APPENDICES

Appendix A

List of instruments used

Instruments	Sources
Adjustable automatic pipette	DLALaboratory, Switzerlands
Analytical balance	A&D Co., Ltd, Japan
Analytical column, Inertsil ODS-3	GL Sciences Inc., Japan.
Autosampler, Varian 9100	Varian, USA.
Guard column, Inertsil ODS-3	GL Sciences Inc., Japan.
Lyophilizer, Lioalpha-10	Telstar, Spain
Magnetic stirrer	Thermolyne Co., USA.
pH meter	BATCO, Thailand
Refrigerate centrifuge	Hanil Sci Industrial Co.,Ltd, Japan.
Solvent Delivery System, Varian 9012Q	Varian, USA.
UV-VIS detector, ProStar 9050	Varian, USA.
UV-160 A spectrophotometer	Shimadzu Co., Japan.
Vortex mixer	Scientific Industries, USA.

Appendix B

List of chemicals and reagents used

Chemicals and reagents	Sources
Albumin, Bovine Serum	Sigma Chemical Co., USA
Acetic acid	Merck, Germany
Acetone	Merck, Germany
Ammonia	BDH, England
Bilirubin oxidase	Sigma Chemical Co., USA
Bilirubin standard	Sigma Chemical Co., USA
Chloroform	Merck, Germany
D-Glucuronic acid lactone	Sigma Chemical Co., USA
Dimethysulphoxide	Merck, Germany
Ethanol	Merck, Germany
Ethylene glycol	Merck, Germany
Glycine	Merck, Germany
Hydrochloric acid	Merck, Germany
Magnesium chloride	Farmitalia carlo erba, Italy
Methanol (HPLC grade)	BDH, England
Oxalic acid	Seelze-hannover, Germany
Potassium phosphate	Sigma Chemical Co., USA
Sodium acetate anhydrous	Fluka, Switzerland
Sodium carbonate	Merck, Germany
Sodium hydroxide	Merck, Germany
Sodium nitrite	Merck, Germany

Chemicals and reagents	Sources
Sodium thiosulfate	M&B, England
Sulfanilic acid	Merck, Germany
Tris (hydroxymethyl)-aminomethane	Merck, Germany
Triton X-100	Sigma Chemical Co., USA
Uridine 5'- diphosphoglucuronic acid	Sigma Chemical Co., USA

Appendix C

List of reagents and buffers preparation

I. Reagents and buffers for standard bilirubin preparation

1. 0.1 mol/L Tris-HCl buffer, pH 7.4

Tris (hydroxy methyl aminomethan)	12.11	g
Distilled water	900	mL
Adjust pH to 7.4 with 1 N HCl		
Adjust volume to 1,000 mL with distilled water		

2. 4 % bovine serum albumin (BSA)

BSA	4.0	g
0.1 mol/L Tris-HCl buffer, pH 7.4	90	mL
Adjust volume to 100 mL with 0.1 mol/L Tris-HCl buffer, pH 7.4		

3. Working 4 % BSA

0.1 mol/L Na_2CO_3	2.0	mL
0.1 N NaOH	1.5	mL
4 % BSA	90	mL
Adjust volume to 100 mL with 4 % BSA		

4. 20 mg/dL bilirubin standard

Bu standard	20	mg
0.1 mol/L Na_2CO_3	2.0	mL
0.1 N NaOH	1.5	mL
Working 4 % BSA	90	mL
Adjust volume to 100 mL with working 4 % BSA		

II. Reagents for bilirubin determination (Jendrassik and Grof, 1938)

1. Caffeine reagent

Caffeine	50	g
Sodium benzoate	75	g
Sodium acetate	125	g
Distilled water (50-60° C)	500	mL
Na_2EDTA	1.0	g

Adjust volume to 1,000 mL with distilled water

2. Reagent A

Sulfanilic acid	5.0	g
Distilled water	500	mL
HCl	15	mL

Adjust volume to 1,000 mL with distilled water

3. Reagent B

$NaNO_2$	500	mg
Distilled water	80	mL

Adjust volume to 100 mL with distilled water

4. Diazo reagent

Reagent A	10	mL
Reagent B	0.25	mL

5. 4 % Ascorbic acid

Ascorbic acid	4.0	g
Distilled water	80	mL

Adjust volume to 100 mL with distilled water

6. Tartrate solution

NaOH	75	g
Sodium potassium tartrate	320	g
Distilled water	500	mL
Adjust volume to 1,000 mL with distilled water		

III. Reagents for bilirubin determination (Malloy and Evelyn, 1937)

1. Sulfanilic acid

Sulfanilic acid	500	mg
Distilled water	70	mL
HCl	1.5	mL
Adjust volume to 100 mL with distilled water		

2. Sodium nitrite: stock solution

$NaNO_2$	25	g
Distilled water	80	mL
Adjust volume to 100 mL with distilled water		

3. 0.5 % Sodium nitrite

Stock $NaNO_2$	0.1	mL
Distilled water	4.9	mL

4. Diazo reagent

0.5 % $NaNO_2$	0.3	mL
Sulfanilic acid	10	mL

5. Diazo blank

HCl	0.5	mL
Distilled water	90	mL
Adjust volume to 100 mL with distilled water		

IV. Buffers for bilirubin retention time determination

1. **0.1 mol/L Glycine-NaOH buffer, pH 10.0.**

Glycine	7.51	g
Distilled water	800	mL
Adjust pH to 10.0 with 1 N HCl or 1 N NaOH		
Adjust volume to 1,000 mL with distilled water		

2. **0.1 mol/L Tris-HCl buffer, pH 8.0**

Tris (hydroxy methyl aminomethan)	12.14	g
Distilled water	800	mL
Adjust pH to 8.0 with 1 N HCl or 1 N NaOH		
Adjust volume to 1,000 mL with distilled water		

V. Solvents for HPLC separation (Spivak and Yuey, 1986)

1. **Solvent A: 0.04 M sodium acetate in methanol**

Sodium acetate anhydrous	3.282	g
Methanol	900	mL
Adjust volume to 1,000 mL with methanol		

