CHAPTER 3

RESULTS

Student population

Forty-seven dentistry students, 37 females and 10 males, with little or no prior exposure to embalming solution, were enrolled in this study. Their average age was 19 $\pm$ 0.6 years old. All students not used breathing masks. Characteristics of the population are shown in Appendix D.

Data from the questionnaire revealed that all students were not only exclusively exposed to embalming solution vapor, but also other agents such as monomer, methyl methacrylate and acrylic. This fact became known during dental practice since beginning of the semester until midterm (about 2 month's exposure). Regarding to X-ray exposure, 7 students (No. 5, 23, 26, 38, 44, 49 and 53) had been exposed X-ray for the last 2 months before buccal cells were collected at the end of the semester. Three students (No. 5, 40 and 57) had been using antibiotics for their acne treatment. Two students (No. 30 and 54) had been using paracetamol and 2 students (No. 49 and 55) had been treated with a high dosage of vitamin A (isotretinoin) during the study.

Fifty five % of students (n = 15) used supplementary food such as vitamin B and C. Approximately 15% of students (n = 7) usually consumed spicy food, 72% sometimes (n = 34) and only 13% hardly ever (n = 6). As indicated in Chapter II, only non-and ex-smokers were included in this study to avoid any possible interference of cigarette smoking in the micronucleus investigation.

Micronuclei determination

Micronuclei were found in the epithelial buccal cells of dentistry students at both before and after exposure to embalming solution (Fig. 17). Six buccal cells appeared to have bi-micronucleus in 10 week-exposure and 2 cells in 15 week-exposure are shown in Fig. 18. Buccal cell with bi-micronucleus was presented only in the sample taken after exposing to embalming solution vapor and only one cell was found in male. The total number of micronuclei in mononucleated buccal cells (MNBC) and the frequency of mononucleated buccal cells with micronuclei (BCMN) in 47 dentistry students are shown in Table 2,3. The results show that the mean of MNBC and BCMN were higher than those of before exposure. The initial high baseline micronucleus was due to a large variation in 1,500 cells of counted for the pre-exposure sampling (0 to 5 micronuclei per 1,500 buccal cells, is shown in Table 2,3). A significant difference ($P < 0.05$) of MNBC and BCMN was found between before and after exposure to embalming solution in all subjects. However, in this study the data are expressed separately for both male and female. A significant difference ($P < 0.05$) of MNBC and BCMN were found in both genders at 10 week-exposure and only in females for 15 week-exposure. On the other hand, in males the differences were not statistically significant ($P = 0.13$). This study found that there were no correlation between gender, X-ray exposure and spicy food consumption and the micronucleus. Students were not exposed to only formaldehyde during the first 10 weeks but also methyl methacrylate before being collected the buccal cells at mid of the semester.

An occasional finding, but less often, was the presence of the broken egg nucleus (Fig. 19). These nuclei were found in 0, 10 and 15 week-exposure. However, at 10 and 15 week-exposure these nuclei were higher than 0 week-exposure. Broken egg nuclei are an interesting phenomenon with unclear significance. Based on this study, the presence of these nuclei cannot be attributed to the exposure of embalming solution. In this study, the subject with highest incidence of micronuclei also had not a relatively high broken egg incidence (Appendix D).

Anucleated (karyolysis) cells (Fig. 20) were found in 2 subjects (No. 8 and 34) after 15 weeks exposure. Only cells with ghost nuclei or no visible nuclei were considered anucleated.

One subject was found a cell with multi-nucleated (Fig. 21) (No. 8) after 15 week exposure.

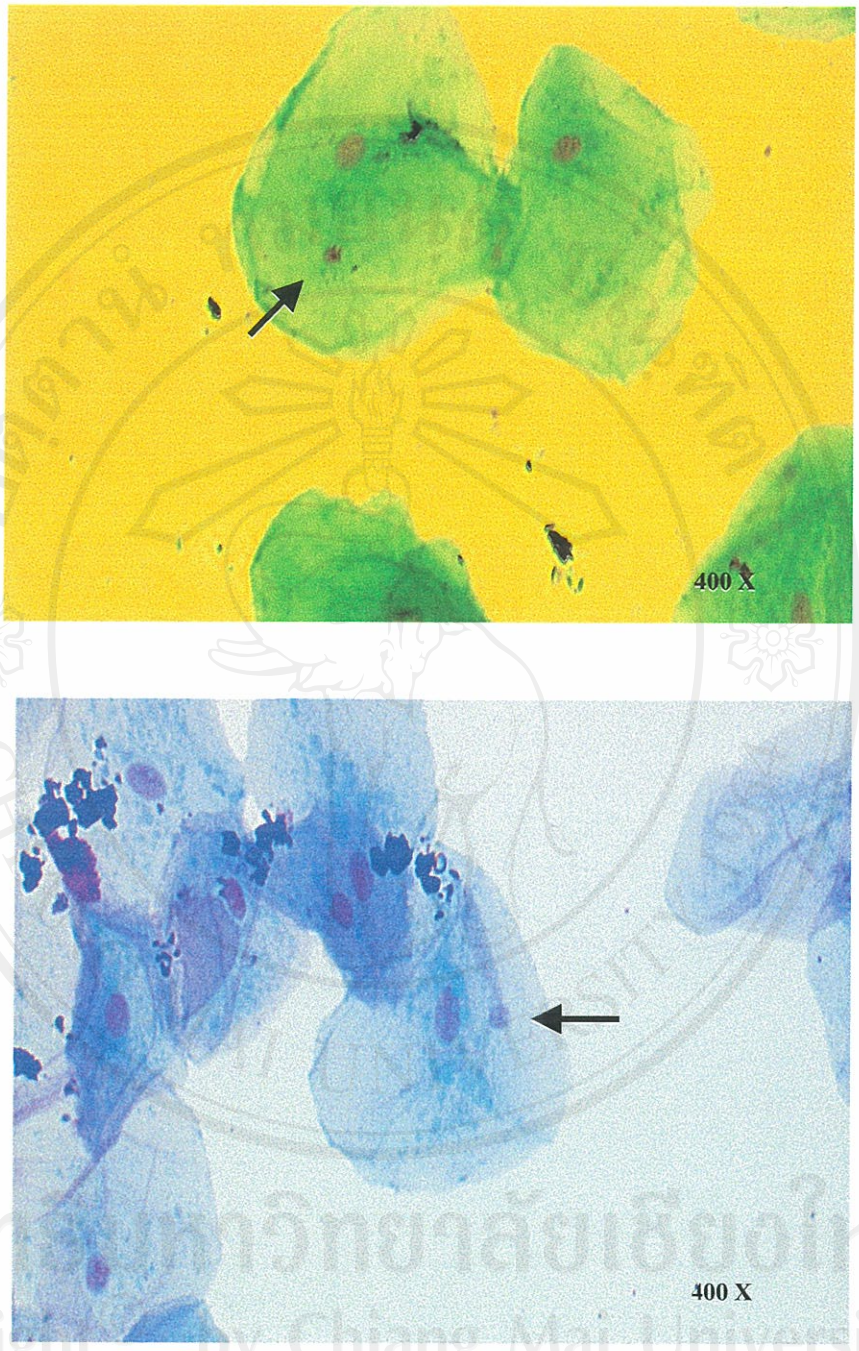

Figure 17 Micronucleus (arrow) found in buccal cells (Feulgen-Fast green nuclear stain) of dentistry students who were taking the human gross anatomy course for 10 weeks.

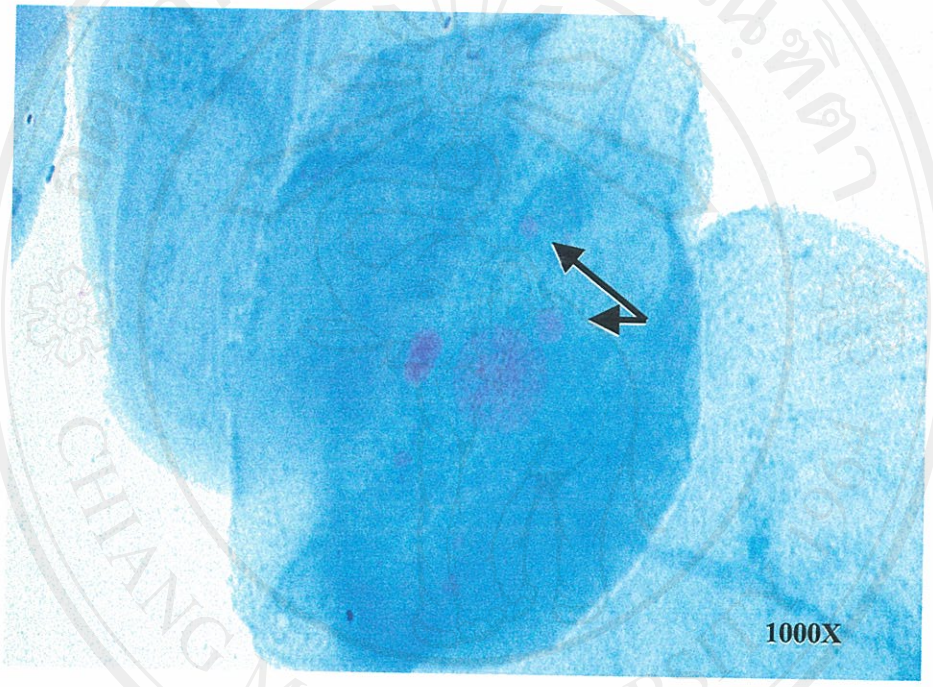

Figure 18 Bi-micronuclei (arrow) in buccal cells (Feulgen-Fast green nuclear stain) found after a 15 week-exposure period of embalming solution in dentistry student who were taking the human gross anatomy course.

