Research Article

Method Validation for the Extraction and Analysis of Spiromesifen in Cabbage, Tomato and Soil by GC and GC-MS

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Abstract

Spiromesifen (3-mesityl-2-oxo-1-oxaspiro [4.4] non-3-en-4-yl 3, 3-dimethylbutyrate) is a novel miticide/ insecticide that belong to the chemical class of spirocyclic phenyl tetronic acid. An analytical method was validated for the analysis of spiromesifen in cabbage, tomato and soil using gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). The sample extraction was carried out using acetonitrile and dispersive solid phase extraction (d-SPE) clean-up was carried out using primary secondary amine (PSA). The recoveries were in the range of 72.56-103.37% with relative standard deviation (RSD) of 2.7-9.4%. The Limit of detection (LOD) was 0.003 µg mL^{-1}. The limit of quantification (LOQ) of the method, the lowest concentration at which the compounds could be analyzed in the samples, was 0.01 mg kg^{-1}, respectively. The measurement uncertainty was within 9.9-14.9%, indicating the true value remained within this range.

Keywords: spiromesifen, QuEChERS, gas chromatography (GC), gas chromatography mass spectrometry (GC-MS), method validation.

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Introduction

Spiromesifen is a non-systemic insecticidal/miticidal compound belonging to the chemical class of spirocyclic phenyl substituted tetronic acid. It is an effective compound for controlling whiteflies (*Bemisia* and *Trialeuroides spp*.) and numerous mite species (*Tetranychus* and *Panonychus spp*.) [1, 2]. It controls insects and mites which are resistance to other insecticide/miticide [3]. It is a lipid biosynthesis inhibitor, acts by inhibiting lipid metabolism enzyme acetyl Co-A carboxylase [4]. It does not exhibit cross resistance to the more commonly used neonicotinoids [5].

Cabbage (*Brassica oleracea*) is an annually grown leafy vegetable. The annual production of cabbage is 3 million metric tons [6]. Cabbage reported to have anti-carcinogenic agent. It is a good source of vitamin C, fiber and beta-carotene [7]. Most common pests of cabbage are aphids [8]. Tomato is the important vegetable consumed throughout the world. It is good source of carotene and lycopene and acts as a natural antioxidant. Tomato reportedly gives preventive control of prostate, head and neck cancer [9]. Psyllid and whiteflies are the common pests of tomato. Spiromesifen can give effective control of psyllid, whiteflies and aphids of cabbage and tomato [10-13]. When the pesticides are used for the pest control on different crops, it may also reach the soil. Soil is the major source of entry of pesticides into food chain.

QuEChERS is the most convenient method for the extraction of the broad range of pesticides. It is a single step buffered acetonitrile extraction and clean-up method using small amount of solvent [14-15]. Method validation is a process to provide the evidence that the method was suitable for the residue analysis of the intended compound. Method validation was carried out as per SANCO (2013) for analysis of spiromesifen in cabbage, tomato and soil [16]. The parameters of the method validation studied were accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), linearity, range, selectivity and measurement uncertainty (MU).

Experimental

Materials and Reagents

Analytical grade spiromesifen of 99% purity were obtained from Sigma Aldrich, India (**Figure 1**). HPLC grade acetonitrile, acetic acid, n-hexane, acetone, magnesium sulphate, sodium sulphate and sodium acetate of analytical grade were procured from Rankem Fine Chemicals Limited, India. Magnesium sulphate was heated at 600º C for 5 hours and kept in desiccator before use. Primary secondary amine (PSA) of particle size 40 µm was procured from Agilent technologies. PTFE membrane filter of pore size 0.2 µm was procured from Phenomenex (Bangalore, India).

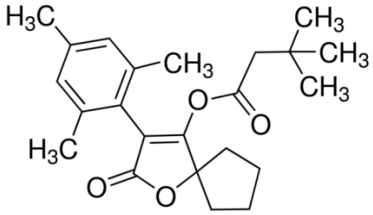

Figure 1 Chemical structure of spiromesifen.

Standard preparation

Stock solution of spiromesifen was prepared by dissolving 10±0.1 mg of spiromesifen in n-hexane: toluene (1:1). Working standards were prepared by appropriate dilutions of stock solution. Calibaration standards of 0.003, 0.01, 0.05, 0.1, 0.5 and 1 µg mL^{-1} were prepared in pure solvent as well as in matrix blank of cabbage, tomato and soil. Matrix matched standards are prepared by using 1 mL of matrix blank of the sample and concentrating it in a Turbo vap® LV concentrator (caliper life sciences, USA). The volume is made upto 1 mL by adding the appropriate concentration of the working standard. Stock and working standards are stored at -20ºC.

