APPENDICES

APPENDIX A

STATISTIC FORMULAE

A-1 Standard deviation [70-71]

The most common measure of the error or a statistical measure of precision in an experimental quantity is standard deviation (SD) of a set of data. The standard deviation defines as a series of n measurements of the same measure and the quantity is characterizing the distribution of the results:

$$SD = \left[\sum\left(X_i - \overline{X}\right)^2 / (n-1)\right]^{1/2}$$

Where X_i is the result of the i measurement

$\overline{X}$ is the arithmetic mean of the n results considered

n is the number of measurement

The definition is estimated the standard deviation for n values of a sample of a population and is always calculated using n-1. If the analysis was repeated several times to produce several sample sets of data, it would be expected that each set of measurements would have a different mean and a different estimate of the standard deviation.

A-2 The detection limit [72]

The detection limit is the minimum concentration of analyte that can be detected and its signals significantly differenced from blank. It can be calculated from following equation.

$$\text{Detection limit} = \frac{3 \times SD}{\text{slope}}$$

Where 3 is *Z*-value at 99% confidence level

SD is the standard deviation of blank

Slope is the slope of calibration curve

A-3 Calculation of precision [70]

Arithmetic mean is given by the equation:

$$\bar{X} = \frac{\Sigma Xi}{N}$$

Where $\bar{X}$ is arithmetic mean or average of total measured value

Xi is an individual measured value

ΣXi is the sum observation values

N is the total number of measurements

Relative standard deviation, RSD, or coefficient of variation, CV, may be expressed as a fraction less than 1 or as a percentage. It is defined as:

$$RSD = \frac{SD}{\overline{X}}$$

$$\%RSD = \frac{SD}{\overline{X}} \times 100$$

Where RSD is the relative standard deviation

SD is the standard deviation

$\overline{X}$ is arithmetic mean or average of the set

This work used the relative standard deviation (RSD) to calculate the relative precision for comparative purposes.

APPENDIX B

PRECISION AND DETECTION LIMIT OF CADMIUM AND LEAD BY FAAS DETECTION

B-1 The detection limit

The detection limit of the method for determination of Cd and Pb were investigated by analyzing blank (1% HNO_3) ten times. The detection limits were calculated by statistical formula in Appendix A and presented in **Table B-1**.

Table B-1 Absorbance obtained from blank solution.

No.	Abs. of 1% HNO_3 for Cd	Abs. of 1% HNO_3 for Pb
1	0.001	0.001
2	-0.001	0.001
3	0.000	0.000
4	-0.002	0.001
5	0.002	0.005
6	0.000	0.003
7	0.001	0.003
8	0.001	0.000
9	0.001	0.002
10	0.001	0.004
Mean	0.0004	0.0020
SD	0.0011	0.0016
Slope	0.1169	0.0079
Detection limit (ppm)	0.03	0.61

Detection limit = (3 x SD) / slope

Detection limit of Cd = (3 x 0.0011) / 0.1169 = 0.03 ppm

Detection limit of Pb = (3 x 0.0016) / 0.0079 = 0.61 ppm

B-2 Precision

For precision studies, the absorbance of ten injection times of 1 μg ml^{-1} standard solution of Cd and 10 μg ml^{-1} standard solution of Pb were detected by FAAS. The precisions were calculated by statistical formula in Appendix A. The results obtained are shown in **Table B-2**.

Table B-2 The precision of the FAAS instrument for Cd and Pb analysis

No.	Abs. of Cd (1 μg ml^{-1})	Abs. of Pb (10 μg ml^{-1})
1	0.180	0.112
2	0.183	0.118
3	0.183	0.117
4	0.184	0.117
5	0.182	0.114
6	0.180	0.112
7	0.178	0.117
8	0.181	0.112
9	0.185	0.114
10	0.181	0.114
Mean	0.1817	0.1147
SD	0.0020	0.0022
%RSD	1.10	1.95

The precision of the FAAS instrument was calculated by:

$$\%RSD = \frac{SD}{\overline{X}} \times 100$$

$$\%\,RSD \text{ of Cd} = (0.0020 / 0.1817) \times 100 = 1.10$$

$$\%\,RSD \text{ of Pb} = (0.0022 / 0.1147) \times 100 = 1.95$$

B-3 The accuracy

For Cd, the method was examined by determining the recoveries of the added 1 and 2 μg ml^{-1} of Cd into studied sample solutions. For Pb, the method was examined by determining the recoveries of the added 10 and 20 μg ml^{-1} of Pb into studied sample solutions. The results are shown in **Table B-3** and **Table B-4**.

Table B-3 The recoveries of Cd analysis

Sample	Concentration of Cd (μg ml^{-1})		% Recovery
	Added	Found*	
1	-	-0.10 ± 0.00	
	1.00	0.77 ± 0.00	87.50
	2.00	1.86 ± 0.01	98.21
2	-	-0.13 ± 0.02	
	1.00	0.76 ± 0.01	89.68
	2.00	1.81 ± 0.01	97.02
3	-	-0.14 ± 0.00	
	1.00	0.72 ± 0.01	86.51
	2.00	1.53 ± 0.01	83.43

* Mean ± SD (N=3)

No. 1 sample,

$$\%\ \text{Recovery} = \frac{\underline{\textit{spike sample result} - \textit{sample result}}}{\textit{spike amount added}} \times 100$$

$$\%\ \text{Recovery} = \frac{0.77 - (-0.10)}{1.00} \times 100$$

$$\%\ \text{Recovery} = 87.50$$

The % recovery of Cd in the other samples can be calculated similar to this way.

