Chapter 5

Determination of Paclobutrazol Residue: Developing and Optimizing GC-MS and SPME condition

5.1 Abstract

Solid-phase microextraction (SPME) is a solvent-free sample preparation technique. An 85 micrometer polyacrylate fiber was used to extract the analytes directly from aqueous samples and then thermal desorption was carried out in the hot injector of GC-MS. Paclobutrazol was detected at a retention time of 17.038 ± 0.2 minutes and the characteristic mass ions of *m/z* 294, 236, and 125. Diclobutrazol was clearly selected to internal standard with a retention time of 17.518 ± 0.2 minutes and the characteristic mass ions of *m/z* 272, 270, and 159. Three mass characteristic ions were chosen because they gave the clear specific integration of paclobutrazol and diclobutrazol. In addition, the optimization of adsorption time, desorption time and cleaning up time of SPME fiber were observed at 60, 6, and 10 minutes, respectively. The validation of SPME optimum conditions were also used for further analyses.

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5.2 Introduction

Solid-phase microextraction (SPME) is a relatively new technique that appears as a convenient and efficient extraction method in contrast with more complex techniques used for pesticide residue analysis based on liquid-liquid and solid phase extraction (Beltran *et al*., 1998). SPME is a fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both. The fiber coating removes the compounds from the sample by absorption in the case of liquid coatings or adsorption in the case of solid coatings (Mani, 1999). The primary parameters influencing analyte absorption into the stationary phase are fiber type, extraction time, ionic strength, pH, temperature, sample volume, and agitation. For SPME-GC, analyte desorption is a function of time and temperature. Conversely, solvent type and volume or time is critical for SPME-HPLC modes (Beltran *et al*., 2000; Krutz *et al*., 2003).

SPME technique has been developed to combine sampling and sample preparation in one step (Wardencki *et al*., 2004). In addition, it is a powerful tool in pesticide residue analysis for both qualitative and quantitative determination (Beltran, *et al*., 2000).

In this work we aim at finding out information about the calibration curve of paclobutrazol, selection of an internal standard, and SPME conditions such as adsorption, desorption, and clean up including the temperature during the extraction of paclobutrazol residue.

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5.3 Materials and methods

The investigation was initiated at Chiang Mai University as part of postharvest ripening analysis, where samples were prepared for residue analysis. Samples were then transported to University of Hohenheim, since the instrument for determining paclobutrazol residue, the gas chromatography-mass spectrometry (GC-MS), was not available at Faculty of Agricultural, Chiang Mai University.

5.3.1 Internal standard selection

Chemicals and reagents

Paclobutrazol (PBZ 99.5 %), Diclobutrazol (DBZ 98.4 %), Hexaconazole (HCZ 98.9 %) and Flutriafol (FTF 96.7 %), analytical standards of purity, were purchased from Sigma-Aldrich GmbH, Germany.

Sodium chloride (analytical grade, Merck, Germany), Acetonitrile (isocratic grade for LC 99.8 %, VWR, Darmstadt, Germany) Celite 503 (silica; Carl Roth GmbH & Co., Karlsruhe, Germany) were ordered from listed suppliers. Helium (99.999 %) was used as the carrier gas.

Materials

Filter paper 2 types:
- Filter papers for Buechner funnel, diameter size 110 millimeter (mm) No.589/3 (Blue ribbon, Microscience GmbH, Dassel, Germany)
- Filter papers for glass funnel, diameter size 70 millimeter (mm) No.595½ (Microscience GmbH, Dassel, Germany)

Rotary evaporator (Büchi, Rotavapor-R)

Water bath with water circulator with magnetic stirrer (IKA-Combimag, RCO)

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GC-MS Conditions *(alteration from Crook, 1999)*

The GC analyses were performed with a Varian model Star 3400 gas chromatography with electronic flow control (EFC) and fitted with Saturn II ion-trap mass spectrometer (Varian Instruments, USA). The mass spectrometer was operated in the electron impact (EI) ionization scan mode. The scanned mass rang was *m/z* 50-300 with a scan rate of 1000 milliseconds, and segment acquire time 25 minutes.

The GC column used was 30 meters of a BPX5 with a 0.25 millimeters internal diameter and a 0.25 micrometers film thickness, which was inserted directly into the ion source of the mass spectrometer. The GC column temperature program used was to hold the temperature initially at 60˚C for 6 minutes (for SPME desorption time). The column was programmed at a rate of 20 ˚C per minute to a final temperature of 280 ˚C. The column was then held at this temperature for 8 minutes. A column head pressure of 11 p.s.i. injector constant temperature of 300 ˚C and detector constant temperature of 300 ˚C were used. Helium (99.999%) was used as carrier gas. Samples were manually injected into SPI/1077 splitless program.

SPME procedure *(alteration from Crook, 1999)*

The polyacrylate (PA) fiber was preconditioned before initial application in the hot port (split) of the gas chromatography, heating at 300 ˚C for 3 hours according to instruction provided by the supplier; this treatment removed the impurities present in the coating and introduced during the manufacture of the fiber.

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SPME parameters: 85 micrometer polyacylate fiber (white) for manual holder from Supelco, USA.
Equilibrium time: 45 minutes with constant agitation.
Desorption time: 6 minutes
Desorption temperature: 300 ˚C

All SPME was made directly from the aqueous sample. The vials were filled with 1.2 milliliters aqueous sample or standard solution and with the addition of 0.4 grams sodium chloride. First, the fiber was exposed to the stirred sample for an optimized absorption time 45 minutes at room temperature (20 ˚C) and then it was removed from sample and introduced into the GC injector where the thermal desorption of the analyte was carried out at 300 ˚C for 6 minutes.

Information about the calibration curve of paclobutrazol (PBZ) and selection internal standard

Four triazole standards were weighed 1.00 milligram (exact weight at 0.001 milligram) and dissolved in 50 milliliters of acetonitrile. The solution was then brought to a volume of 100 milliliters in a volume metric flask using distilled water. From this stock solution, dilutions were made of standard solution concentrations of 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 micrograms per milliliter. After diluting, 1.2 milliliters of each concentration was transferred to a vial containing 0.4 grams sodium chloride and shaken well until complete dissolution of the sodium chloride occurred. Then the analysis of the standards at different concentration was performed by GC using mass selective detection in the electron impact (EI) ionization scan mode.

Integration and Quantitation of PBZ chromatograms

To obtain the results of the previous experiment, "Information, calibration curve of paclobutrazol (PBZ) and selection internal standard", three modes of integration and quantification were programmed. They were:

1. Specification of parameter was used RICs (Recalculated Ion Current or Reconstructed Ion Chromatograms)
2. Specification of parameter was used only one characteristic ion number 236
3. Specification of parameter was used 3 characteristic ions number 294, 236 and 125

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Internal standard

Paclobutrazol standard solutions were prepared at the concentrations of 0.5, 0.4, 0.2, 0.1, 0.05, 0.01, 0.005, 0.001 micrograms per milliliter, respectively. Each concentrations of paclobutrazol solution were combined with 0.05 micrograms per milliliter of diclobutrazol (internal standard). After thoroughly mixing, 1.2 milliliters of each solution was transferred to a vial containing 0.4 grams sodium chloride, shaken well until complete dissolution of the sodium chloride occurred. Then all solutions were analyzed by GC using mass selective detection in the electron impact (EI) ionization scan mode.