Sample preparation and spiking

Cabbage and tomato sample were grown at the experimental field of Indian Institute of Horticultural Research (IIHR), Bangalore without application of pesticides. The samples (2 kg) was cut into small pieces and homogenized thoroughly using high volume robot coupe homogenizer (Blixer ® 6 V.V, France). Fifteen gram of the homogenized sample was weighed in a 50 mL polypropylene centrifuge tubes. Soil samples with no history of pesticide were collected from Indian Institute of Horticultural Research (IIHR). The samples were spiked at four concentrations, 0.01, 0.05, 0.1 and 0.5 mg kg^{-1} with 6 replications each. Extraction and purification of the sample were carried out as per the quick easy cheap effective rugged safe (QuEChERS) method. To the tubes containing 15g of sample 15 mL of 1% acetic acid in acetonitrile was added and vortexed for about 1 min. Magnesium sulfate (6 g) and sodium acetate (1.5 g) were added to the tubes and shaken for 2 min and centrifuged at 4100 rpm for 10 min. The supernatant layer of about 3 mL was taken and d-SPE clean-up was done using 150 mg magnesium sulfate and 50 mg of primary secondary amine (PSA) sorbent. The tubes were vortexed and centrifuged. The samples were filtered through PTFE membrane filter of pore size 0.2 µm. one mL of the extract were taken and concentrated to dryness using LV concentrator and re-constituted with n-hexane : acetone (9:1) for analysis by GC and GC-MS.

Twenty gram soil samples were weighed in 100-mL polypropylene centrifuge tubes, 30 mL of acetonitrile: water (2:1) was added and mixed thoroughly. The tubes were centrifuged at 10,000 rpm for 10 minutes. Upper acetonitrile layer was transferred to a measuring cylinder with stopper and 10 mL of saturated sodium chloride solution was added and mixed thoroughly. Three mL of upper acetonitrile layer was subjected to d-SPE clean-up using 450 mg magnesium sulphate and 150 mg PSA. Two mL of the sample was concentrated to dryness using LV concentrator and reconstituted with n-hexane: distilled acetone (9:1) for analysis by GC and GC-MS.

GC and GC-MS analysis

Analysis of spiromesifen in cabbage, tomato and soil were carried out using GC-450 equipped with electron capture detector (ECD). DB-5MS (30 m × 0.25 mm i.d. × 0.25 µm; packed with 5% phenyl and 95% polysilaxone (stationary phase) were used for the separation of the analyte. Injection volume of the sample was 1 µL. The injector was kept at

split mode with split ratio of 20. Carrier gas, ultra high pure nitrogen (99.99%), flow rate was maintained at 1 mL min^{-1}. Injector and detector temperatures were maintained at 280^0 C and 300^0 C, respectively. The column temperature started at 80º C with a hold time of 1 min; increased at 15ºC min^{-1} to 150º C with a hold time of 5 min; 4º C min^{-1} to 250º C and 10º C min^{-1} to 280º C with a hold time of 17 min. The retention time of spiromesifen was 39 min (**Figure 2**).