Table 2 Mean total number of micronuclei in mononucleated buccal cells (MNBC) found in 47 dentistry students, before, during and after taking human gross anatomy course.

Subject	N	MNBC								
		Before exposure (mean ± SD)	Min	Max	10 weeks exposure (mean ± SD)	Min	Max	15 weeks exposure (mean ± SD)	Min	Max
All	47	1.57 ± 1.51	0	5	5.26 ± 3.91*	0	16	2.53 ± 1.72*	0	9
Female	37	1.59 ± 1.54	0	5	5.38 ± 4.03*	0	16	2.59 ± 1.80*	0	9
Male	10	1.50 ± 1.51	0	5	4.80 ± 3.61*	0	11	2.30 ± 1.42	0	5

* $P < 0.05$

Min = Minimum number

Max = Maximum number

Table 3 Mean frequency of mononucleated buccal cells with micronuclei (BCMN) found in 47 dentistry students, before, during and after taking human gross anatomy course.

Subject	N	BCMN								
		Before exposure (mean ± SD)	Min	Max	10 weeks exposure (mean ± SD)	Min	Max	15 weeks exposure (mean ± SD)	Min	Max
All	47	1.57 ± 1.51	0	5	5.13 ± 3.70*	0	15	2.49 ± 1.69*	0	9
Female	37	1.59 ± 1.54	0	5	5.24 ± 3.80*	0	15	2.54 ± 1.77*	0	9
Male	10	1.50 ± 1.51	0	5	4.70 ± 3.47*	0	11	2.30 ± 1.42	0	5

* $P < 0.05$

Min = Minimum number

Max = Maximum number

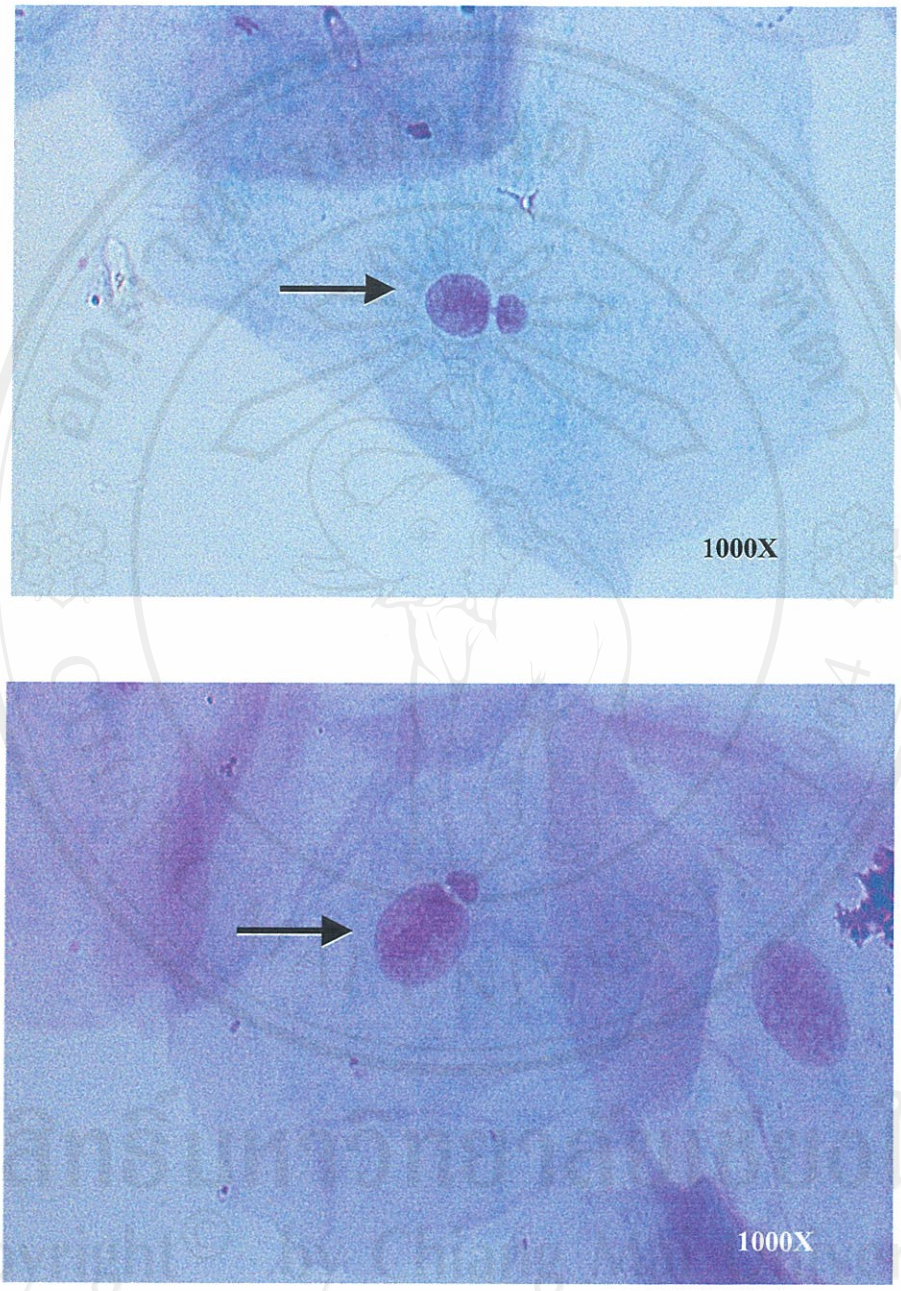

Figure 19 Broken egg nucleus (arrow) in buccal cells of dentistry student that found higher frequency after exposure than before exposure to embalming solution. The broken egg nucleus is a nucleus divided into portions as of it had the band connecting the nuclei fragment, which one usually larger than the other one. (Feulgen-Fast green stain)

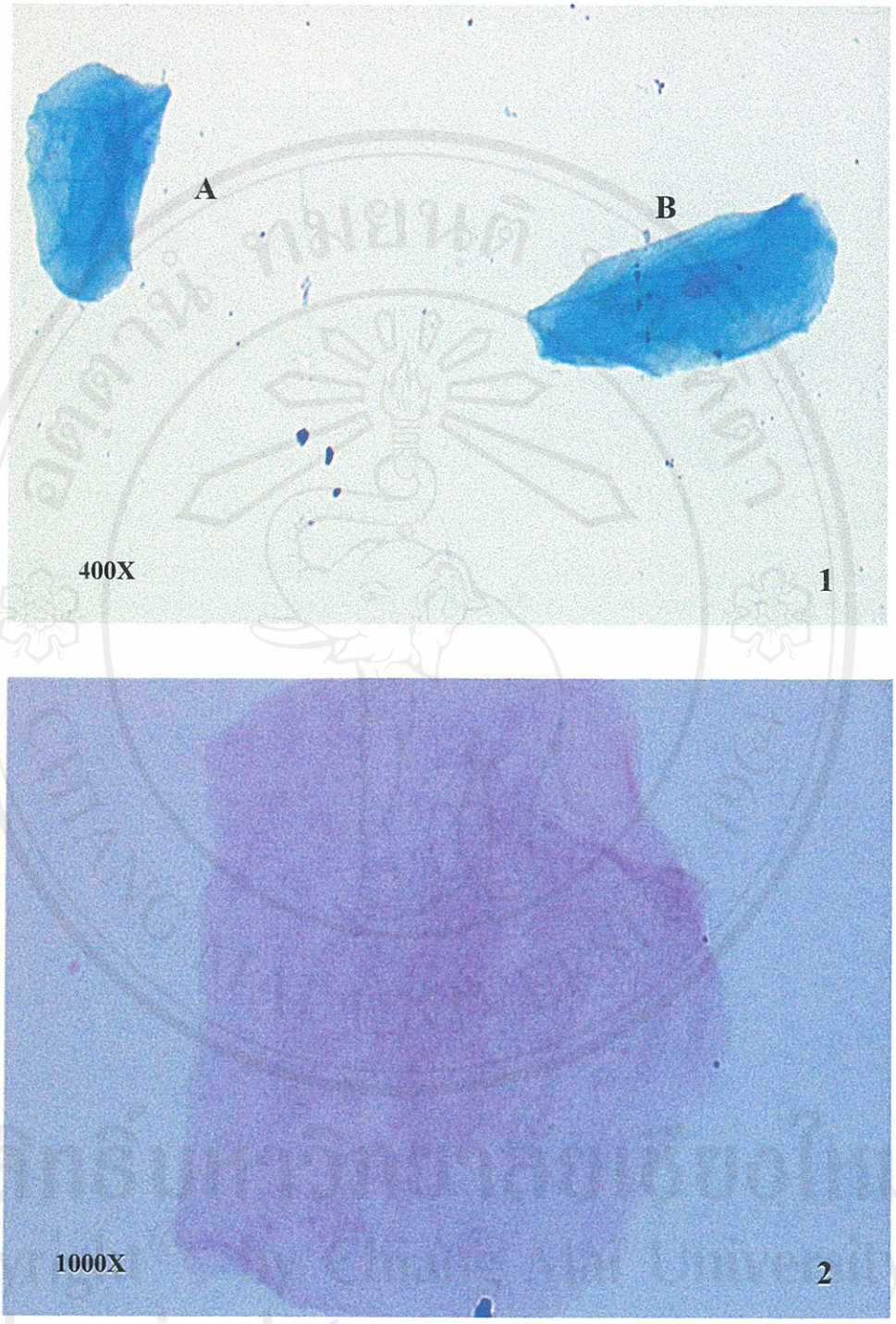

Figure 20 Buccal cells with out nucleus (karyolysis cells or anucleated cell, Feugen Fast green nuclear stain)

