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GC-MS/SIM ANALYTICAL METHOD FOR DETERMINATION OF PENDIMETHALIN RESIDUE IN TOBACCO MATRIX

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Pendimethalin, a dinitroanilin group pesticide, is being widely used as herbicide in tobacco cultivation. However, apprehensions related to accumulation of residues of crop protection agents (CPAs) at toxic levels in the final produce drives the safety regulations to become more and more stringent in most of the countries. This necessitates continuos monitoring of CPA residues in final produce by employing standard analytical methods. Development of an analytical method for determination of pendimethalin residue important for monitoring pendimethalin accumulation in tobacco leaf. We proposed a new GC-MS/SIM analytical method for extraction and analysis of pendimethalin residue in tobacco leaf matrix. The new method with good linearity (R^2>0.99) over the concentration range of 0.015-0.250 mg kg^{-1} had limit of determination (LOD) and limit of quantification (LOQ) values of 0.004 and 0.01 mg kg^{-1}, respectively. The method with 84-108% recovery showed compliance with international specification. The GC-MS/SIM analytical method, based on quantifier-qualifier ions (252, 253 & 281) ratio method, had been successfully employed to estimate the pendimethalin residues in some field samples. Results showed that in the majority of field samples, pesticide residue levels were below the GRL value set by CORESTA.

Keywords: Pendimethalin residue, Tobacco, GC-MS/SIM analysis, Ion ratio method.

INTRODUCTION

In India, tobacco is cultivated in about 0.4 M ha yielding 0.76 mt of dry leaf produce (FAO, 2012). India is the world's second largest producer (after China) and exporter (after Brazil) of tobacco. In countries like India, with tropical-humid climate, the incidences of pest and diseases are frequent and application of pesticides for their management is almost obligatory. Apprehensions related to accumulation of pesticide residues at toxic levels in the final produce drives the safety regulations to become more and more stringent in most countries. In spite of several awareness campaigns about the imminent potential health problems associated with tobacco, millions of people, particularly in lower and middle-income societies still indulge in cigarette smoking (WHO, 2011). Presence of pesticide residues in tobacco further aggravates the health risk not only to the smoker, but also to those subject to passive inhalations. Thus, monitoring of pesticide residues in tobacco is an important issue of critical concern from public health and safety point of view demanding implementation of stringent regulatory policies (CORESTA, 2013).

Like any other crop, tobacco (*Nicotiana tabacum L.*), is also prone to pest attacks, and the farmers do apply various pesticides as a control measure. The residues of pesticides applied on tobacco during its cultivation may remain in the final product (Lowman, 1972; Morris, 1972; Rahman *et al.* 2013, Ghosh *et al.* 2014a, 2014b; Khan *et al.* 2014). To protect the consumers by controlling pesticide residue levels in tobacco, the Guidance Residue Levels (GRL) of 118 pesticides have been issued by the Agro-Chemical Advisory Committee (ACAC) of the Cooperation Center for Scientific Research Relative to Tobacco (CORESTA, 2013). The GRL list for tobacco contains different classes of pesticides, such as organochlorine, organophosphorus, pyrethroids etc. Pendimethalin, a pesticide of the dinitroaniline class, is widely used as a herbicide in tobacco cultivation across the world, including India. However, its use as a suckericide for sucker control in tobacco has drawn attention of the scientific community as well as of the exporting agencies. Hence, determination of pendimethalin residue in tobacco is an urgent need as there is no report on Indian tobacco.

The objective of the present study was to develop an effective, sensitive and economic analytical method for pendimethalin in tobacco using a single quadrupole GC-MS instrument.

MATERIALS AND METHODS

Collection of tobacco samples and chemicals

The cured leaf samples of Flue-cured tobacco were collected from the Nalgonda (17.0600 N 79.3 E) district of Telengana, West Godavari (16.57 N 82.15 E) and Guntur (16.3008 N 80.4428 E) districts of Andhra Pradesh and Mysore (12.31 N 76.29 E) district of Karnataka, India. The representative leaf samples (0.5 kg) were oven dried at 60°C for 2 h. The dried leaves after removing the midribs were powdered, homogenized, passed through 1-mm sieve and utilized for subsequent analyses.

Certified reference standard of pendimethalin (>98% purity) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Acetonitrile, the extraction solvent was of specially dried residue analysis grade and purchased from Thomas Baker (Mumbai, India). The adsorbent, florisil, was received from Sisco Research Laboratories (Mumbai, India). The other reagents viz., sodium chloride and anhydrous sodium sulphate were of Analytical Reagent grade and purchased from Merck (Mumbai, India).