2. **Solvent B: 1% ammonium acetate**

Acetic acid	10	mL
Distilled water	900	mL
Adjust pH to 4.5 with concentrated NH_3 (28-30%)		
Adjust volume to 1,000 mL with distilled water		

VI. Buffers for liver biosynthesis (Wu *et al.*, 1980)

1. **0.05 mol/L potassium phosphate buffer, pH 7.4 ± 0.05**

K_2HPO_4	8.71	g
Distilled water	900	mL

Adjust pH to 7.4 with 1 N HCl

Adjust volume to 1,000 mL with distilled water

2. **0.1 mol/L potassium phosphate buffer, pH 6.8 ± 0.05**

K_2HPO_4	17.42	g
Distilled water	900	mL

Adjust pH to 6.8 with 1 N HCl

Adjust volume to 1,000 mL with distilled water

VII. Reagents for glucuronic acid determination (Blumenkrantz and Asboe-Hansen, 1973)

1. **Meta-hydroxydiphenyl solution**

3-hydroxybiphenyl	0.15	g
0.5 % NaOH	80	mL

Adjust volume to 100 mL with 0.5 % NaOH

2. **H_2SO_4 / $Na_2B_4O_7 \cdot 10\ H_2O$ solution**

Sodium tetraborate	0.477	g
H_2SO_4 (95-98 %)	80	mL

Adjust volume to 100 mL with H_2SO_4 (95-98 %)

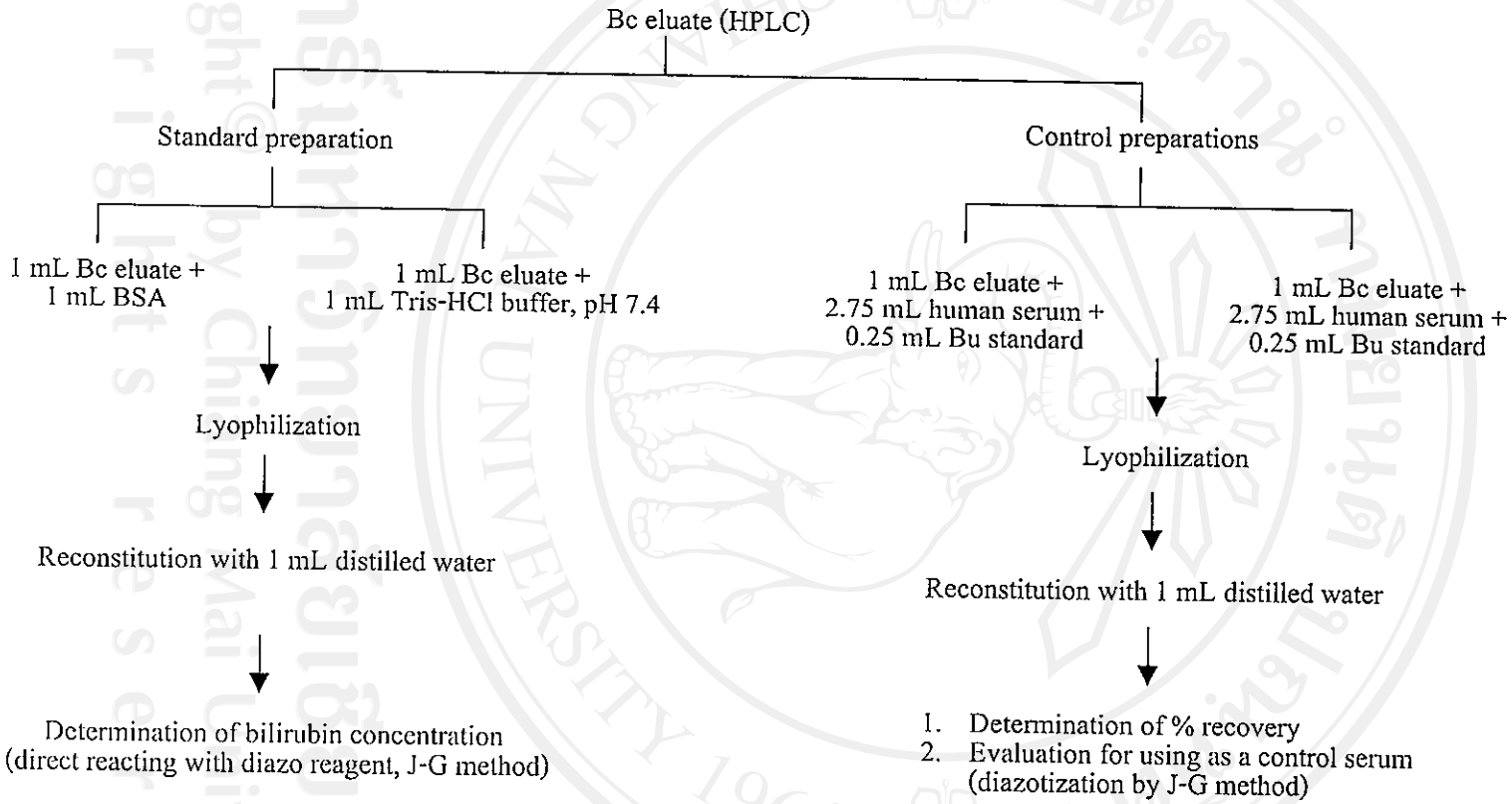
3. **Stock 50 µg glucuronic acid / 0.2 mL standard**