Table B-4 The recoveries of Pb analysis

Sample	**Concentration of Pb (μg ml^{-1})**		**% Recovery**
	Added	**Found***	
1	-	-0.90 ± 0.10	
	10	9.38 ± 0.15	102.78
	20	19.69 ± 0.39	102.95
2	-	-0.31 ± 0.09	
	10	9.76 ± 0.18	100.69
	20	19.10 ± 0.34	97.05
3	-	-1.46 ± 0.09	
	10	6.77 ± 0.00	82.29
	20	18.72 ± 0.10	100.87

* Mean ± SD (N=3)

No. 1 sample,

$$\% \text{ Recovery} = \frac{\textit{spike sample result} - \textit{sample result}}{\textit{spike amount added}} \times 100$$

$$\% \text{ Recovery} = \frac{9.38-(-0.90)}{10.00} \times 100$$

$$\% \text{ Recovery} = 102.78$$

The % recovery of Pb in the other samples can be calculated similar to this way.

B-4 Determination of cadmium and lead in human hair samples by FAAS

A 0.50 g of human hair samples was digested by microwave digestion and the sample solution was adjusting to 50.00 ml in volumetric flask. A 5.00 ml of sample solution was preconcentrated by cloud point extraction and determination the amount of Cd and Pb by FAAS. The results are shown in **Table B-5** and **Table B-6**.

Table B-5 The concentration of Cd in human hair samples

Sample	**Cd**			**Average**	**SD**
	Replications	**Abs.**	**Conc. (μg ml^{-1})**		
A-1	1	0.000	-0.061	-0.056	0.006
	2	0.002	-0.048		
	3	0.000	-0.061		
B-1	1	0.003	-0.042	-0.038	0.006
	2	0.003	-0.042		
	3	0.005	-0.029		
C-1	1	0.009	-0.004	-0.002	0.003
	2	0.009	-0.004		
	3	0.010	0.002		

Table B-5 (Continued)

Sample	Cd			Average	SD
	Replications	Abs.	Conc. (μg ml^{-1})		
C-2	1	0.013	0.021	0.023	0.008
	2	0.012	0.014		
	3	0.015	0.033		
C-3	1	0.018	0.052	0.048	0.003
	2	0.017	0.046		
	3	0.017	0.046		
D-1	1	0.024	0.089	0.093	0.011
	2	0.023	0.083		
	3	0.027	0.108		
D-2	1	0.029	0.121	0.123	0.003
	2	0.030	0.127		
	3	0.029	0.121		
D-3	1	0.028	0.114	0.116	0.008
	2	0.030	0.127		
	3	0.027	0.108		
D-4	1	0.030	0.127	0.131	0.006
	2	0.030	0.127		
	3	0.032	0.139		
D-5	1	0.024	0.089	0.100	0.011
	2	0.025	0.096		
	3	0.028	0.114		
D-6	1	0.028	0.114	0.121	0.005
	2	0.029	0.121		
	3	0.030	0.127		
D-7	1	0.034	0.152	0.175	0.032
	2	0.045	0.220		
	3	0.034	0.152		
D-8	1	0.037	0.171	0.166	0.003
	2	0.036	0.164		
	3	0.036	0.164		

Calibration curve: $y = 0.1601x + 0.0097$

$R^2 = 0.9972$

Table B-6 The concentration of Pb in human hair samples

Sample	Pb			Average	SD
	Replications	Abs.	Conc. (μg ml^{-1})		
A-1	1	-0.001	-0.012	-0.012	0.000
	2	-0.001	-0.012		
	3	-0.001	-0.012		
B-1	1	0.000	0.105	0.105	0.000
	2	0.000	0.105		
	3	0.000	0.105		
C-1	1	-0.001	-0.012	0.066	0.055
	2	0.000	0.105		
	3	0.000	0.105		
C-2	1	-0.002	-0.128	-0.167	0.145
	2	-0.004	-0.360		
	3	-0.001	-0.012		
C-3	1	-0.003	-0.244	-0.167	0.055
	2	-0.002	-0.128		
	3	-0.002	-0.128		
D-1	1	-0.001	-0.012	-0.205	0.145
	2	-0.003	-0.244		
	3	-0.004	-0.360		
D-2	1	-0.004	-0.360	-0.089	0.198
	2	-0.001	-0.012		
	3	0.000	0.105		
D-3	1	-0.001	-0.012	0.066	0.055
	2	0.000	0.105		
	3	0.000	0.105		

Table B-6 (Continued)

Sample	Pb			Average	SD
	Replications	**Abs.**	**Conc. (μg ml^{-1})**		
D-4	1	0.000	0.105		
	2	-0.003	-0.244	-0.050	0.145
	3	-0.001	-0.012		
D-5	1	-0.002	-0.128		
	2	-0.002	-0.128	-0.050	0.110
	3	0.000	0.105		
D-6	1	-0.001	-0.012		
	2	-0.001	-0.012	0.027	0.055
	3	0.000	0.105		
D-7	1	0.001	0.221		
	2	-0.002	-0.128	-0.050	0.198
	3	-0.003	-0.244		
	1	-0.002	-0.128		
D-8	2	-0.004	-0.360	-0.244	0.095
	3	-0.003	-0.244		