Active ingredient in the commercial paclobutrazol product 10%WP

Ten milligrams (exact weight at 0.001milligram) of commercial paclobutrazol was weighed and dissolved in 50 milliliters of acetonitrile and then adjusted the volume in 100 milliliters volumemetric flask with distilled water. This stock solution was used to make 0.01 micrograms per milliliter of paclobutrazol by dilution with distilled water and 0.05 micrograms per milliliter DBZ was added into the solution and mixed well. After mixing, 1.2 milliliters of the solution was transferred to a vial containing 0.4 grams sodium chloride, shaken well until complete dissolution of the sodium chloride occurred. Finally, all of the solutions were analyzed by GC using mass selective detection in the electron impact (EI) ionization scan mode.

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5.3.2 Optimal SPME condition

Instrumentation

The GC-MS analysis was performed by a Varian model Star 3400 gas chromatograph equipped with Electronic Flow Control (EFC) and fitted with a Saturn II ion trap mass spectrometer (Varian Instruments, Walnut Creek, CA, USA).

The GC chromatographic column consisted of a BPX5 capillary column (SGE GmbH, Darmstadt, Germany), length 30 meters, internal diameter (I.D.) 0.32

millimeters and containing 5% phenyl-polysilphenylen-siloxane with a phase thickness of 0.5 micrometers connected to the splitless injector (The I.D. and thickness were changed from the previous test (I.D.=0.25 millimeters, thickness = 0.25 micrometers). The carrier gas was helium (99.999 %).

Gas chromatographic (GC) and mass spectrometry (MS) detection conditions
(alteration from Crook, 1999)

The temperature program of GC was to hold the temperature initially at 60 ˚C for 6 minutes to a final temperature of 280 ˚C at a rate of 20 ˚C per minute and then remain at this temperature (280 ˚C) for 8 minutes. A column head pressure of 11 p.s.i. and an injector temperature of 300 ˚C were used. The injector was operated by manual holder into splitless mode (SPI/1077) for 6 minutes, the lapse of time for SPME fiber desorption and set at a fixed constant temperature of 300 ˚C. The GC transfer line was maintained at continual 300 ˚C. The mass spectrometer was operated in the electron-impact ionization (EI) scan mode with a source temperature of 300 ˚C. Ionization mode was obtained at fixed mode. The electron energy was 70 eV and the filament current 10 μA. The manifold temperature was set at 180 ˚C. The electron multiplier voltage was established at 1800 volts. The amplitude voltage (A/M) was 4.0 volts. The external event 1 was turned on.

Chromatograms were acquired in 'scan' mode scanning the mass range from *m/z* 50 to *m/z* 300 (with a scan rate 1000 milliseconds), with a back ground mass of *m/z* 45 segment acquire time 25 minutes. In order to improve the peak identification, three fragment ions were monitored from the spectrum of each compound to quantify the response in the selected-ion monitoring (SIM) mode. The mass spectrum of *m/z* 125, 236 and 294 for paclobutrazol (Retention time 17.038 ± 0.2 minutes) and *m/z* 159, 270 and 272 for Diclobutrazol (Internal standard, Retention time 17.518 ± 0.2 minutes) were ion monitored as references. In this way they could be easily identified.

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Solid-phase microextraction fiber (SPME)

The SPME holder and fiber assembly for manual sampling were provided by Supelco (Bellefonte, PA, USA) and used without modification. The silica fiber coated with an 85 micrometers (µm) thick polyacrylate (PA) film. Prior to the measurements, the fiber was conditioned in the injector for 3 hours at 300 ˚C, with the split vent opened, to fully remove any contaminant or impurities present in the coating and introduced during the manufacture of the fiber which might have caused very high baseline noise and injected into the GC system until interfering peaks disappeared. This was according to instruction provided by the supplier.

Solid-phase microextraction (SPME) analytical procedure
(alteration from Crook, 1999)

All SPME was made directly from an aqueous sample. The vials were filled with 1.2 milliliters aqueous sample or standard solution with the addition of 0.4 grams sodium chloride (NaCl, Merck). The vials were then sealed with the hole caps and Teflon-faced silicone septa (Supelco, USA). The samples were stirred for 15 minutes to dissolve the salt. Then, the vials were placed in a water bath which circulated water via magnetic stirrer (IKA-Combimag RCO.) in order to control the temperature at 35 ˚C, stirring again at 15 minutes to adjust the temperature of aqueous sample to 35 ˚C. The speed of stirring was set at level 3.5. Next, the fiber was exposed to aqueous phase for an appropriate time period of 60 minutes, with the stirring of the sample set at 35 ˚C (in order to improve mass transfer from the aqueous sample into the fiber coating). After the extraction, the fiber was removed from the sample and directly introduced into the hot injector of the GC system for analysis (desorption) where the thermal desorption of the analysis was carried out at 280 ˚C for 6 minutes. After the desorption, the fibers were reused after cleaning by inserting them into the split port for 10 minutes at 300 ˚C and allowing helium (He) gas flow at 11 p.s.i. Then, the next sample could be continually operated.

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Preparation of standard solutions

To prepare individual stock standard solutions (paclobutrazol, PBZ and diclobutrazol, DBZ) at the concentration of approximately 2.8 milligrams per 100 grams, all standards was prepared in acetonitrile (99.8%) with distilled water (final ratio 3.5%:96.5% (w/w)). Approximately 2.8 milligrams (± 0.00001) of PBZ or DBZ standard was weighed and dissolved in a 100 milliliter volumetric flask with 3.5 (± 0.00001) grams of acetonitrile. Distilled water was then added to the flask but not reaching the mark. Then, the flask was stored at 20 ˚C for about 20 minutes and the volume was readjusted to 100 milliliters with distilled water using a pasture pipette and thoroughly shaken. A stock solution was used by spiking (paclobutrazol) and internal standard (diclobutrazol). The concentration of PBZ or DBZ solution was maintained at 0.7 milligrams per kilogram sample after spiking into the samples.

