Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Capillary gas chromatographic determination of dimethachlon residues in fresh tobacco leaves and cut-tobacco*

LIU Hong-cheng[1], LI Qi-wan[†‡1], TANG Li-bin[2]

([1]*Supervision and Testing Center for Farm Product Quality, Ministry of Agriculture (Kunming), Yunnan Academy of Agriculture Science, Kunming 650223, China*)

([2]*Kunming Institute of Physics, Kunming 650223, China*)

[†]E-mail: cmliu_0@sina.com

Received June 14, 2006; revision accepted Oct. 17, 2006

Abstract: Simple procedures for extraction and chromatographic determination of dimethachlon residues in fresh tobacco leaves and cut-tobacco are described. The determination was carried out by capillary gas chromatography (GC) with electron capture detection (ECD) and confirmed by GC-MS. The mean recoveries and relative standard deviation (*RSD*) were 93.2%~112.9% and 3.5%~6.7%, respectively at levels ranging from 0.01 to 0.1 mg/kg. The limit of determination was 0.001 mg/kg. Tobacco samples in routine check were successfully analyzed using the proposed method.

Key words: Dimethachlon residues, Tobacco, Gas chromatography (GC)
doi:10.1631/jzus.2007.B0272 **Document code:** A **CLC number:** O657.7$^+$1

INTRODUCTION

Myzus persica (Sulzer) is a serious disease caused by *Alternaria alternata* (Fries) Keissler affecting the leaves of tobacco, resulting in important economic losses. No effective treatment against *Myzus persica* has so far been devised, although the fungicide dimethachlon [N-(3,5-dichlorophenyl)-succinimide] (Fig.1) seemed the most suitable chemical for controlling the disease (Zhang *et al*., 2003; Li *et al*., 2003; Ma *et al*., 2002; Chen *et al*., 2001; Shi *et al*., 2000), in China. Dimethachlon is mainly used in Yunnan, a Chinese province, which is the largest producer of tobacco in China. This has led to the problem of declining tobacco quality due to the soils' dimethachlon residues. So it is imperative to search for a simple method to determine the dimethachlon residues in tobacco. Dimethachlon's physical, chemical and toxicological properties are summarized in a pesticide manual (Wang and Zhang, 1994). Effective analytical methods for determineation of many pesticides are more developed (Jánská *et al*., 2006; Huang *et al*., 2006; Lehotay *et al*., 2005; Mastovska and Lehotay, 2004; Mastovska *et al*., 2004), Zhang *et al*.(2006) determines 405 pesticides residues. But no method has been published for determining dimethachlon residues in tobacco or other plants. Shi (1996) reported the analysis procedure for dimethachlon by GC-flame ionization detection (FID), which is used to evaluate the purity of commercial pesticide containing dimethachlon (95%).

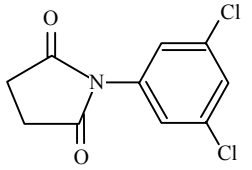

Fig.1 Structure of dimethachlon

In this work, simple analysis procedures have been developed for determining dimethachlon in tobacco samples (fresh or dry). The method includes

‡ Corresponding author

* Project (No. 2006NG01) supported by the Agriculture Breakthrough Program of Yunnan Province, China

solvent extraction, solid phase extraction (SPE) clean-up and determination by GC with electron capture detection (ECD). Our work aimed at (1) developing a simpler, faster and sensitive methodology for determining dimethachlon residues in tobacco by GC-ECD, considering aspects such as quantification, recoveries and identification problems and also characterization by GC-MS; (2) studying the applicability of the proposed method in routine analysis of tobacco samples containing dimethachlon residues.

EXPERIMENTAL DETAILS

Reagents

Dimethachlon certified standards were provided by Chemservice (West Chester, PA, USA). Standard stock solutions in acetone (1 mg/ml) were prepared and stored at −18 °C for three months. Various standards for standard curved (0, 0.005, 0.01, 0.05, 0.1 and 0.5 μg/ml) were prepared from the standard stock solution by serial dilutions. All the solvents used were of analytic-grade and redistilled.

Cartridges repacked with SPE-Florisil (SUPELCO, USA) 1.0 g/6 ml.