Glucuronic acid	0.025	g
0.1 mol/L potassium phosphate buffer, pH 6.8	80	mL

Adjust volume to 100 mL with 0.1 mol/L potassium phosphate buffer, pH 6.8

Appendix D

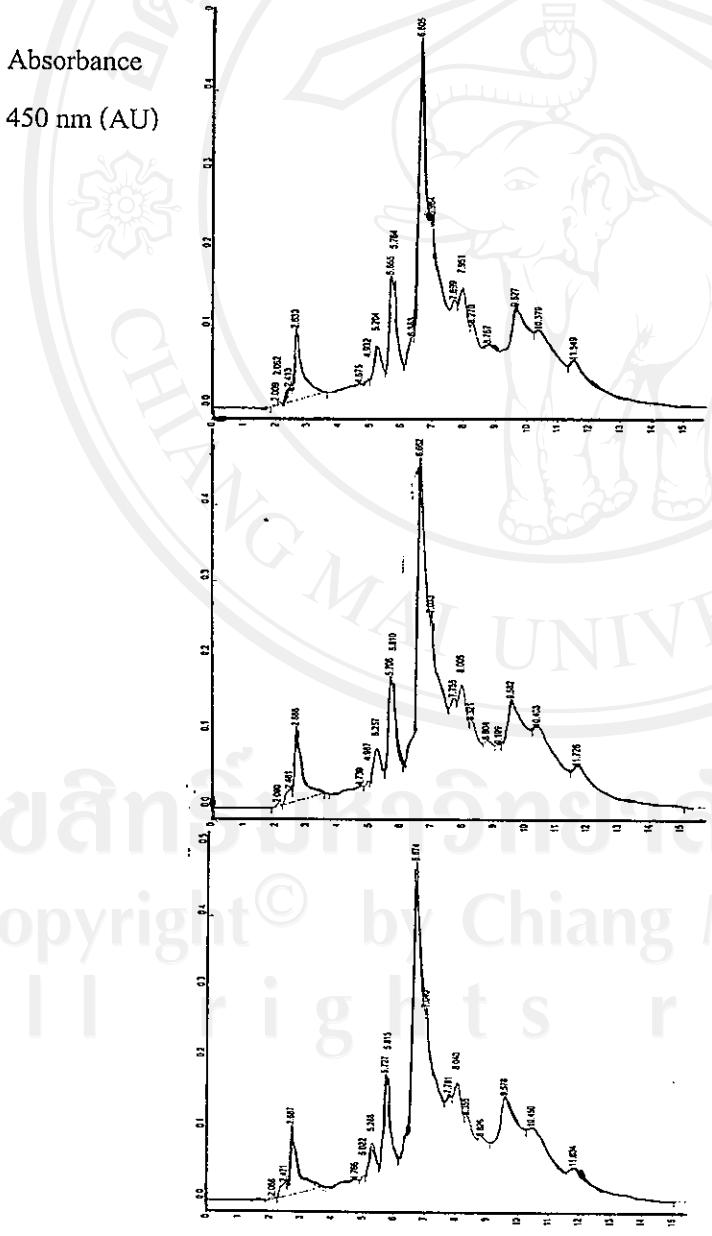
Outline of preparation of control serum



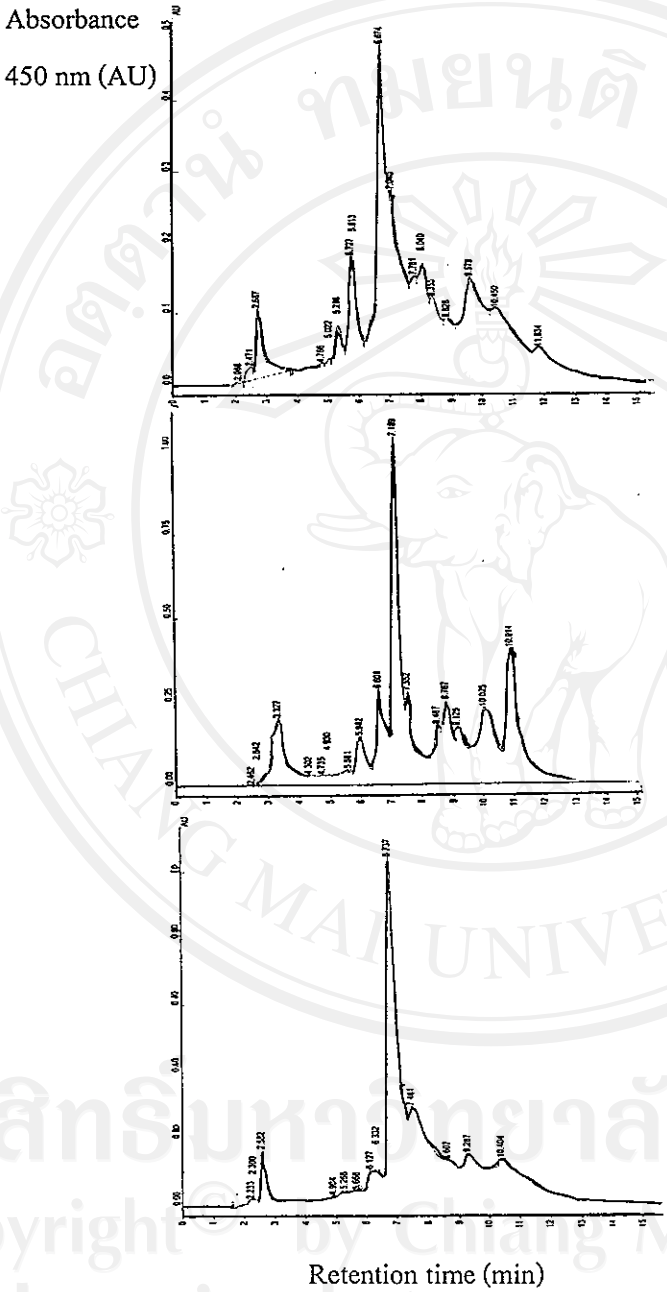