Optimal Absorption time of SPME (Extraction time)

A time profile of paclobutrazol absorption onto the 85 micrometer polyacrylate was determined in order to assess the optimum SPME sampling period. 1.2 milliliter aliquots of 0.05 microgram per milliliter were combined with diclobutrazol 0.05 micrograms per milliliter standard solution and were analysed by SPME using mass selective detection with sampling periods of 30, 40, 45, 50, 55, 60, 65, 75, and 90 minutes from the stirred solution. Desorption time was set at 300 ˚C for 6 minutes for these experiments. Then 0.4 gram of sodium chloride of was added to each vial which was placed in a thermostatized water bath at 35 ˚C and with constant stirring (speed in level 3.5, IKA-Combimag RCO) for 15 minutes to allow the salt dissolution as well as to adjust the solution temperature. During extraction, the SPME fiber holder was fixed with a stand assembly. Then, the fiber was removed and placed into the injection port of a GC for desorption. The compounds were analyzed under the conditions as described above. The clean up of fiber was set at 1 hour at 300 ˚C by split port. In this the experiment, the same fiber was used for all the optimization processes (without the injection of a blank).

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Optimal desorption time of SPME

A desorption time was established to prevent carry over of analyte from one analysis to the next. 1.2 milliliters aliquots of 0.05 micrograms per milliliter combined with diclobutrazol 0.05 micrograms per milliliter standard solution were analysed by SPME using mass selective detection with sampling period of 45 minutes and 60 minutes with continuous stirring. Then 0.4 gram of sodium chloride was added to each vial which was kept in a thermostatized water bath at 35 ˚C and continuously stirred (speed in level 3.5, IKA-Combimag RCO) for 15 minutes to allow the salt dissolution as well as to adjust the solution temperature. Desorption times of 2, 4, 6, 7, 8, 9, 10, 12, and 14 minutes were investigated. During extraction, the SPME fiber holder was fixed with a stand assembly. Then, the fiber was removed and placed into the injection port of a GC for desorption. The compounds were analyzed under the conditions as described above. Subsequent to each sample injection, a blank injection of the fiber was made in order to determine if any carry over occurred. The clean up of fiber was set to 1 hour at 300 ˚C by split port.

Clean up time of the SPME fiber

A clean up time for the fiber eradicated influence of particulate matter, especially when real samples are analyzed. Aliquots 1.2 milliliters containing PBZ 0.1 microgram per milliliter and diclobutrazol 0.05 micrograms per milliliter standard solution were analysed by SPME using mass selective detection with sampling period of 60 minutes (as previously observed) while stirring solution. A 0.4 gram amount of sodium chloride was added to each vial which was placed in a thermostatized water bath at 35 ˚C under stirring mode (speed in level 3.5, IKA-Combimag RCO) at 15 minutes to allow the salt dissolution and adjustment of the solution temperature. During extraction, the SPME fiber holder was fixed by a stand assembly. The fiber was then removed and placed into the injection port of a GC for desorption. The compounds were analyzed under the conditions as described above. Desorption time was set for 6 minutes at 300 ˚C (as previously observed). Clean up times of 0, 5, 10, 30, and 60 minutes at 300 ˚C in split port were investigated.

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5.4 Results

5.4.1 Internal standard selection

Investigation of paclobutrazol and internal standards

The results in an experiment calibration curve of paclobutrazol (PBZ) and selection internal standard showed that after injection each concentration of the four triazole standards. The response graphs obtained illustrated the peaks of paclobutrazol, diclobutrazol; flutriafol and hexaconazole which showed rather similar retention times (Table 5.1). The mass spectrum found corresponded to the research which has been done by Sandra, *et al*., 2003. Data referring to mass spectrum of paclobutrazol was **294**, **236**, 238, 167, **125**; Diclobutrazol was **272**, **270**, **159**, 83; Flutriafol was **219**, **164**, **123**, 83; and Hexaconazole was **216**, **214**, **83**, and 82. The bold numbers were selected as the fragment ions which were identified from standard chemistry. The three orders of fragment ions, maximum, secondary and third order of high abundance percentage were used. The full scan mass spectra of paclobutrazol presented peaks at *m/z* 294 which corresponds to the pseudo molecular ion $[M + H]^+$ with the characteristic isotopic pattern in agreement with the presence of a chlorine atom. Therefore, paclobutrazol molecular weight was about 294 g/mol.

Paclobutrazol molecular weight was about 294.0 grams per mole. The retention time of diclobutrazol was detected and was higher than paclobutrazol and also different to that of flutriafol and hexaconazole. Thus, it was made the internal standard. The mass spectrum of paclobutrazol and diclobutrazol are shown in Figure 5.1B, 5.1C. The calibration curve of paclobutrazol was determined after a clear retention time, mass spectrum of paclobutrazol and those of the internal standard, diclobutrazol, were given. The peak areas were monitored by RICs (Recalculated Ion Current or Reconstructed Ion Chromatograms) mode. The linear regression was more than 0.990 as shown in Figure 5.2C. The addition of ion chromatographs obtained under RICs mode after SPME extraction of 0.05 micromilliliters spiked solution is exhibited in Figure 5.1A.

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Table 5.1 Summary of retention time by GC-MS analysis

Standard pesticide	Retention time (minutes)	Main EI fragment ions (*m/z*)
Paclobutrazol	17.038 ± 0.200	125, 236, 294
Diclobutrazol	17.518 ± 0.200	159, 270, 272
Flutriafol	17.215 ± 0.200	123, 164, 219
Hexaconazole	17.269 ± 0.200	83, 214, 216

For the experiment of integration and quantization of chromatograms, selective specification ions mode were used as a programming analysis. The results are shown in Figure 5.2. Only for ion number 236 was it found that the coefficient of the determination value of the peaks area was lower than RICs and the 3 characteristic ions number 294, 236 and 125. The values identified were not good when compared with the coefficient of determination value of peaks area of RICs and three characteristic ions number 294, 236 and 125. Therefore, the three characteristic ions number 294, 236 and 125 were selected in this experiment because they were more specific than the RICs parameter.

