

Development and Validation of QuEChERS Method for Estimation of Propamocarb Residues in Tomato (*Lycopersicon esculentum* Mill) and Soil

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Abstract

An easy, simple and efficient analytical method was standardized and validated for the estimation of residues of propamocarb in tomato and soil. QuEChERS method included extraction of the sample with ethyl acetate and cleanup by treatment with PSA and graphitised carbon. Final clear extracts of ethyl acetate were concentrated under vacuum to almost dryness and reconstituted into hexane. The residues of propamocarb were estimated using gas chromatograph-mass spectrometry (GC-MS). Propamocarb presented a distinct peak at retention time of 8.962 min. Consistent recoveries of propamocarb ranging from 87 to 92 percent were observed when samples were spiked at 0.10, 0.50 and 1.00 mg·kg⁻¹ levels. The limit of quantification (LOQ) of this method was determined to be 0.10 mg·kg⁻¹.

Keywords: QuEChERS, Propamocarb, Tomato, Soil, GC-MS

1. Introduction

Propamocarb hydrochloride [propyl 3-(dimethylamino) propylcarbamate hydrochloride] shown in **Figure 1**, a systemic fungicide with protective action against phycomycetous diseases (*Pythium*, *Phytophthora* spp.), is used on a wide variety of mainly greenhouse vegetables [1]. Propamocarb was introduced into European markets for control of oomycete pathogens in ornamental crops and certain vegetables [2]. It has been widely used as a soil drench against *Phytophthora* and *Pythium* diseases of numerous crops [3-6]. More successfully, it has been used to control potato late blight by *Phytophthora infestans*, where metalaxyl-resistant populations presented cause a serious problem [7,8]. Due to its lack of adverse effects on beneficial microorganisms such as mycorrhizae and *Trichoderma* species, propamocarb has been considered as a good component of IPM programs [9,10].

Gas chromatographic determination of propamocarb in agricultural products have appeared in the literature [11] where the analyte was extracted with acetone-water and cleaned up by liquid-liquid partition into diethyl ether and analysed by GC. The object of this study has been the development of a QuEChERS technique for fast, ac-

curate and direct determination of propamocarb in commercial pesticides. The method can be used in the quantitative analysis of the analyte in different agricultural products, also reducing as much as organic solvents fulfilling the purposes to establish a wider acceptability of the methodology.

2. Material and Methods

2.1. Standards and Reagents

The technical grade analytical standard of propamocarb

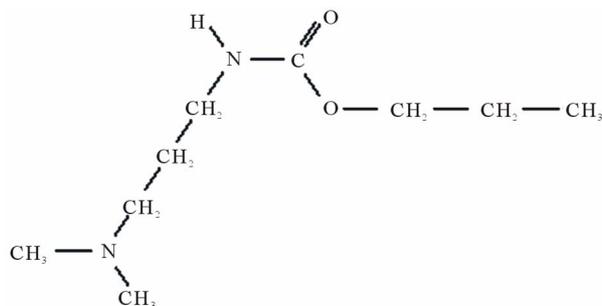


Figure 1. Structure of propamocarb.

(purity 96.8 %) was supplied by M/s Bayer CropScience India Ltd., Mumbai, India and stored at -10°C in a deep freezer. Solvents like ethyl acetate and hexane were obtained from E. Merck (India) Limited, Mumbai. Sodium chloride was also obtained from E. Merck (India) Limited, Mumbai. Sodium sulfate anhydrous was from S. D. Fine Chemicals, Mumbai. Activated anhydrous MgSO_4 was also obtained from E. Merck (India) Limited, Mumbai. Primary Secondary Amine (PSA) Sorbent and graphitised carbon decolorizing powder were obtained from Sigma-Aldrich, Mumbai, India. All common solvents were redistilled in all-glass apparatus before use. The suitability of the solvents and other chemicals were ensured by running reagent blanks before actual analysis.

2.2. Apparatus

Estimation of propamocarb residues were carried out on a GC (Shimadzu 2010) coupled with mass detector (Fisons MD-800, quadrupole mass detector) equipped with capillary column (Rtx-5 Sil MS, 30 m \times 0.25 mm i.d. \times 0.25 μm film thickness). Rotary vacuum film evaporator (Heidolph Labrota 4002) was supplied by Heidolph, Germany was used for concentration of sample. A high speed homogenizer (Heidolph Silent Crusher -M[®]) was used for homogenization of sample.

