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Water Based Microwave Assisted Extraction of Thiamethoxam Residues from Vegetables and Soil for Determination by HPLC

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Abstract A microwave assisted extraction (MAE) method for determination of thiamethoxam residues in vegetable and soil samples was standardized. Insecticide spiked vegetable and soil samples were extracted by MAE using water as an extraction solvent, cleaned up by solid phase extraction and analysed by high performance liquid chromatography on photodiode array detector. The recoveries of the insecticide from various vegetable (tomato, radish, brinjal, okra, French been, sugarbeet) and soil (sandy loam, silty clay loam, sandy clay loam, loamy sand) samples at 0.1 and 0.5 μg g^{-1} spiking levels ranged from 79.8% to 86.2% and from 82.1% to 87.0%, respectively. The recoveries by MAE were comparable to those obtained by the conventional blender and shake-flask extraction techniques. The precision of the MAE method was demonstrated by relative standard deviations of <3% for the insecticide.

Keywords Microwave assisted extraction · Thiamethoxam · Vegetables · Soil · HPLC

Thiamethoxam [(*EZ*)-3-(2-chloro-1, 3-thiazol-5-ylmethyl)-5-methyl-1, 3, 5-oxadiazinan-4-ylidene (nitro) amine], is a neonicotinoid insecticide with broad spectrum activity against insect pests for use in various crops including vegetables (Maienfisch et al. 2001). Residues of this insecticide in agricultural samples have been analysed by High performance liquid chromatography (HPLC) with photodiode array (PDA) detector (Ying and Kookana 2004). The sample preparation for residue analysis is a multistep analytical procedure involving extraction of the insecticide from matrix with organic solvent, concentration, partitioning and cleanup for separation of analyte from interfering coextractives prior estimation by HPLC (Karmakar and Kulshrestha 2009). Thiamethoxam residues were extracted from vegetable and soil samples using polar solvent by blending and shake-flask method, respectively (Karmakar et al. 2005). Partitioning with non polar solvent has proved efficient cleanup method in analysis of this insecticide. In general, these methods are lengthy, time consuming and make use of organic solvent which is of great environmental concern.

The microwave-assisted extraction (MAE) has been found useful for the extraction of organochlorine and few other pesticides from environmental samples (Concha-Grana et al. 2003; Barriada-Pereira et al. 2005) using organic solvents. Water has been suggested as a good alternative to organic solvents as the extractant for MAE of triazines from soil (Xiong et al. 1999). The solid-phase extraction (SPE) cartridges for neonicotinoid insecticides demonstrated higher cleanup efficiencies and smaller organic solvent consumptions than classical methods of liquid–liquid partitioning or column chromatography (Obana et al. 2003).

The paper here describes an effective MAE of thiamethoxam from various vegetable and soil samples using water as an extraction solvent followed with SPE cleanup and estimation by HPLC. This work represents first research report on the development of the method using water as an extractant for MAE of thiamethoxam.

Materials and Methods

The analytical standard of thiamethoxam (>98% purity) was procured from M/s Syngenta India Ltd. The solvents

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and reagents used were analytical grade. The HPLC grade water and acetonitrile; and Lichrolute® RP-18 solid phase extraction cartridges (3 mL) were purchased from Merck, India Ltd. Distilled deionized water was used for extraction in MAE. A stock solution of thiamethoxam was prepared in acetonitrile (100 μg mL^{-1}) and was used to prepare working standards of different concentrations by serial dilution.

Vegetables viz. tomato, radish, brinjal, okra, French bean and sugarbeet; and sandy loam soil samples were collected from control experimental plots of the farm at Indian Agricultural Research Institute (IARI), New Delhi. Remaining three soils (silty clay loam, sandy clay loam and loamy sand) were obtained from untreated agricultural fields from Bangalore, Pantnagar and Punjab, respectively in India. The vegetable/soil samples (50 g) in Erlenmeyer flasks were spiked with acetone solution of thiamethoxam (50 μg mL^{-1}) to obtain the desired concentration and allowed to stand for 4 h prior analysis.