1 = A; karyolysis cell, B; normal nuclear cell

2 = karyolysis cell

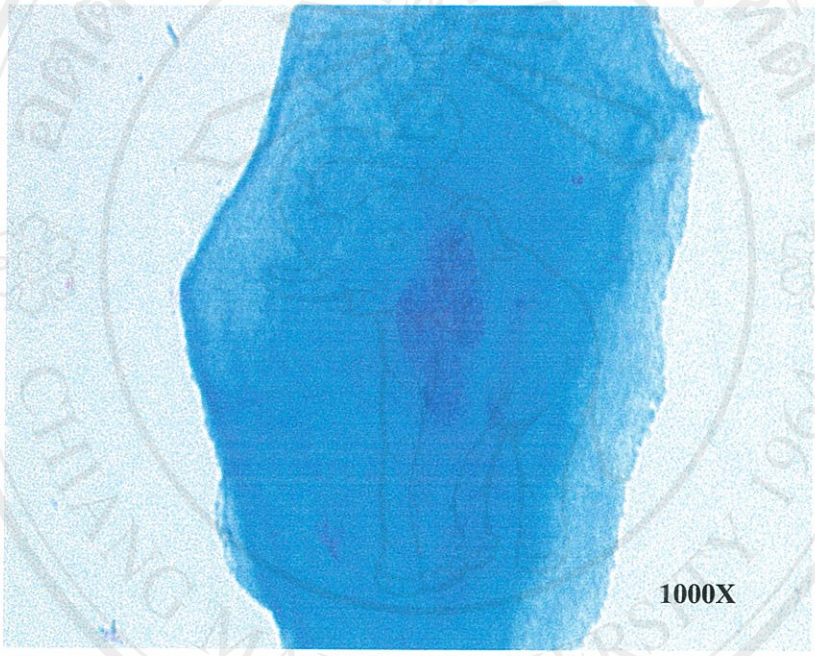

Figure 21 Buccal cell with multinuclei (Feulgen Fast green nuclear stain) only one cell found after taking human gross anatomy laboratory

Formaldehyde concentration in indoor air of autopsy room

Synthesized DNPHo purity

Synthesized, solid, dried of 2,4-dinitrophenylhydrazone (DNPHo) was found as 99.807% purity after analyzed by using Differential Scanning Calorimeter (DSC)

Analysis of DNPHo by HPLC

HPLC chromatogram of the DNPHo standard is shown in Fig. 22. The calibration curve of DNPHo was performed with 7 different concentrations ranging from 0.78-100 ppm. The curve is linear and shown in Fig. 23 with a correlation coefficient (r) of 0.9989.

In this study, standard DNPHo solutions were scanned to investigate the absorption spectra at 190 to 367 nm. Absorption spectrum of DNPHo demonstrated characteristic between spectra within the range of absorbance at 220 to 330 mm. The pattern of espected DNPHo in the air sample was very similar to the spectrum of DNPHo standard (Fig. 24). HPLC analysis of air sample sorbing tube which collected from human gross anatomy room were using the concentration curve displayed in Fig. 23. Extractive fractions of DNPHo in sample sorbing tube were rapidly separated by HPLC in 20 min compared to standard shown in Fig. 25. There were several peaks detected in the air sample. The DNPHo spiked sample was also operated to ensure that the peak at retention time of 4.61 min was exactly the DNPHo. The concentration of DNPHo obtained from the sample was shown in Table 4. DNPHo contents in the control blank tube was also measured and be subtracted the amount of DNPHo in the sample tube.

Quantitative analysis is performed using the synthesized formaldehyde hydrazone as an external standard and then converting results to concentration of formaldehyde using the formula indicated in Chapter 2. The formaldehyde exposure levels in the anatomy laboratory were illustrated in the Table 5. The highest concentration of formaldehyde (0.31 ppm) was found at the first week of semester which student studied on breast pectoral region and axilla and the lowest (0.11 ppm) formaldehyde concentration was found at the fourth week which student had studied. The average $\pm$ standard derivation of formaldehyde concentration was at 0.19 $\pm$ 0.06 ppm or 0.23 $\pm$ 0.08 mg/m^3.

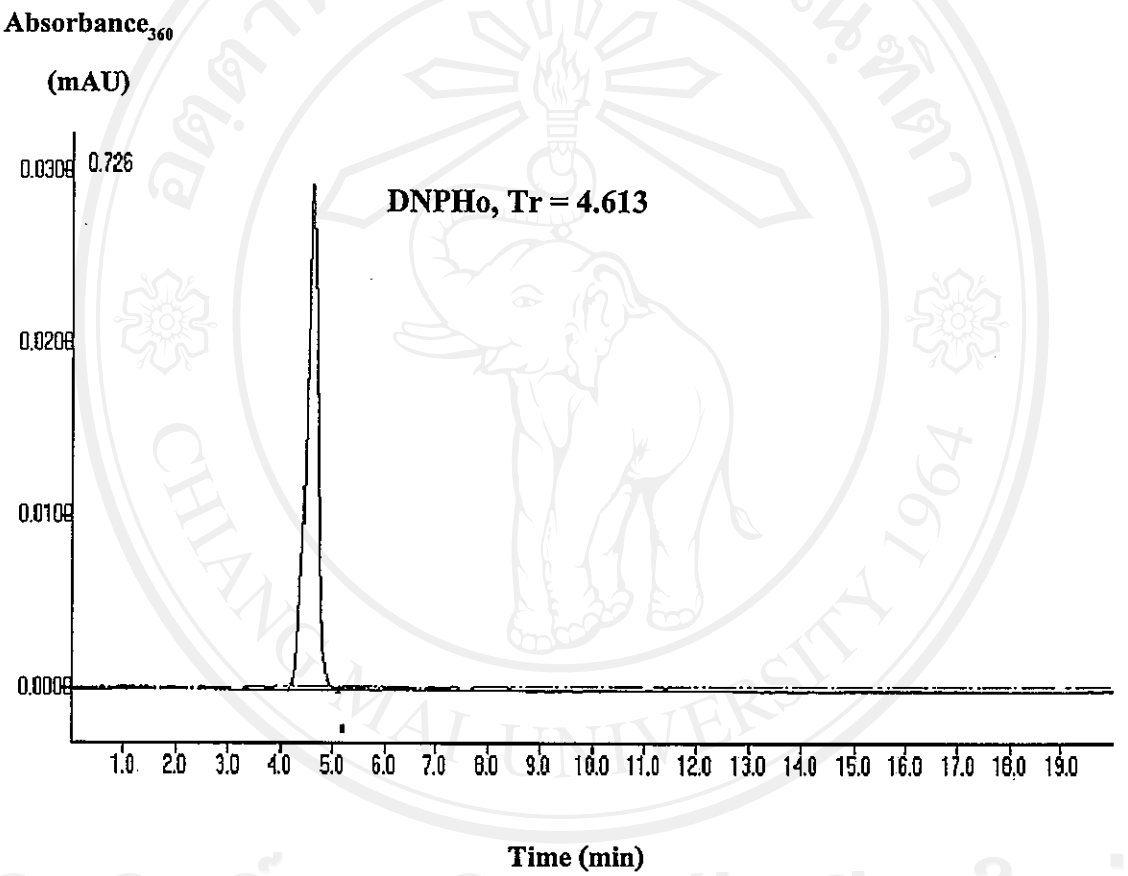

Figure 22 A typical HPLC-UV chromatogram of 2,4-dinitrophenylhydrazone (DNPHo) determined at 360 nm using C_{18} column and water:acetonitrile as a mobile phase. Retention time of the DNPHo standard was at 4.613 min.