2.3. Standard Solution

A standard stock solution of propamocarb (1 mg/mL) was prepared in hexane. The standard solutions required for preparing a calibration curve (0.10, 0.25, 0.50, 1.00, 1.50 and 2.00 $\mu\text{g}/\text{mL}$) were prepared from stock solution by serial dilutions with hexane. All standard solutions were stored at -4°C before use.

3. QuEChERS Sample Preparation

The tomato and soil samples were prepared by following QuEChERS method for the determination of propamocarb residues shown in **Figure 2**.

3.1. Tomato

A sub sample of 15 g tomato was weighed into a 50 mL polypropylene tube and added 30 mL ethyl acetate. The sample was homogenized using high speed homogenizer (Heidolph Silent Crusher-M[®]) for 2 - 3 min at 14 - 15,000 rpm. Anhydrous sodium chloride (NaCl) 10 ± 0.1 g was added to homogenized sample for phase separation. The contents were centrifuged at 25 - 3,000 rpm for 3 min. An aliquot of 15 mL ethyl acetate layer was transferred over 10 ± 0.1 g sodium sulfate (Na_2SO_4) in a test

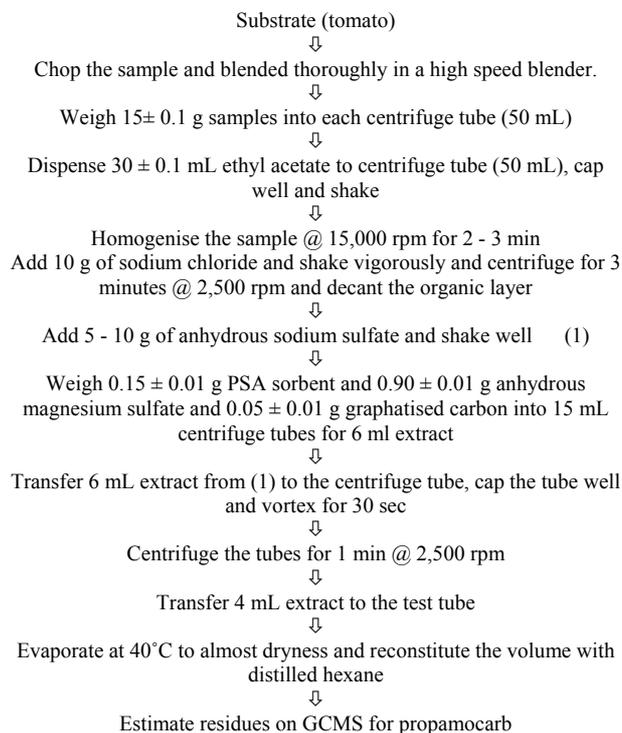


Figure 2. Flow chart of extraction and cleanup methodology for propamocarb.

tube. The ethyl acetate extract subjected to cleanup by dispersive solid phase extraction (DSPE). An aliquot of 6 mL acetonitrile was taken in a test tube containing 0.15 ± 0.01 g PSA sorbent, 0.90 ± 0.01 g anhydrous MgSO_4 and 0.05 ± 0.01 g graphitised carbon and the content was thoroughly mixed on vortex shaker. Again centrifuged at 25 - 3,000 rpm for 1 min. 4 mL aliquot of this ethyl acetate extract was evaporated to dryness using low volume evaporator to dryness using low volume evaporator at 40°C . Volume was made with distilled hexane.

3.2. Soil

A sub sample of 10 g soil was weighed into a 50 mL centrifuge tube and water was added before the initial extraction to get a total of 10 mL water and added 20 mL ethyl acetate. The sample was homogenized using high speed homogenizer (Heidolph Silent Crusher-M[®]) for 2 - 3 min at 14 - 15,000 rpm. Anhydrous sodium chloride (NaCl) 3 ± 0.1 g was added to homogenized sample for phase separation. The contents were centrifuged at 25 - 3000 rpm for 3 min. An aliquot of 15 mL ethyl acetate layer was transferred over 10 ± 0.1 g sodium sulfate (Na_2SO_4) in a test tube. The ethyl acetate extract subjected to cleanup by dispersive solid phase extraction (DSPE). An aliquot of 6 mL acetonitrile was taken in a test tube containing 0.15 ± 0.01 g PSA sorbent, $0.90 \pm$

0.01 g anhydrous MgSO_4 and 0.05 ± 0.01 g and the content was thoroughly mixed on vortex shaker. Again centrifuged at 25 - 3000 rpm for 1 min. 4 mL aliquot of this ethyl acetate extract was evaporated to dryness using low volume evaporator to dryness using low volume evaporator at 40°C . Volume was made with distilled hexane.