For MAE, the spiked/control vegetable/soil sample (10 g) was taken in a glass beaker (100 mL) to which 40 mL distilled deionized water was added and the contents mixed with a glass rod. The beaker covered with a petriplate was placed in the centre of the platform of the commercial household microwave oven (Samsung model CE118KF) operated with a 60 Hz output of 1,000 W. The contents in the beaker were exposed to microwave energy for 30 s at 50% power setting. After exposure, contents of the beaker were cooled by keeping in refrigerator and again exposed to microwave energy for 30 s. Finally the contents were cooled to room temperature and centrifuged at 3,000 rpm on a centrifuge (Remi Model R 24) for 10 min. An aliquot (10 mL) of the supernatant after centrifugation equivalent to 2.5 g of substrate was used for cleanup by hexane partitioning or solid-phase extraction (SPE) cartridge. The aqueous solution (10 mL) was transferred to a separatory funnel and partitioned with hexane (3 × 20 mL). The hexane fraction was discarded. For solid-phase extraction, SPE cartridge was first conditioned with acetonitrile (2 mL) followed by equilibration with distilled deionized water (3 mL). The extract (10 mL) was loaded on the cartridge column with a dropper at a slow speed. The loaded cartridge was washed with distilled water (5 mL) and eluted with acetonitrile making the final volume to 1 mL. Triplicate samples were taken for each analysis.

For conventional extraction method, the spiked/control vegetable sample (20 g) mixed with anhydrous sodium sulfate (10 g) was blended with acetone (50 mL) for 5 min. The contents were filtered under suction. The extraction was repeated twice (2 × 50 mL) more. The combined extract was concentrated on a rotary vacuum evaporator under suction to ~5 mL. The soil sample (20 g) in an Erlenmeyer flask was extracted with acetone (3 × 50 mL) by shake-flask method on a horizontal shaker for 30 min, filtered using vacuum and the combined extract was concentrated on a rotary vacuum evaporator (30°C) under suction to ~5 mL. The concentrate was transferred with the help of aqueous sodium chloride solution (15%, w/v) to a separatory funnel and partitioned with hexane (3 × 20 mL). The hexane fraction was discarded. The aqueous layer was then partitioned three times with dichloromethane (3 × 20 mL). The organic phase was passed through anhydrous sodium sulphate (5 g) and evaporated on rotary vacuum evaporator (30°C) to dryness. The residue was dissolved in acetonitrile and volume made prior injection in HPLC. Triplicate samples were processed for each analysis.

A reverse phase LC system (Hewlett Packard model 1100) equipped with quaternary pump, degasser, a photodiode array detector set at λ 254 nm, a computer (model Vectra) and a Rheodyne injector was used. Lichrosphere on RP-18 packed stainless steel column (25 cm × 4 mm i.d.) at ambient temperature was used as stationary phase. The mobile phase was acetonitrile : water gradient maintained at a flow rate of 1.0 mL min^{-1}. Gradient programming starting from 85% water : acetonitrile to 60% water : acetonitrile within 10 min followed by 10% water : acetonitrile was used for the best resolution and separation of parent compound and peaks of coextractives in plant extracts, if any. A 20 μL aliquot from each sample solution was injected in the HPLC, chromatograms recorded in a Window 95 based HP Chemstation programme and the detector response was measured in terms of peak areas. The concentration of the insecticide in the extract was determined by comparing the peak area with that of analytical standard. Mass spectral analysis was performed using Agilent 1100 series LC-MS (VWD-G1314A, ALS-G1313A) instrument with ESI (+) mode. The LC conditions were similar to the HPLC.