Appendix E

HPLC separation profiles of bilirubin species in chicken bile

I. Repeated specimen



II. Different specimens

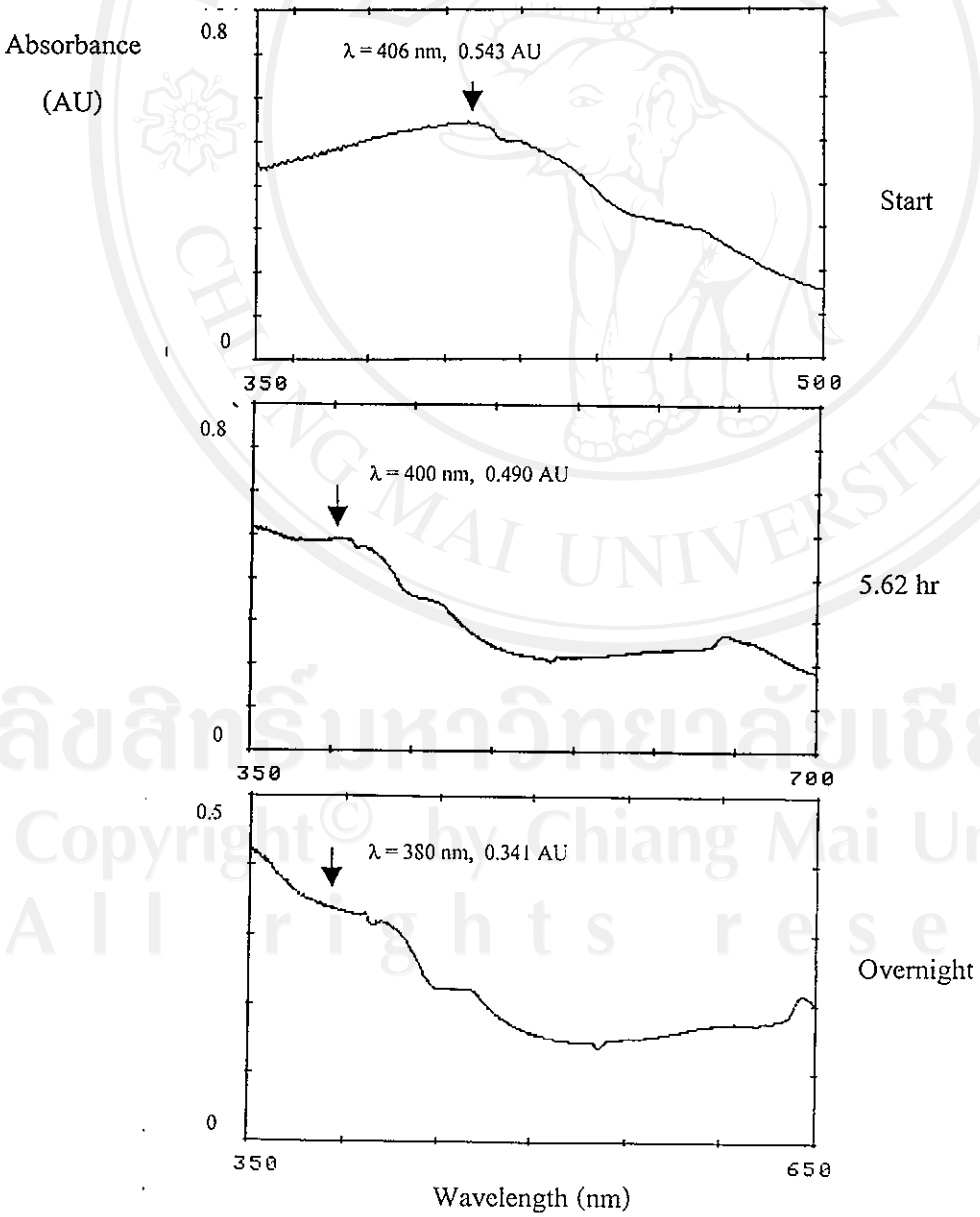


Appendix F

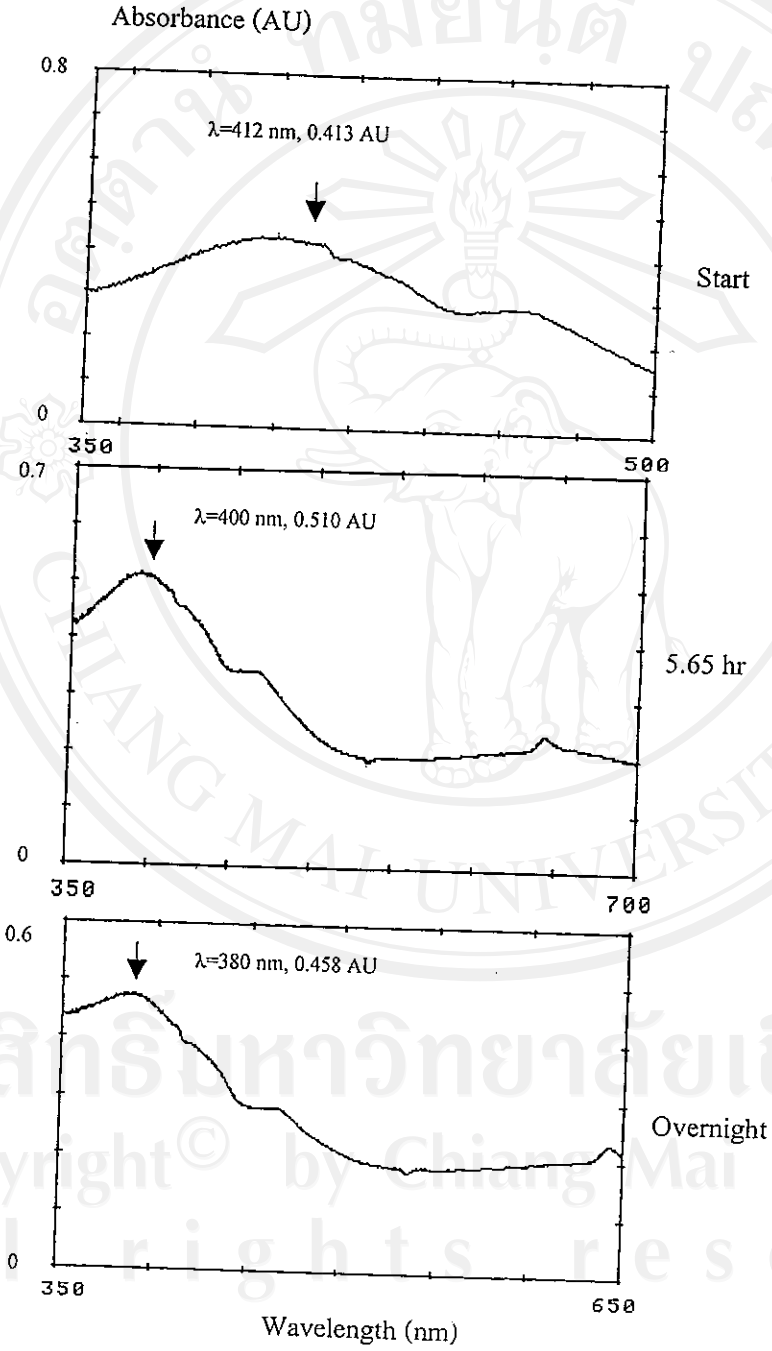
Physical properties of reconstituted Bc prepared in different matrix