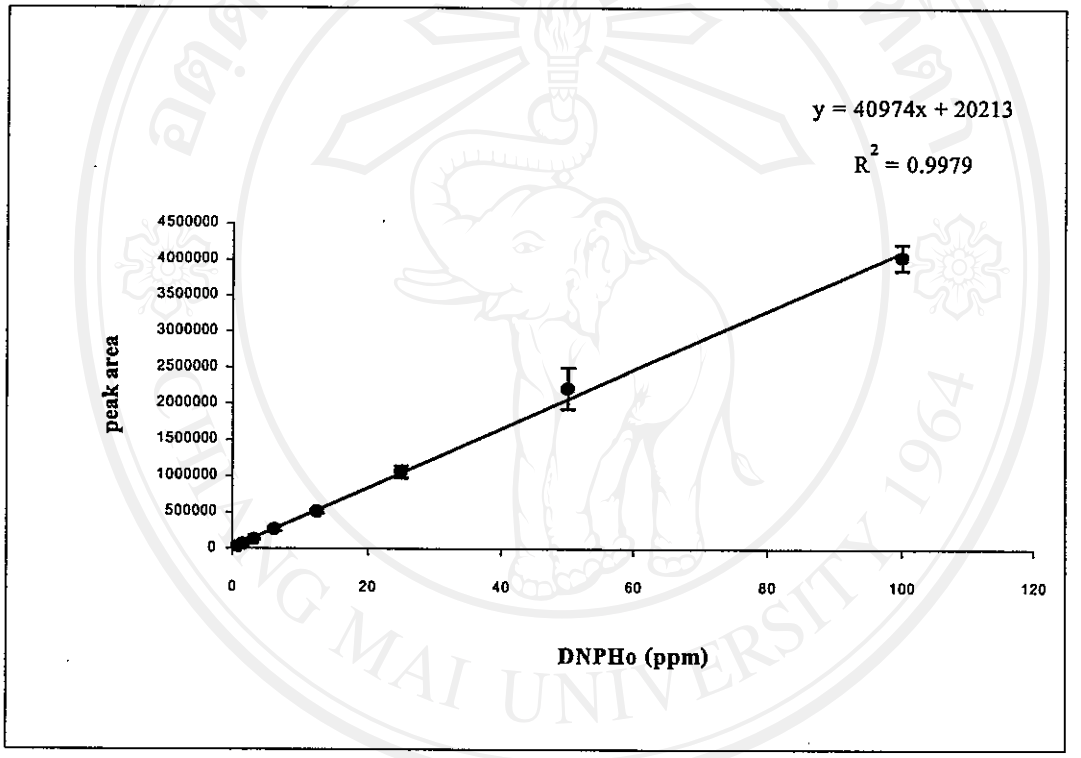

Figure 23 A typical calibration curve of standard 2,4-dinitrophynylhydrazone (DNPHo) plotted with concentrations ranging from 0.78 to 100 ppm and the peak area (r = 0.9989).

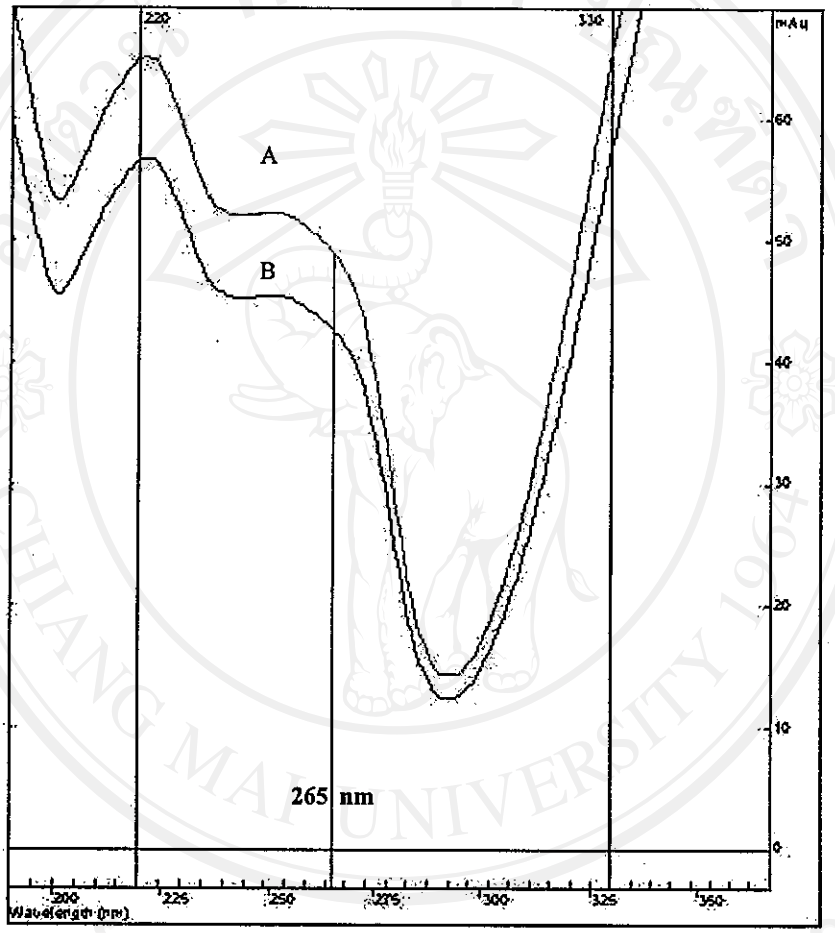

Figure 24 The absorption spectra of 2,4-dinitrophenylhydrazone (DNPHo) scanning with HPLC-Diode Array detector.

A = DNPHo in air sample collected from the autopsy room

B = DNPHo standard

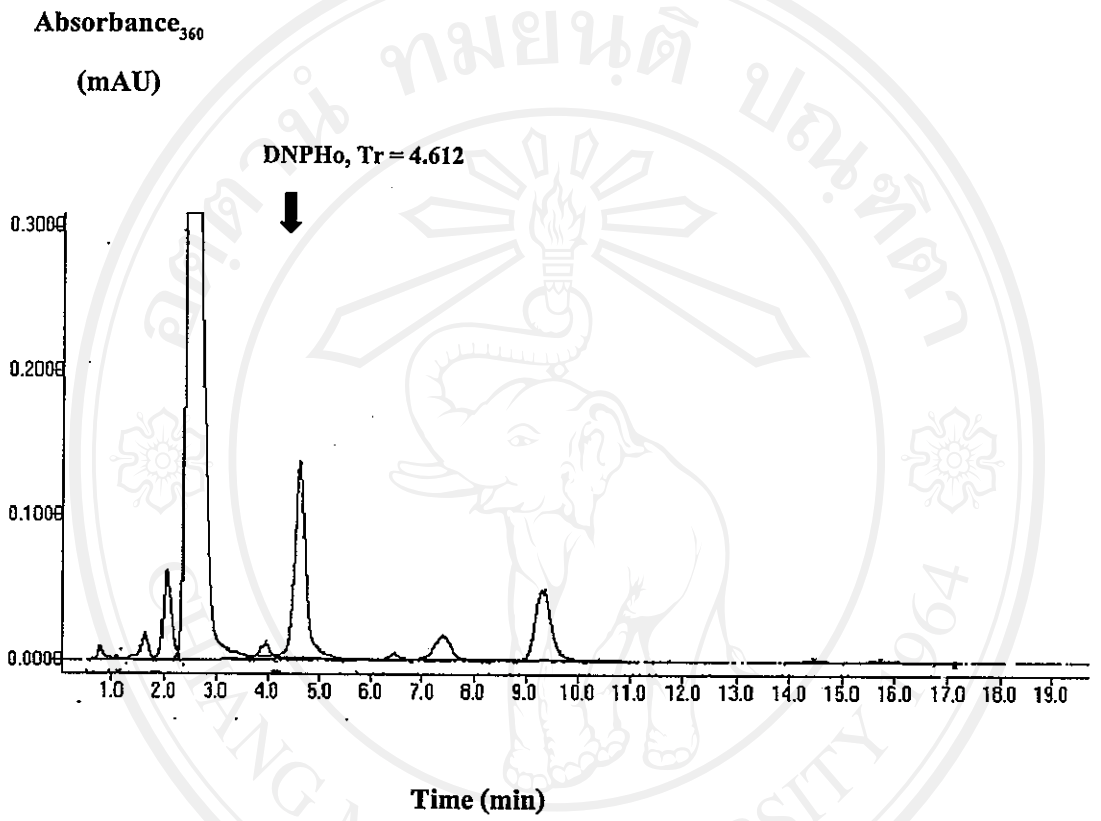

Figure 25 A typical HPLC chromatogram of DNPHo in air sample extract from sorbent tube for collection of formaldehyde. The retention time of DNPHo was at 4.612 min.