The Instrumental detection limit (IDL), the minimum concentration of insecticide that could be reliably detected by the HPLC system under the stated conditions of analysis, for thiamethoxam was estimated through 10 repetitive injections of a standard solution containing 0.5 mg L^{-1} as follows:

$$\begin{aligned}&\mathrm{IDL}(\mathrm{mg\,L^{-1}})\\&\quad=[\mathrm{SD}\times t_{95}\times\text{concentration of standard injected}]/\\&\qquad\text{average peak area of ten repetitive injections}\end{aligned}\tag{1}$$

Where, SD is the standard deviation of the peak areas for the replicate injections and t_{95} is the student's t at 95% level of confidence. The calculated value was confirmed by injecting a standard solution of IDL concentration. Sensitivity (S) was calculated from IDL taking into account the amount (20 μL) injected in the HPLC.

The estimated method detection limits (EMDL), the minimum concentration of a pesticide that can be determined from a particular matrix by a particular method, for thiamethoxam was estimated from IDL as follows:

$$\mathrm{EMDL}\left(\mathrm{mg\,kg^{-1}}\right) = (\mathrm{IDL} \times \mathrm{V} \times 100)/\mathrm{M} \times \%\mathrm{R} \qquad (2)$$

Where, M is the mass of the sample (g), % R is the average percent recovery of the pesticide in the method for the matrix and V is the final volume made for analysis. The calculated EMDL values were further confirmed by actually spiking triplicate samples of vegetable/soil at these levels and analyzing by the MAE procedure described.

Results and Discussion

Under the described HPLC conditions, thiamethoxam resolved as a separate sharp peak at retention time (R_t) 6.40 min. The Instrumental detection limit (IDL) was estimated as 0.02 μg mL^{-1} of thiamethoxam solution. The peak obtained was reliable having a signal to noise ratio 3:1. The sensitivity of the instrument was found to be 0.4 ng of thiamethoxam.

MA extraction was carried out in a commercial household microwave oven in a glass beaker covered with a petriplate. MAE was tried for 30, 60, 90 and 120 s at 50 and 70% power. Heating at 50% power was preferred because the higher power setting often caused a rapid boiling and spilling that resulted in the loss of the sample. The time required to achieve quantitative recoveries was chosen as 2 × 30 s and the results were comparable with those obtained at longer times. Therefore, the choice of 2 × 30 s at 50% power setting for microwave extraction was selected for thiamethoxam from these two matrices under study. The blank sample of thiamethoxam in water on similar microwave exposure (30 + 30 s) followed by HPLC analysis showed a single peak in the chromatogram at R_t 6.40 min. MS spectra of this sample gave molecular ion peak at 291.8 (M^+) with other fragment ion peaks at 245.8 (M^+–NO_2), 211.0 (M^+–Cl and –NO_2) and 132.0 (M^+–$C_4N_4O_3H_7$) confirming that microwave exposure did not cause degradation of the parent compound. Stability of thiamethoxam at these microwave conditions was earlier reported (Singh et al. 2004).

Initially, microwave extraction conditions were optimized, by spiking control samples of tomato and sandy loam soil with insecticide at 1 μg g^{-1} level and MA extraction with water followed with conventional hexane partitioning clean up. The mean recoveries of thiamethoxam from these two matrices were 72.2% and 76.8%, respectively. Recoveries ranging from 57.6% to 102% for carbamate and urea pesticides; and from 87.7% to 100% for neonicotinoid insecticides from fresh and processed tomatoes have been reported using MAE with acetone (Singh et al. 2004; Paiga et al. 2009). In the present study, water proved an effective MAE extraction solvent in place of organic solvent. MAE using an aqueous medium and cleanup by partition on hexane has been earlier reported for determination of pesticides in agricultural soil (Fuentes et al. 2006). In our subsequent study, MAE using water was carried out at two fortification levels of 0.1 and 0.5 μg g^{-1} where cleanup by partition on hexane was replaced with SPE cleanup. This improved the insecticide recovery by 10% to 12% and recoveries ranged from 82.9% to 86.2% for tomato and 82.1% to 87.3% for sandy loam soil. The developed MAE was found efficient and rapid sample preparation technique for determination of thiamethoxam in tomato and soil.

