CHAPTER 3

RESULTS

3.1 Suitable GC-MS conditions for determination of AM and MA

3.1.1 GC-MS conditions

The suitable GC-MS conditions for determination of AM and MA are shown in Table 3.1. A total ion scan chromatogram of AM, MA, MDA, MDMA, MDE, and ketamine to a final concentration of 20, 5, 50, 5, 20 and 20 µg/ml, respectively, is shown in Figure 3.1. Each compound showed its peak separately without any overlapping of the chromatogram. This indicated that the GC-MS condition was appropriate for separation of these substances, although background noises were detected. Figure 3.2 and Figure 3.3 show the total ion chromatogram and relative mass spectrum of AM and MA, respectively. Retention time of amphetamine derivatives is demonstrated in Table 3.2.

Table 3.1 GC-MS parameter

Operation	**Condition**
1. Injection	SPME, Splitless mode
2. Injector temperature	250 °C
3. Column	HP-5MS 30 m ×0.25 mm×0.25 µm
4. Column temperature program	60 °C (hold2 min) - 250°C (20 °C/min, hold 1 min)
5. Carrier gas	Helium 1.0 ml/min
6. Transfer line	280 °C
7. MS source	230 °C
8. MS quadrupole	150 °C
9. MS mode	Scan m/z 35-550

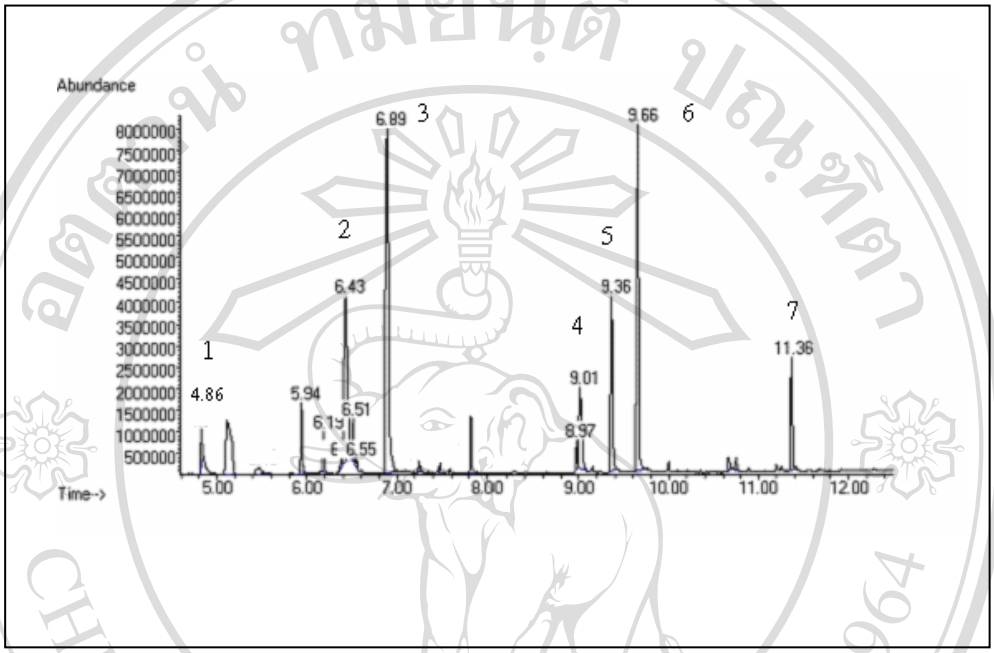


Figure 3.1 Scan mode chromatogram of mixed standard solution of amphetamine derivatives and ketamine using HS-SPME-GC-MS.

Peak identification: 1) INS 2) AM 20 µg/ml 3) MA 5 µg/ml 4) MDA 50 µg/ml 5) MDMA 5 µg/ml 6) MDE 20 µg/ml 7) ketamine 20 µg/ml

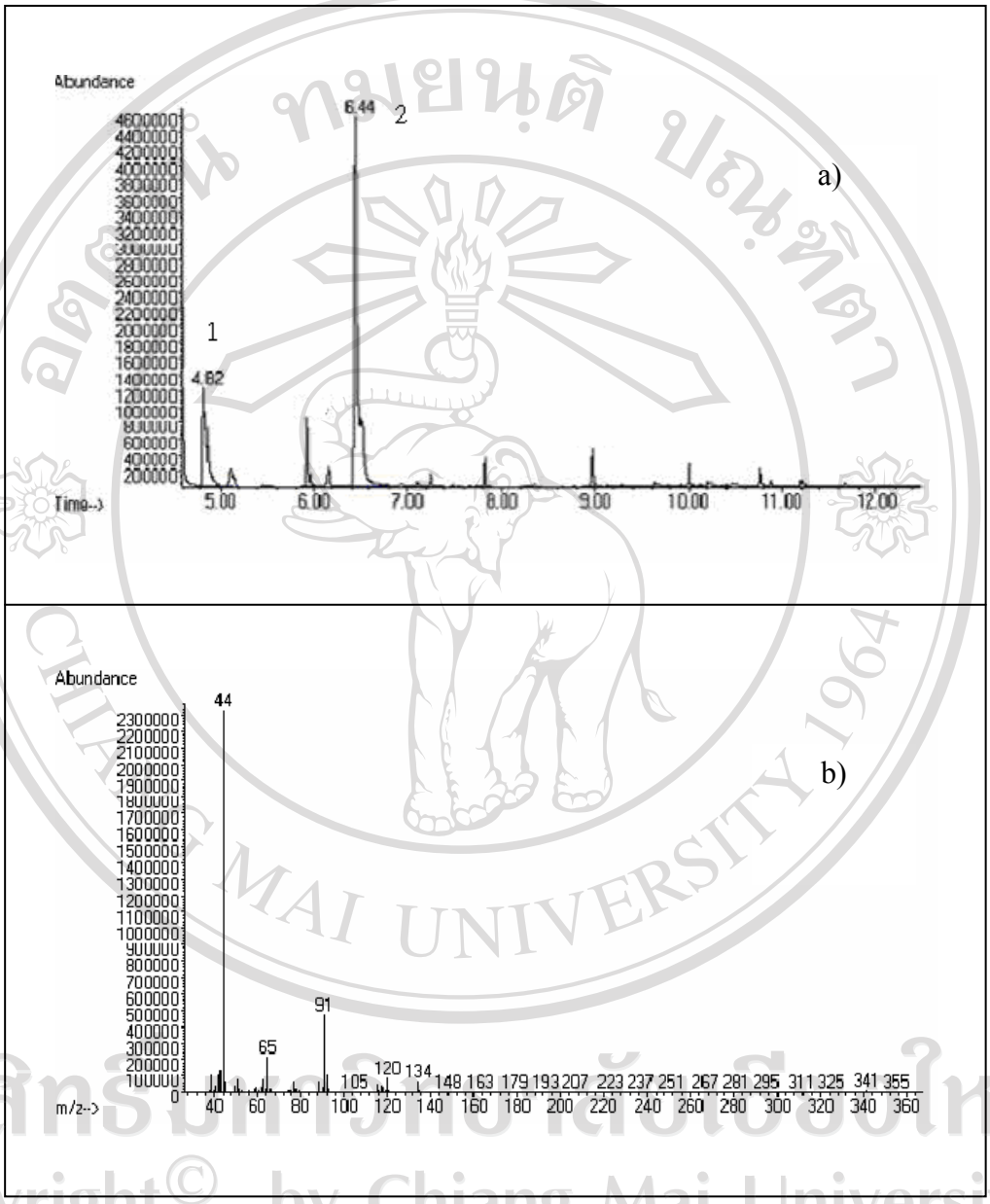


Figure 3.2 a) Scan mode chromatogram of AM standard

b) Mass spectrum of AM by using scan mode.