Table 4 DNPHo concentration eluted from the sorbing tube for collecting formaldehyde from indoor air in autopsy or human gross anatomy room which was higher than permissible exposure limit (0.75 ppm)

Sample No.	Week	Sample collected date	2,4- dinitrophenylhydrazone DNPHo (ppm)			
			Front section of sorbent tube	Back section of sorbent tube	Total DNPHo	Total DNPHo (subtract blank[1])
1	1	13-Jun 03	40.78	0.00	40.78	40.42
2	2	20-Jun 03	27.48	0.00	27.48	27.12
3	3	27-Jun 03	21.65	0.00	21.65	21.29
4	4	4-Jul 03	13.96	0.80	14.76	14.40
5	5	11-Jul 03	18.36	0.34	18.70	18.34
6	8	8-Aug 03	25.05	0.73	25.78	25.42
7	11	29-Aug 03	21.41	1.50	22.91	22.55

[1] concentration of DNPHo in cartridge blank was 0.36 ppm

Table 5 Formaldehyde levels in indoor air of the autopsy room for 7 weeks during the human gross anatomy laboratory practice of the dentistry students

Topic of study	Formaldehyde	
	4 hr exposure (ppm)	4 hr exposure $(mg/m^3)^1$
• Breast pectoral region and axilla	0.31	0.38
• Triangles of the neck, cervical viscera and root of the neck	0.21	0.26
• Face, temporal, parotid and infractemporal regions	0.16	0.20
• Scalp, cranial meninges and sinuses	0.11	0.14
• Cranial fossae and cranial nerves	0.14	0.17
• Mouth	0.20	0.24
• Pericardium and heart, anterior, superior and posterior mediastina	0.17	0.21
Mean	0.19	0.23
SD	0.06	0.08

[1] 1 ppm = 1.23 mg/m^3

Methanol concentration in indoor air of autopsy room

Gas chromatogram of methanol standard is shown in Fig. 26. The calibration curve of methanol is linear as expected and is shown in Fig. 27 with correlation coefficient (r) of 0.9982. Concentrations of methanol in both sections of sampling tube was extracted and calculated using the standard curve. The result is shown in Table 6. The methanol exposure levels in indoor air of the anatomy laboratory room were illustrated in Table 7. The highest concentration of methanol (105.65 ppm) was found at the first week of the semester and decreased concentration down to 1.12 ppm at the 14^{th} week of the study. Fig. 28 illustrated GC chromatogram of methanol extracted from the air samples sorbing tube. Retention times of methanol are at 1.545 min. Confirmation of the methanol peak was initially operated by GC-FID. GC-FID chromatographic patterns of methanol standard and methanol in the air sample are shown in Fig. 29. They were illustrated at the same retention time.

Identification was carried out by GC-MS in order confirm genuine methanol in the sample. Analysis was obtained under optimum conditions of the SPME-GC-MS as described in Chapter 2. GC-MS chromatogram with the ion-mass fragment patterns of methanol is shown in Fig.30. The major ion fragmentation of methanol was at m/z 29,31 and 32.

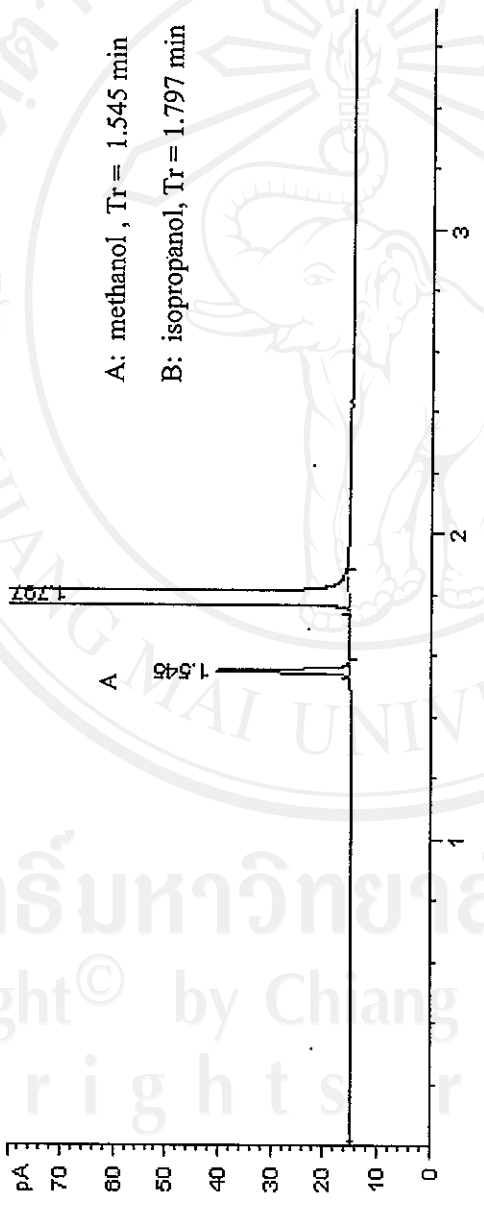

Figure 26 A gas chromatogram of methanol standard at the concentration of 200 ppm. The retention time of methanol (A) and isopropanol (B) is at 1.545 and 1.797 min, respectively.

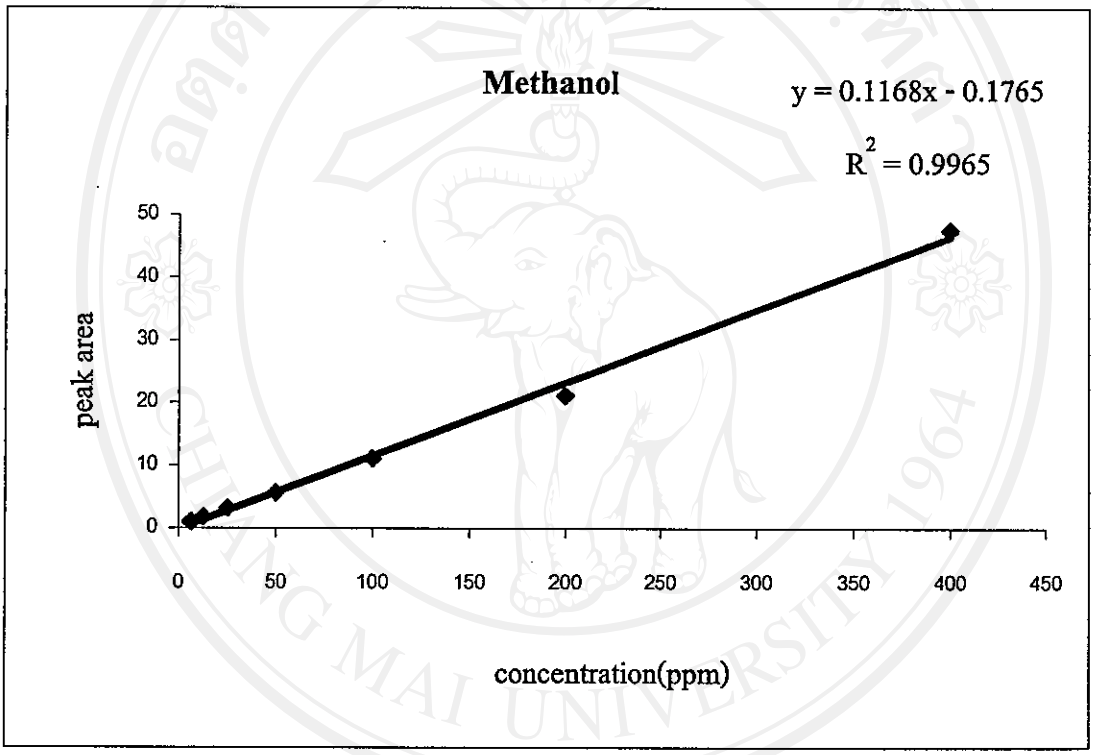

Figure 27 A typical calibration curve of methanol plotted with concentrations ranging from 6.25 to 400 ppm and the peak area (r = 0.9982).

Table 6 Methanol concentrations in the sorbing tube collected from indoor air in autopsy room for 4 weeks

Sample No.	Week	Sample collected date	Methanol (ppm)			
			Front section of sorbent tube	Back section of sorbent tube	Total	Total (subtract blank[1])
1	6	18-Jul 03	270.19	70.38	340.57	332.27
2	9	15-Aug 03	114.42	46.89	161.31	153.01
3	12	5-Sep 03	15.12	4.05	19.17	10.87
4	14	19-Sep 03	8.49	3.31	11.81	3.51

[1] concentration of methanol in the blank cartridge was 8.3 ppm

Table 7 Methanol concentration levels at 4 weeks during the study.