Table 1 summarizes the recoveries of thiamethoxam from spiked tomato, radish, brinjal, okra, French been and sugarbeet at the 0.1 and 0.5 μg g^{-1} levels. The mean recoveries of the pesticide by MAE for various vegetables ranged from 79.8% to 86.2%, respectively. Table 1 also shows that recovery of thiamethoxam in vegetables by MAE favourably compared to those obtained by the conventional blender extraction technique. The recoveries obtained from tomatoes, radish, brinjal, okra, French been and sugarbeet spiked at 0.1 and 0.5 μg g^{-1} levels by the later technique ranged from 80.5% to 92.1%. Using similar MAE conditions and extraction with acetone, recoveries of thiamethoxam and imidacloprid from the fortified vegetable (cabbage, tomato, chilies, potatoes and peppers) samples ranged from 76.3% to 106% and were comparable to those obtained (76.1%–97.5%) by the conventional blender extraction technique (Singh et al. 2004). The blender extraction method is considered similar to those normally used for the routine residue analysis of this pesticide from vegetable crops. However, when compared to MAE, the blender extraction method is time consuming and uses a considerably large volume of solvent.

As seen in Table 2, the recoveries of thiamethoxam after MAE from sandy loam, silty clay loam, sandy clay loam and loamy sand at 0.1 and 0.5 μg g^{-1} levels ranged from 82.1% to 87.3%. The recoveries obtained from sandy loam, silty clay loam, sandy clay loam and loamy sand spiked at 0.1 and 0.5 μg g^{-1} levels by the pesticide and subjected to shake-flask extraction ranged from 83.2% to 93.6%. MAE was reported as effective as shake-flask method for the extraction of sulfonylurea pesticides from agricultural soil for HPLC analysis (Singh and Kulshrestha 2007). The results in Tables 1 and 2 illustrate the effectiveness of MAE in obtaining satisfactory recoveries of this pesticide from the vegetable and soil samples used in this study. Overall, the precision can be described by a relative standard deviation (RSD) of <3% for thiamethoxam used in this study.

Table 1 Recovery of thiamethoxam from spiked vegetable samples using MAE and blender extraction method

Vegetable	Spiking level ($\mu g\ g^{-1}$)	Average percent recovery ± SD (%RSD) (n = 3)[a]	
		MAE	Blender extraction
Tomato	0.1	82.88 ± 0.9 (1.07)	88.28 ± 1.4 (1.59)
	0.5	86.23 ± 0.5 (0.58)	91.88 ± 0.5 (0.54)
Radish	0.1	80.34 ± 0.7 (0.87)	86.17 ± 0.8 (0.93)
	0.5	82.88 ± 1.0 (1.21)	88.13 ± 1.0 (1.13)
Brinjal	0.1	81.15 ± 1.05 (1.23)	90.07 ± 0.7 (0.78)
	0.5	84.44 ± 1.0 (1.18)	87.23 ± 1.0 (1.15)
Okra	0.1	79.76 ± 1.6 (1.99)	88.12 ± 0.8 (0.91)
	0.5	80.45 ± 2.0 (2.40)	80.50 ± 2.0 (2.48)
French bean	0.1	81.56 ± 0.4 (0.49)	83.64 ± 0.8 (0.96)
	0.5	82.83 ± 1.0 (1.21)	81.83 ± 1.0 (1.22)
Sugarbeet	0.1	80.12 ± 0.65 (0.78)	92.13 ± 1.2 (1.30)
	0.5	81.70 ± 2.0 (2.29)	82.20 ± 2.0 (2.43)

%RSD percent relative standard deviation

[a] Number of replicates; *SD* standard deviation

Table 2 Recovery of thiamethoxam from spiked soil samples using MAE and shake- flask extraction method