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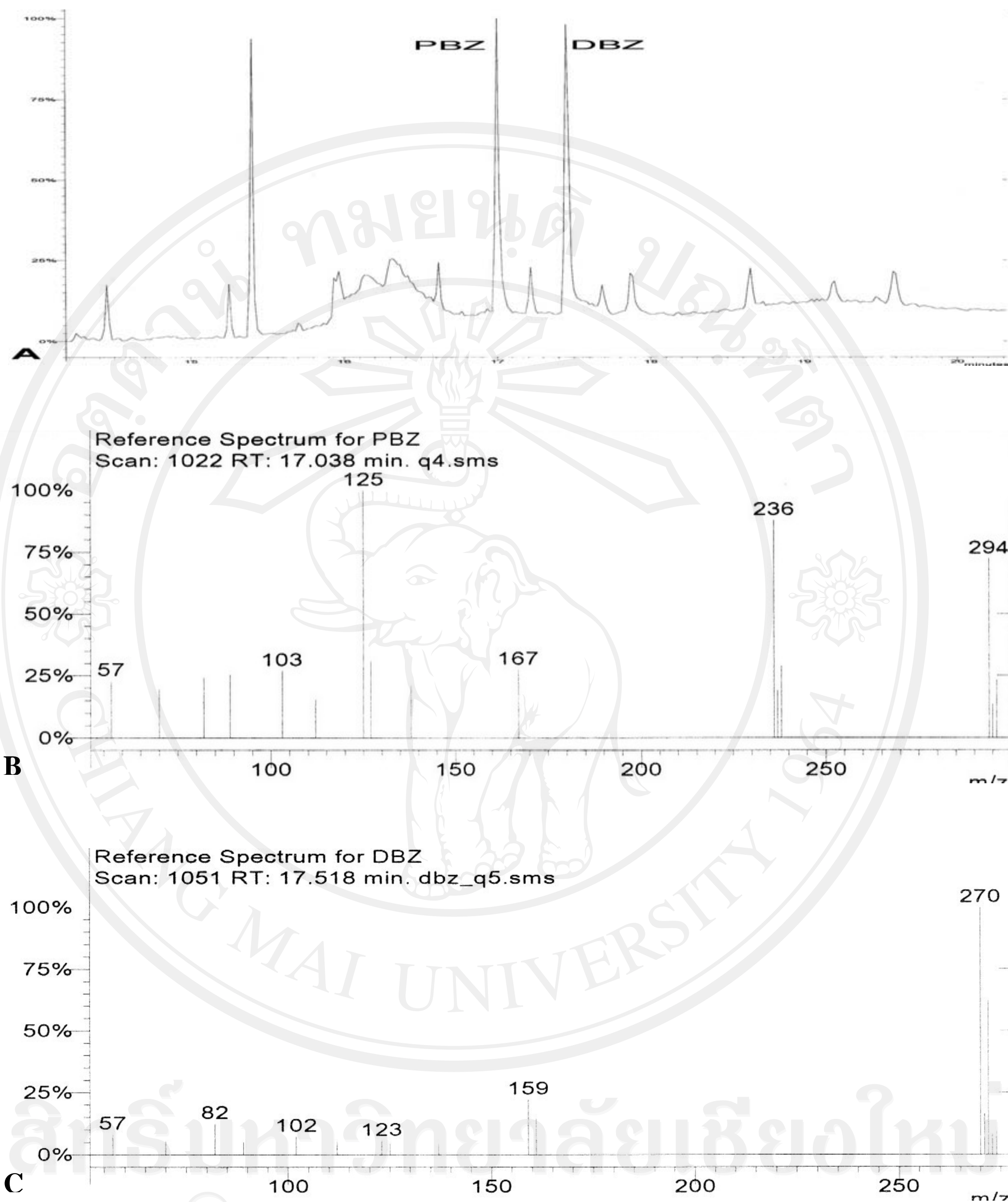

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Figure 5.1 *A*. GC-MS ion chromatograms obtained for PBZ and DBZ (internal standard) by RICs mode. *B*. mass spectrum of PBZ in library. *C*. mass spectrum of DBZ in library.

An experiment internal standard, a graph of the response obtained the peaks of paclobutrazol and diclobutrazol were shown at the same retention time when compared with the result from the experiment information, calibration curve of paclobutrazol (PBZ) and selection internal standard. The peak areas of paclobutrazol increased followed by the increasing of standard concentrations. However, the peak areas of diclobutrazol (internal standard) changed slightly because the effect of the used fiber after several extractions. The performance of the fiber was diminished by particulate matter that interferes the peak areas. However, in the present work the value was acceptable. Afterwards, all values of the paclobutrazol peak area were used to produce the standard curve which found that the coefficient of determination value was the same as the experiment integration and quantitation of paclobutrazol chromatograms as shown in Figure 5.1B. This regression curve was more than 0.990 (Figure 5.3). Then, the calculation of the ratio of PBZ/DBZ peak area and concentration were used to produce the curve which found that the coefficient of determination value was the same to paclobutrazol (Figure 5.3B). Diclobutrazol was shown at the same retention time when compared to the result from the experiment information, calibration curve of paclobutrazol (PBZ) and selection internal standard. The peak areas were rather similar as well.

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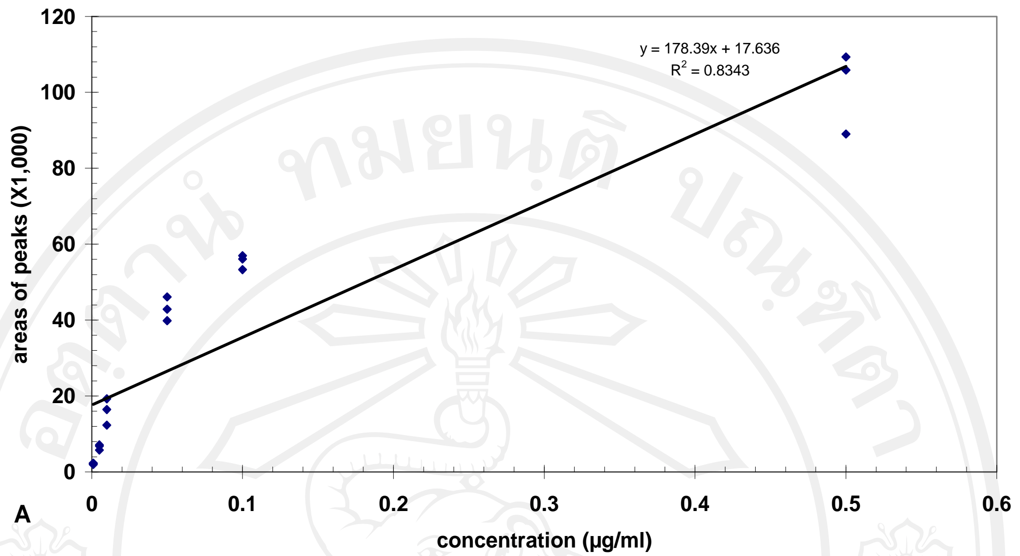