Peak identification: 1) INS 2) AM 20 µg/ml

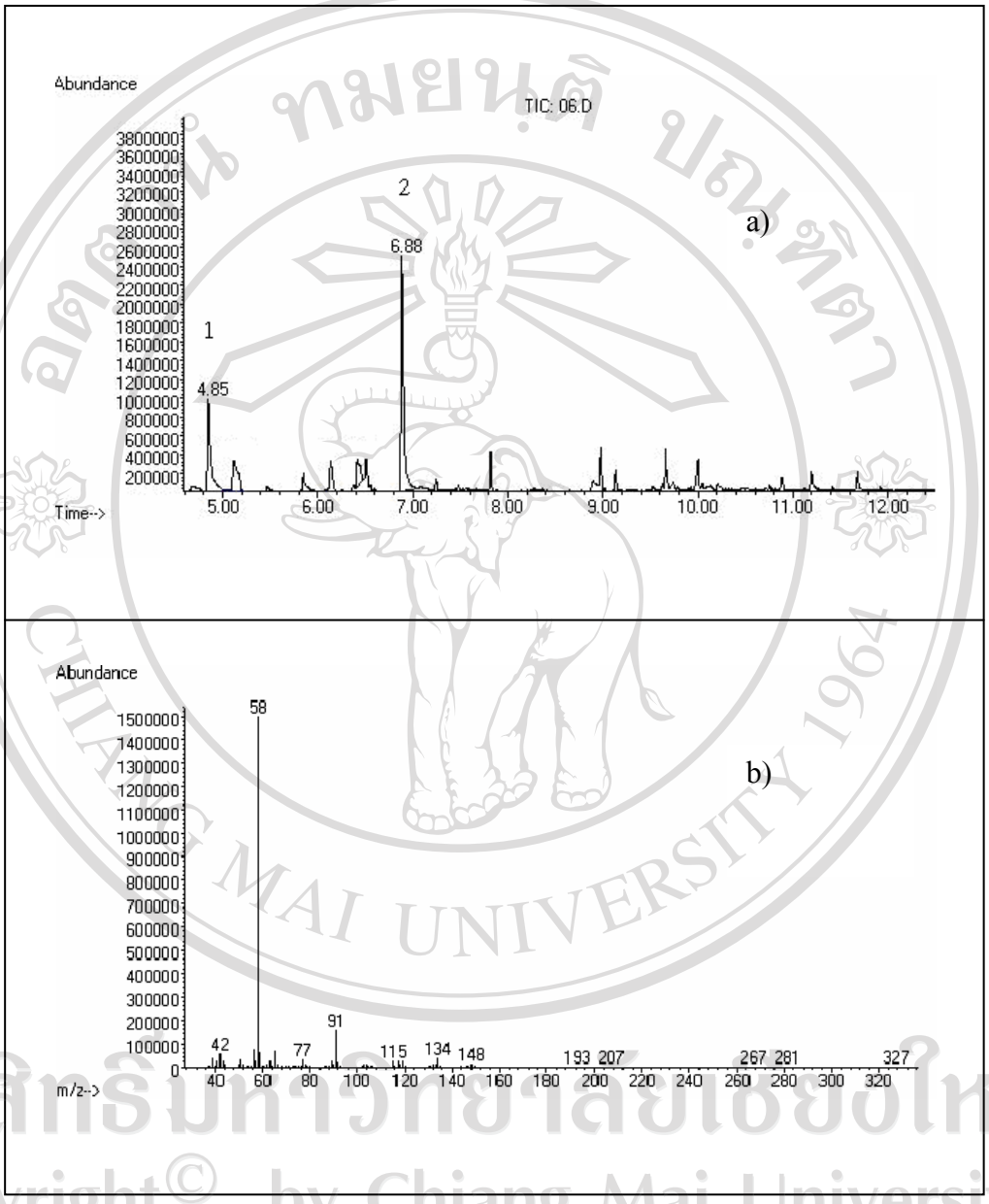


Figure 3.3 a) Scan mode chromatogram of MA standard

b) Mass spectrum of MA by using scan mode.

Peak identification: 1) INS 2) MA 2 µg/ml

Table 3.2 Retention time (RT) and selected ion monitoring (SIM) of amphetamine derivatives and ketamine

Analyte	RT (min)	Target Ion	Qualifier Ions	Mass ratio
AM	6.43	44	91, 65	100, 20,8
MA	6.90	58	91, 65	100, 10, 5
MDA	9.04	44	136, 135	100, 50, 45
MDMA	9.37	58	135, 77	100, 10, 5
MDE	9.66	72	44, 135	100, 15, 10
Ketamine	11.36	180	182, 209	100, 32, 27

Compounds were identified using the RT and relative abundance of three confirming ions, 1 target and 2 qualifier ions. Qualitative data were identified by the relative abundance of the target ion and 2 qualifier ions that do not exceed ±20% of a standard substance [48]. From this result, the retention time of AM was 6.44, the target ion was 44 (base peak) and the 2 qualifier ions were 91and 65. The mass ratio of these 3 ions was 100, 20 and 8. For MA, the target ion was 58 (base peak) and the 2 qualifier ions were 91and 65. The mass ratio of these 3 ions was 100, 10 and 5. The RT, target and qualifier ions of compounds shown in Table 3.2 are identified and used in SIM mode. The selected ions for AM and MA in this experiment agreed with selected ions reported by other studies [51].

SIM chromatograms of mixed standard solutions are shown in Figure 3.4. Each substance demonstrated its peak at a different RT. The background noise in SIM mode is less than that found in total ion scan mode. Figure 3.5 and Figure 3.6 show the SIM chromatogram and relative mass spectrum of AM and MA, respectively.

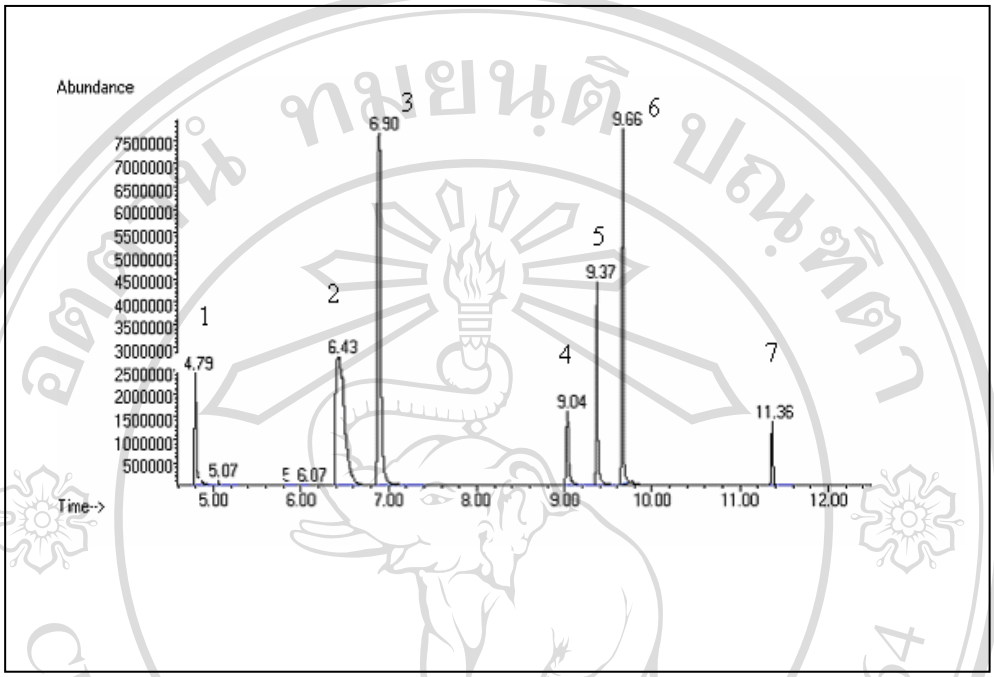


Figure 3.4 SIM mode chromatogram of mixed standard solutions of amphetamine derivatives and ketamine using HS-SPME-GC-MS.

Peak identification: 1) INS 2) AM 20 µg/ml 3) MA 5 µg/ml 4) MDA 50 µg/ml 5) MDMA 5 µg/ml 6) MDE 20 µg/ml 7) ketamine 20 µg/ml

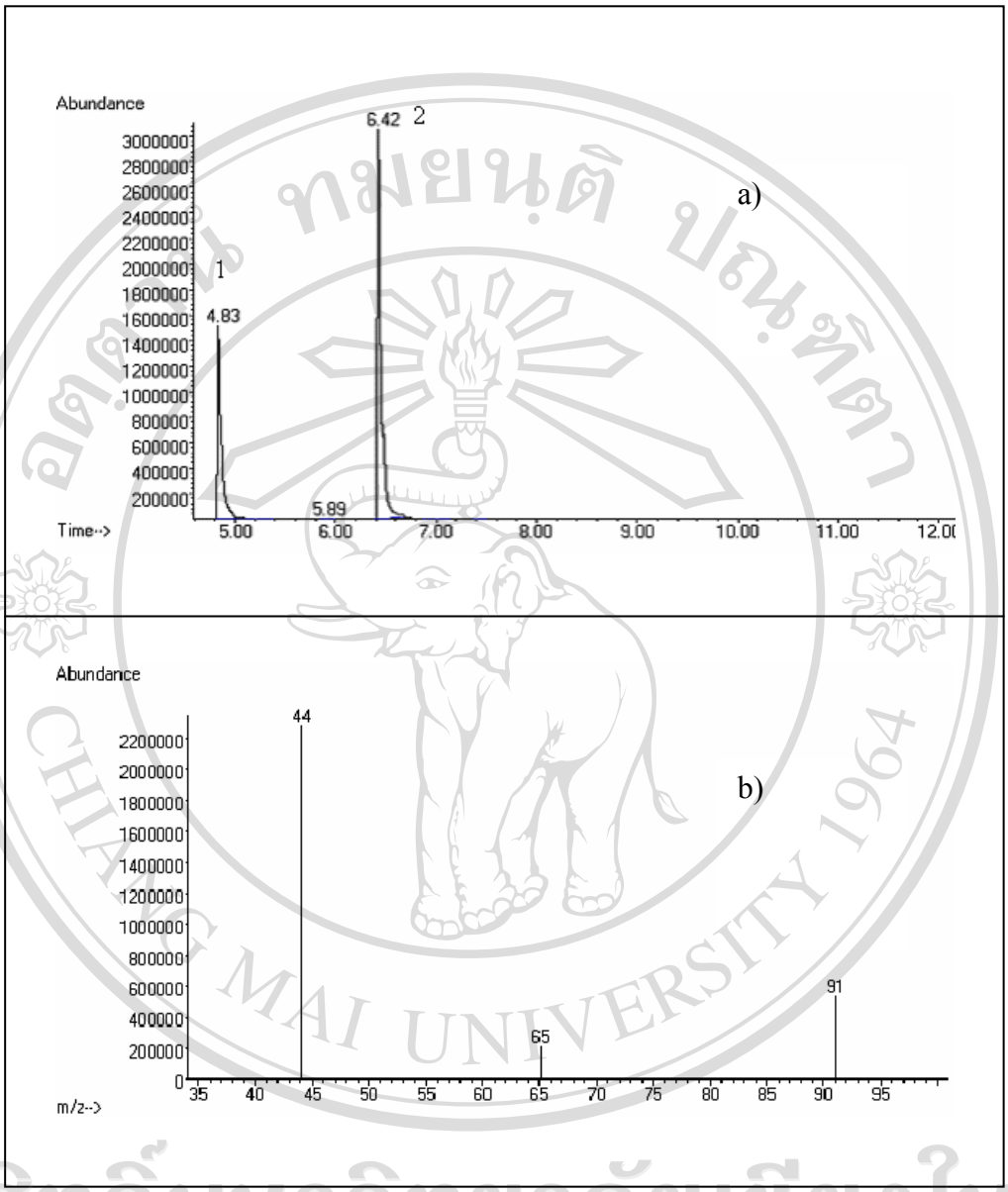


Figure 3.5 a) SIM mode chromatogram of AM standard

b) Mass spectrum of AM by using SIM mode.