Instrumentation

Analysis was carried out using an Agilent 6890N GC system consisting of a 7683 automatic liquid samples tray 100, a micro electron capture detector (*m*-ECD). Data acquisition and processing were performed with an HP computer using Agilent Technologies Chemstation Software (A.09.03, Windows 2000).

Mass spectrometric analysis was carried out using a Shimadzu GC/MS-QP2010 system, with DI-2010 automatic liquid samples. Data acquisition and processing were performed with a computer using GC-MS solution (Ver. 2, Windows 2000).

Preparation of samples

Fresh tobacco leaves chopped in a high speed blending jar were homogenized for 2 min and stored at −18 °C. To analyze the cut-tobacco samples, a 100 g sample was first cut into pieces in a high speed blending jar and homogenized for 5 min, then the sample dropped into 200 ml water for 10 min before extraction.

Extraction

Twenty-five grams samples were homogenized by high-speed refiner with 50 ml acetonitrile for 3 min; the mixture was filtered through a 8.9 cm high speed filter paper into a 100 ml graduated flask (with tap) into which had been placed 10 g sodium chloride. The graduated flask was shaken vigorously for 5 min and was left to stand for 30 min until the filtrate was transparent. Ten milliliters organic phase was accurately transferred to a 250 ml breaker. The acetonitrile phase was evaporated nearly to dry by blowing nitrogen on an 80 °C water-bath. The residues were dissolved in 1 ml of *n*-hexane.

Clean-up

The residues were then transferred to the LC-Florisil (1 g/6 ml), which was conditioned with 3 ml acetone:*n*-hexane (1:10, v:v) and 3 ml *n*-hexane. The pesticides were eluted with 10 ml of acetone: *n*-hexane (1:10, v:v), and collected in a 10 ml graduated test tube with a tap. The elution was concentrated to 5 ml by nitrogen evaporator (N-EVAP III, Organomation Associates Inc., USA) on a 40 °C water-bath, and its volume was accurately made up to 5 ml with *n*-hexane. A 1 μl portion was analysed by GC.

GC determination: ECD

The operating conditions were as follows: capillary column DB-1 (0.25 μm film×0.25 mm i.d.×30 m length, J&W Scientific, USA). The detector and injection temperatures were 320 °C and 200 °C, respectively. The injection volume was 1 μl.

The oven temperature was maintained at 150 °C for 2 min after injection, then programmed at 6 °C/min to 270 °C, which was held for 8 min, and after 8 °C/min to 280 °C, which was held for 5 min. The makeup gas was nitrogen (99.999%) with flow rate of 1 ml/min, and splitless.

Confirmation by GC-MS

Mass spectrometry analysis was used to verify the dimethachlon in the extract. Mass spectra were obtained on a GC/MS-QP2010, a capillary column DB-1 (0.25 μm film×0.25 mm i.d.×30 m length, J&W Scientific, USA). The oven temperature was maintained at 50 °C for 1 min after injection, then programmed at 20 °C/min to 150 °C, which was held for 2 min, and after 8 °C/min to 250 °C, which was held

for 3 min. The MS operation conditions were 70-eV electron ionization (EI, 200 °C) at scan mode, scanning between *m/z* 45 to 475. Helium at flow rate of 1 ml/min was used. The injection temperatures was 250 °C, and injection volume was 1 μl and splitless.

Recovery studies

The proposed samples preparation method was validated by carrying out a recovery study on fresh tobacco leaves and cut-tobacco samples, which were not treated with the pesticide studied, and fortified following the procedure described above with 1 ml of the working standard mixture solution. These values represent 0.01~0.1 mg/kg of dimethachlon in the samples. The recovery assays were replicated five times.

RESULTS AND DISCUSSION

A different chromatographic profile was obtained for fresh tobacco leaves and cut-tobacco, resulting in the most complex chromatogram. Fig.2 shows typical chromatograms of standard dimethachlan, fresh tobacco leaves and cut-tobacco samples fortified with dimethachlon at 0.1 mg/kg and the spectral matches were obtained at 18.474 min, the retention time of dimethachlon. The background level for cut-tobacco was more complicated than that at fresh tobacco leaves, but it did not disturb the determination of dimethachlon, the other peak was 18.598 min; two peaks could be completely separated. It was important to drop cut-tobacco in water for 10 min before it was extracted by acetonitrile, because the tissue of cut-tobacco is soluble in water and the content extracted by acetonitrile is little. It was also important that the graduated flask was shaken vigorously for 5 min and left in place >30 min until the filtrate was transparence. This can ensure that the ratio of extraction was more than 95% and that the acetonitrile completely evaporated.