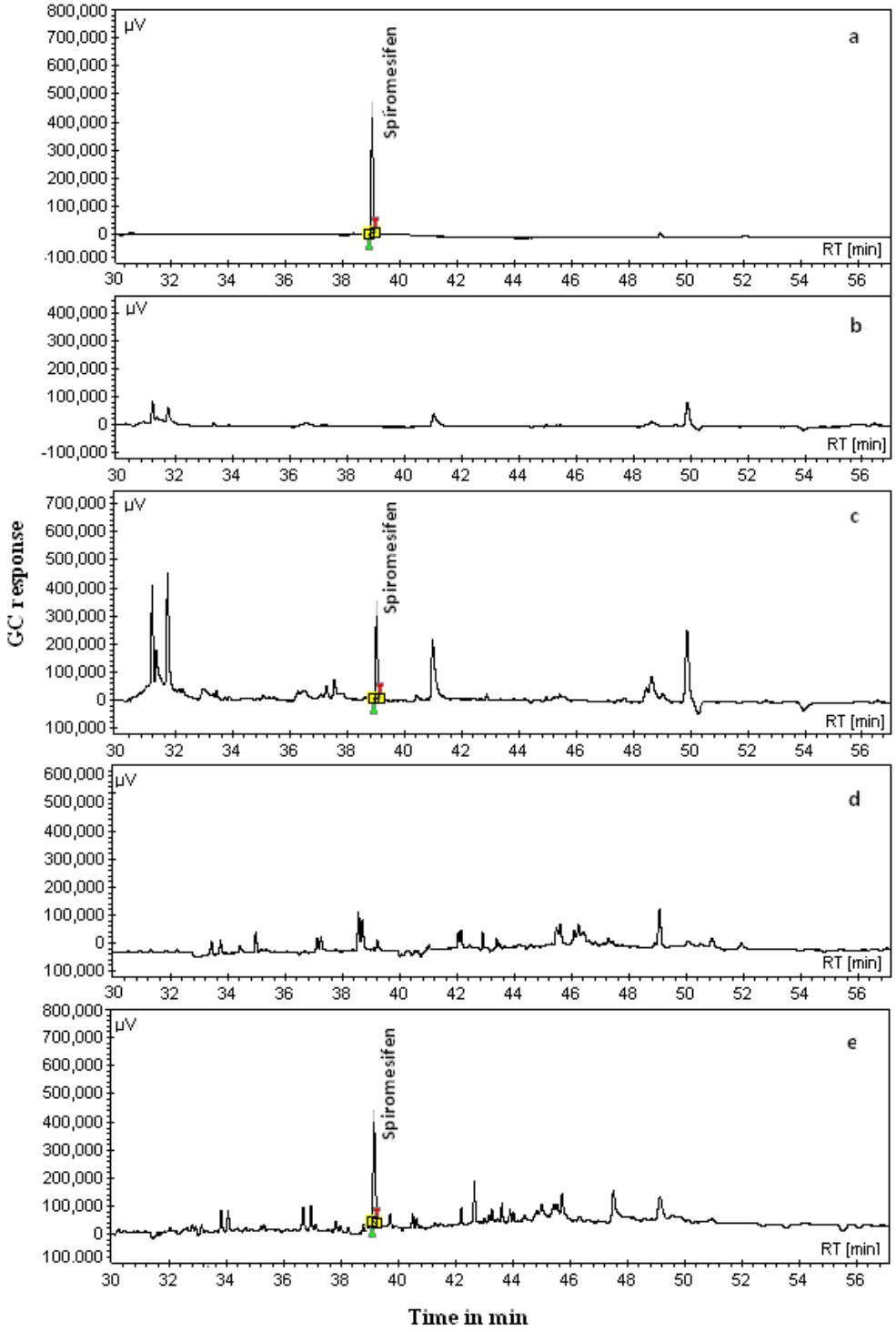

Figure 2 GC Chromatograms (a) spiromesifen standard at 0.1 µg mL^{-1}, (b) cabbage control, (c) cabbage spiked at 0.1 mg kg^{-1}, (d) tomato control, (e) tomato spiked at 0.1 mg kg^{-1}.

GC-MS with QP2010 plus detector (Shimadzu) operated in electron ionization mode (EI, 70eV) was used for the confirmation of spiromesifen in cabbage, tomato and soil. The chromatographic separation of the analyte was carried out using a Restek column, Rtx®-1701 (30m × 0.25mm, 0.25µm film thickness; stationary phase, 14% cyanopropylphenyl/ 86% dimethyl polysiloxane). Helium (99.99% purity) was used as carrier gas with the flow rate of 1 mL min^{-1}. Injector and ion source temperature were 250ºC and 200ºC, respectively. Temperature programme of the column was similar to that of the GC. MS confirmation was carried out in selective ion monitoring (SIM) mode. Under the above operating conditions the retention time of spiromesifen was 36.4 min (**Figure 3**).

