a commercial insecticide and a TMX metabolite (de Oliveira et al., 2010; Nauen, Ebbinghaus-Kintscher, Salgado, & Kaussmann, 2003). TMX and CLO are structurally similar; however, CLO differs in the TMX oxadiazinane CH_2OCH_2 moiety, which is metabolically labile to methylene hydroxylation leading to partial conversion of TMX to CLO (Ford & Casida, 2006). CLO has a higher lipophilicity than TMX based on water solubility and the partition coefficient (Environmental Protection Agency, 2003).

Information on residue determinations of TMX and CLO in leafy vegetables is very scarce. Watanabe, Kobara, Baba, and Eun (2013) established a water-based extraction and cleanup procedure with two solid-phase extraction cartridges to recover nine hydrophilic pesticides, including acetamiprid, clothianidin, dinotefuran, flonicamid, imidacloprid, methomyl, pymetrozine, thiacloprid, and thiamethoxam from tomatoes, green peppers, and spinach using high performance liquid chromatography-diode array detection. In our previous study, we detected CLO and its metabolites (MNG, TMG, TZMU, and TZNG) in three minor crops, including crown daisy, sedum, and amaranth (Kim et al., 2012). Most research on neonicotinoid residues considers CLO as a parent analyte and does not consider it a metabolite.

The dissipation curves reported in the literature are valid only for a given crop under specific conditions (Hem et al., 2011). Many factors contribute to pesticide deposit and residue dissipation; however, the crop (morphology, cuticle characteristics, stage of growth at treatment, and growth rate) and application method (formulation, rate, water volume, pressure, nozzle type, and boom height above the canopy) are the most important under the same environmental conditions (Ebeling, 1963). Therefore, the maximum residue limit (MRL) regulations require a pre-harvest interval to ensure a decline of pesticide residues to below the proposed MRL at harvest time (Karmakar & Kulshrestha, 2009). The National Agricultural Products Quality Management Service (Gimcheon, Republic of Korea) has set pre-harvest residue limits (PHRL) in relation to pesticide MRLs (established by the Korea Food and Drug Administration) to prevent the distribution of unsuitable agricultural products that may exceed those MRLs (Chang et al., 2011). The PHRL assesses the amount of pesticide residues sprayed during the pre-harvest period and predicts the amount of pesticide residue at harvest time by computing the biological half-life and the decay constant (Chang et al., 2011).

The aim of this study was to detect the residue levels of TMX and its metabolite CLO using the quick, easy, cheap, effective, rugged, safe (QuEChERS) extraction method and liquid chromatography–tandem mass spectrometry (LC/MS/MS) to analyse Swiss chard grown under greenhouse conditions. The dissipation pattern of both analytes was examined in two different areas (Gwangju and Naju) over 14 days to estimate the PHRL.

2. Experimental

2.1. Reagents and chemicals

Standard thiamethoxam (3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1, 3,5-oxadiazinan-4-ylidene (nitro) amine) guanidine; 99.5%) and its metabolite CLO ((E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine; 99.5%), were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC grade acetonitrile (ACN) was obtained from Burdick and Jackson (SK Chemical, Ulsan, Republic of Korea). Analytical-grade anhydrous magnesium sulphate ($MgSO_4$), sodium acetate (NaOAc), and acetic acid were obtained from Junsei Chemicals Co., Ltd. (Tokyo, Japan). Primary secondary amine (PSA), and C_{18} dispersive sorbent was supplied by Agilent Technologies, (Santa Clara, CA, USA). Water was purified through a Milli-Q apparatus (Ultima Duo 200 (COMBI), Balmann Tech, Daegu, Republic of Korea) prior to use.

Stock standard solutions (100 mg/L) of TMX and its metabolite (CLO) were prepared individually in ACN. An intermediate

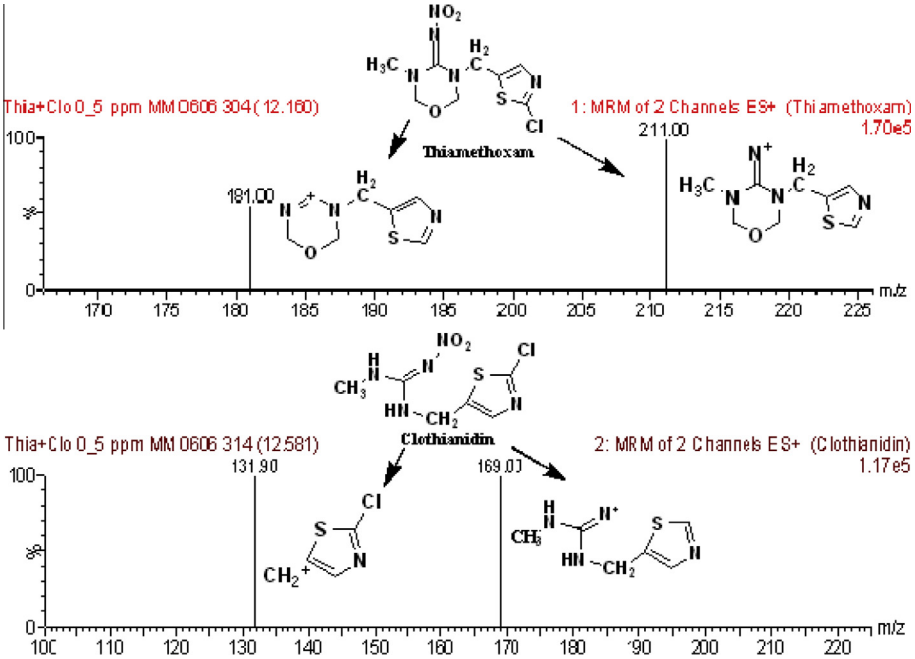

Fig. 1. Fragmentation pattern of thiamethoxam and its metabolite, clothianidin.