Mass spectrometric study

The presence of dimethachlon in the extracts of samples was confirmed by GC-MS in scan mode as shown in Fig.3. We could not find the dimethachlon in the standard datum (NIST datum). The molecular ion peak was *m/z* 243, corresponding to $[M\text{-}H]^+$, the isotope ions *m/z* 243:245:247=9:6:1, included two chlorines. A fragmentation pattern at *m/z* 215 corresponding to $[M\text{-}CO]^+$, *m/z* 187 corresponding to $[M\text{-}COC_2H_4]^+$, the split ions *m/z* 152 were the ions *m/z* 187 leaking out one chlorine (Cl), the split ion *m/z* 124 was then leaking out carbon oxide (CO). Through the split ions peaks, we confirmed that the molecular particle was the dimethachlon.

Linearity, reproducibility and recovery

The linear dynamic range was checked for tobacco extracts fortified with dimethachlon by injecting an absolute amount of 0.005~0.5 ng and the calibration graph showed good linearity with a

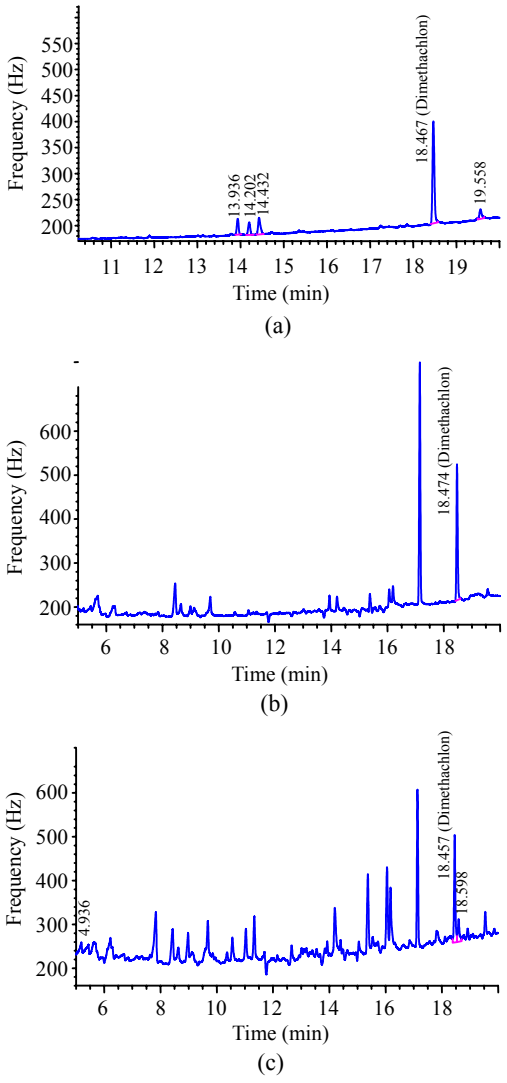

Fig.2 Chromatogram of standard (a), tobacco leaves (b) and cut-tobacco (c) fortified with dimethachlon