Peak identification: 1) INS 2) AM 20 µg/ml

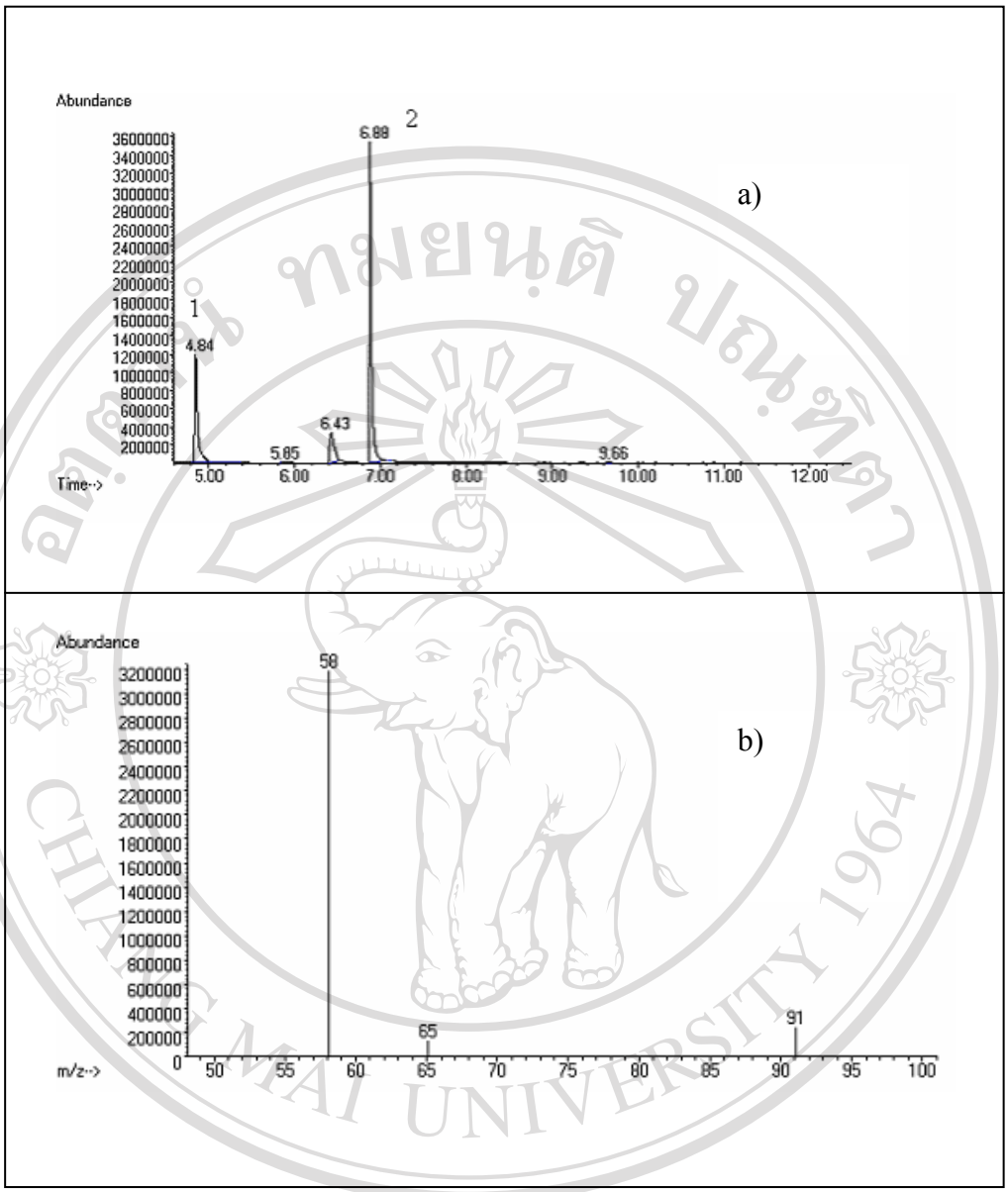


Figure 3.6 a) SIM mode chromatogram of MA standard

b) Mass spectrum of MA by using SIM mode.

Peak identification: 1) INS 2) MA 2 µg/ml

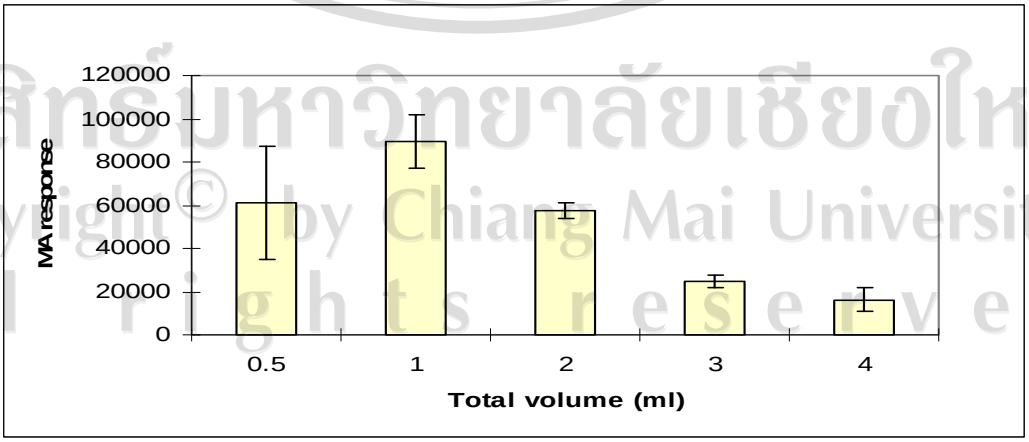
3.2 Optimization of SPME conditions

3.2.1 Optimization of total volume of solution preparation

To evaluate a proper total volume in a tested vial, 0.5, 1, 2, 3 and 4 ml of volume preparation were determined. The results in Table 3.3 and Figure 3.7 show that a different total volume of preparation gave a different response ($p < 0.001$, ANOVA). One ml of total volume yielded a higher response, but also high variability. The total volume of 2 ml in the vial showed less response than 0.5 and 1 ml, but it also showed less variability. The total volume of 2 ml was selected for further analysis.

Table 3.3 Comparison of different total volume (n=5)

Sample volume (ml)	**Peak area MA**	
	Mean	**(±SD)**
0.5	61236	26277
1.0	89237	12369
2.0	**57413**	**3658**
3.0	24741	2688
4.0	16191	5359



$p < 0.001$, ANOVA

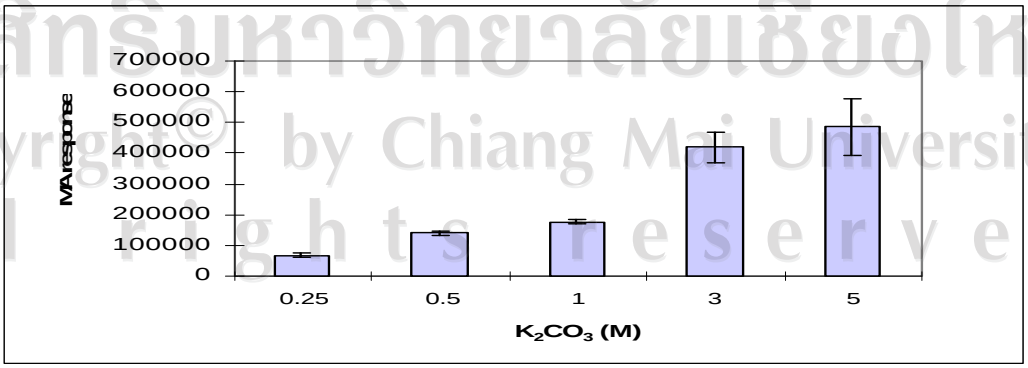
Figure 3.7 Comparison of different total volumes of preparation.

3.2.2 Optimization of potassium carbonate concentration

To increase the recoveries of analysis, K_2CO_3 was used as a salting-out. The proper concentration of K_2CO_3 was determined in this experiment. The results in Table 3.4 and Figure 3.8, show that there was a significant difference in using different concentrations of K_2CO_3. The highest concentration of K_2CO_3 yielded the highest response in MA analysis, but also high variability. When the pH of solutions was measured, the samples that added 5, 3 and 1 M, had a pH equal to 12.5, 12 and 11.5, respectively. Although the preparation that added 1 M K_2CO_3 showed less MA response, it had less variability, and the matrix pH closed the pKa of MA. Therefore, 1 M of K_2CO_3 was used in this study.