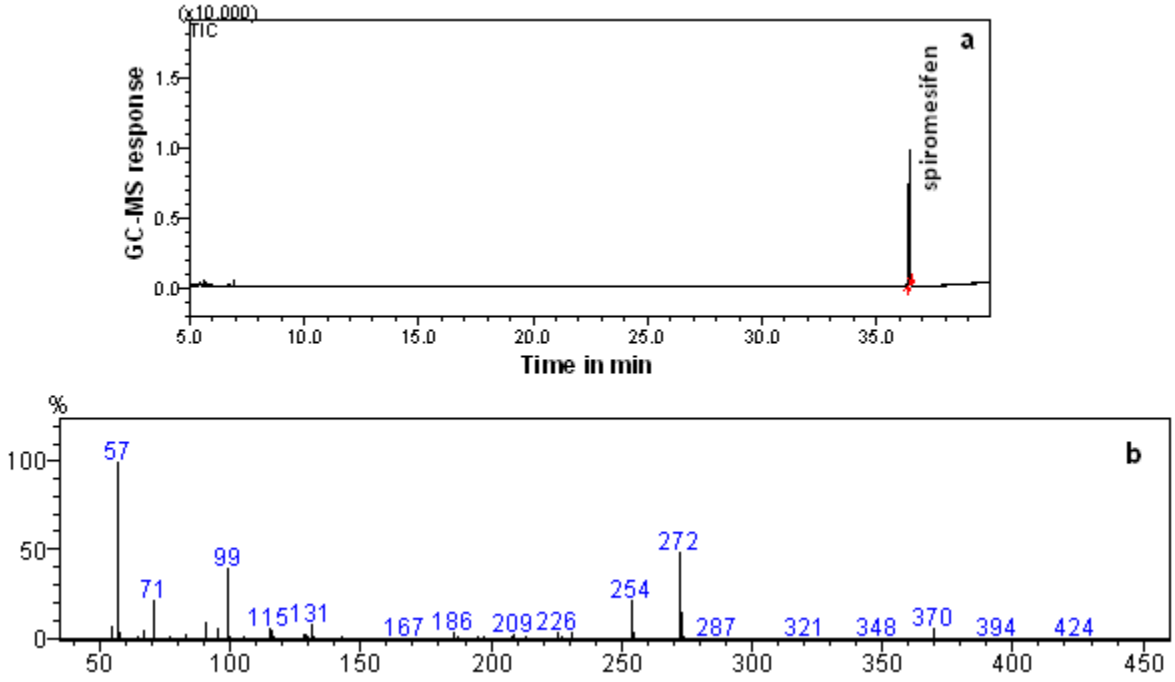

Figure 3 (a) GC-MS chromatogram of cabbage containing Spiromesifen, (b) mass spectrum of Spiromesifen.

Method validation

QuEChERS method used for the extraction of spiromesifen in cabbage, tomato and soil was validated by studying various parameters of method validation. The parameters studied were accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), linearity, range, selectivity and measurement uncertainty. Accuracy and precision of the analytical method was carried out by conducting the recovery experiments at four concentration levels in the range of 0.01, 0.05, 0.1, 0.5 mg kg^{-1}. Control samples of cabbage, tomato and soil were spiked with spiromesifen at 4 concentrations. Each spiking concentration was carried out with six replicates. Limit of detection (LOD) of the analytical method was determined by injecting the calibration standards from 0.003- 1 µg mL^{-1}. Lowest concentration of analyte at which peak could be reliably detected with the signal to noise ratio of 3:1 was considered as the LOD. Lowest concentration of analyte at which chromatographic peak could be easily identified with the signal to noise ratio of 10:1 was considered the LOQ. Linearity of the method was determined by analyzing the pure standards as well as with matrix blank of cabbage, tomato and soil in the range of 0.003-1 µg mL^{-1}. Selectivity of the method was studied by analyzing the blank and spiked samples from low to high concentrations. Matrix matched standards are used for the quantification of the residues in the sample.

Measurement uncertainty (MU)

Measurement uncertainty is the deviation from the true value or expected value. The factors that contribute towards measurement uncertainty are: recovery, sample concentration, sample volume measure, temperature, purity of reference standard, mass of reference standard used, volume of standard solution prepared and mass of sample. The

individual uncertainties were combined to get a combined uncertainty at 95% confidence level (coverage factor, K=2).

The combined uncertainty is calculated by using the formula

$$\sqrt{\left\{\left(\frac{U\,rec}{Mean}\right)^2+\left(\frac{U\,Sample\,conc}{Mean}\right)^2+\left(\frac{U\,Volume}{Sample\,volume}\right)^2+\left(\frac{U\,Conc\,of\,ref\,std}{Purity\,of\,ref\,std}\right)^2+\left(\frac{U\,volume\,of\,ref\,std}{Volume\,of\,solvent\,used}\right)^2+\left(\frac{U\,mass\,of\,ref\,std}{weight\,of\,ref\,std\,taken}\right)^2+\left(\frac{U\,mass\,of\,sample}{Weight\,of\,sample\,taken}\right)^2\right\}}$$

Where, Urec - uncertainty from recovery, Usample conc - uncertainty from sample concentration, Uvol sample vol measure and temp - uncertainty from sample volume measure and temperature, Uconc of ref std - uncertainty from concentration of reference standard, Uvol of ref std - uncertainty from volume of reference standard, Um ref std - uncertainty from mass of reference standard, Um of sample - relative uncertainty from mass of sample.