Soil	Spiking level ($\mu g\ g^{-1}$)	Average percent recovery ± SD (%RSD) (n = 3)[a]	
		MAE	Shake-flask extraction
Sandy loam	0.1	82.05 ± 1.3 (1.55)	89.03 ± 0.2 (0.22)
	0.5	87.27 ± 0.4 (0.49)	90.30 ± 0.3 (0.33)
Silty clay loam	0.1	83.20 ± 0.4 (0.51)	83.22 ± 0.5 (0.60)
	0.5	87.03 ± 0.8 (0.92)	89.00 ± 1.0 (1.12)
Sandy clay loam	0.1	82.21 ± 0.4 (0.49)	88.12 ± 0.8 (0.91)
	0.5	84.04 ± 0.8 (0.89)	84.43 ± 1.0 (1.18)
Loamy sand	0.1	82.12 ± 1.0 (1.20)	87.10 ± 0.7 (0.80)
	0.5	83.50 ± 0.8 (0.89)	93.60 ± 1.0 (1.07)

%RSD percent relative standard deviation

[a] Number of replicates; *SD* standard deviation

A commercial household microwave unit was used in our study. The main advantage of using MAE, particularly in the developing countries, will be the low cost of the equipment. Earlier it has been shown that MAE extraction using a household microwave unit is a viable alternative to soxhlet extraction (SE) of chlorothalonil in coffee beans (Negeri et al. 2000), fenitrothion in white and black beans (Diagne et al. 2002) and polychlorinated biphenyls (PCBs) and chlorobenzenes (CBzs) in fly ash (Yifei et al. 2005). The SE method, however, when compared to MAE, is more time consuming and uses a large volume of solvent. It was also reported that the use of MAE and accelerated solvent extraction (ASE) in the extraction of chlorothalonil from coffee beans was more convenient, faster, and less solvent intensive relative to SE (Negeri et al. 2000). However, the high cost of the ASE instrument, particularly in the developing countries, was considered prohibitive for laboratories budgets. The extraction time and organic solvent consumption are greatly reduced with MAE compared with SE. This makes MAE a viable alternative to the commonly used SE for the extraction of pesticide residues from matrix low in moisture content.

Table 3 Estimated method detection limit (EMDL) of thiamethoxam for different vegetables and soils by HPLC

Vegetable	EMDL ($\mu g\ g^{-1}$)	Soil	EMDL ($\mu g\ g^{-1}$)
Tomato	0.030	Sandy loam	0.028
Okra	0.052	Silty clay loam	0.029
French bean	0.038	Sandy clay loam	0.028
Radish	0.028	Loamy sand	0.030
Brinjal	0.029		
Sugarbeet	0.038		

Table 3 shows estimated method detection limits (EMDLs) of thiamethoxam for various vegetables and soils calculated at 0.1 $\mu g\ g^{-1}$ level. By comparison with the pure standard solutions, the vegetable/soil-containing samples showed matrix diminishment effects for the pesticide under study. To prove the practicality of the EMDL, triplicate sub-samples of each untreated control vegetable and soil sample was spiked at the MDL, the spiked sample was extracted by the described MAE procedure and analysed on the HPLC instrument. Well defined chromatographic peaks proved the validity of the calculated MDL. Samples with pesticide concentrations lower than the MDLs were considered not quantifiable. The codex maximum residue limits (MRLs) of thiamethoxam for all vegetables are not available. US EPA has established MRL of 0.2 $\mu g\ g^{-1}$ for okra and brinjal; and Japan has fixed MRL of 0.5 $\mu g\ g^{-1}$ for tomato for export reasons (US FAO 2004; Karmakar and Kulshrestha 2009). The MDLs of the vegetables were well below their prescribed MRLs, suggesting suitability of the MAE method for routine analysis of thiamethoxam residues in vegetables.

The method presented here demonstrated that MAE is an efficient tool for extraction of thiamethoxam residues from vegetable and soil samples without showing strong matrix effects normally observed in blender extraction and SE. It suggested water, as a cheap, safe and environmentally friendly solvent that can be a good alternative to organic solvent as MAE extractant for these matrices. MAE process described here is simple, cost effective and

less time consuming procedure. This method provides an approach with detection limits at sub parts per million concentrations and could be extended to additional crops and pesticides present at very low concentrations.

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