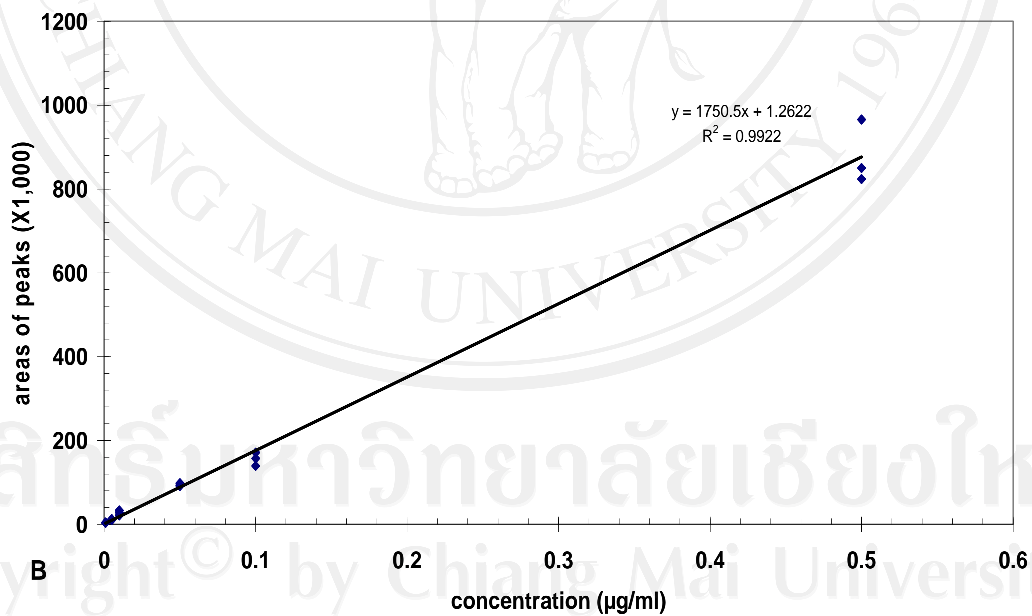

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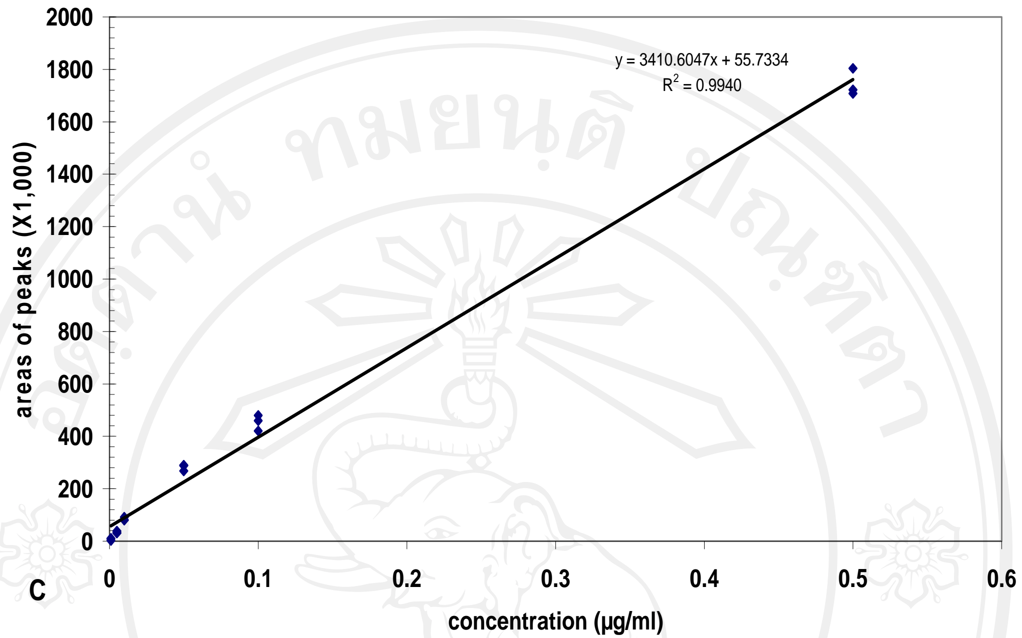

Figure 5.2 Area of peak in each parameter showing linear correlation. *A*. The only ion number 236. *B*. Three characteristic ions number 294, 236 and 125. *C*. RICs parameter.

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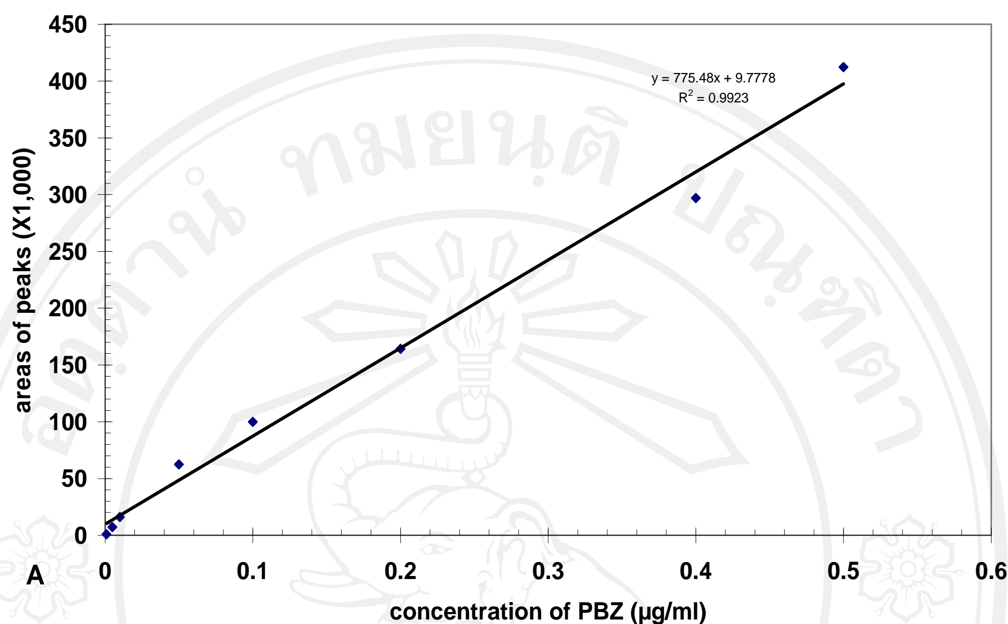

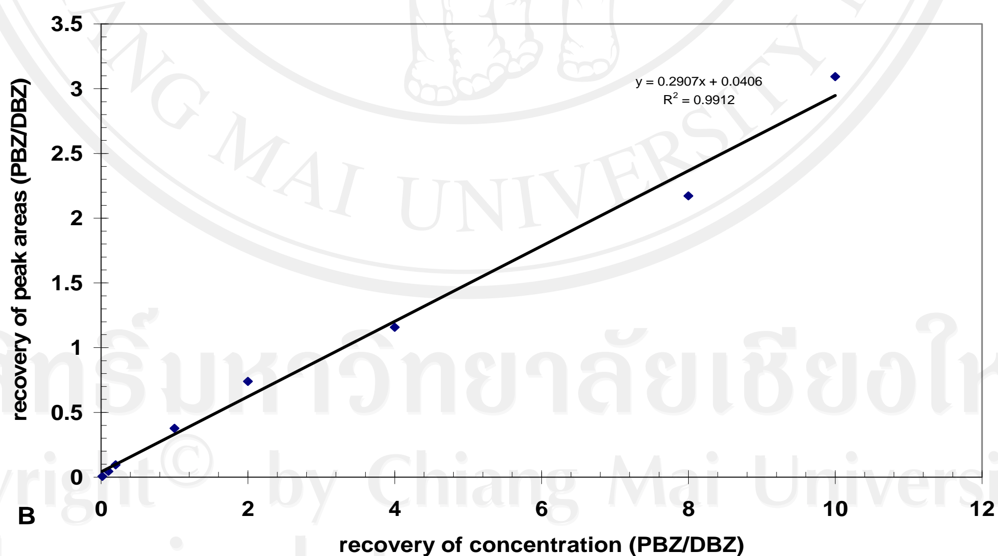

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Figure 5.3 *A*. Area of PBZ peaks integrated by three characteristic ions number 294, 236, and 125. *B*. recovery PBZ/DBZ ratio of peak area and concentration.