Preparation of solvent and matrix matched standards

The stock solution of pendimethalin standard was prepared by dissolving 10 (± 0.01) mg in a volumetric flask in 10 mL ethylacetate and stirred. An intermediate mixture of 10 mg/L was prepared by mixing appropriate quantities of the stock solutions followed by volume make up with ethyl acetate and stored at -20 (±2)°C. A working standard mixture of 1 µg/mL was prepared by diluting the intermediate stock solution, from which the calibration standards (0.005-0.25 mg/mL) were prepared by serial dilution with ethyl acetate. Matrix matched standards at the same concentration levels were made by extracting control tobacco, and spiking the extract with appropriate volumes of the working standard solutions.

Procedure for extracting tobacco matrix

In a 150 mL Erlenmeyer conical flask dry powdered sample (1 g) was mixed with 20 mL of acetonitrile : water (1:1) mixture. The mixtures were agitated for 45 min over an orbital shaker at 150 rpm and filtered. The filtrate was partitioned with 40 mL of hexane and the colored hexane fraction was collected for clean-up. A column was prepared by fabrication of a column bed which was made of 2 g florisil (60/100 mesh) sand wiched between two layers of anhydrous sodium sulfate (2 g each layer). Before clean-up, the column was eluted with pure hexane followed by passing the coloured hexane extract through the column. Clear and colorless hexane fraction were collected from the column and evaporated to dryness under reduced pressure. The residuem was re-dissolved in a volume of 2.5 mL ethylacetate and analyzed by a QP-2010 Plus GC-MS (single quadrupole, Shimadzu Corporation, Kyoto, Japan) in SACN mode with reference standards (on the basis of retention time) and in SIM mode with quantifier and qualifier ions (*m/z*).

Instrument condition

The GC-MS system (GC 2010 Plus) was equipped with ZB-5 (5% diphenyl, 95% dimethylpolysiloxane, 30 m (l) x 0.25 mm (id), 0.1 µm film thickness) capillary column and autosampler. Ultra-pure grade helium (INOX Limited, Hyderabad) was used as the carrier gas. The GC-MS separation of pesticides was achieved by formulating an optimized oven temperature program that started from an initial temperature of 100 C (hold for 0.5 min), ramped at the rate of (@) 30 C / min up to 180 C (hold 1 min), @ 10 C / min up to 240 C (hold for 2 min), @ 10 C min^{-1} up to 250 C / min (hold for 1 min), @ 10 C / min to 260 C (hold 2 min) and finally @ 40 C / min up to 320 C (hold for 10 min). This program resulted in a run time of 18.67 min. The injector temperature was maintained at 250 C in a splitless mode and 2 µl of the sample volume was injected. The carrier gas (helium) flow was maintained at 3 ml / min at the linear velocity of 64.4 cm / s.

Method performance

The calibration curves for pendimethalin in pure solvent and matrix were obtained by plotting

the peak area against the concentration of the corresponding calibration standards at six calibration levels ranging between 0.015-0.25 mg/ml. The sensitivity of the method was evaluated in terms of limit of quantification (LOQ). The LOQ is the concentration at which the S/N is e" 10 in matrix extract (ME). Recovery of the pesticide from tobacco matrix was studied at 0.05 mg/kg level of fortification with six replications. The matrix effect was evaluated by spiking untreated tobacco samples with the pesticide mixture at 0.05 mg/kg level. The peak area response of pendimethalin in hexane was compared with that of the corresponding response in the matrix matched standard at the same concentration level. A negative value of ME (%) indicates matrix induced signal suppressions, whereas a positive value indicates enhancement in the signal.

RESULTS AND DISCUSSION

Pendimethalin had a retention time (R_t) of 8.95 min with the newly developed analytical method. The calibration curve with R^2 = 0.999 for the test compound indicated good linearity of the method. The LOD and LOQ values for pendimethalin were 0.004 and 0.1 mg kg^{-1} which were much below the GRL value (5 mg / kg) of CORESTA. The dilution factor of the newly developed method was 5. The present method showed 85-108% recovery range with relative standard deviation (RSD) less than 15% which met the internationally accepted recovery criteria (CORESTA 2013, Khan, 2014, Ghosh *et al.*, 2014a). The matrix induced enhancement was observed for pendimethalin (Fig. 1) and, therefore, matrix-matched standards were used for quantification. The matrix matched calibration curve with R^2 = 0.9929 showed good linearity of the method (Fig. 2).

The method employed in the present study enabled the separation and detection of the pesticides in a tobacco matrix fortified at 0.05 mg kg^{-1} level as (Fig.1). Pendimethalin had a retention time (R_t) of 8.95 min. Notably, any plant matrix compound with different retention time than that of the target pesticide, can be detected correctly on the basis of retention time. However, false detection may occur if both the target pesticide and plant matrix compound are having the similar retention time and the concentration of plant matrix compound is more prevalent than that of the pesticide.