Calibration curve: $y = 0.0086x - 0.0009$

$R^2 = 0.9996$

B-5 The amount of cadmium and lead in human hair samples

Table B-7 The amount of Cd and Pb in human hair samples

Sample	Cd content (μg/g) *	Pb content (μg/g) *
A-1	ND	ND
B-1	ND	10.50 ± 0.000
C-1	ND	6.60 ± 0.055
C-2	2.30 ± 0.008	ND
C-3	4.80 ± 0.003	ND
D-1	9.30 ± 0.011	ND
D-2	12.30 ± 0.003	ND
D-3	11.60 ± 0.008	6.60 ± 0.055
D-4	13.10 ± 0.006	ND
D-5	10.00 ± 0.011	ND
D-6	12.10 ± 0.005	2.70 ± 0.055
D-7	17.50 ± 0.032	ND
D-8	16.60 ± 0.003	ND

*Mean ± SD (N=3)

ND = Not detectable

The calculation for the concentrations of Cd and Pb in human hair samples are shown as follow:

D-3 sample (from **Table B-5**)

The amount of Cd = 0.116 μg ml^{-1}

The amount of Cd in 1.00 ml sample solution = 0.116 μg

The amount of Cd in 5.00 ml sample solution = 0.116 μg x 5 ml

= 0.58 μg

The amount of Cd in 50.00 ml sample solution = $\left[\frac{0.58 \times 50.00}{5.00}\right]$μg

The amount of Cd in 50.00 ml sample solution = 5.80 μg

Since a 50.00 ml sample solution came from a 0.50 gram of human hair sample. Hence, the amount of Cd in 0.50 g human hair sample = 5.80 μg

Therefore,

The amount of Cd = $\frac{5.80\mu g}{0.5g}$

The amount of Cd = 11.60 μg/g

The amount of Cd and Pb in the other samples can be calculated similar to this way.

APPENDIX C

CALIBRATION CURVE BY FAAS DETECTION

Table C-1 Calibration equation and R^2 used for study on cloud point extraction of Cd from FAAS

Parameters	**Calibration equation**	**Correlation coefficient (R^2)**
Complexing time	y = 0.1248x + 0.0032	0.9981
PAN concentration	y = 0.1078x + 0.0149	0.9960
Triton X-114 concentration	y = 0.1072x + 0.0124	0.9976
pH	y = 0.1169x + 0.0299	0.9943
Vol. of acetate buffer solution pH 3	y = 0.1493x - 0.002	0.9996
Vol. of MeOH in 0.1 mol l^{-1} HNO_3	y = 1.113x + 0.0597	0.9931
Determination of Cd in human hair samples	y = 0.1601x + 0.0097	0.9972