Topic of study	Methanol	
	4 hr exposure (ppm)	4 hr exposure (mg/m^3)[1]
• Ear, facial nerve and orbit	105.65	138.45
• Preverebral region, pharynx and larynx	48.65	63.76
• Abdominal wall and cavity, abdominal viscera I	3.46	4.53
• Urogenital system of both sexes	1.12	1.46
Mean	39.72	52.05
SD	49.10	64.34

[1] 1 ppm = 1.310 mg/m^3

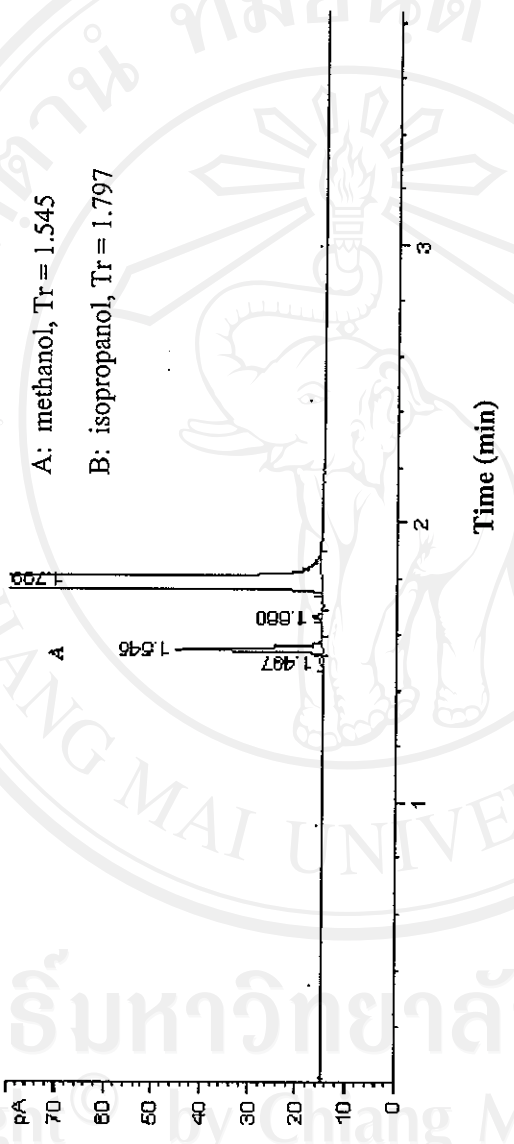

Figure 28 A typical gas chromatograms of methanol in air sample collected from indoor air of the autopsy room.

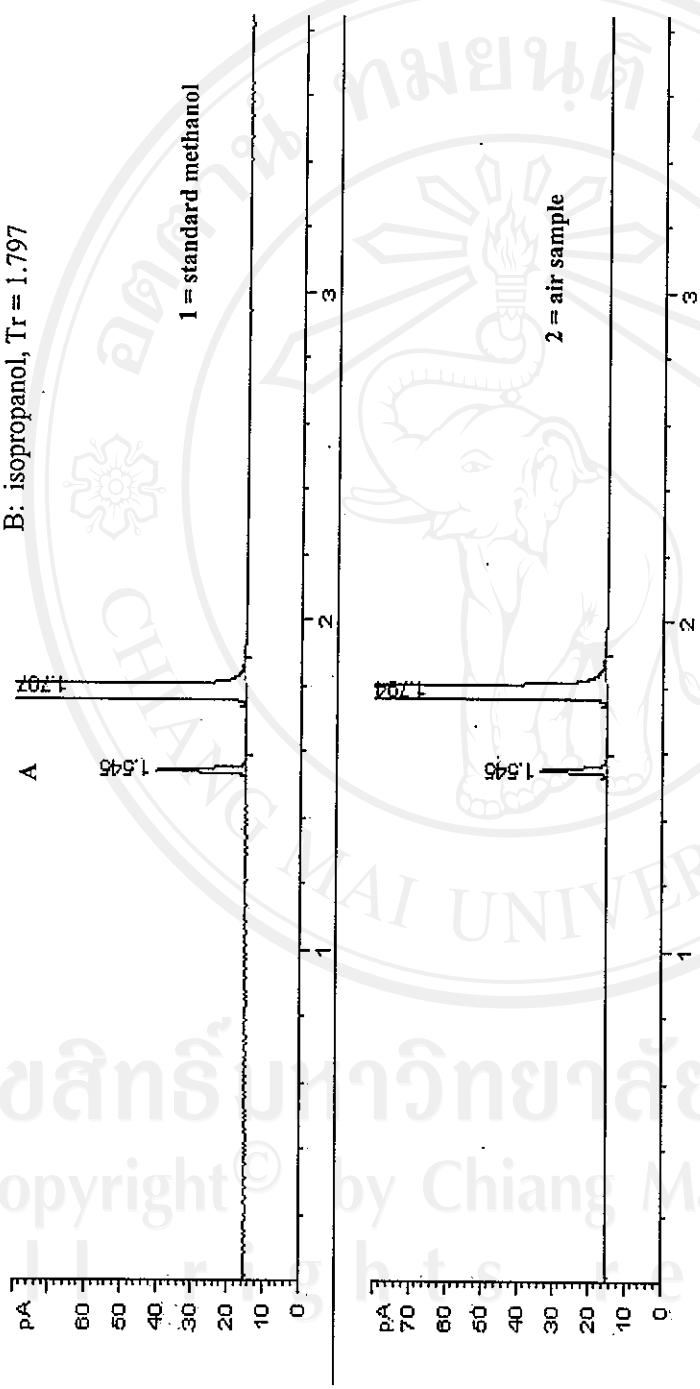

Figure 29 Typical GC chromatograms of methanol standard (1) comparison to the chromatogram detected from the air sample collected from indoor air of the human gross anatomy room (2).

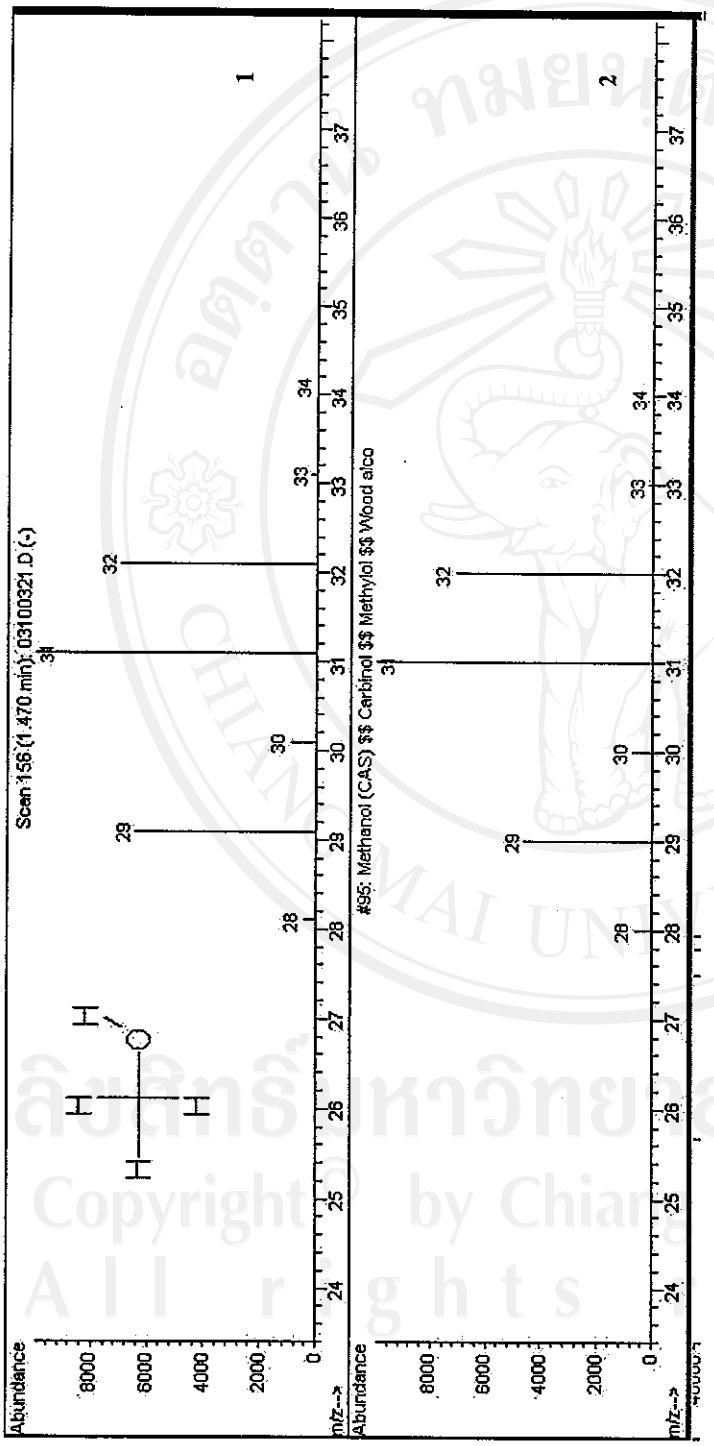

Figure 30 Mass spectra of methanol in air sample (1) comparison to standard methanol spectrum (2).

Phenol concentration in indoor air of autopsy room

Gas chromatogram of phenol standard is shown in Fig. 31. The liner calibration curves of phenol is shown in Fig. 32 with a correlation coefficient (r) of 0.999. Concentration of phenol found in both sections of sampling tube is shown in Table 8. The phenol concentration levels in anatomy laboratory room were illustrated in Table 9. The highest concentration of phenol was found at the third week (the 13th week of the study) in which students studied on abdominal viscera and posterior abdominal viscera system. The lowest concentration of phenol was found at 0.03 ppm. Which was the last phenol air sampling Fig. 33 illustrated the GC chromatogram of phenol extracted from air sample sorbing tube. The retention time of phenol is at 4.20 min. Confirmation of the phenol peak was initially obtained by GC-MS. The GC-FID chromatographic pattern of phenol standard and phenol in the sample are shown in Fig. 34.

Analysis was obtained under optimum conditions as described in Chapter 2. GC-MS chromatogram with the ion-mass fragmentation of phenol is shown in Fig.35. The characteristic of the ion fragmentation of phenol was shown at m/z 39, 66 and 94.