From the results of the last experiment, an active ingredient in the commercial paclobutrazol product 10 % (WP) showed that the amount of active ingredient in commercial paclobutrazol was higher than 10 % which was labeled at the bottle side. It was detected at 11.55 percent (Table 5.2).

Table 5.2 Summary of an active ingredient in commercial paclobutrazol 10 % (WP)

Items		Percentage of active ingredient
Calculate from (y = 0.4896x + 0.0539)	1	12.149
	2	10.952
Average		**11.551**
CV (%)		7.33
S.D.		0.85

5.4.2. Optimal SPME condition

The optimization of SPME

The effects of various parameters (e.g., adsorption time, desorption time, and clean up time of fiber) on the SPME process efficiency were optimized under the selected ion monitoring (SIM) acquisition mode. In addition, the sodium chloride addition of 0.4 gram was already discussed by Crook (1999); the temperature of extraction at 35 ˚C was used. The procedure can be summarized as follows: the first step to be optimized was the time required for the analytes to reach equilibrium between the aqueous and the stationary phase. Then, the absorption time profile was evaluated by monitoring the area counts versus the extraction time, the fiber was exposed to standard solution of the analytes in a concentration of 0.05 micrograms per milliliter combine with diclobutrazol 0.05 micrograms per milliliter for considering different extraction time from 30, 40, 45, 50, 55, 60, 65, 75, and 90 minutes. All the extractions were carried out at a temperature of 35 ˚C and with continuous stirring in order to ensure that the aqueous sample is perfectly agitated and to reduce the equilibration times. Sodium chloride, 0.4 grams was added. After the absorption process, the analytes were thermally desorbed into the injection port of a gas

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chromatograph at 300 ˚C for 6 minutes. The results found that the paclobutrazol reached the maximum extraction yield in 60 and 90 minutes, when comparing the mean values, as shown in Figure 5.4. However, further experiments were performed at 60 minutes and so on because the comparison between the all of peak areas was not significantly different and clearly showed that the responsibility was rather stable after 60 minutes. The addition of this can be time saving, and was no benefit when longer absorption periods were used.

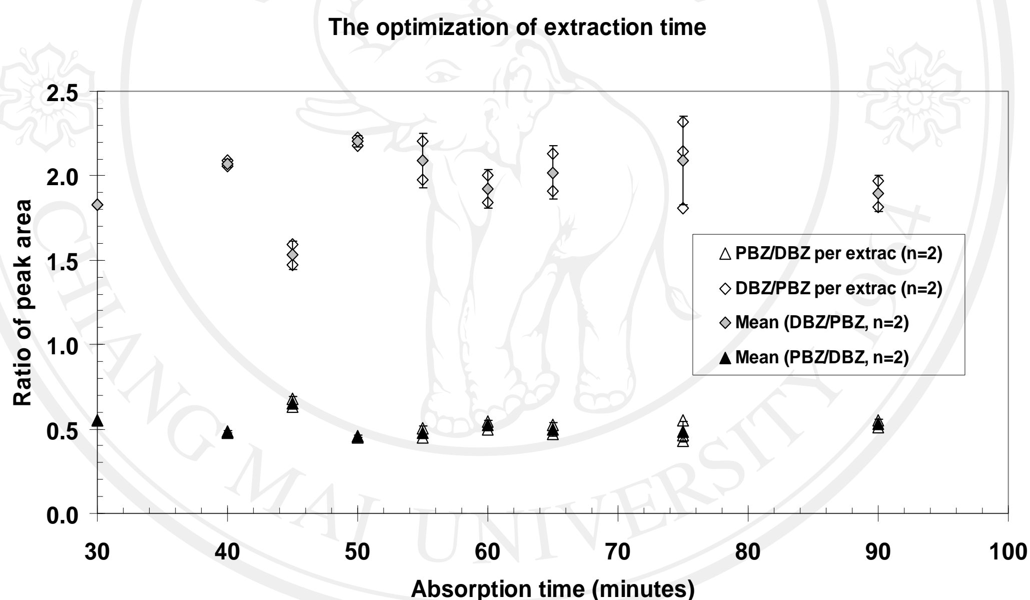

Figure 5.4 Absorption time profile (interval time 30 to 90 min) for paclobutrazol combined with diclobutrazol by direct-SPME using 85µm PA fiber was analysed.

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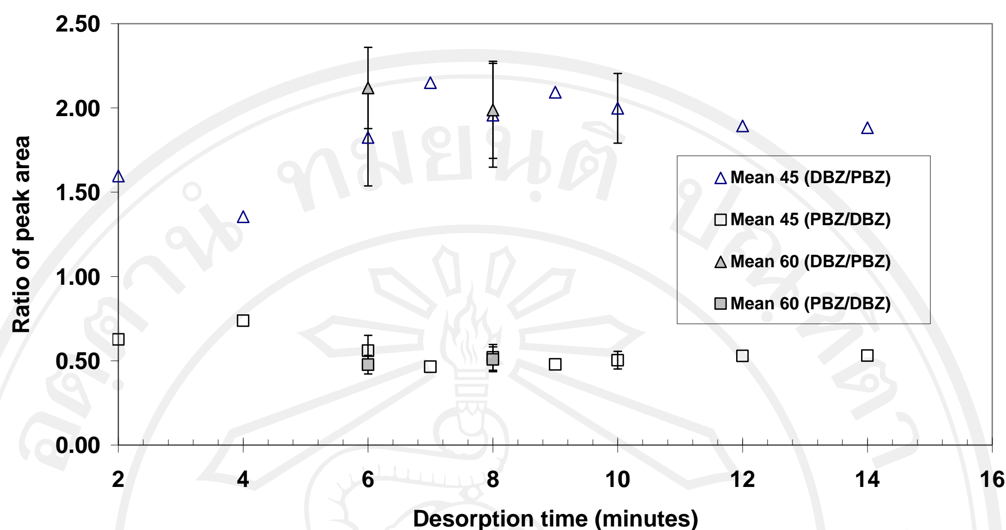

Figure 5.5 Desorption time profile (interval time 2 to 14 minutes) for paclobutrazol combined with diclobutrazol by direct-SPME using PA fiber was analysed. Two differences of absorption time were at 45 and 60 minutes.