4. Estimation of Propamocarb Residues

The estimation of propamocarb residues was done by GC-MS. Helium was used as a carrier gas with flow rate of 1 ml^{-1} . The injector temperature was maintained at 260°C , Interface and ion source temperatures were maintained at 220°C and 200°C , respectively. Injection volume was $1 \mu\text{l}$ in split less mode. Detector voltage was maintained at 0.9 KV in SIM mode. The samples were injected and confirmed on electron ionization (EI) mode. The compounds were identified based on m/z ratio of total ion chromatograph (TIC) and fragmentations of selective ions monitoring (SIM) compared with fragmentations of different mass numbers obtained with standard propamocarb. The compounds were identified both in total scan and SIM mode based on m/z ratio. The sensitivity of the instrument increased in SIM mode as the most abundant fragment ions, characteristics of the analyte, were counted. The mass spectra of standard propamocarb showed the most abundant ions at m/z 72, 129, 143, 188 and base peak at 58. These ions values were compared with the tomato and soil samples spiked with propamocarb and tomato samples collected from treated plots for quantitation and confirmation of propamocarb residues.

5. Results and Discussions

5.1. GCMS Chromatograms of Propamocarb

GCMS detection has proven to be a good for propamocarb determination because no derivation step is needed. Under the chosen conditions, propamocarb showed a retention time of 8.962 min. The mass spectra of standard propamocarb showed the most abundant ions at m/z 72, 129, 143, 188 and base peak at 58 shown in **Figure 3**.

5.2. Validation of the Method

As the quantitative determination of propamocarb in tomato and soil is directly related to the evaluation and interpretation of data, a reliable method is required which is reproducible and can be applicable to different commodities. The method was fully validated according to bio analytical method recommendations described in the

Food and Drug Administration (FDA) guidelines in terms of selectivity, linearity, precision (repeatability), precision (reproducibility), and accuracy for both detection systems [12,13].

5.3. Linearity

For most chromatographic procedures a linear relation is observed between detector response (y) and analyte concentration (x). This can be expressed as a linear regression equation: $y = a + bx$. The parameters obtained by the selected chromatographic conditions for propamocarb correspond to: $y = 27005x + 11414$, $R^2 = 0.999$ was shown in **Figure 4**. The linearity of a method is a measure of range within which the results are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [14].

5.4. Limit of Detection and Limit of Quantitation

The limit of detection (LOD) is the lowest concentration of analyte detectable by an analytical method and is expressed in concentration units. The limit of quantification (LOQ) is the lowest solute concentration that can be determined with acceptable precision and accuracy, under the stated experimental conditions. It is also expressed in concentration units. The residues of propamocarb were estimated by comparison of peak height of standard and that of the unknown samples run under identical conditions. Fairly good response *i.e.* about 10% of full scale deflection was observed by injecting 0.2 ng of propamocarb. When tomato sample (15 g) was processed and 4 mL aliquot was concentrated to a final volume 2 mL from which $2 \mu\text{L}$ was injected (equivalent to 2 mg sample) into the instrument, there was no base line noise. In case of soil samples (10 g), was processed and 4 mL aliquot was concentrated to a final volume 2 mL from which $2 \mu\text{L}$ was injected (equivalent to 2 mg sample) into the instrument, there was no base line noise. Therefore, the limit of quantification (LOQ) was observed to be $0.1 \text{ mg}\cdot\text{kg}^{-1}$ for both the substrates. The limit of detection (LOD) was determined as the concentration having peak area three times higher in relation to the noise of the base line at the retention time of the peak of interest. Therefore, the LOD was calculated to be $0.03 \text{ mg}\cdot\text{kg}^{-1}$ for propamocarb.