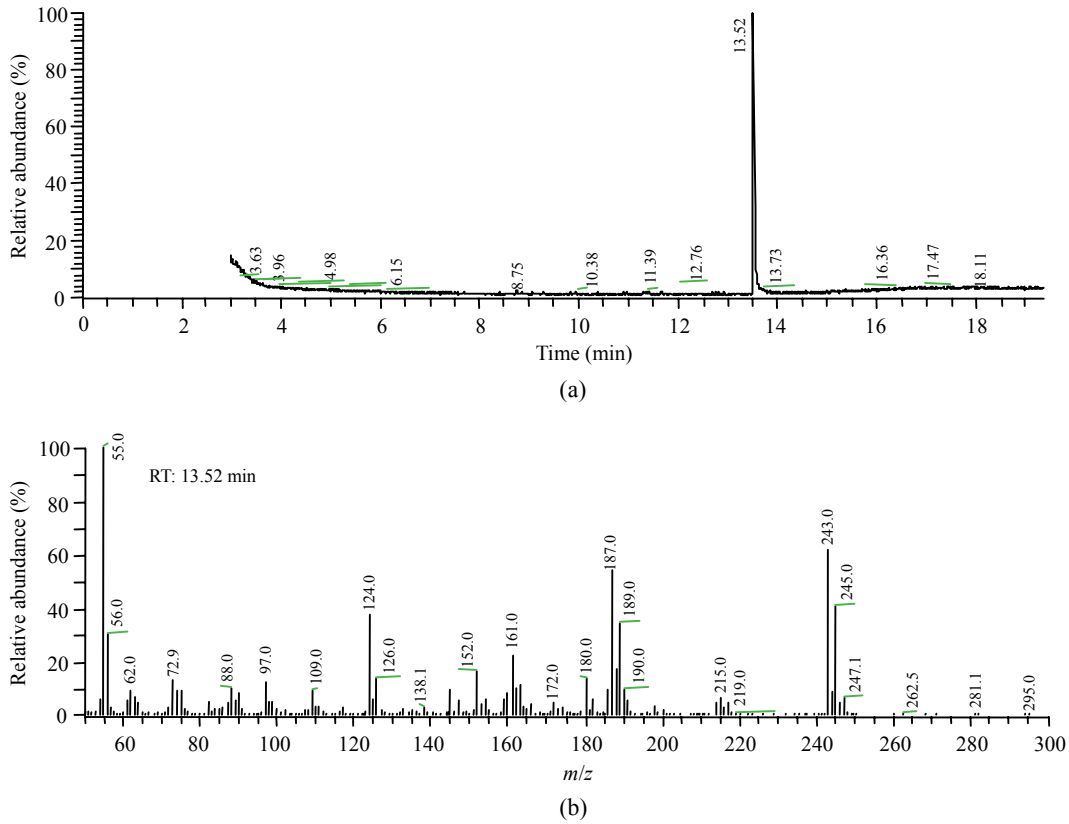

Fig.3 Mass spectrum of dimethachlon. (a) Scan mode; (b) Bar chromatogram

correlation coefficient of 0.9997; y=2475.5035× Amt+194.25589. Non-fortified samples were also analyzed in order to ensure that no peak was obtained at the retention time of the analyte. A concentration of 0.001 mg/kg can be considered as the detection limit of the method as lower concentrations did not always allow a proper definition of the spectrum. This level was low enough for monitoring dimethachlon in tobacco.

The retention time was 18.474 min and the reproducibility of the retention time was tested by analyzing five fortified tobacco samples.

The procedure was validated by carrying out a recovery study on tobacco samples, fortified at three levels, 0.01, 0.05 and 0.1 mg/kg. Table 1 shows the average recovery and relative standard deviation (*RSD*) obtained in the application of the proposed procedures on the fresh tobacco leaves and cut-tobacco sample spiked with dimethachlon. The recovery of dimethachlon was complete and independent of the commodity and fortification level, and the average recovery and *RSD* were replicated five times respectively at levels ranging from 0.01 to 0.1 mg/kg.

Table 1 Recovery and *RSD* obtained on fresh tobacco leaves and cut-tobacco sample after applying the methods (n=5)

Sample	Fortification (mg/kg)	Dimethachlon	
		Recovery (%)	*RSD* (%)
Tobacco leaves	0.01	93.2	5.2
	0.05	96.7	3.5
	0.10	98.5	4.6
Cut-tobacco	0.01	112.9	6.7
	0.05	105.6	3.8
	0.10	102.3	5.4

Application of the method to real samples

Routine analysis of 50 tobacco leaves and 50 cut-tobacco samples (from three areas in Yunnan Province, China) were selected to evaluate the effectiveness of the proposed method. Residues of di-

methachlon were found in 5 tobacco leaves in the range of 0.01~0.08 mg/kg. Fig.4 shows the chromatograms of sample with 0.06 mg/kg dimethachlon residue.