The probabilities of false detection in samples become crucial, in case of only plant matrix compound is present which sustains the same retention time of pesticide despite its absence. To avoid the chances of false detection, a new GC-MS selective ion monitoring (SIM) method with confirmative identification on the basis of the quatifier-qualifier ions (m/z) ratio was employed. Based on the molecular breakdown recorded in the detector, five ions (m/z) namely, 162, 191, 252, 253 and 281 were selected (Fig. 3a). However, this selection resulted in complex chromatogram and the base ion, 162, was found to present in several other molecules. Hence, the ion 162 was dropped and rest four ions (191, 252, 253 and 281) were

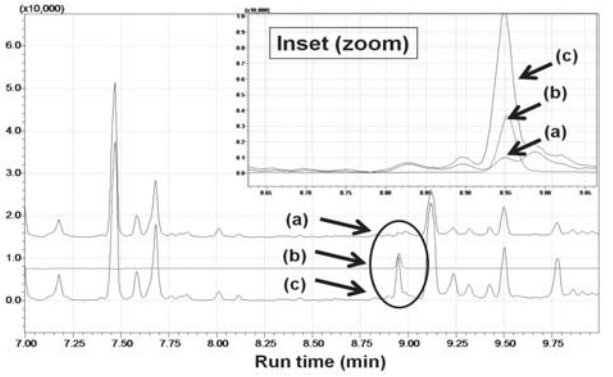

Figure 1: Matrix effect on determination of pendimethalin, (a) control tobacco matrix, (b) 0.05 ppm pendimethalin solution standard and (c) tobacco matrix fortified at 0.05 ppm pendimethalin

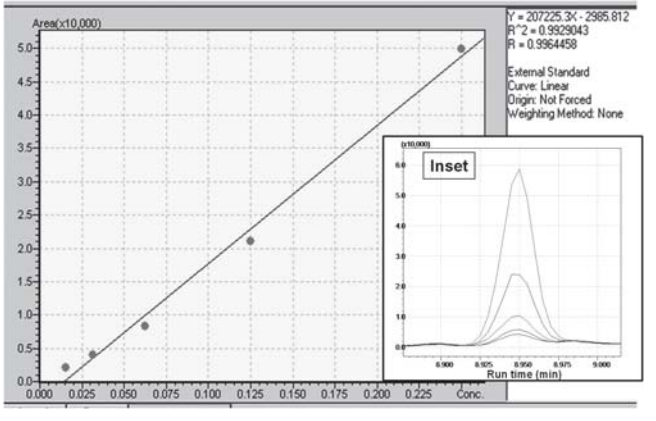

Figure 2: Matrix matched calibration curve, Inset: Different concentrations of pendimethalin at retention time of 8.95 min.

selected (Fig. 3b). In this case also, it was observed that selection of 191 ion as a base peak improved the identification of pendimethalin as compared to 162 ion. Finally, 252 ion was selected as quantifier ion coupled with 253 and 282 ions as qualifier ions.