5.5. Precision

5.5.1. Precision (Repeatability)

Precision (repeatability) reflects the variation in results when repetitive analyses are made on the same condi-

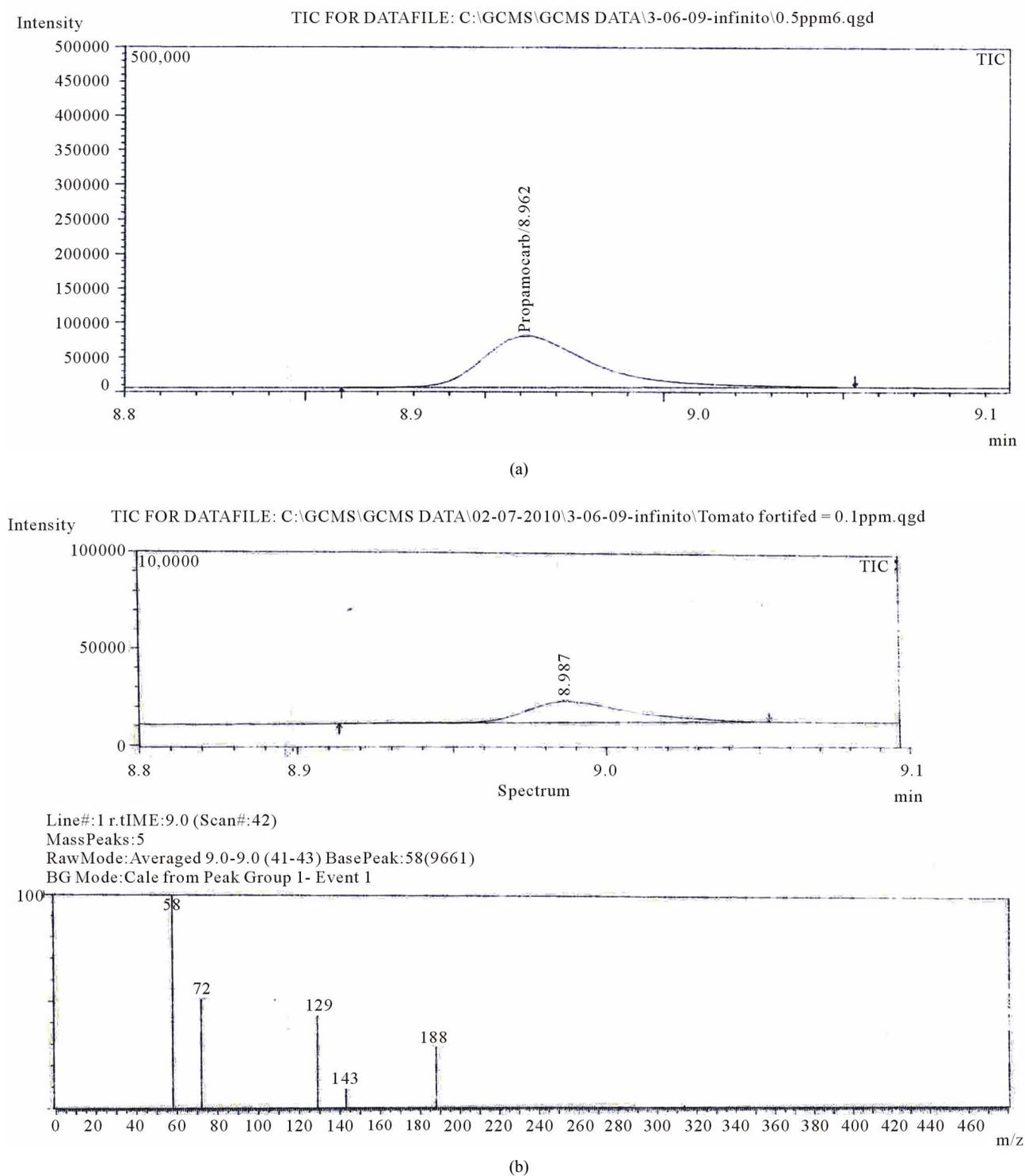


Figure 3. GC-MS chromatograms of (a) Standard propamocarb and (b) Fortified tomato sample and its mass ions.

tions. The numerical value used is the relative standard deviation for repeatability (RSD_r). Repeatability of the developed analysis method was determined by adding propamocarb in different concentrations to blank sam-

ples. The within-batch recovery and repeatability (RSD_r) of spiked samples at the levels of 1.00, 0.50 and 0.10 $mg \cdot kg^{-1}$ for propamocarb are summarized in **Table 1**. The precision (repeatability) in tomato range from 2.22%