Results and Discussion

The method validated for residue analysis of spiromesifen in cabbage, tomato and soil was suitable for the intended purpose. The accuracy and precision of the method was evaluated by spiking the cabbage, tomato and soil at four concentrations in the range of 0.01-0.5 mg kg^{-1}. The recoveries of spiromesifen in cabbage, tomato and soil were within the acceptable range of 70-120% (**Table 1**). The recoveries of cabbage were within 85.44-100.21%; tomato, 72.56-92.78% and soil, 88.04-103.37%. Precision of the method was determined by calculating the relative standard deviation (RSD) of the spiked sample at four concentrations with six replicates each (n=6). The RSD of spiromesifen were in the range of 5.3-9.4% (cabbage), 2.7-9.4% (tomato) and 3.2-6.9% (soil). The Precision of the method was with the acceptable range of ≤20% [16].

Table 1 Recovery and RSD of spiromesifen from cabbage, tomato and soil.

Spiking level (mg kg^{-1})	Average recovery (%)± SD*					
	Cabbage		Tomato		Soil	
	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)
0.01	85.44 ± 8.5	9.4	72.56 ± 6.8	9.4	88.04±6.1	6.9
0.05	91.13 ± 7.7	8.5	87.39 ± 6.0	6.9	93.87±5.4	5.7
0.10	97.94 ± 6.4	6.6	89.81 ± 5.3	5.9	98.44±5.5	5.6
0.50	100.21 ± 5.3	5.3	92.78 ± 2.5	2.7	103.37±3.3	3.2

LOD of the analytical method was determined by injecting the spiromesifen from lower to higher concentration in the range of 0.003-1 µg mL^{-1}. The LOD of the method was found to be 0.003 µg mL^{-1}, the concentration at which the peaks could be reliably identified with the signal to noise ratio of 3:1. LOQ of the method was 0.01 mg kg^{-1} is the lowest spiking concentration at which the peak could be reliably identified with acceptable precision and accuracy. The LOQ of spiromesifen was below the European Union regulatory maximum residue limit (MRLs) of cabbage (0.02 mg kg^{-1}) and tomato (1 mg kg^{-1}) [17]. The LOQ level achieved in this study was less than the reported LOQ of 0.05 mg kg^{-1} [18]. Calibration curve was linear in the range of 0.003-1 µg mL^{-1} with the correlation coefficient >0.99. Matrix matched standards used for the quantification could nullify the matrix effect.

Selectivity of the method was evaluated by comparing the blank sample with spiked samples and there was no interference at the time when spiromesifen peak was eluted. The presence of spiromesifen was confirmed by analysis using GC-MS. Analysis of spiromesifen was initially carried out by scan mode and the fragmentation pattern was obtained. The base ion (57) and the 2 major ions (257, 254) were selected. Spiromesifen analysis was further carried out in SIM mode. Analysis of spiromesifen in SIM mode was highly selective. The MU of the method was 11.3-

14.9% for cabbage analysis, 9.6–14.9% for tomato analysis and 9.9-12.6% for soil analysis (**Table 2**). The uncertainties were higher at the low concentration level and decreased with increase in the spiking concentration.

Table 2 Uncertainty of measurements.

Spiking level (mg kg^{-1})	Cabbage	Tomato	Soil
0.01	14.9	14.9	12.6
0.05	14.0	12.6	11.6
0.10	12.3	11.7	11.4
0.50	11.3	9.6	9.9

Method application

The method was used to monitor the residue level of spiromesifen in real samples of cabbage and tomato collected from markets of Bangalore city (20 each). Extraction and purification of the samples was carried out as per the above method and analysis was carried out using GC and GC-MS. Spiromesifen residues were <LOQ in all the samples.

Conclusions

The QuEChERS method validated in conjunction with GC and GC-MS/MS for analysis of spiromesifen gave satisfactory results for all parameters studied. The recoveries were in the range of 72.56-103.37% with the RSD of 2.7-9.4 %. The LOD and LOQ of the analytical method for the analysis of spiromesifen were 0.003 µg mL^{-1} and 0.01 mg kg^{-1}. The method was linear with the relative coefficients of 0.99. Method could be applied for the rapid analysis of spiromesifen in real samples.

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