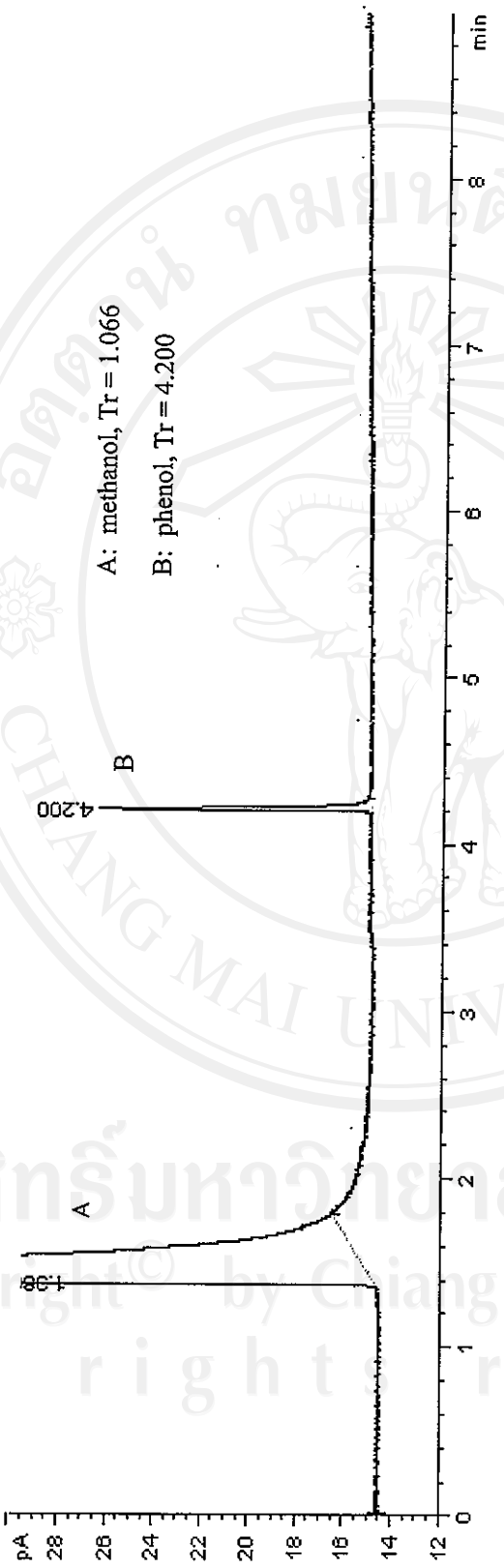

Figure 31 A typical GC-FID chromatogram of phenol standard in methanol, at concentration of 25 ppm.The retention time of phenol is 4.2 min.

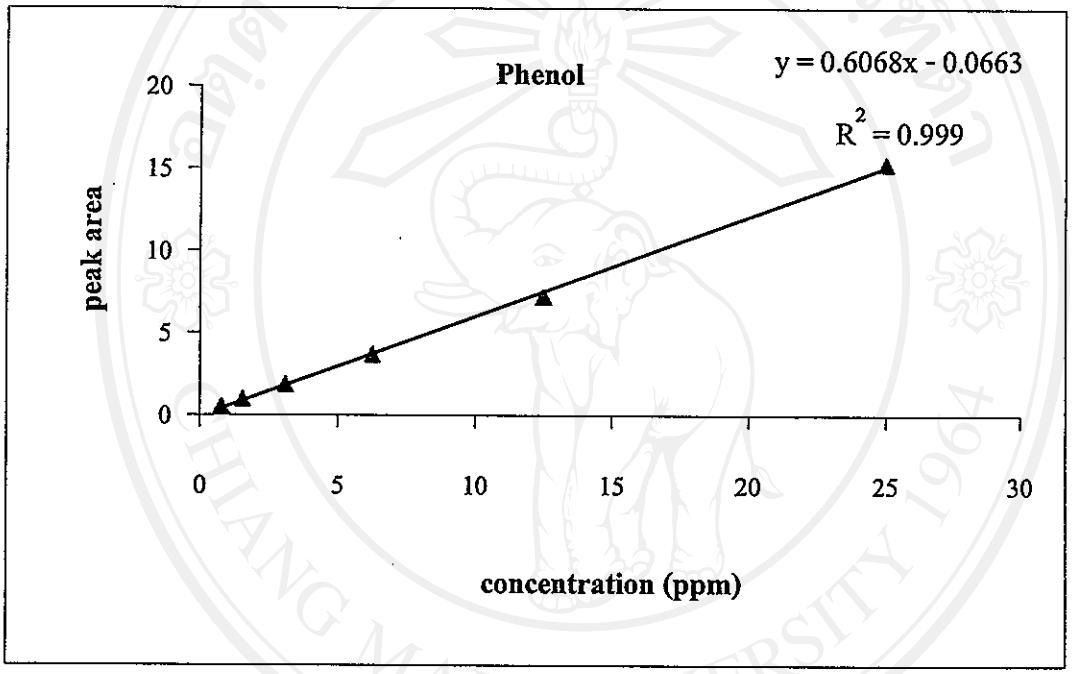

Figure 32 A typical calibration curve of phenol plotted with concentrations ranging from .0.78 to 25 ppm and the peak area (r = 0.999).

Table 8 Phenol concentration in the in door air of the autopsy room determined four times during the study

Sample No.	Week No.	Sample collected date	Phenol (ppm)			
			Front section of sorbent tube	Back section of tube	Total	Total (subtract blank[1])
1	7	25-Ju 03	1.47	0.05	1.52	1.52
2	10	22-Aug 03	3.49	0.07	3.56	3.56
3	13	12-Sep 03	3.18	0.12	3.31	3.31
4	15	26-Sep 03	0.72	0.05	0.78	0.78

[1] concentration of phenol in the blank cartridge was 0 ppm

Table 9 Phenol concentration at 4 weeks during the study .

Topic of study	Phenol	
	4 hr exposure (ppm)	4 hr exposure (mg/m^3)[1]
• Nasal cavity, maxillary nerve and pterygopalatine, fossa	0.05	0.20
• Thoracic wall, pleura and general mediastinum, trachea, bronchi and lung	0.12	0.47
• Abdominal viscera II and posterior abdominal viscera	0.11	0.44
• Final examination	0.03	0.10
Mean	0.08	0.31
SD	0.05	0.18

[1] 1 ppm = 3.85 mg/m^3

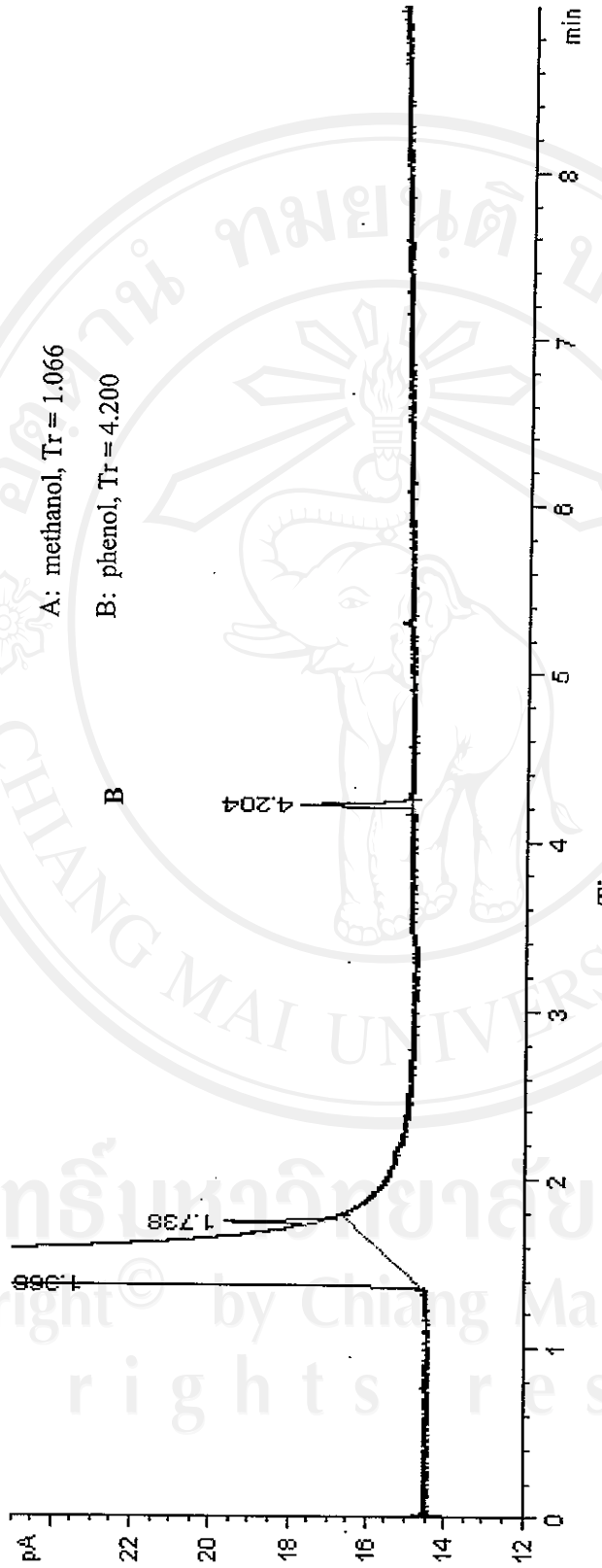

Figure 33 Typical GC-FID chromatogram of phenol in air sample. The retention time of phenol is 4.2 min.