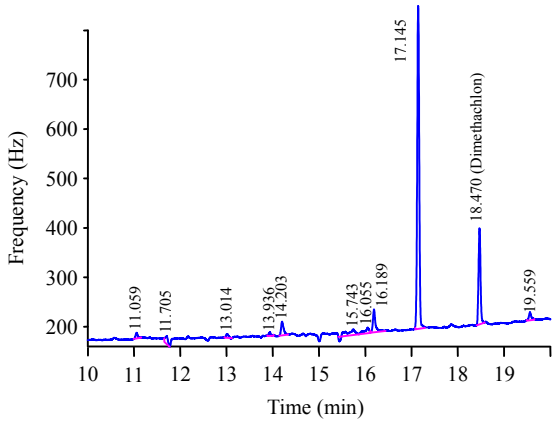

Fig.4 Chromatograms of sample with dimethachlon residue

References

Chen, A.D., Yan, W.Z., Yang, J.L., 2001. Measurement of tobacco losses of quality and output caused by tobacco spot disease. *Chin. J. Plant Prot.*, **27**(6):11-13 (in Chinese).

Huang, B.Y., Pan, C.P., Wang, Y.R., Cao, Z., Zhang, W., Li, W.M., Jiang, S.R., 2006. Rapid determination of pesticide multiresidues in vegetables by GC/MS and compensation by matrix effect with protection. *Chem. J. Chin. Univ.*, **27**(2):227-232 (in Chinese).

Jánská, M., Lehotay, S.J., Mastovská, K., Hajslová, J., Alon, T., Amirav, A., 2006. A simple and inexpensive "solvent in silicone tube extraction" approach and its evaluation in the gas chromatographic analysis of pesticides in fruits and vegetables. *J. Sep. Sci.*, **29**(1):66-80. [doi:10.1002/jssc.200500237]

Lehotay, S.J., Mastovska, K., Lightfield, A.R., 2005. Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *J. AOAC Int.*, **88**(2):615-629.

Li, Y.J., Chen, H.M., Zhou, L., 2003. Experiment on mixed pesticides for controlling tobacco brown spot and *Myzus Persicae* (*Sulzer*). *Chin. J. Tobacco Sci. Technol./Dis. Pest Control*, **10**(2):45-47 (in Chinese).

Ma, J., Zheng, R., Xu, L., Wang, S., 2002. Differential sensitivity of two green algae, *Scenedesmus obliqnus* and *Chlorella pyrenoidosa*, to 12 pesticides. *Ecotoxicol. Environ. Saf.*, **52**(1):57-61. [doi:10.1006/eesa.2002.2146]

Mastovska, K., Lehotay, S.J., 2004. Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues. *J. Chromatogr. A*, **1040**(2):259-272. [doi:10.1016/j.chroma.2004.04.017]

Mastovska, K., Hajslova, J., Lehotay, S.J., 2004. Ruggedness and other performance characteristics of low-pressure gas chromatography-mass spectrometry for the fast analysis of multiple pesticide residues in food crops. *J. Chromatogr. A*, **1054**(1-2):335-349. [doi:10.1016/j.chroma.2004.08.061]

Shi, J.H., 1996. Gas chromatographic determinate the pesticide of dimethachlon. *Chin. J. Pest*, **35**(6):23-26 (in Chinese).

Shi, Z.Q., Zhou, M.G., Ye, Z.Y., 2000. Resisitance of *Sclerotinaia Sclerotiorum* to carbendazim and dimethachlon. *Chin. J. Oil Crop Sci.*, **22**(4):54-57 (in Chinese).

Wang, H.M., Zhang, Z.M., 1994. A New Pesticide Manual. Agricultural Press, Beijing, China, p.362-363 (in Chinese).

Zhang, X.L., Sun, X.M., Zhang, G.F., 2003. Preliminary report on the mentoring of the resistance of *Sclerotinia libertinia* to carbendazim and its internal management. *Chin. J. Pest. Sci. Adm.*, **24**(6):18-22 (in Chinese).

Zhang, J.J., Cao, Y.Z., Li, X.M., Li, Z.Y., Wu, Y.P., Guo, T.T., 2006. Simultaneous determination of 405 pesticide residues in grain by accelerated solvent extraction then gas chromatography-mass spectrometry or liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.*, **384**(5):1366-1408.