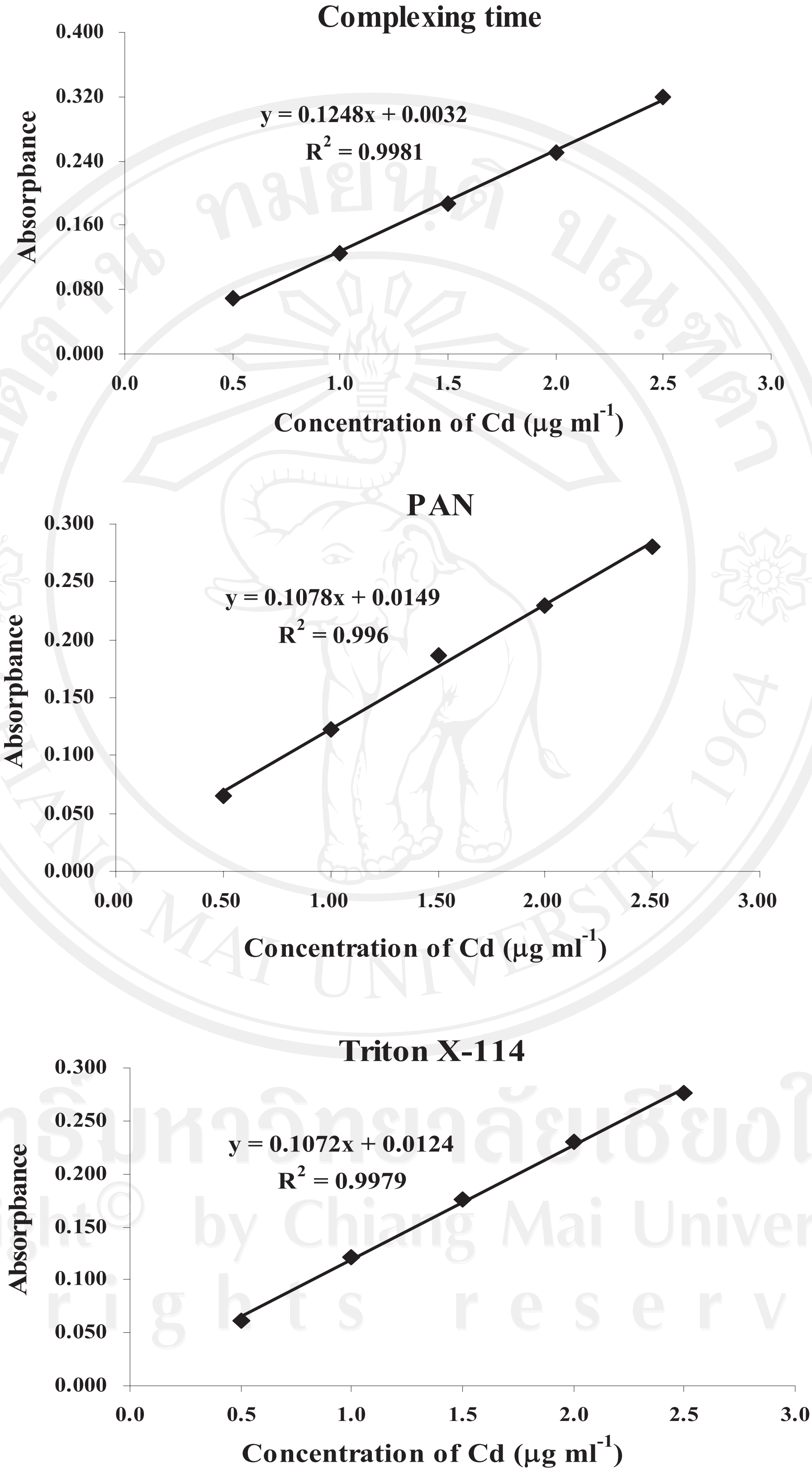
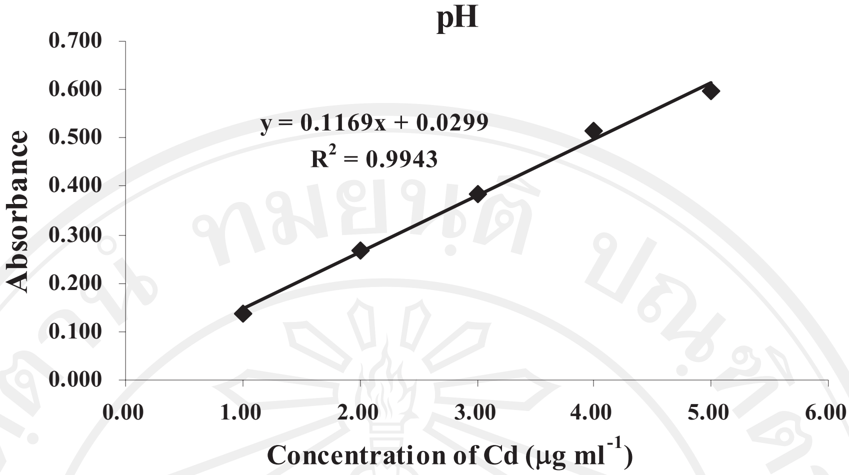
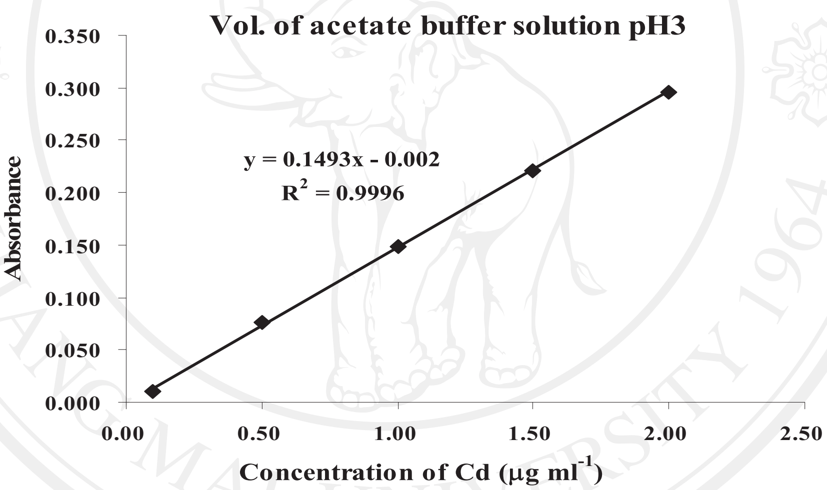


Figure C-1 Calibration curve of Cd for cloud point extraction





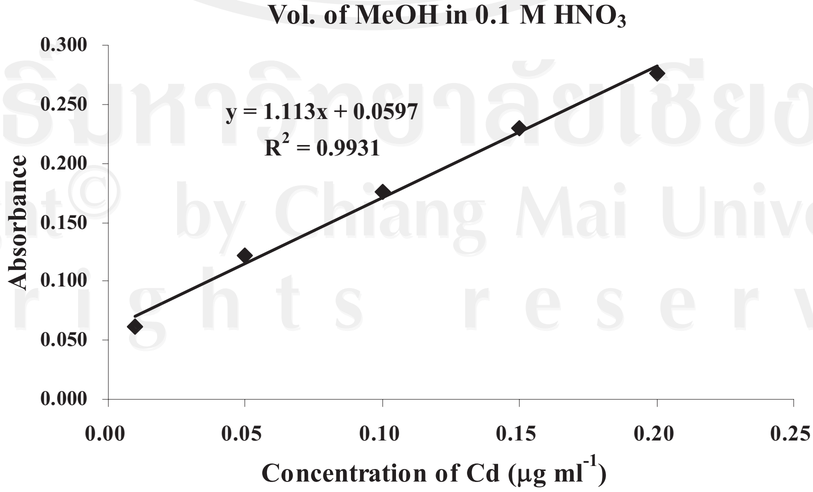


Figure C-1 (Continued)