I. Photodegradation

1. Absorption spectra of Bc in 0.1 mol/L Tris-HCl buffer, pH 7.4

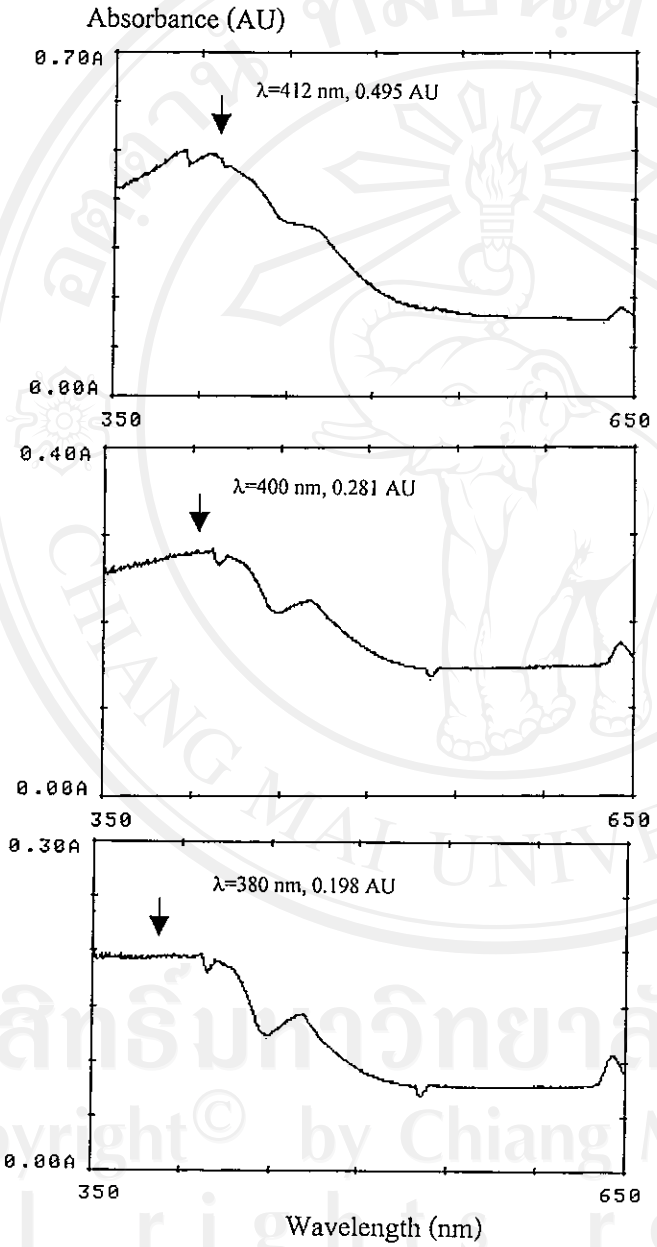


2. Absorption spectra of Bc in 4 % BSA

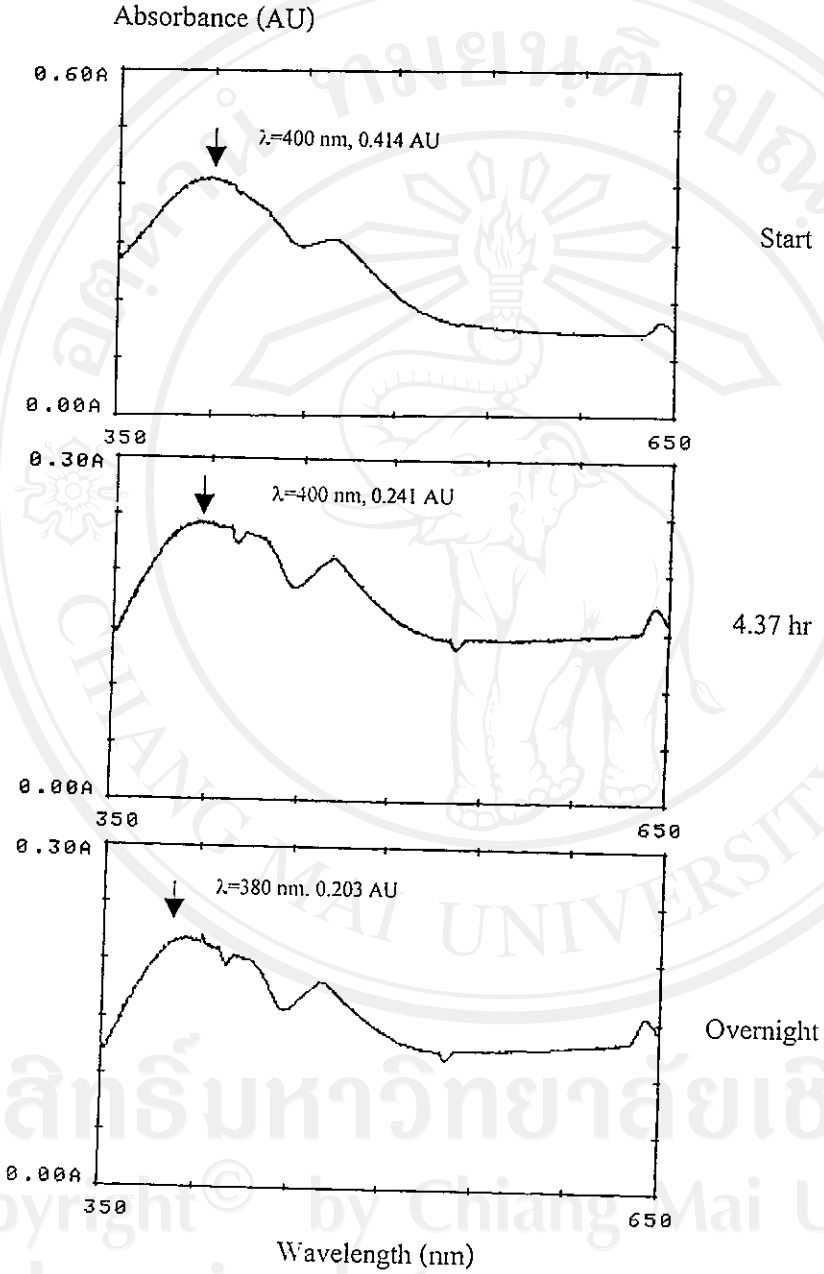


II. Oxidation by oxygen

1. Absorption spectra of Bc in 0.1 mol/L Tris-HCl buffer, pH 7.4

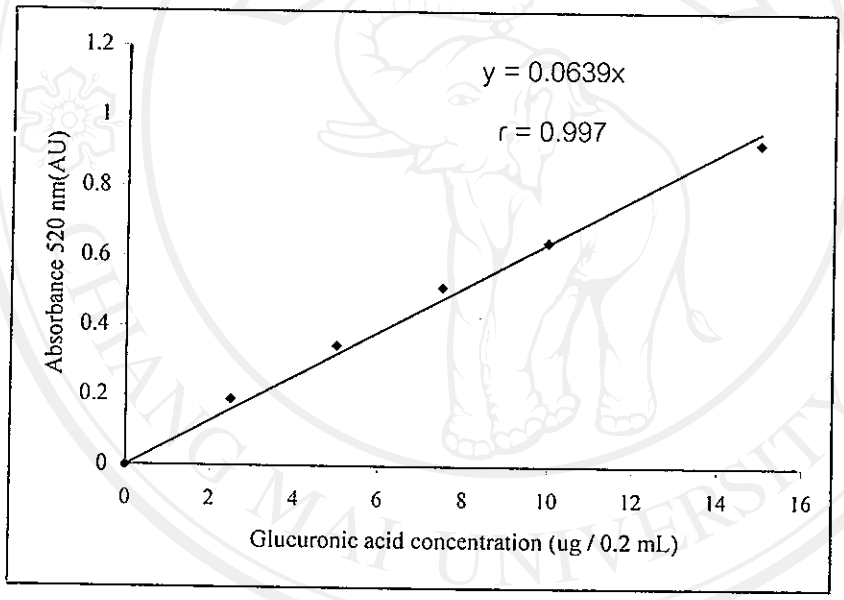


2. Absorption spectra of Bc in 4 % BSA