Table 3.4 Comparison of potassium carbonate concentration (n=5)

K_2CO_3 (molar)	**Peak area MA**	
	Mean	**(±SD)**
0.25	68143	8991
0.50	140946	6810
1.00	**176233**	**7553**
3.00	418685	47724
5.00	484837	91427



$p < 0.001$, ANOVA

Figure 3.8 Comparison of potassium carbonate concentration.

3.2.3 Comparison of SPME fibers

Two kinds of SPME fibers; 100 µm polydimethylsiloxane (PDMS) and 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) were compared by extraction of AM 20 µg/ml, MA 5 µg/ml, MDA 50 µg/ml, MDMA 5 µg/ml, MDE 20 µg/ml and ketamine 20 µg/ml. The results in Table 3.5 and Figure 3.9 show that using PDMS/DVB fiber yielded a higher AM and MA response than using PDMS fiber, but for MDA, MDMA, MDE and ketamine, PDMS fiber showed better responses than PDMS/DVB. This experiment focused on AM and MA analysis, therefore, PDMS/DVB fiber was selected for this study.

Table 3.5 Comparison of PDMS and PDMS/DVB fibers on amphetamine derivatives and ketamine analysis (n=1)

Analyte	**RT**	**Peak area**		**Ratio**
		PDMS	**PDMS/DVB**	**PDMS:PDMS/DVB**
AM	6.44	509687	17165648*	0.03
MA	6.90	791956	3352165*	0.24
MDA	6.04	2191154	1037259	2.11
MDMA	9.40	4024269	2685091	1.50
MDE	9.66	2341863	1953801	1.20
Ketamine	11.37	563217	550310	1.02

(* $p < 0.001$ by student t-test)

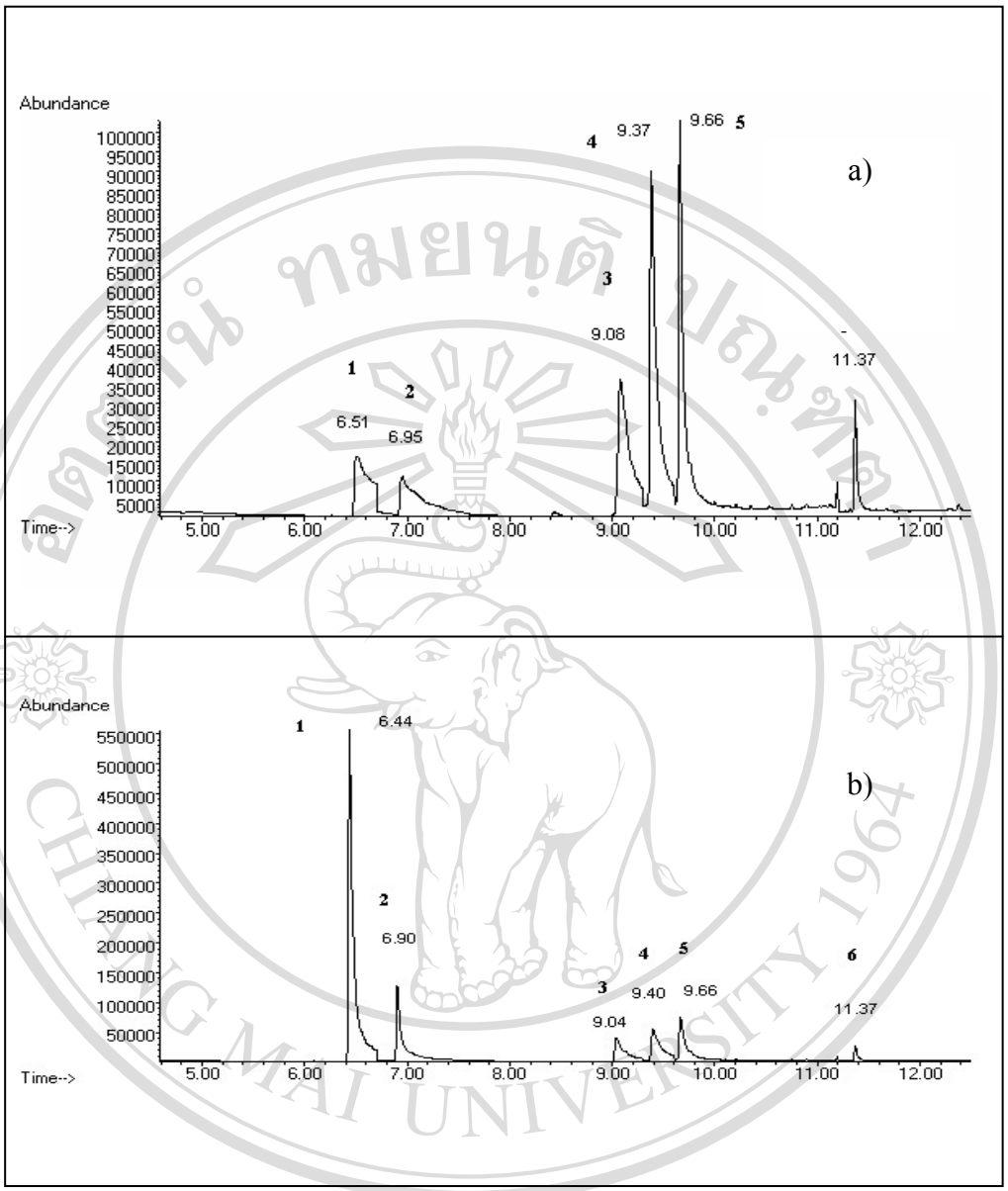


Figure 3.9 a) SIM chromatogram of mixed standard solutions of amphetamine derivatives and ketamine using PDMS with HS-SPME-GC-MS

b) SIM chromatogram of mixed standard solutions of amphetamine derivatives and ketamine using PDMS/DVB with HS-SPME-GC-MS.

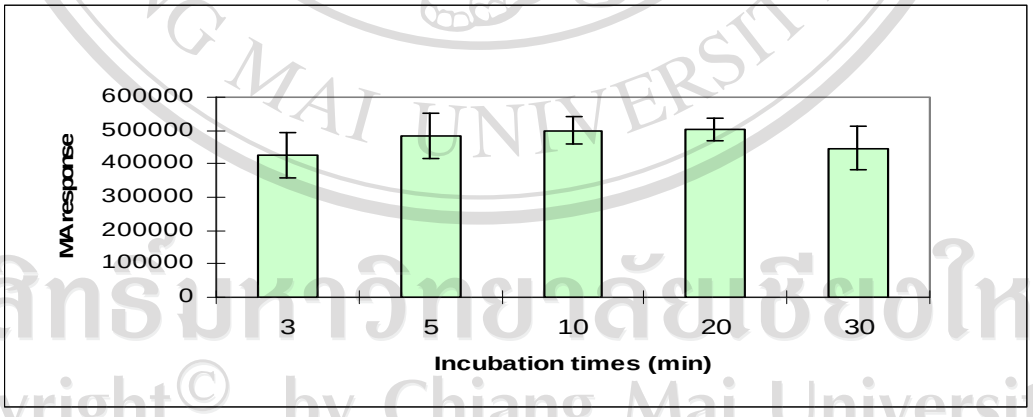
Peak identification: 1) AM 20 µg/ml 2) MA 5 µg/ml 3) MDA 50 µg/ml 4) MDMA 5 µg/ml 5) MDE 20 µg/ml 6) ketamine 20 µg/ml

3.2.4 Optimization of incubation time

To determine a suitable incubation period, 3, 5, 10, 20 and 30 min incubation times were used for MA analysis. The results in Table 3.6 and Figure 3.10 show that there was no significant difference in MA response between different incubation times. A 5 min incubation time was used for further experiments, since it yielded marginally higher responses than the 3 min incubation period.

Table 3.6 Comparison of incubation time (n=3)

Incubation time (min)	**Peak area MA**	
	Mean	**(±SD)**
3	426687	67839
5	**482091**	**67438**
10	500140	42726
20	502812	33814
30	447037	67181



p = 0.295, ANOVA

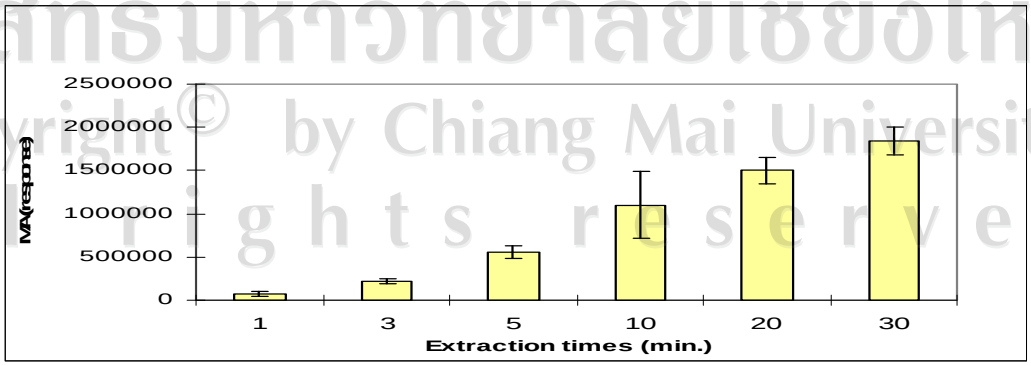
Figure 3.10 Comparison of incubation time.