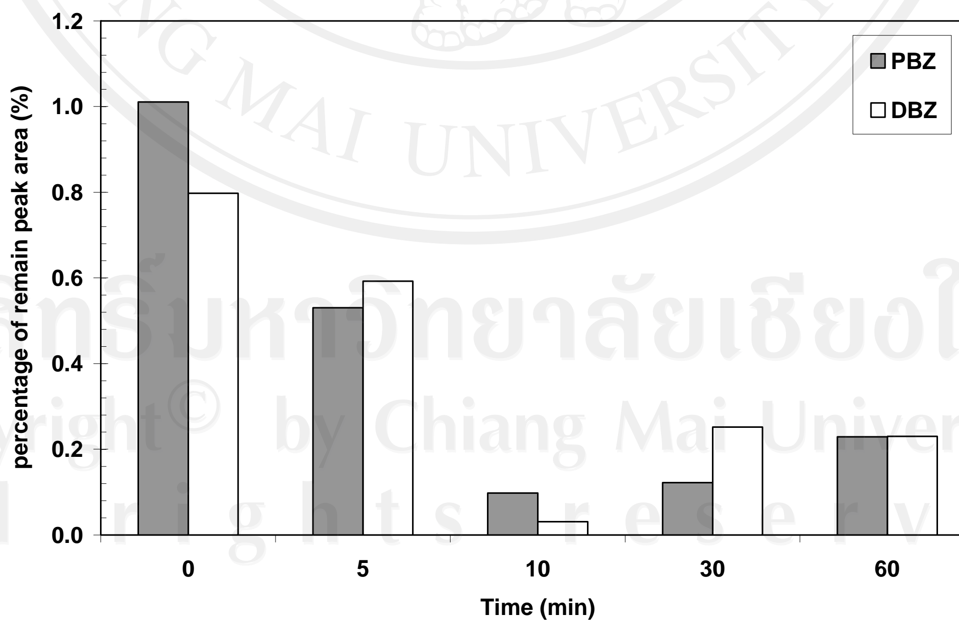

Figure 5.6 Clean up time of SPME fiber at different interval time from 0 to 60 minutes with calculates in percentage of remain peak areas was evaluated.

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The next experiment was to optimize desorption time. This parameter was determined by maintaining the absorption time of the fiber to the aqueous sample at 45 and 60 minutes. The other experimental parameters were the same as the conditions above described. The studied was carried out by varying desorption time in the range from 2 to 14 minutes at 300 ˚C inside the injector port. It was observed that at 6 minutes, higher mean values were exhibited in both absorption times (45 and 60 minutes) than desorption time at 8 minutes. The mean value at the head arrow position was shown in Figure 5.5. Moreover, the maximum value of diclobutrazol was encouraged. Therefore, desorption time was chosen at 6 minutes as the optimum for further analysis. The results obtained after 8 minutes can be explained that the exposure of peak areas were not raised, which as mean value rather identical.

The clean up of fiber is also the most important for reducing the disturbance particulate matter of several extractions, especially when real samples are analyzed. The clean up time of 0, 5, 10, 30, and 60 minutes at 300 ˚C in split port was investigated. GC-MS conditions for analysis were the same as above described. The finding showed that at 10 minutes was exhibited very low remnant paclobutrazol and diclobutrazol in the fiber. It was less than 0.1 percent (Figure 5.6) in comparison between the peak areas of absorbed fiber and after desorbed fiber. The results of time were cleared for cleaning up fiber.

5.5 Discussion

5.5.1 Internal standard selection

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The mass spectra of paclobutrazol were similar to those of Sancho *et al.* (2003) in Figure 5.7, Sharma and Awasthi (2005) in Figure 5.8, 5.9, Bolygo and Atreya (1991) and Sannino *et al.* (2004). They were reported that the full scan mass spectra were presented at *m/z* 296, 294, 236 and 125. Diclobutrazol mass ions were at *m/z* 82, 159, 270, 272 and 292 (Hirahara *et al.*, 2005; Bolygo and Atreya, 1991; Sandra *et al.*, 2003). Whereas, it may be also due to the matrix and instrument interference, the abundance percentages of mass fragments were a little bit varied.

Therefore, only one ion (highest abundance percentage) was used for indicative ion monitoring. However, a different retention time was shown because of the different conditions of the GC or GC-MS and other factors including the dissimilar machine types, extraction of method, or the characteristic of the detectors. The active ingredient occurred higher than 10 percent, as labeled on the bottle side. This exhibited that the efficiency of fiber with absorption was not stable. On the other hand, it was probably due to the temperature the fiber was exposed to during sample stirring for an optimized absorption time 45 minutes at room temperature (20-24 ˚C) that made the absorption unstable.

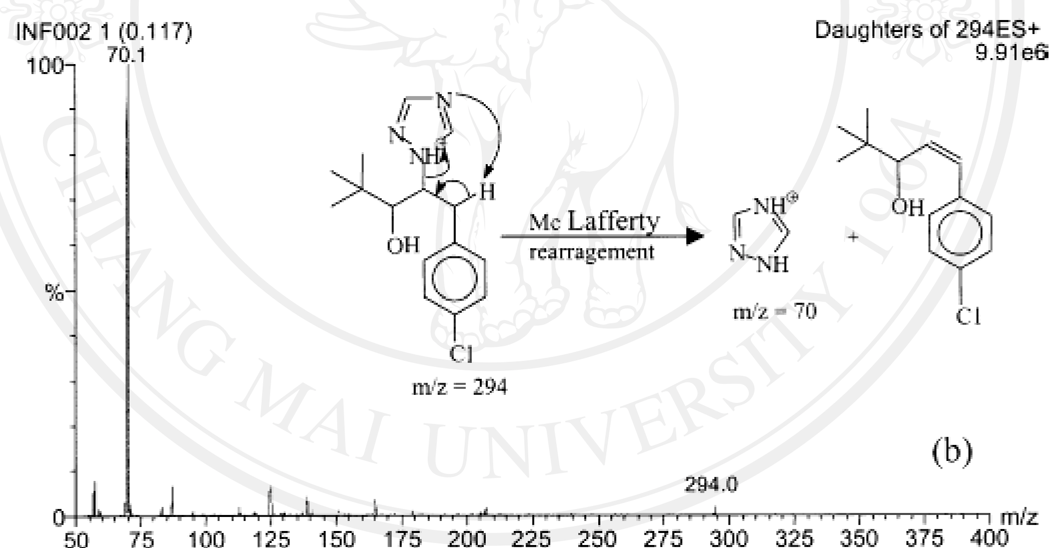

Figure 5.7 The full scan mass spectra of paclobutrazol by Sancho *et al*. (2003).

