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Analytical Methods

Validation of a GC–MS method for the estimation of dithiocarbamate fungicide residues and safety evaluation of mancozeb in fruits and vegetables

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ABSTRACT

A sensitive and rugged residue analysis method was validated for the estimation of dithiocarbamate fungicides in a variety of fruit and vegetable matrices. The sample preparation method involved reaction of dithiocarbamates with Tin(II) chloride in aqueous HCl. The CS_2 produced was absorbed into an isooctane layer and estimated by GC–MS selected ion monitoring. Limit of quantification (LOQ) was $\leqslant$40 μg kg^{-1} for grape, green chilli, tomato, potato, brinjal, pineapple and chayote and the recoveries were within 75–104% (RSD < 15% at LOQ). The method could be satisfactorily applied for analysis of real world samples. Dissipation of mancozeb, the most-used dithiocarbamate fungicide, in field followed first + first order kinetics with pre-harvest intervals of 2 and 4 days in brinjal, 7 and 10 days in grapes and 0 day in chilli at single and double dose of agricultural applications. Cooking practices were effective for removal of mancozeb residues from vegetables.

1. Introduction

Dithiocarbamate (DTC) group represents one of the largest consumed fungicides in agriculture, chiefly because of their broad spectrum biological activity, low production costs and safe mammalian and environmental toxicity profile. Furthermore, due to the possibility of combination with new generation systemic fungicides, on-farm applications of these compounds remain quite successful in management of disease resistance especially in horticultural crops (Rai, Keshavayya, & Rai, 2012; Tripathi, Raja, & Hanif, 2011). Frequent and excess application of such fungicides might result in apprehensions related to their residue accumulations in the commodities at harvest. It is therefore very important to monitor and control the residues of DTC fungicides in food to avoid any hazard to the consumers (http://www.inchem.org/documents/ehc/ehc/ehc78.htm).

Due to the inherent insolubility in common extraction solvents and poor stability, DTCs are not amenable to multi-residue extraction with other group of pesticides. Therefore, its analysis involves conversion into carbon disulfide (CS_2) in an acid medium and subsequent estimation using spectroscopy (Caldas, Conceicao, Miranda, de Souza, & Lima, 2001; Keppel, 1971; Schwack, & Nyanzi, 1994), and head space gas chromatography (GC) (Ahmad et al., 1996; Perz, Lishaut, & Schwack, 2000). A microwave assisted extraction method has also been reported (Vryzas, Papadakis, Papadopoulou-Mourkidou, 2002).

Several authors reported estimation of DTCs using GC with specific detectors like electron capture detector (ECD), flame atomic absorption spectroscopic detector (FAAS) and flame photometric detection (FPD with sulfur filter) (Dubey, Heberer, & Stan, 1997; Pizzutti, Vareli, Da Silva, & de Kok, 2008; Qin, Qiao, Wang, & Zhao, 2010; Türker and Sezer, 2005). In these methods, DTC residues are decomposed to CS_2 by reaction with $SnCl_2$–HCl solution. The liberated CS_2 is absorbed in hexane, and then determined by GC with selective detectors. However, on the basis of only retention time criterion, these methods cannot distinguish the signal of CS_2 from any interfering signals originating from matrix and therefore may lead to ambiguous or false positive results. Although the methods for selective determination of DTC residues is reported using LC-APCI-MS (Blasco, Font, & Picó, 2004), DART–TOFMS (Cajka, Riddellova, Zomer, Mol, & Hajslova, 2011), and LC-diode array detection (López-Fernández, Rial-Otero, González-Barreiro, & Simal-Gándara, 2012), the DTC residues are prone to degradation and such methods are difficult to adopt for routine analysis due to inherent complexity.

Looking at the drawbacks of the methods described above, it was felt necessary to optimise a sample preparation method followed by estimation using GC–MS commonly available in most

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of the food testing laboratories across the world. The applicability of the method was tested in a variety of fruits and vegetable matrices viz. grape, green chilli, potato, tomato and brinjal (eggplant) at the National Referral Laboratory (NRL), NRC Grapes, Pune, India. The reproducibility of the method was further assessed in pineapple, tomato and chayote at the residue laboratory in the Centro de Investigación en Contaminación Ambiental (CICA) located at the University of Costa Rica. The validated method was successfully applied to study the dissipation of mancozeb in grapes, brinjal and green chilli in field conditions. This study will ensure accurate measurement of dithiocarbamate residues and help in generating the pre-harvest intervals (PHIs) of these fungicides in different crops.

2. Materials and methods

2.1. Chemicals

Certified reference standard of thiram (99.5% purity for calibration purpose) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Residue analysis grade ethyl acetate (dried) was obtained from Thomas Baker (Mumbai, India). Carbon disulfide (CS_2) with 99.9% purity was obtained from Sigma–Aldrich, Poole, UK. Tin(II) chloride (assay:min 97%) was obtained from Fisher Scientific, Powai, Mumbai. Hydrochloric acid (35% GR) and isooctane (assay $\geqslant$99.5%) were obtained from Merck India Ltd., Mumbai.

2.2. Apparatus

The equipments used in sample preparation included a precision balance (ADGR-202, Adair Dutt, Tokyo, Japan), high speed refrigerated centrifuge (6500 Kubota, Tokyo, Japan), water bath (BW-20G Jeio Tech, Kyunggi-dQ, Korea), and a micro-centrifuge (Microcentrifuge Pico, Kendro, D-37520, Osterode, Germany).