3.2.5 Optimization of extraction time

The extraction time for PDMS/DVB fiber exposed to the substance on the headspace of solution was determined. The results in Table 3.7 and Figure 3.11 show that there was a significant difference in MA response in different extraction times ($p < 0.001$ by ANOVA). The longer the extraction time, the more the MA was detected in this system. Using a post-hoc test, there was no significant response between 10, 20 and 30 min extraction time. Therefore, the 10 min extraction time was used in this experiment. The 20 or 30 min extraction time, which yielded higher detection of MA, was not selected because the analytical cycle time would take too long and it might not be applicable to daily laboratory work.

Table 3.7 Comparison of extraction time (n=3)

Extraction time (min)	**Peak area MA**	
	Mean	**(±SD)**
1	77366	27172
3	220017	30664
5	560668	17851
10	**1101442**	**384951**
20	1499095	158434
30	1843192	159390



$p < 0.001$, ANOVA

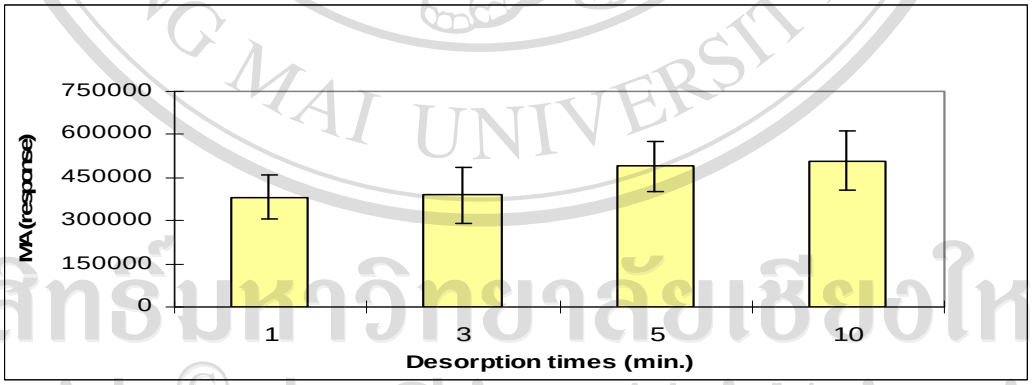
Figure 3.11 Comparison of extraction time.

3.2.6 Optimization of desorption time

When an SPME needle is injected into a GC injection port, the analyte released from the fiber gets into the GC. This desorption time was determined. The results in Table 3.8 and Figure 3.12 show that there was no significant difference in MA response in various desorption times. A desorption time of 5 min yielded higher MA detection than 1 and 3 min, if not statistically significant. For a proper analytical cycle time, 5 min desorption time was selected for this study.

Table 3.8 Comparison of desorption time (n=3)

Desorption time (min)	Peak area MA	
	Mean	(±SD)
1	382266	78249
3	388724	97080
5	**488596**	**89144**
10	507676	102557



p = 0.393, ANOVA

Figure 3.12 Comparison of desorption time.

3.2.7 Determination of carry over effect

The fiber was kept in a bake out station at 270 °C for 5 min before the next round of analysis. Three high concentrations of MA (10,000, 20,000 and 30,000 ng/ml) were used to determine the carry over effect. MA was detected in a blank vial after using the 20,000 and 30,000 ng/ml vials, but not the 10,000 ng/ml vial (Table 3.9). Therefore, the carry over could be observed in a sample that had an MA higher than 20,000 ng/ml.

Table 3.9 Determination of carry over effect

Concentrations (ng/ml)	peak area (mean)	blank area (mean)	% carry over
10,000	53487967	ND	ND
20,000	69266242	68950	0.08
30,000	77279570	216260	0.28

3.2.8 Optimal condition for SPME in this experiment

After optimizing each parameter of SPME, the condition of SPME for further analysis is concluded in Table 3.10.

Table 3.10 Optimal condition for SPME

Operation	**Condition**
Sample preparation	20 mg of hair sample
INS	150 µl of 0.0001% v/v benzaldehyde
K_2CO_3	1650 µl of 1 M K_2CO_3
SPME fiber	PDMS/DVB
Incubation and extraction temperature	90 °C
Incubation time	5 min
Extraction time	10 min
Desorption temperature	250 °C
Desorption time	5 min
Bake out temperature	270°C
Bake out time	5 min

3.3 Method validation

Method validation included assessments of linearity, accuracy and precision, limit of detection (LOD) and limit of quantitation (LOQ).

3.3.1 Linearity

Using the AM concentration of 2.5, 4, 5.5, 7, 8.5, 10 ng/mg of hair, the linear graph was observed (Figure 3.13). The coefficient of correlation (r^2) of the AM standard curve was higher than 0.98 in both intra-day and inter-day analysis (Table 3.11). For MA, a good linear calibration curve was observed in the range 0.5-10 ng/mg (Figure 3.14). The correlation coefficient of MA analysis was higher than 0.99 in both intra-day and inter-day experiments (Table 3.12). Within these concentrations of AM and MA, the standard curves of both were linear with a good correlation coefficient.

3.3.2 Accuracy and precision

When adding 200 µl of 250, 500 and 1,000 ng/ml of AM into 20 mg of negative hair, an expected concentration of 2.5, 5 and 10 ng/mg of hair was yielded. The accuracy of AM analysis is demonstrated as %RR in Table 3.13. The %RR of AM analysis for intra-day and inter-day was between 101.08-104.68 and 98.49-111.22, respectively. All %RR were less than 15% as suggested in the FDA guideline [48]. The precision, indicated as %CV, is shown in Table 3.13. The %CV of AM analysis within a day was between 7.68 and 10.08 of three AM concentrations. The %CV of AM analysis for inter-day was between 3.14 and 13.09. These %CVs fell within 15%, as mentioned in the FDA guideline [48].

The 200 µl of 100, 250 or 500 ng/ml of MA was spiked into 20 mg of negative hair, yielding a final concentration of 1, 2.5 and 5 ng/mg of hair. The

accuracy and precision of MA analysis was indicated by %RR and %CV, respectively, as demonstrated in Table 3.13. The %RR of MA analysis within a day was between 98.39 and 103.75 of their expected concentrations. The %RR of MA analysis was between 93.50 and 98.06 for inter-day analysis. The %CV of MA analysis was between 9.53 and 12.92, and 9.07 and 11.23 for intra-day and inter-day analysis, respectively. All %RR and %CV were within the FDA guideline [48].

3.3.3 Limit of detection and limit of quantitation

Limit of detection (LOD) values were selected based on the percentage of coefficient of variation, relative recovery and relative abundance (mass ratio). Adding 200 µl of 150, 200 and 250 ng/ml of AM to 20 mg of negative hair yielded the expected concentration of 1.5, 2, and 2.5 ng/mg of hair, respectively.

Adding 200 µl of 25, 30 and 50 ng/ml of MA to 20 mg of negative hair yielded the expected concentration of 0.25, 0.3, and 0.5 ng/mg of hair, respectively.

Overlay chromatogram of AM and MA at each concentration is shown in Figure 3.15 and 3.16, respectively. At the three low concentrations of AM used in this experiment, the concentration of 1.5 ng/mg of hair showed a mass ratio of over ±20%, but the concentration of 2.0 ng/mg of hair (109.67 for %RR and 12.11 for %CV) was within the LOD criteria. For MA, the concentration of 0.25 ng/mg of hair showed a mass ratio of over ±20% and could not be detected, but that was not the case with 0.3 ng/mg of hair (112.94 for %RR and 4.78 for %CV). From this result, 2.0 ng of AM/mg of hair and 0.3 ng of MA/mg of hair were considered as LOD.

LOQ is a concentration that can be measured accurately and precisely. LOQ of AM and MA were 2.5 and 0.5 ng/mg, respectively (Table 3.14).