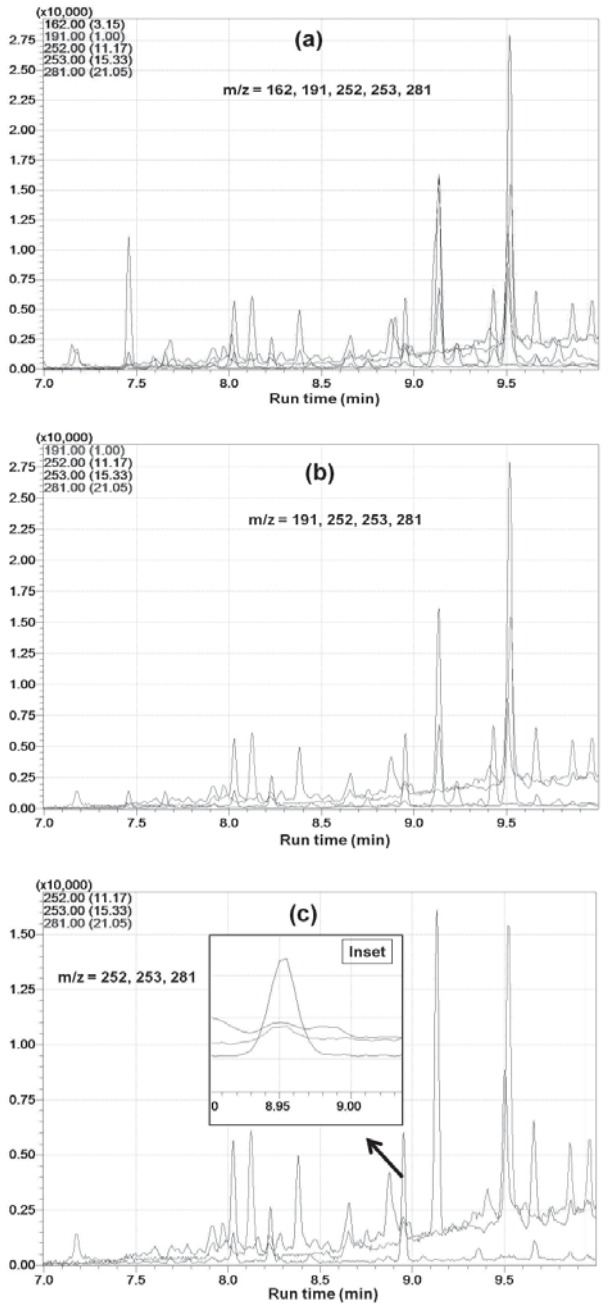

Figure 3: Selection of ions for selective determination of pendimethalin, (a) 162, 191, 252, 253 & 281 ions, (b) 191, 252, 253 & 281 ions and (c) 252, 253 & 281.

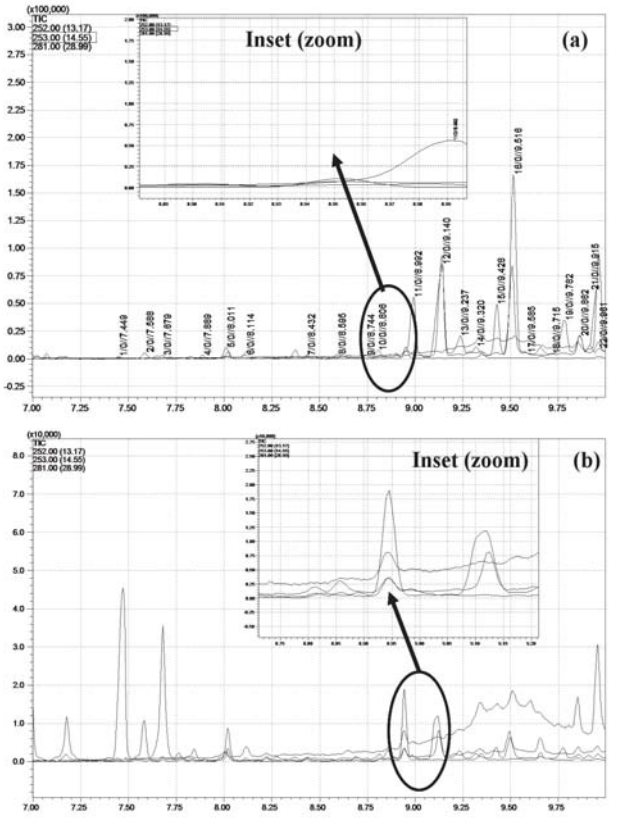

Figure 4: Field sample analysis for estination of pendimethalin with the newly developed method, (a) field sample without any residue and (b) field sample with pendimethalin residue

The developed method was employed for analysis of some field samples (Figure 4). The results (Table 1) indicated that only in few samples, pendimethalin residues were detected. The detected residue concentration (0.077-0.129 mg/kg) were far below the CORESTA (2013) GRL limit of pendimethalin (5 mg/kg).

The present method with internationally acceptable recovery level and matrix effect ensures sensitive and true detection of pendimethalin residue at trace levels. This is the first report on estimation of pendimethalin residues in Indian FC tobacco. Results showed that in the majority of samples, pesticide residue levels were below the GRL value set by CORESTA. Based on the results obtained, we conclude that the levels of pendimethalin residues in FC tobacco grown in India are very low and negligible.

Table 1: Field sample analysis for pendimethalin residue by the newly developed GC-MS method

Sample identity	Sample type	Location	Pendimethalin concentration (mg/kg)
1 IT	Barley tobacco	Nalgonda, Telengana (17.0600 N 79.3 E)	0.094
4 IT	Barley tobacco	Guntur, A.P. (16.3008 N 80.4428 E)	BLQ*
D 1	NLS Tobacco	West Godavari, A.P. (17.03 N 81.37 E)	0.127
D 3	NLS Tobacco	West Godavari, A.P. (17.03 N 81.37 E)	BLQ
T-1	NLS Tobacco	Mysore, Karnataka (12.31 N 76.29 E)	BLQ
T-11	NLS Tobacco	Mysore, Karnataka (12.31 N 76.29 E)	BLQ
T-2	NLS Tobacco	Mysore, Karnataka (12.31 N 76.29 E)	0.086
T-22	NLS Tobacco	Mysore, Karnataka (12.31 N 76.29 E)	BLQ
T-3	NLS Tobacco	Mysore, Karnataka (12.31 N 76.29 E)	0.077
T-33	NLS Tobacco	Mysore, Karnataka (12.31 N 76.29 E)	0.078
T-4	NLS Tobacco	Mysore, Karnataka (12.31 N 76.29 E)	0.129
T-44	NLS Tobacco	Mysore, Karnataka (12.31 N 76.29 E)	0.08

*BLQ= below the limit of quantification

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