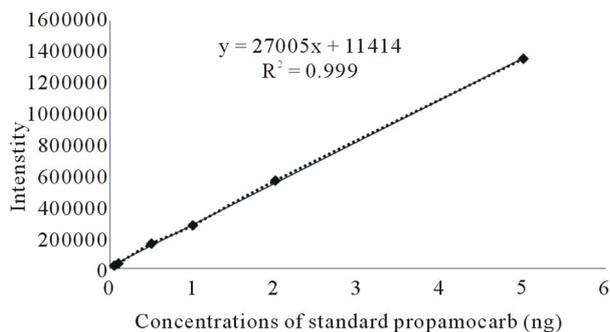


Figure 4. Calibration curve of different concentrations of propamocarb.

Table 1. Recovery of propamocarb in tomato and soil (n = 6).

Substrates	Level of fortification (mg·kg ⁻¹)	Propamocarb		
		Recovery %	^a SD	^b RSD _r %
Tomato	1.00	91.67	4.16	4.54
	0.50	90.00	2.00	2.22
	0.10	87.33	2.52	2.89
Soil	1.00	92.35	2.57	2.78
	0.50	86.67	2.08	2.40
	0.10	86.67	1.15	1.33

^aSD = Standard deviation; ^bRSD_r = Relative standard deviation for repeatability.

to 4.54% for propamocarb, in soil ranging from 1.3% to 2.78% for propamocarb. The results are fairly good for the concentration levels investigated.

5.5.2. Precision (Reproducibility)

Precision (reproducibility) is the degree of agreement obtained by the analysis of the same sample under various test conditions. The usually numerical value used is the relative standard deviation for reproducibility (RSD_R). The reproducibility of this analytical method was determined by analyzing spiked samples under various test conditions (different analysts, different instruments and different days). The between-batch recoveries and reproducibility (RSD_R) investigated at several levels are given in **Table 2**. The RSD_R values for propamocarb residues in tomato and soil were very good and well within 15% at all concentrations.

5.6. Accuracy

The accuracy of an analytical method is the agreement between the true value of analyte in the sample and the value obtained by analysis. Accuracy is usually ex-

Table 2. Recovery and RSD values obtained from analyses of samples spiked with propamocarb.

Sample	Day	Propamocarb		
		Recovery %	^a RSD _r %	^b RSD _R %
Tomato	1	87.33	2.89	
	2	91.04	3.11	7.88
	3	86.29	2.65	
Soil	1	86.67	1.33	
	2	88.72	3.64	9.87
	3	93.12	2.37	

^aRSD_r = Relative standard deviation for repeatability; ^bRSD_R = Relative standard deviation for reproducibility.

pressed as the recovery by the assay of known, added amounts of analyte [14]. The recovery tests were carried out on three replicates at each spike level. Results are the average from three injections. The average recoveries obtained for propamocarb at all concentrations and conditions investigated (**Tables 1 and 2**) were determined as 87.85% and 87.52% in tomato and soil, respectively, for propamocarb, which are very satisfactory.

6. Application of the Method to Real Samples

Initially, the representative samples of tomato and soil were extracted in acetonitrile, but the recoveries were observed to be very poor. A representative 15 g sample of tomato was spiked with propamocarb at 0.10, 0.50 and 1.00 mg·kg⁻¹ level, extracted with ethyl acetate. The results were astonishing as the percent recoveries ranged from 86.67 to 92.35. The same method was applied to tomato and soil the results were found to be excellent. The percent recoveries of propamocarb from different vegetable substrates spiked at 0.10, 0.50 and 1.00 mg·kg⁻¹ are reported in **Table 1**. Each value is the mean ± standard deviation of six replicate determinations. The results were encouraging and suggested that the method could be extended to more substrates. Moreover, it is simple, efficient, and easy to adopt in laboratories engaged in pesticide residue analysis.

7. Conclusions

We used a highly sensitive, easy, less time consuming and cost effective method for quantifying pesticide residues in tomato and soil samples. However, as the extraction was done with ethyl acetate, the method can be suitable apply for quantifying propamocarb in other ag-

ricultural products and also for other pesticides simultaneously as incase propamocarb is an active component in the formulation mixtures of Infinto.

8. References

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