Table 3.11 Linearity and correlation coefficient of AM

Analyte	Day	Set	Slope	Intercept	r^2
AM	1	1	0.4744	1.0930	0.9820
		2	0.5077	1.1469	0.9887
		3	0.4713	1.0506	0.9882
	2	1	0.4668	0.9589	0.9813
		2	0.5074	1.2343	0.9817
		3	0.4832	1.1674	0.9801
	3	1	0.4921	1.2403	0.9952
		2	0.4811	1.2283	0.9936
		3	0.4269	1.1201	0.9881
		Mean	0.4789	1.1378	0.9865
		SD	0.02	0.09	0.0056

Table 3.12 Linearity and correlation coefficient of MA

Analyte	Day	Set	Slope	Intercept	r^2
MA	1	1	1.7330	0.3706	0.9997
		2	1.6699	0.5483	0.9982
		3	1.9516	0.0106	0.9985
	2	1	1.7458	0.3124	0.9933
		2	1.8451	0.4098	0.9979
		3	2.0080	0.2291	0.9995
	3	1	1.7291	0.0344	0.9976
		2	1.9986	0.6299	0.9976
		3	1.8677	0.4779	0.9942
		Mean	1.8388	0.3259	0.9974
		SD	0.13	0.23	0.0022

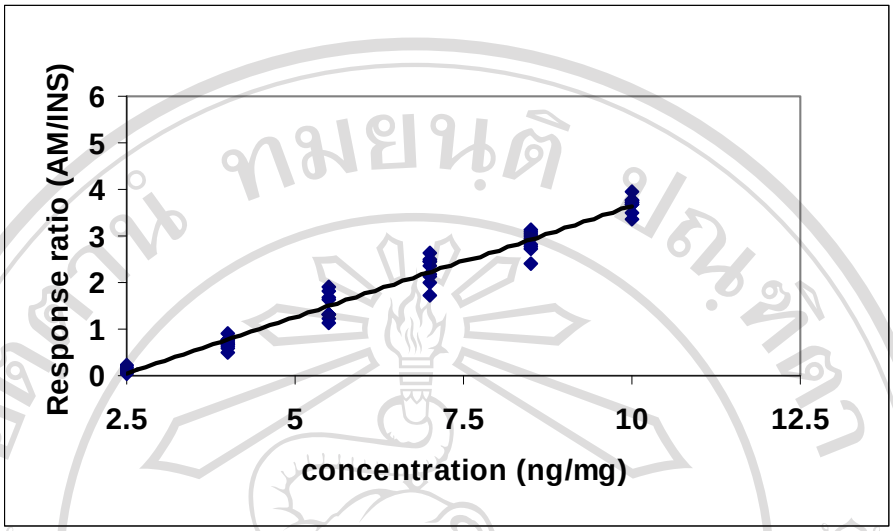


Figure 3.13 Linearity of AM.

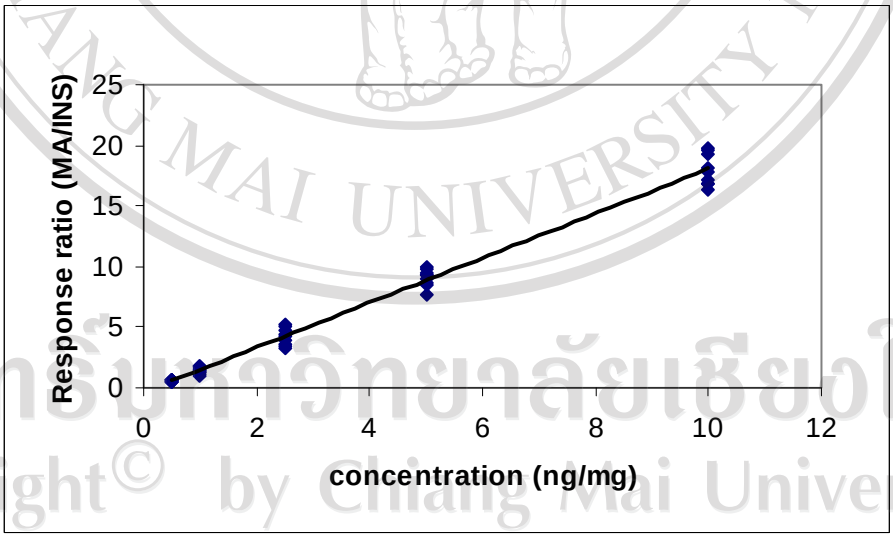


Figure 3.14 Linearity of MA.

Table 3.13 Accuracy and precision of AM and MA

Analyte	Expected conc. (ng/mg)	Measured concentration (ng/mg) (± SD)		% Relative Recovery		% CV	
		Intra-day n = 7	Inter-day n = 12	Intra-day n = 7	Inter-day n = 12	Intra-day n = 7	Inter-day n = 12
AM	2.5	2.62 (±0.20)	2.78 (±0.09)	104.68	111.22	7.68	3.14
	5	5.05 (±0.51)	4.92 (±0.64)	101.08	98.49	10.08	13.09
	10	10.18 (±0.93)	10.26 (±1.18)	101.79	102.30	9.18	11.45
MA	1	1.04 (±0.09)	0.93 (±0.08)	103.75	93.50	8.54	9.07
	2.5	2.46 (±0.23)	2.42 (±0.20)	98.39	96.71	9.53	10.72
	5	4.94 (±0.44)	4.90 (±0.55)	98.90	98.06	12.92	11.23

Table 3.14 Limit of detection and limit of quantitation of AM and MA

Analyte	Expected conc. (ng/mg)	Measured concentration (ng/mg) (± SD)	% Relative Recovery	% CV
AM	2.5	2.59(±0.18)	103.41	6.95
	2	2.19(±0.27)	109.67	12.11
	1.5	ND	-	-
MA	0.50	0.51(±0.03)	102.39	5.46
	0.30	0.34(±0.02)	112.94	4.78
	0.25	ND	-	-

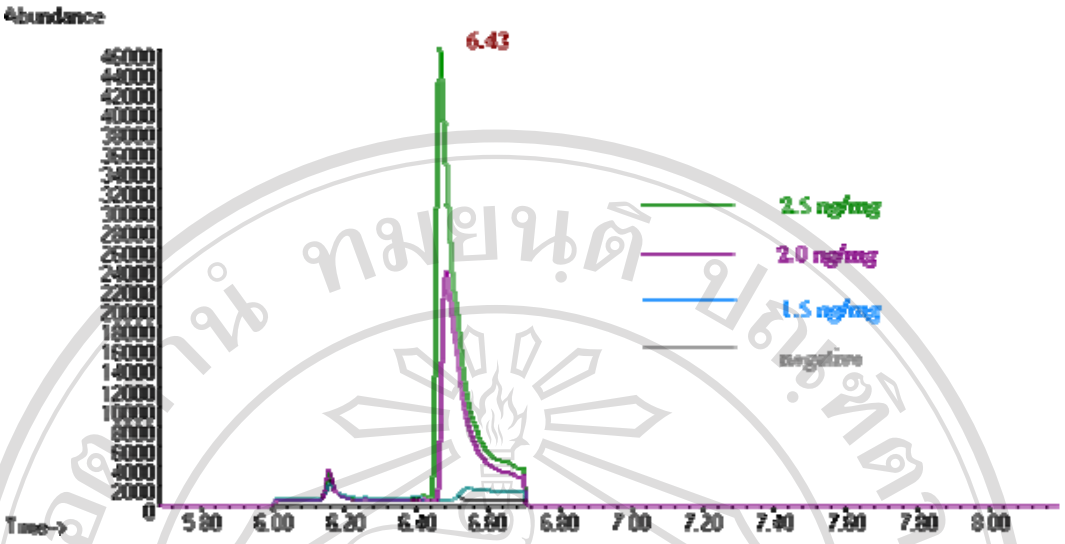


Figure 3.15 Overlay chromatogram of AM at 1.5, 2.0, 2.5 ng/mg of hair and negative hair.

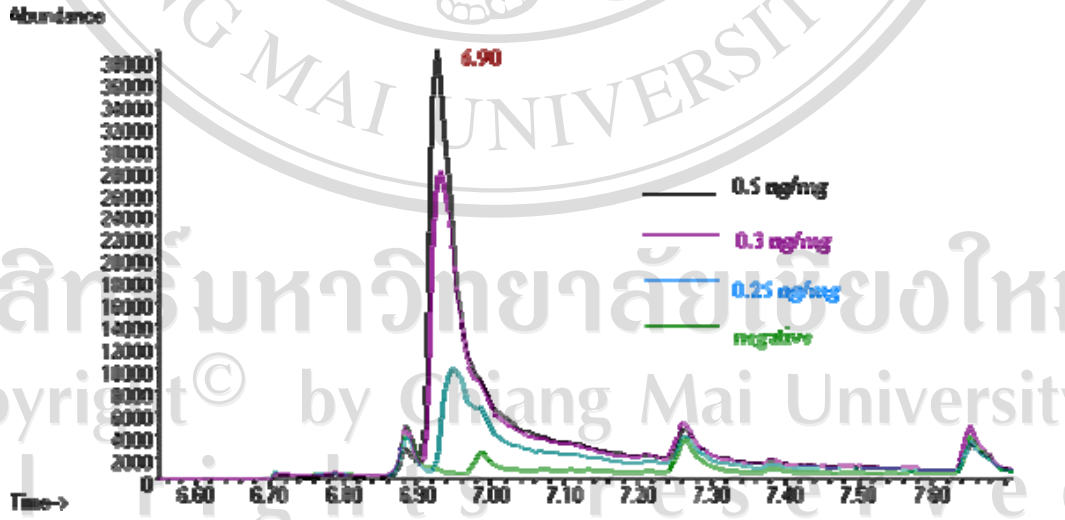


Figure 3.16 Overlay chromatogram of MA at 0.25, 0.3, 0.5 ng/mg of hair and negative hair.

3.4 Analysis of hair samples from drug abusers

Hair samples were obtained from the "Reducing Youth Drug-Related HIV/STD Risk Behaviors in Thailand: Phase II Intervention project". The history of previous use, frequency of use and amount of use were also recorded from the subjects' self-report. The frequency of use was recorded as the number of days on which the drug was used, as follows:

- Once a month or less = 1 day/month
- 2-3 days a month = 2.5 days/month
- About once a week = 4 days/month
- 2-3 days a week = 10 days/month
- 4-6 days a week = 20 days/month

The average number of YABA tablet used from the self-reports was also recorded as the number of YABA tablets used per day, as follows:

- < 1 tablet or 1/2 tablet = 0.5 tablet/day
- 1 tablet = 1 tablet/day
- > 1 but < 2 tablets = 1.5 tablets/day
- 2 tablets = 2 tablets/day
- > 2 tablets = 3 tablets/day

The number of YABA tablets used per month by each individual was then calculated by the number of days the drug was used per month multiplied by the average number of YABA tablet used per day, for example, 1 day/month x 0.5 tablet /day = 0.5 tablet/month.