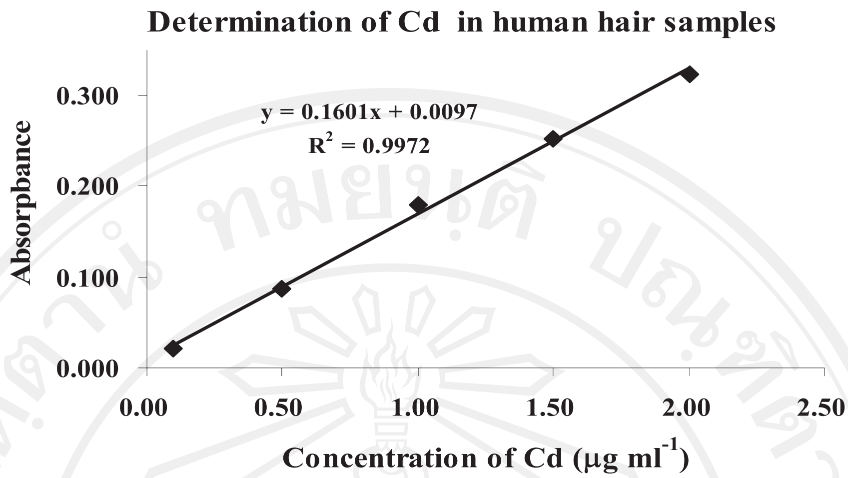


Figure C-2 Calibration curve of Cd for human hair samples

Table C-2 Calibration equation and R^2 used for study on cloud point extraction of Pb from FAAS

Parameters	Calibration equation	Correlation coefficient (R^2)
Complexing time	y = 0.0066x + 0.008	0.9820
PAN concentration	y = 0.007x + 0.0062	0.9879
Triton X-114 concentration	y = 0.0062x + 0.0064	0.9985
pH	y = 0.0072x – 0.0038	0.9977
Vol. of acetate buffer solution pH 3	y = 0.0056x + 0.0006	0.9985
Vol. of MeOH in 0.1 mol l^{-1} HNO_3	y = 0.0062x + 0.0112	0.9945
Determination of Pb in human hair samples	y = 0.0086x - 0.0009	0.9996