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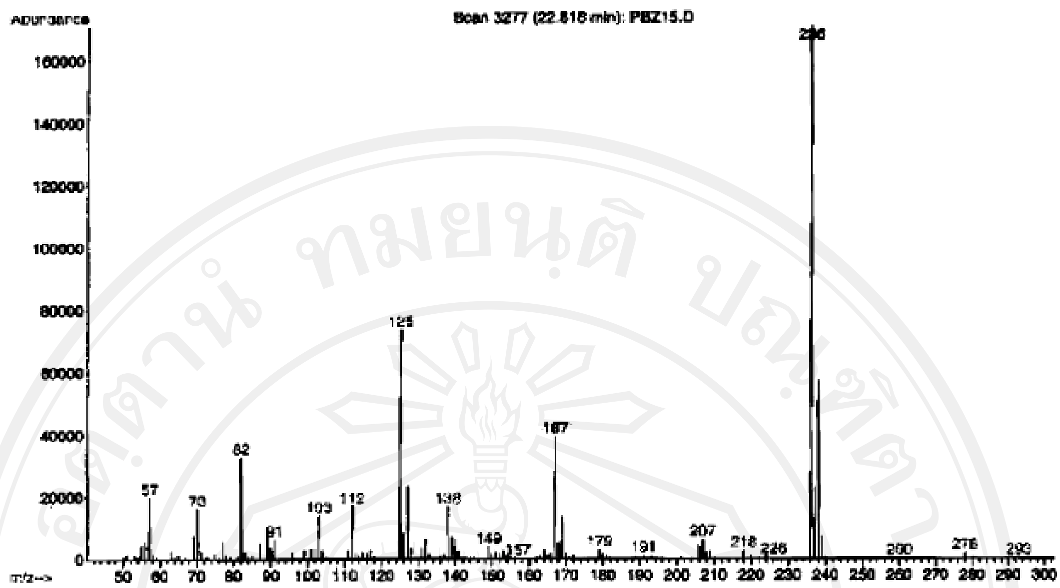

Figure 5.8 The spectra of paclobutrazol standard by Sharma and Awasthi (2005)

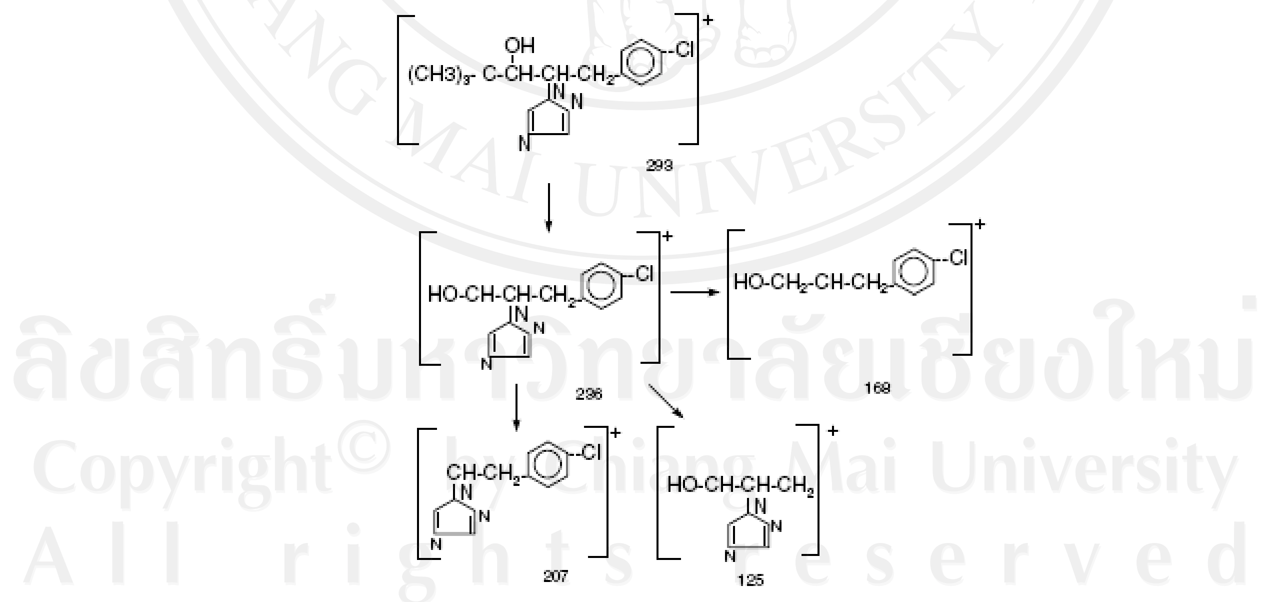

Figure 5.9 The mass fragments of paclobutrazol by Sharma and Awasthi (2005)

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5.5.2. Optimal SPME condition

Initially, information was obtained about the time used for the extraction of paclobutrazol and internal standard at 45 minutes at room temperature (20 ˚C) of adsorption according to Crook (1999). The results and the daily standard check showed that fluctuations in the data were found when later samples were injected. Thus, in the second experiment, the optimization of SPME was controlled at a temperature of 35 ˚C during adsorption or extraction period with SPME by placed the sample vials in water bath with circulator. After more injections, the data were rather stable because it improved mass transfer from the aqueous sample into the fiber coating. Besides the effect of temperature, magnetic stirring is widely used for agitation (Anonymus, 1998; Kataoka *et al*., 2000) and to reduce the equilibration time (Aguilar *et al*., 1998) as well. A desorption time of 6 minutes at 300 ˚C was similar to that was found by Crook (1999). Paclobutrazol is slightly volatile (USEPA, 2000) so that the thermal desorption was also appropriate and retained the advantages; solvents are completely eliminated, blanks are greatly reduced, and desorption time can be reduced to a few minutes. However, the current methods require extensive modification of the gas chromatographic injector or the addition of a desorption module (Arthur and Pawliszyn, 1990). Therefore, for SPME-GC, analyte desorption is a function of time versus temperature (Krutz *et al*., 2003). A part of the adsorption time was altered at the beginning 45 to 60 minutes because it exhibited higher peak areas than at other times. Therefore, although at 45 and 90 minutes gave high relative peak areas, at 50 minutes that was decreased somewhat with an increase again at 55 minutes. Otherwise, the relative peak response after 60 minutes was reduced. The clean up time at 10 minutes was clearly chosen. However, all treatments showed that the residue less than 1.0 percent. Whereas, it could be acceptable if the fibers are reused without cleaning. However, when the fiber was repeatedly adsorbed with the paclobutrazol at a high concentration (>1 milligram per kilogram), it was necessary to clean. On the other hand, if the analysis started at a very low concentration, the fiber should contain minimum residuals. Cleaning the fiber also reduced the effect of carry over. It was checked by a blank desorption running. In addition, the shorter fiber's life time at 1 hour of clean up at the beginning was apparent.

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5.6 Conclusion

Paclobutrazol was detected at a retention time of 17.038 ± 0.2 minutes, with the characteristic ions of *m/z* 294, 236, and 125. Diclobutrazol was clearly distinguished from paclobutrazol that was selected to make the internal standard with the retention time 17.518 ± 0.2 minutes and the characteristic masses of *m/z* 272, 270, and 159. The three characteristic ions (294+236+125) were used to integrate or screen the chromatograph in selected ion monitoring (SIM) mode. The optimization of an SPME procedure was found the absorption time at 60 minutes, desorption time at 6 minutes and the clean up time of fiber at 10 minutes under the GC-MS program described above. The condition used within this study can also be suitable for validating the method of paclobutrazol residue in soil and mango as described in the next chapter.

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