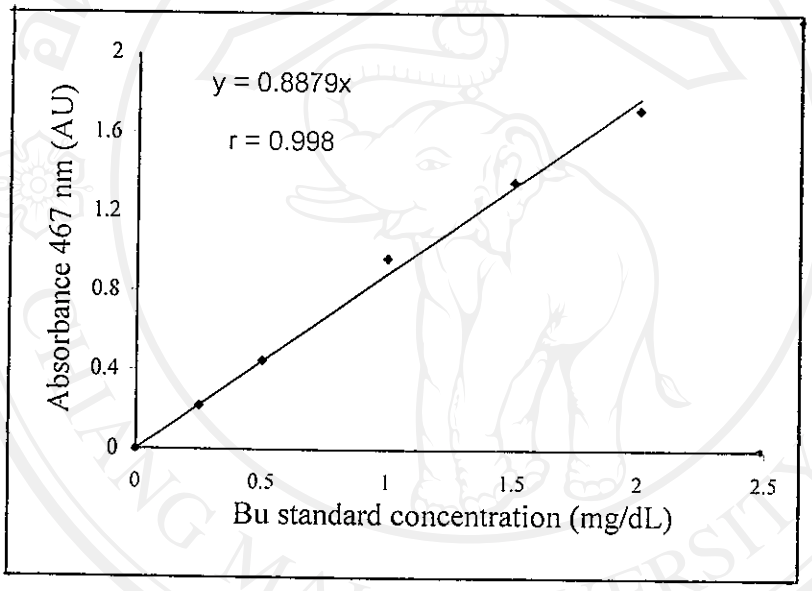
Appendix G

Standard graph of glucuronic acid in 0.1 mol/L potassium phosphate buffer, pH 6.8



Appendix H

Standard graph of Bu standard in 4 % BSA based on direct spectrophotometry



Appendix I

Distribution of bilirubin values in prepared control serum (Fig. A- B) and commercial control serum (Randox, UK) (Fig. C- D)