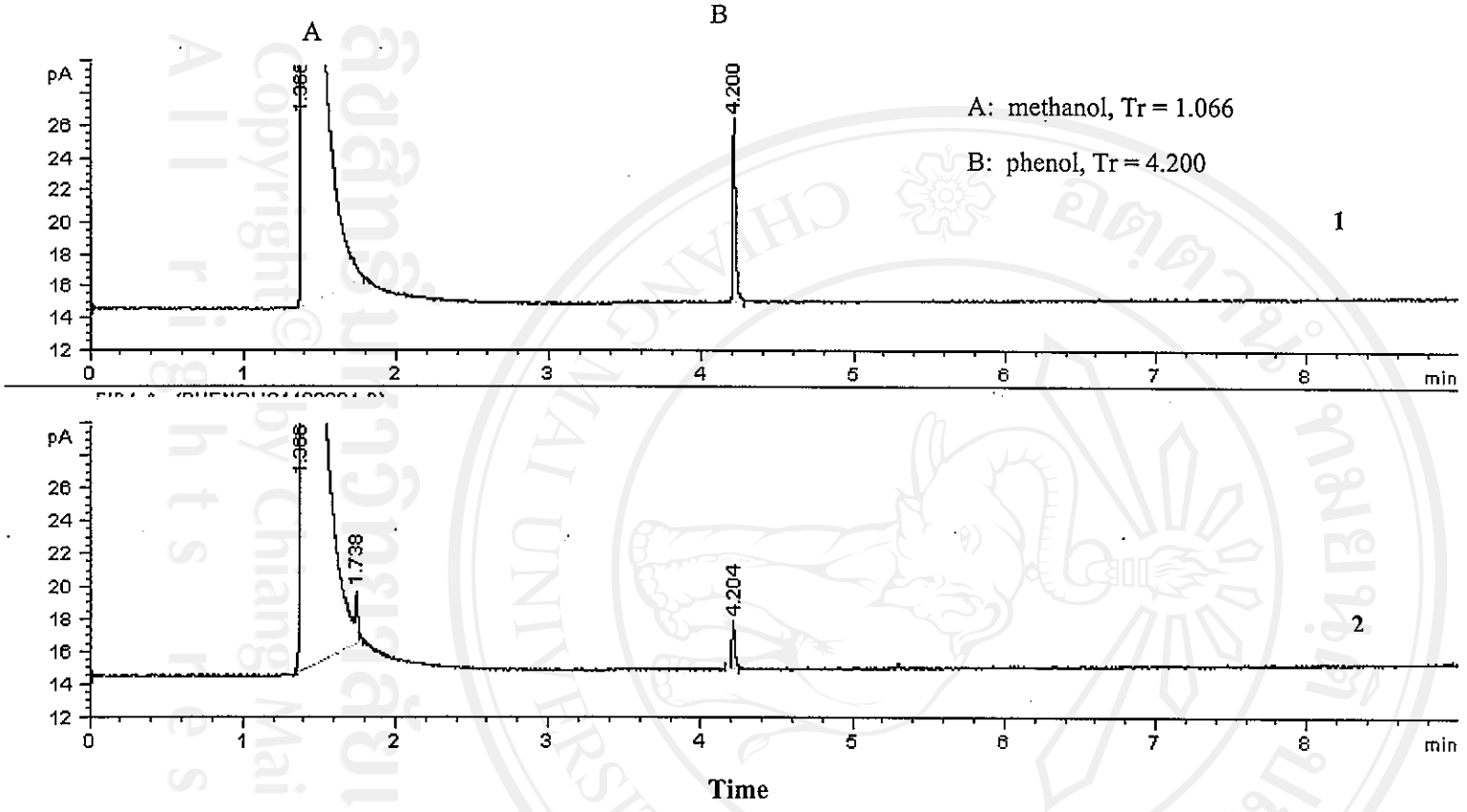

Figure 34 GC chromatograms of phenol standard (1) comparison to the chromatogram detected from the air sample collected from indoor air of the human gross anatomy room (2).

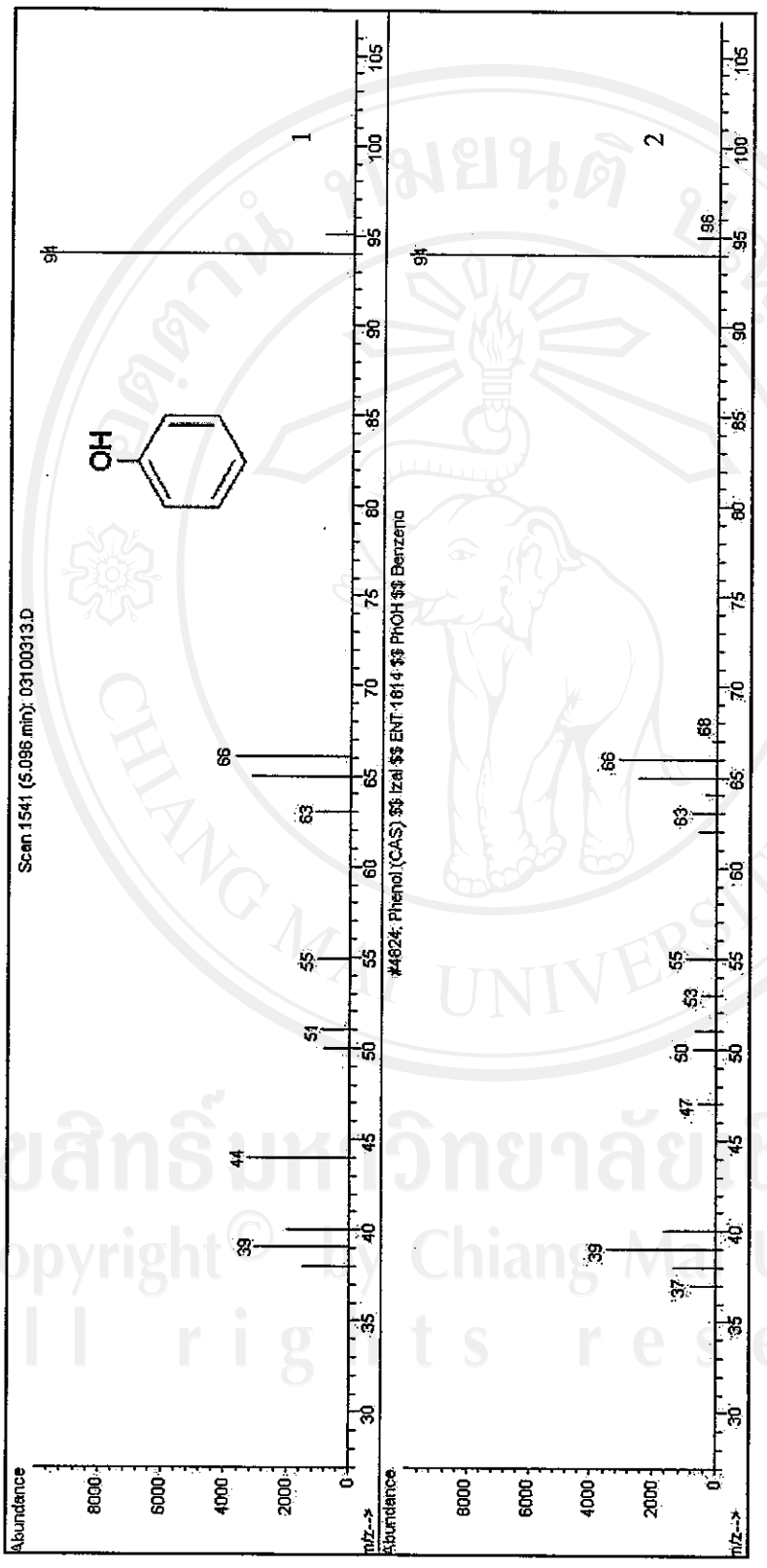

Figure 35 Mass spectra of phenol in indoor air and the sample (1) standard phenol (2).

A significant ($P< 0.05$) increase in micronucleus frequency was found during the study period, from 1.57 ± 1.51/1,500 cells pre-exposure to 5.26 ± 3.92/1,500 cells and 2.53 ± 1.72/1,500 cells after 10 and 15 week-exposure, respectively.

Air sample analysis indicated that concentrations of formaldehyde, methanol and phenol were lower than the OSHA's permissible exposure limit (formaldehyde PEL = 0.75 ppm, methanol PEL = 200 ppm and phenol PEL = 5 ppm). However, only formaldehyde level in the autopsy room was higher than the NIOSH's recommend exposure limit (0.016 ppm) and short term exposure limit (0.1 ppm).