A Trace GC Ultra quipped with Triplus autosampler and hyphenated to ITQ 900 mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA) was used for analysis. The separation of CS_2 was performed on a DB-5MS (5% diphenyl/95% dimethylpolysiloxane, 30 m $\times$ 0.25 mm ID, 0.25 μm film thickness) capillary column. Ultra-pure grade helium was used as carrier gas and maintained at flow rate of 1 ml min^{-1}. The oven temperature program was started from an initial temperature of 40 °C (hold for 5 min), ramped at the rate of 40 °C min^{-1} up to 200 °C (hold 5 min),with a total run time of 14 min. A typical GC–MS batch consisted of matrix-matched calibration standards, samples, one matrix blank and one recovery sample for performance check after a set of every six samples. The detector voltage was set at 1500 V and the data acquisition was carried out in selected ion monitoring (SIM) mode with compound specific *m/z* of 76 and 78 with ion ratio of 100:10 for confirmatory identification of CS_2. Other parameters included transfer line temperature of 285 °C and ion source temperature of 230 °C. The ion source temperature was varied in the range of 170 and 230 °C for signal optimisation of the selected ions. It was observed that at 170 and 200 °C, the *S*/*N* were 67% and 70%, respectively, in comparison to 230 °C and therefore, this temperature was selected as the optimised ion source temperature. The damping gas flow was set at 0.6 ml min^{-1} and emission current was 250 μA. The acquisition was stopped after 2 min in order to increase the life span of the filament.

The temperature program in the programmable temperature vaporizer (PTV) started from 40 °C (hold for 0.10 min at 100 kPa injection pressure) and ramped at 10 °C s^{-1} up to 80 °C (hold 0.3 min, at evaporation pressure of 200 kPa). In the transfer phase, the temperature was ramped at 10 °C s^{-1} up to 110 °C (hold time 0.50 min) and finally in the cleaning phase @ 14.5 °C s^{-1} to 290 °C. The split flow was maintained @ 20 ml min^{-1} with sampling time of 0.5 min. The solvent vent valve was open until 0.17 min, after which the valve was closed for 4 min and then kept open for rest of the run time.

In split mode, the injection was done at 150 °C at the split flow 20 ml min^{-1} maintaining split ratio of 20. The solvent valve temperature was 100 °C. In case of PTV and split mode, a sample volume of 4 μL was injected.

In the pesticide residue laboratory at CICA, Costa Rica, a 6890 N Network GC System and 5975B inert Series MSD equipped with a 7683B autosampler (Agilent Technologies, Wilmington, DE, USA) was used for the reproducibility study of the method. The separation of CS_2 was performed on a HP-5MS (5% phenyl/95% dimethylpolysiloxane, 30 m $\times$ 0.25 mm ID, 0.25 μm film thickness) capillary column coupled with a 5 m guard column. Ultra-pure grade helium (99.999%) was used as carrier gas and maintained at a flow rate of 1.1 ml min^{-1}. The oven temperature program and the typical GC–MS batch used by the NRL of India were directly replicated at CICA. The quadrupole was also operated in SIM mode as described above. The mass detector voltage was set at 1647 V. The transfer line temperature was 290 °C. The solvent delay was set to 0.0 min and the acquisition was stopped after 3 min. The initial settings of the splitless method included the injection volume of 1 μL, injector temperature 150 °C, saver time 2 min and head pressure 56 kPa.

2.3. Preparation of standard solutions and reaction mixture

2.3.1. Carbon disulphide standard solution

The stock solution of CS_2 (2000 μg ml^{-1}) was prepared by accurately pipetting out 79 μL of CS_2 into a volumetric flask (certified A class, 50 ml) containing approximately 45 ml of isooctane, which was made up to 50 ml with isooctane. The CS_2 stock solution was kept in refrigerator at −20 °C and used within two days of preparation. CS_2 working standard solutions of 200 and 20 μg ml^{-1} concentrations (10 ml each) were prepared by serial dilution of the stock solution with isooctane.

2.3.2. Standard solution of thiram

Thiram 10 (±0.05) mg was weighed into a 10 ml volumetric flask (certified A class) and dissolved in ethyl acetate up to the mark to get a stock solution of 1000 μg ml^{-1} concentration. A 100 μg ml^{-1} thiram working standard was prepared from the stock solution by dilution.

2.3.3. Preparation of reaction mixture

Tin(II) chloride (30 g) was accurately weighed in a 1000 ml volumetric flask (certified A class) to which 1000 ml of concentrated HCl (35%) was added. Then the solution was gradually added to 1000 ml water with continuous stirring to get a clear solution.

2.4. Calibration

Calibration standard solutions of CS_2 at six different concentration levels (0.04, 0.08, 0.16, 0.32, 0.64 and 1.3 μg ml^{-1}) were prepared by appropriate dilutions of CS_2 working standard (20 μg ml^{-1}) in isooctane. Matrix matched standards at the same concentrations were prepared by spiking the isooctane extract of fresh control materials of the test commodities (all organically grown) obtained using the procedure as described in Section 2.6 with appropriate volumes of 20 μg ml^{-1} CS_2 working standard.

2.5. Field studies on persistence and dissipation of mancozeb

Field trials were conducted on grape, brinjal and green chilli to evaluate the dissipation kinetics of mancozeb. These crops were