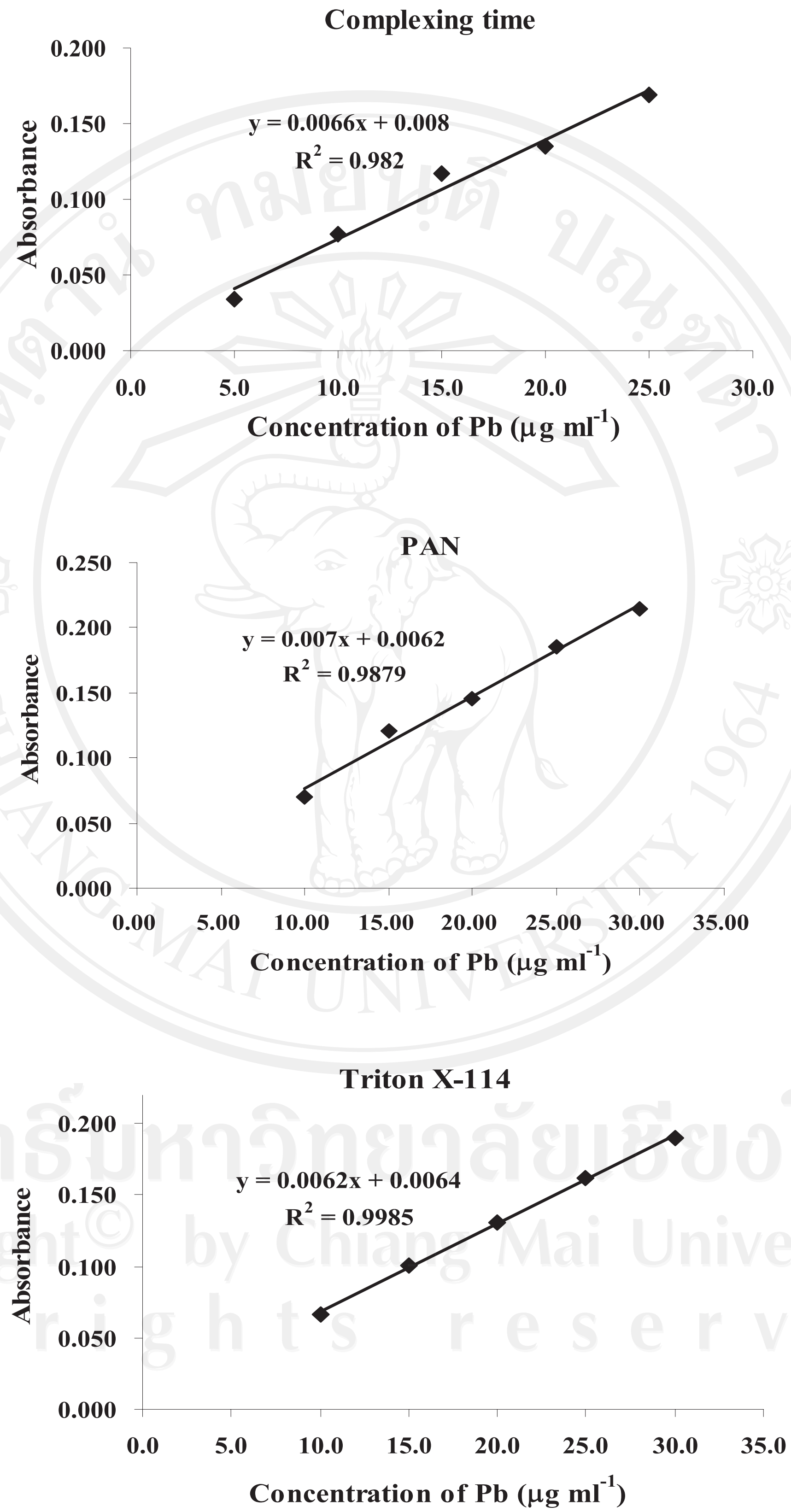
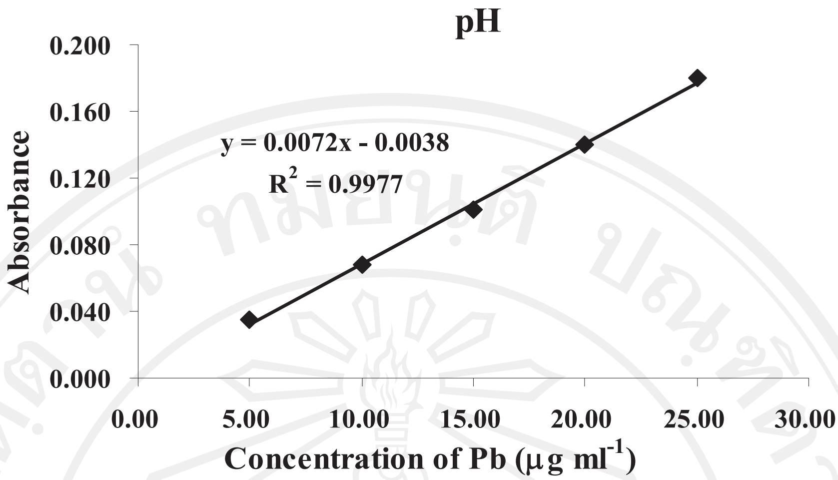
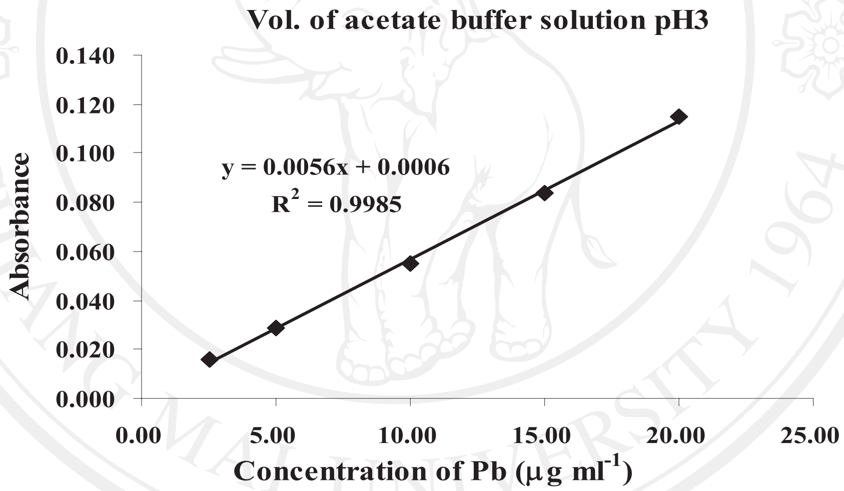


Figure C-3 Calibration curve of Pb for cloud point extraction





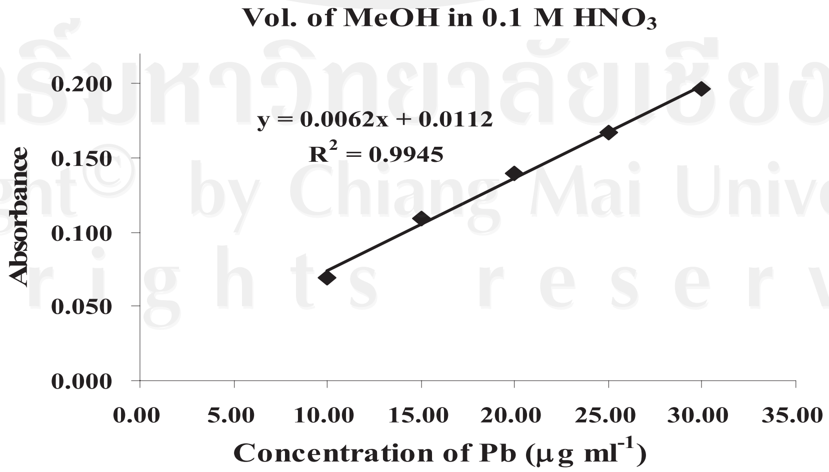


Figure C-3 (Continued)