Hair samples from hair roots were cut into 3 sections of 1 cm in length. The first section was closest to the scalp and the second and third sections were further from the scalp, consecutively. Since the average hair growth is approximately 1 cm per month, these 3 cm long pieces of hair represented 3 months of possible drug abuse. After being washed and extracted, the hair was analysed by HS-SPME-GC-MS following the validated protocol. The chromatogram of negative and positive hair for MA is shown in Figure 3.17 and 3.18, respectively.

3.4.1 Comparison of drug abuse history and the hair analysis for MA

The study group comprised 22 males and 8 females. The average age was 19.9± 2 years, with a range of 18-25 years. The drug use history of the study group is shown in Table 3.15. The control comprised 4 male and 26 female subjects with no history of drug abuse. The average age was 30.4±12.2 years, with a range of 15-60 years.

MA and AM were detected in hair segments of the study group, as shown in Table 3.16. In the first section of hair, which represented the period of one month before, MA was detected in 17 out of 30 subjects, but no AM was detected in this section. In the second section of hair that represented a longer period of time, the number of MA detections increased to 19 out of 30 cases. In the last section (section 3) of studied hair, which represented a period of approximately 3 months before, MA was detected in 20 out of 30 cases, and the mean concentration of MA in section 3 was higher than that in section 2 and 1. AM, on the other hand, was detected in only a couple of subjects. There was no MA or AM identified in the hair of the control subjects.

Table 3.17 demonstrates the number and percentage of MA positives in hair samples from various YABA users. There was a high percentage of MA detection in all sections if the last YABA use was within 30 days. The percentage of MA positives reduced with a longer period from last use.

The number of YABA tablets used was divided into 2 groups, 0.5-4 tablets/month and more than 4 tablets/month. Data from a 3 month interview reported the amount of YABA use in 23 cases. Table 3.18 shows a high percentage of MA positives in the hair of subjects using more than 4 YABA tablets per month. Yet, this observation was not statistically significant. A similar trend also was observed in data from a 30 day interview (Table 3.19). The more YABA tablets reportedly used, the higher the percentage of MA positives (Table 3.20.).

Table 3.15 Descriptive analysis of YABA abuse history in the study group

	Study group	Control group
number of subjects	30	30
male : female	22 : 8	4 : 26
age range (year)	18-25	15-60
mean age (year)	19.9±2	30.4±12.2
range of last drug use (day)	1-223	None
calculated number range of YABA tablets used per day (tablets)	0.5-3	None
calculated frequency range of use per month (day/month)	1-20	None
mean (±SD) frequency of use per month (day/month)	5.4 (±5.59)	
calculated number range of YABA tablets used per month (tablets)	0.5-40	None
mean (±SD) calculated number of YABA tablets used per month (tablets)	9.73 (±12.19)	

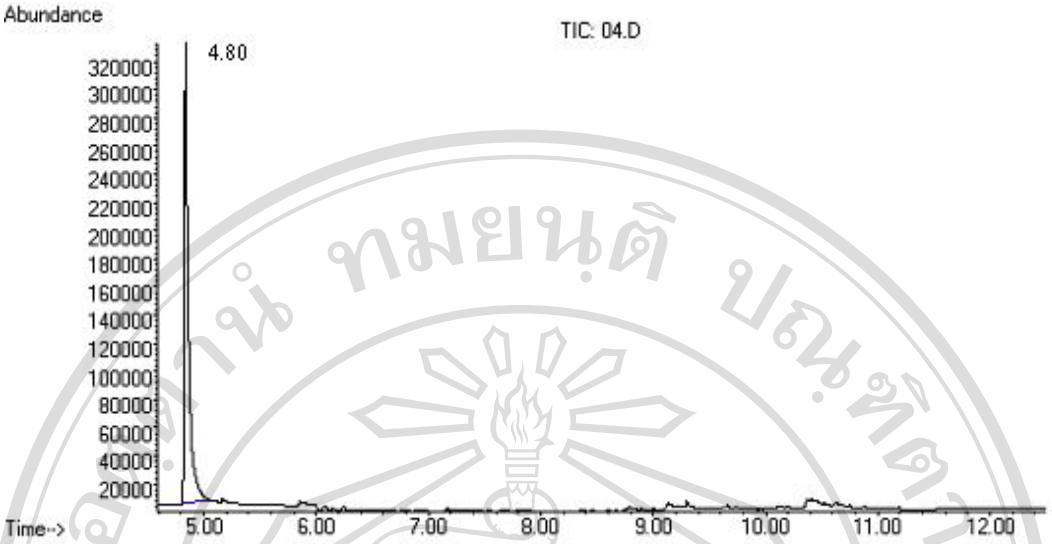


Figure 3.17 Chromatogram of an MA negative hair sample.

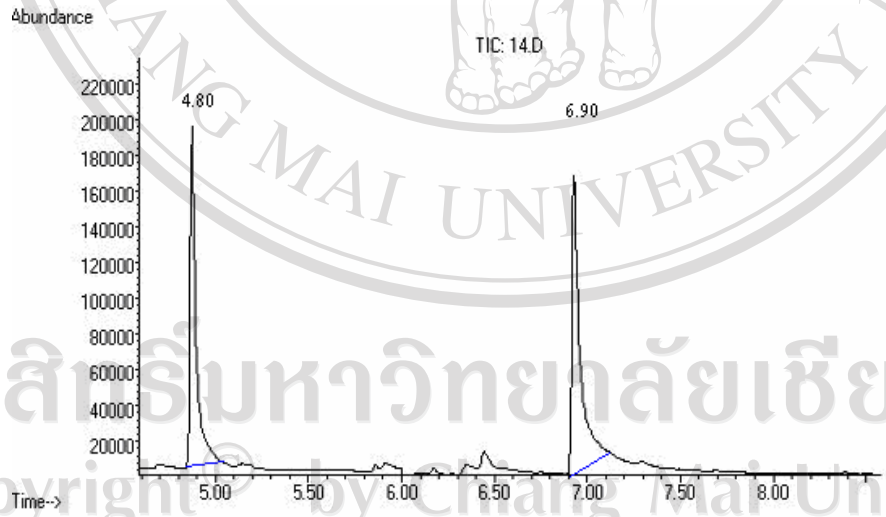


Figure 3.18 Chromatogram of an MA positive hair sample.

Table 3.16 Analysis of hair samples in 3 sections (n=30)

Hair sections	MA			AM		
	positive	range	mean(±SD)	Positive	Range	mean(±SD)
Section 1	17	0.52-21.88	3.03(±4.85)	0	-	-
Section 2	19	0.83-28.49	4.47(±7.40)	3	6.19-14.10	4.57(±4.12)
Section 3	20	0.43-57.59	5.21(±11.43)	2	1.48-9.25	10.15(±5.59)

$p > 0.05$, Fisher-Exact test

Table 3.17 Last use of YABA and percentage of MA positives in hair samples

Time of last use	n	Section 1		Section 2		Section 3	
		positive (number)	positive (%)	positive (number)	Positive (%)	positive (number)	positive (%)
≤ 30 days	21	14	82.35	16	84.21	16	76
31-60 days	1	1	5.88	1	5.26	1	100
61-90 days	2	1	5.88	1	5.26	1	50
> 91 days	6	1	5.88	1	2.26	2	33
Total	30	17	100	19	100	20	100

$p > 0.05$, Fisher-Exact test

Table 3.18 YABA tablet use per month (from 3 months data) and percentage of positives in hair samples

group	N	Section 1		Section 2		Section 3	
		positive (number)	positive (%)	positive (number)	positive (%)	positive (number)	positive (%)
ND	7	2	28.57	2	28.57	3	42.86
0.5-4 tablets/month	14	7	50.00	8	57.14	9	64.29
> 4 tablets/month	9	8	88.89	9	100.00	8	88.89

Chi square test: Section 1 p = 0.053, Section 2 p = 0.008, Section 3 p = 0.145

Table 3.19 YABA tablet use per month (from data 30 days) and percentage of positives in hair samples

group	N	Section 1		Section 2		Section 3	
		positive (number)	positive (%)	positive (number)	positive (%)	positive (number)	positive (%)
ND	10	4	40	4	40	5	50
0.5-4 tablets/month	10	5	50	6	60	7	70
> 4 tablets/month	10	8	80	9	90	8	80

Chi square test: Section 1 p = 0.266, Section 2 p = 0.089, Section 3 p = 0.500

Table 3.20 Last use of YABA tablets per month and percentage of positives in hair samples

Last use	Tablet/Month	n	Hair lab result					
			Section 1		Section 2		Section 3	
			pos	%	Pos	%	Pos	%
≤ 30 days	ND	1	1	100	1	100	1	100
(30 days)	0.5-4	10	5	50	6	60	7	70
	> 4	10	8	80	9	90	8	80
≤ 30 days	ND	1	1	100	1	100	1	100
(3 months)	0.5-4	11	5	45	6	55	7	64
	> 4	9	8	89	9	100	8	89
31-60 days	ND	0	-	-	-	-	-	-
	0.5-4	1	1	100	1	100	1	100
	> 4	0	-	-	-	-	-	-
61-90 days	ND	0	-	-	-	-	-	-
	0.5-4	2	1	50	1	50	1	50
	> 4	0	-	-	-	-	-	-
> 91 days	ND	6	1	17	1	17	2	33
	0.5-4	0	-	-	-	-	-	-
	> 4	0	-	-	-	-	-	-

p > 0.05

3.4.2 Correlation between MA concentration in hair samples and the number of YABA tablets used

A correlation between MA concentration in each segment of hair and the self-reported number of YABA tablets used was plotted.