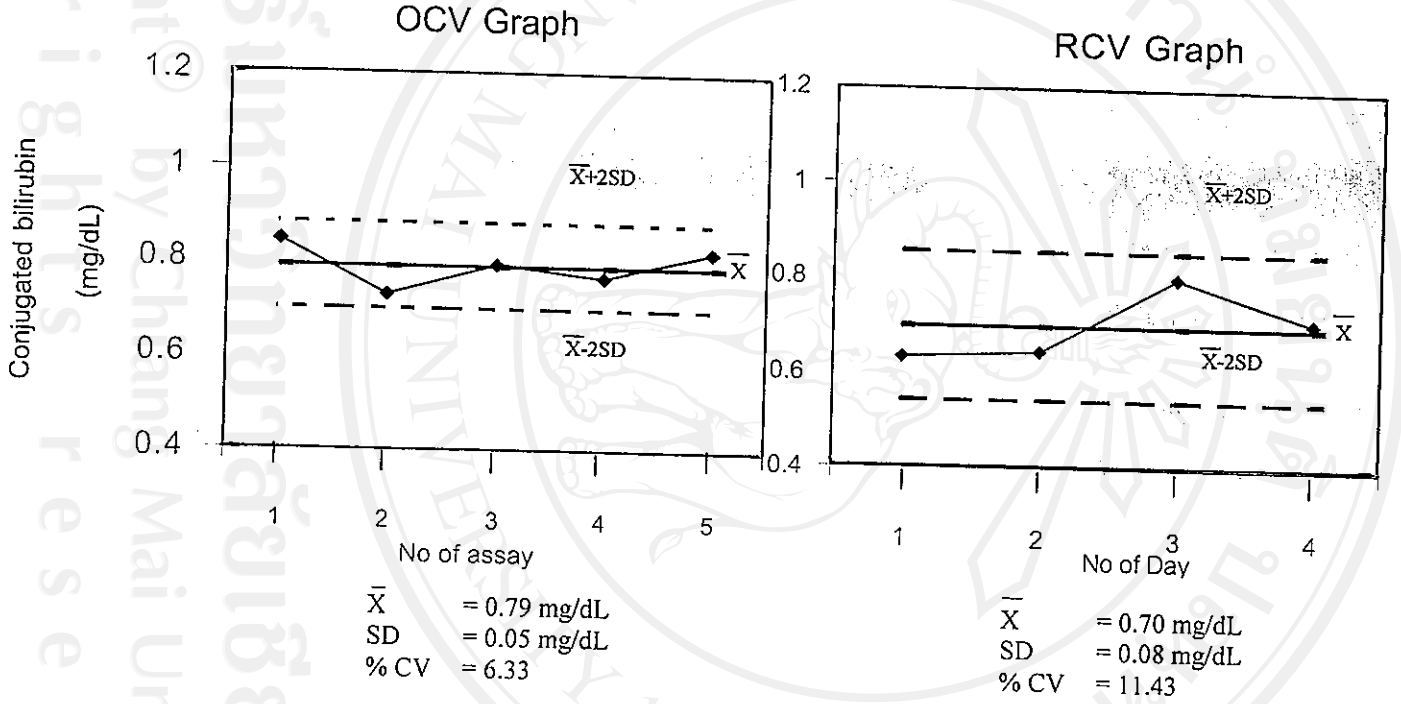


Figure A. Precision of direct bilirubin in control serum using Jendrassik and Grof method.

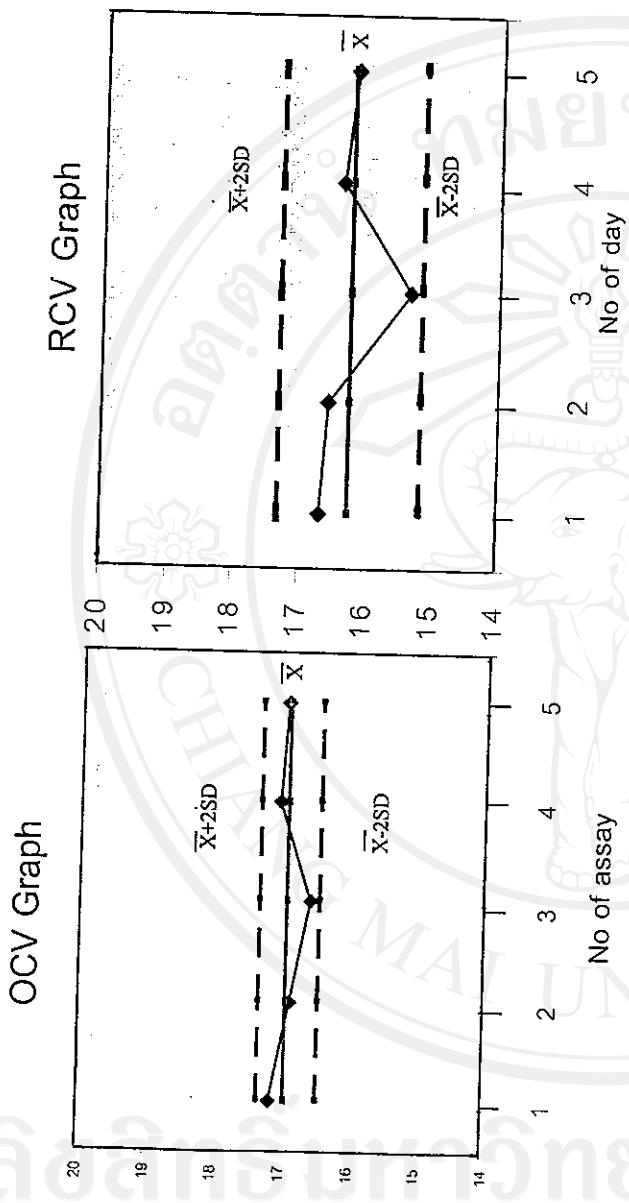


Figure B. Precision of total bilirubin in control serum using Jendrassik and Grof method.