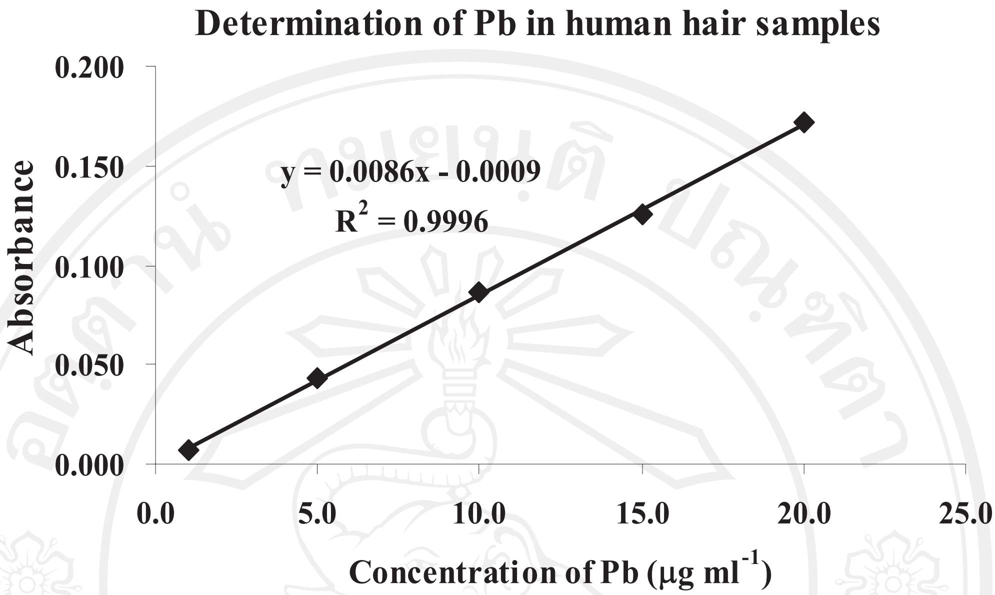


Figure C-4 Calibration curve of Pb for human hair samples

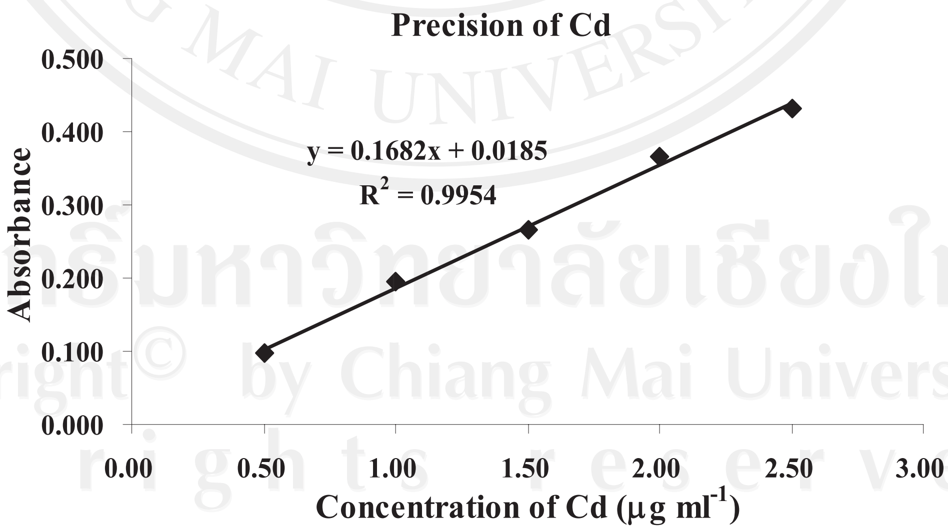


Figure C-5 Calibration curve of Cd for precision analysis

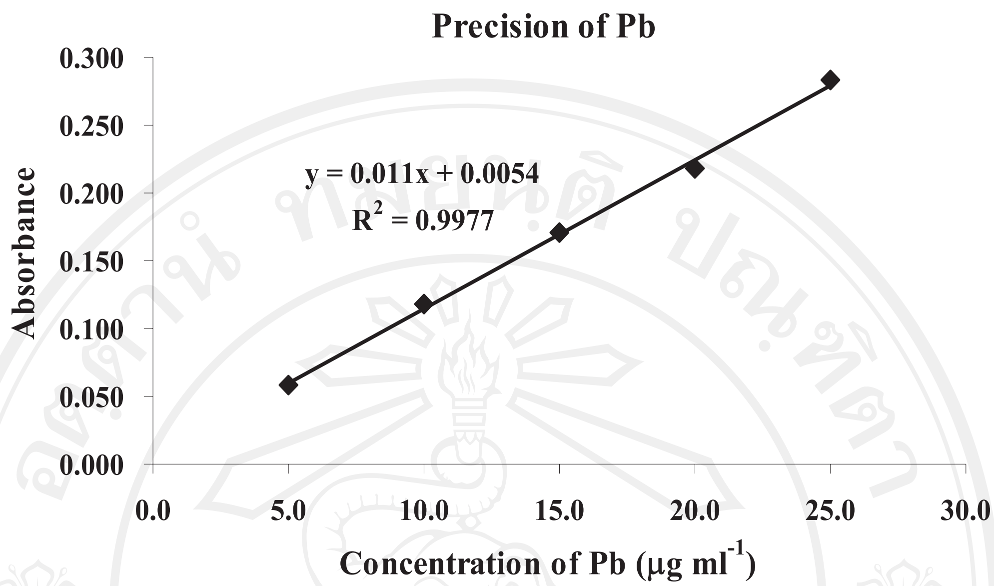


Figure C-6 Calibration curve of Pb for precision analysis

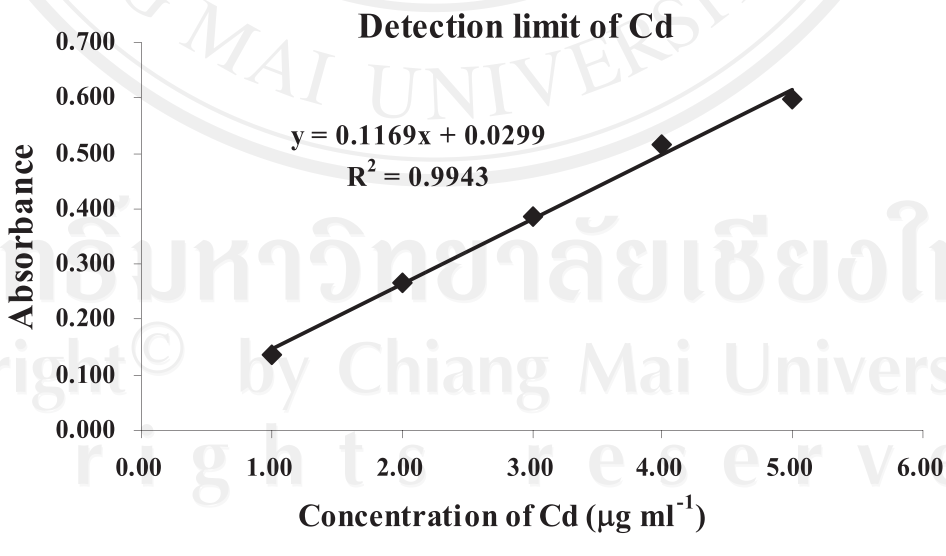


Figure C-7 Calibration curve of Cd for detection limit analysis

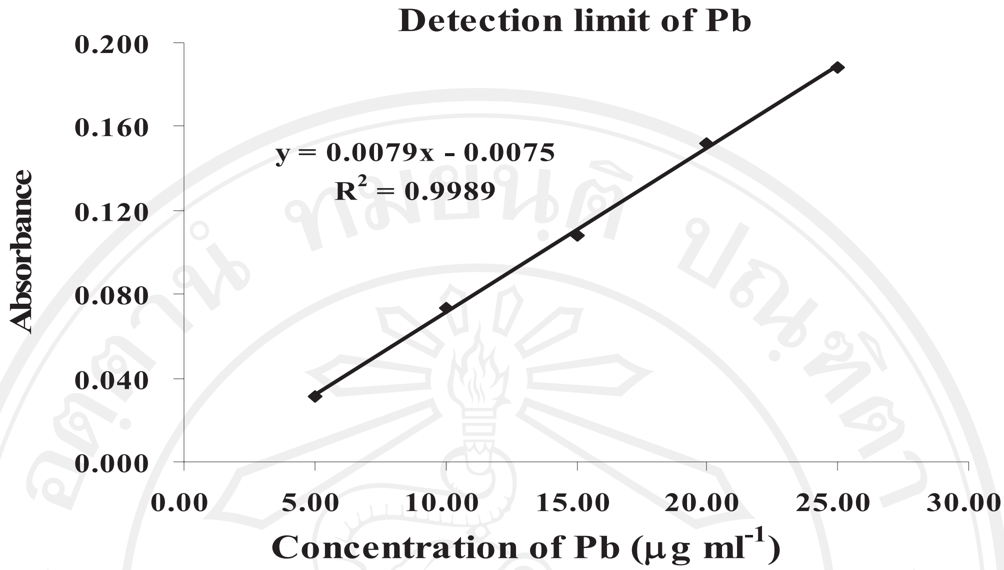


Figure C-8 Calibration curve of Pb for detection limit analysis

APPENDIX D

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Cadmium and lead are classified as toxic elements which tend to be accumulated in environmental systems and humans. Cadmium is known to damage human organs such as the kidneys, liver and lungs. While lead has been demonstrated to be accumulated within bone and in some soft tissues such as liver, kidneys and brain. Nowadays, human hair has attracted the interest of researchers from medical, environmental and forensic science increasingly. Because the toxics of heavy metal can be accumulated within hair for a long time, therefore, an alternative way is to use human hair as a screening tool for diagnosing and proving the cause of death or disease. Hair is a valuable biological material in forensic investigations, but its preparation for toxicological analysis involves many steps and is time consuming, so this material may be a challenge in developing suitable sample preparation methods. Taking into account the advantages of microwave-assisted digestion and cloud point extraction, as well as the increasing concern of the scientific community about the environmental impact of scientific activity, these sample preparation techniques are worth for further exploration. Therefore, the analysis of trace elements in human hair sample has progressively become important.

In this work, the concentration of cadmium and lead were studied using microwave-assisted digestion for sample preparation and using cloud point extraction for preconcentration of cadmium and lead. For the determination of cadmium and lead were detected by flame atomic absorption spectrophotometry. The information of this work will be useful for evaluation hazardous level of cadmium and lead from human hair. Also, the proposed method provides significantly shorten and simplify process for analysis trace elements in human hair, as well as the information increases the role of hair in medical, environmental and forensic examination. Another important point is that requirement of more investigation for application of microwave-assisted digestion and cloud point extraction to analysis of solid samples. Solid samples are essential materials for toxicological analysis. So far, no extraction methodology for solid biological samples is completely satisfactory, especially in terms of extraction time, toxic solvent consumption and reproducibility. The information from this research is therefore especially attractive and useful for development of toxicological analysis in human hair and other solid biological samples.

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