In Figure 3.19 and 3.20 there was no correlation between MA concentration and the amount of YABA used in section 1 of hair. A similar observation was found in section 2 of the hair samples (Figure 3.21). In the third section, some degree of correlation was found between MA concentration and the number of YABA tablets used (Figure 3.22). The Pearson correlation was 0.459 (p=0.028).

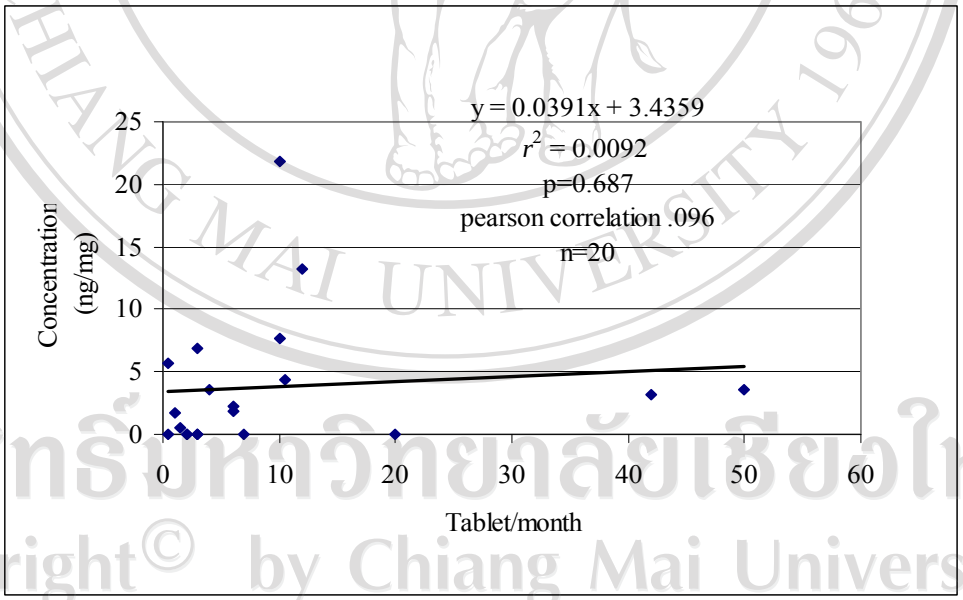


Figure 3.19 Correlation between the concentration in section 1 of hair and the number of YABA tablets used per month from 30 days data.

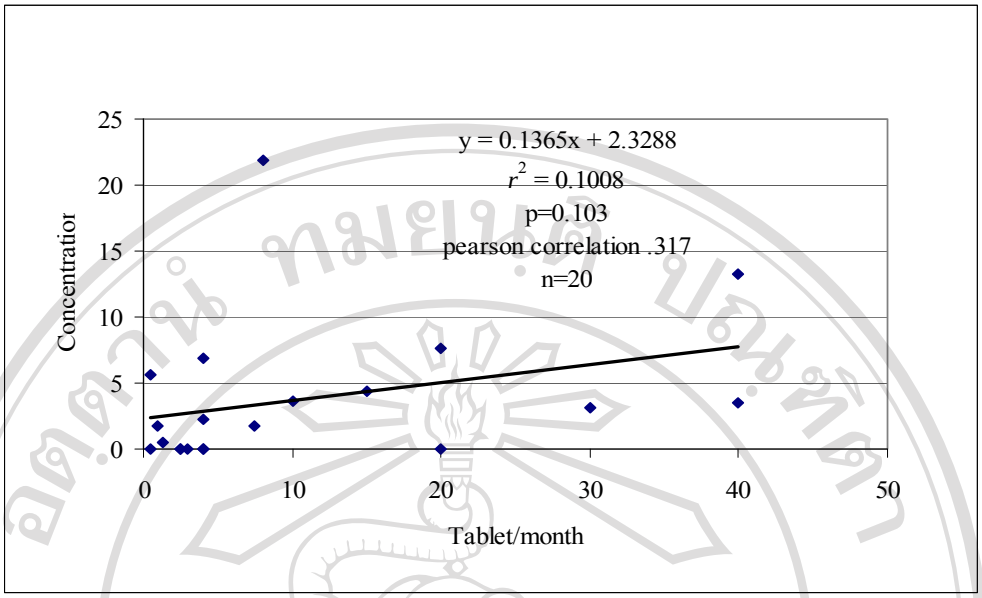


Figure 3.20 Correlation between the concentration in section 1 of hair and the number of YABA tablets used per month from 3 months data.

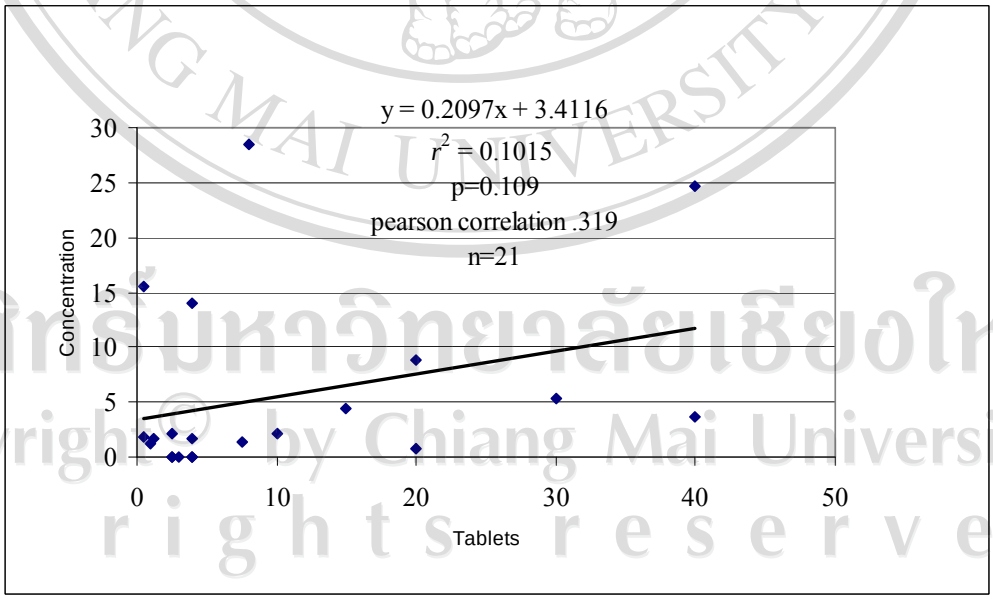
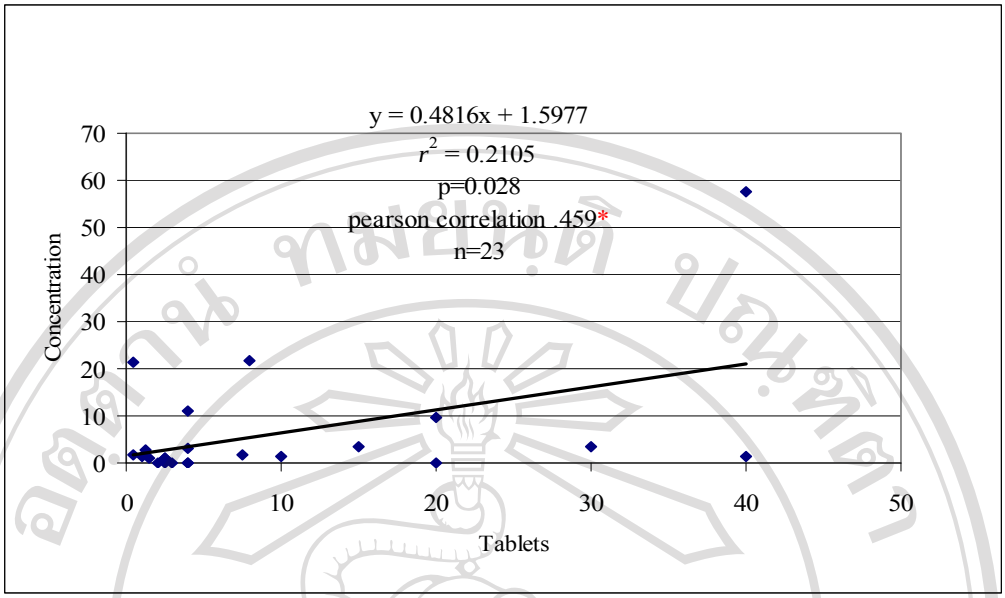


Figure 3.21 Correlation between the concentration in section 2 of hair and the number of YABA tablets used per month.



* $p < 0.05$ (two-tailed).

Figure 3.22 Correlation between the concentration in section 3 of hair and the number of YABA tablets used per month.