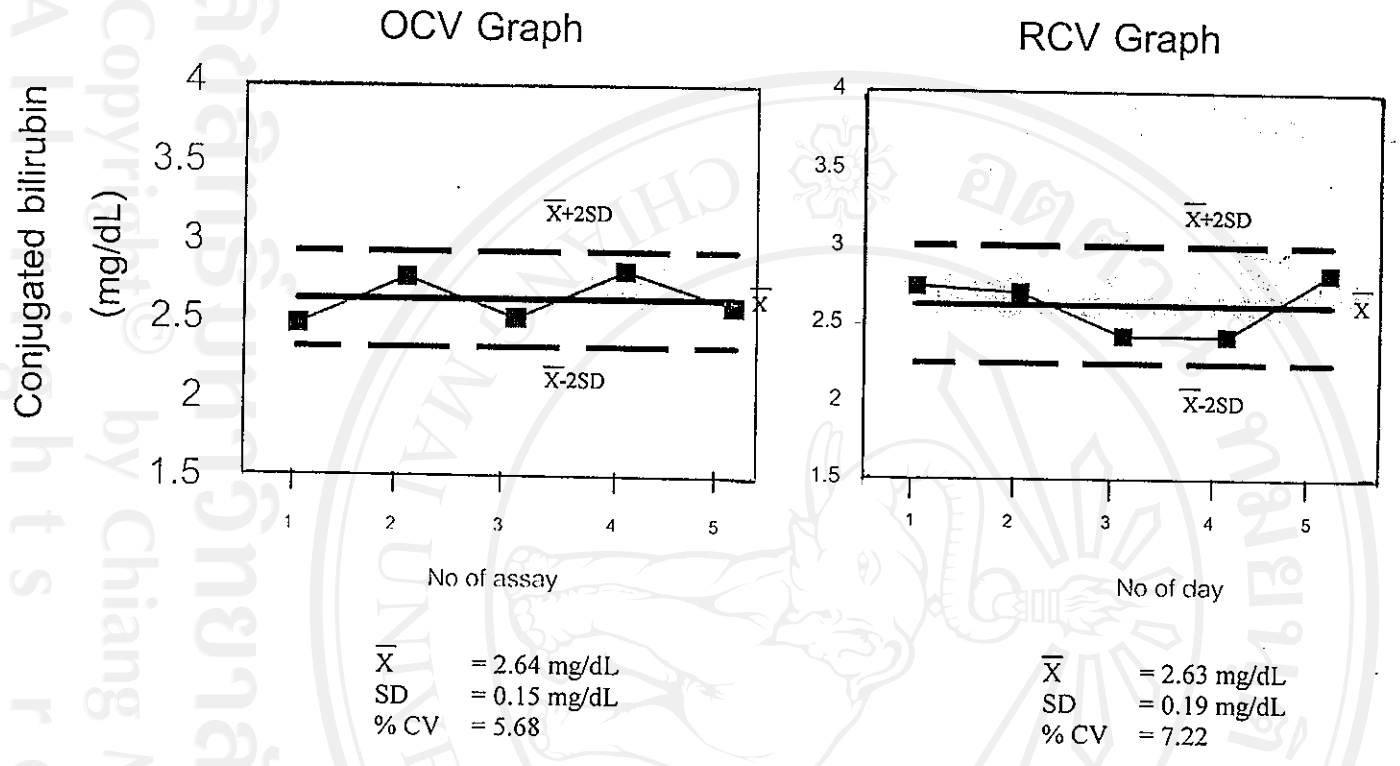


Figure C. Precision of direct bilirubin in commercial control serum (Randox, UK) using Jendrassik and Grof method.

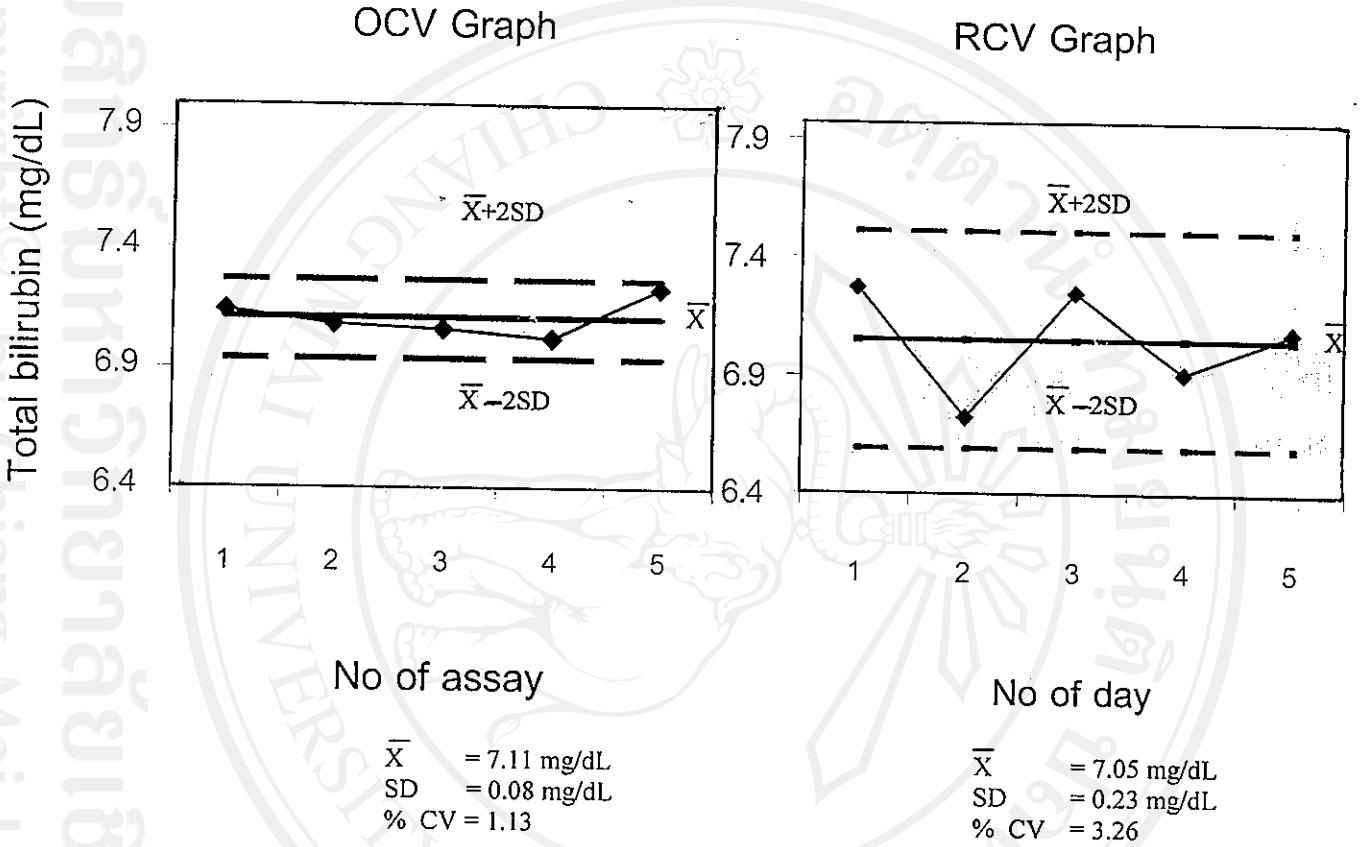


Figure D. Precision of total bilirubin in commercial control serum (Randox, UK) using Jendrassik and Grof method.