APPENDIX A

AIDS defining symptom

Candidiasis;

Cervical cancer, invasive;

Coccidioidomycosis;

Cryptococcosis;

Crytosporidiosis

Diarrhea more than 1 month;

Cytomegalovirus;

Cytomegalovirus retinitis;

HIV encephalopathy or HIV dementia,

AIDS dementia or subacute HIV encephalitis;

Herpes simplex more than 1 month;

Histoplasmosis;

Isosporiasis and diarrhea more than 1 month;

Kaposis's sarcoma;

Lymphoma, Burkitt's (or equivalent term);

Lymphoma, immunoblastic (or equivalent term);

Lymphoma, primary in brain;

Mycobacterium avium complex or M. kansasii;

Mycobacterium, other species or unidentified species, disseminated or extrapulmonary; Mycobacterium tuberculosis;

Pulmonary or extrapulmonary;

Pneumonia recurrent (Bacteria) more than 1 time/year;

Pneumocystis carinii pneumonia;

Penicillium marneffei infection;

Progressive multifocal leukoencephalophathy;

Salmonella septicemia;

Toxoplasmosis in brain;

Wasting syndrome;

Nocardiosis;

Rhodococcosis and serious bacterial infection.

APPENDIX B

Cronbach's alpha coefficiency

The reliability is the extent to which the measurements of a test remain consistent over repeated tests of the same subject under identical conditions. An alternative way of computing the reliability of a sum scale is Cronbach's coefficient alpha (α), formula as follows;

$$\alpha = [k/(k-1)] * [1- (S^2_i)/(S^2_{sum})]$$

Where;

k is the number of items.

S_i is the variances for the k individual items.

S_{sum} is the variance for the sum of all items.

Cronbach's alpha will generally increase when the correlations between the items increase. For this reason the coefficient is also called the internal consistency or the internal consistency reliability of the test. Cronbach's alpha can be used both dichotomous and ordered polychromous data and requires only one test administration.

APPENDIX C

Results of reliability for frequency food questionnaires by calculating Cronbach's alpha coefficiency

RELIABILITY ANALYSIS - SCALE (ALPHA)

Item-total Statistics

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Alpha if Item Deleted
F1	37.9167	173.9058	.2860	.8718
F2	39.7083	182.7373	-.0047	.8734
F3	39.0417	177.6938	.2911	.8709
F4	39.5417	176.8678	.4860	.8692
F5	39.4583	180.9547	.0992	.8733
F6	39.3333	184.8406	-.1504	.8765
F7	39.3750	179.2011	.2467	.8715
F8	36.8333	171.8841	.3140	.8717
F9	38.3750	170.1576	.4593	.8679
F10	39.0000	172.6087	.5633	.8670
F11	39.6250	177.7228	.4840	.8697
F12	39.7083	179.5199	.4205	.8708
F13	38.1250	179.5054	.0524	.8790
F14	39.5417	183.5634	-.0654	.8776
F15	39.4583	177.0417	.3607	.8701
F16	39.4167	176.5145	.3402	.8702
F17	39.7500	182.8043	-.0118	.8732
F18	39.4167	174.4275	.5274	.8680
F19	39.4583	174.2591	.5502	.8677
F20	39.3333	180.4928	.1484	.8726
F21	38.9167	181.2971	.1505	.8724
F22	39.5000	175.2174	.4986	.8685
F23	39.7083	179.4330	.4321	.8707
F24	39.2500	170.8043	.4562	.8680
F25	38.9167	175.6449	.3075	.8708
F26	39.1250	180.5489	.1545	.8725
F27	39.0833	175.1232	.5052	.8684
F28	39.1250	172.3750	.6803	.8661
F29	38.1667	172.4058	.3705	.8698
F30	39.5417	178.3460	.3588	.8705
F31	39.0833	179.1232	.2770	.8712
F32	39.7083	177.1721	.7356	.8688
F33	39.5833	176.7754	.5288	.8690
F34	39.3750	179.6359	.2142	.8719
F35	39.5833	180.7754	.1643	.8723
F36	39.0000	172.8696	.4542	.8682
F37	39.0417	176.6504	.3565	.8701
F38	39.4167	171.0362	.6694	.8655
F39	39.6250	177.2880	.5275	.8693
F40	39.2917	176.0417	.4780	.8689
F41	39.7500	183.8478	-.2003	.8740

RELIABILITY ANALYSIS - SCALE (ALPHA)

Item-total Statistics

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Alpha if Item Deleted
F42	39.5417	177.3025	.3670	.8701
F43	39.2917	173.6069	.5679	.8673
F44	39.1667	171.8841	.6177	.8662
F45	38.4583	168.8678	.5188	.8666
F48	39.6667	181.5362	.0527	.8741
F49	39.6250	183.8098	-.1137	.8746
F50	38.7083	179.4330	.1322	.8737
F51	38.7500	170.0217	.4297	.8686
F52	39.5417	172.2591	.7338	.8658
F53	39.5000	177.0435	.3220	.8705
F54	39.7083	179.4330	.4321	.8707
F55	39.0833	171.2101	.3004	.8727
F56	38.0833	171.6449	.3512	.8705
F57	39.6250	179.4620	.2393	.8716
F58	35.9583	182.7373	-.0099	.8737
F59	36.4167	169.6449	.3338	.8721

Reliability Coefficients

N of Cases = 24.0 N of Items = 57

Alpha = .8727

APPENDIX D

Questionnaire form

การบริโภคอาหารของผู้ติดเชื้อเอชไอวี

คำชี้แจง

แบบสอบถามนี้มีวัตถุประสงค์เพื่อศึกษาการได้รับจุลสารอาหารในกลุ่ม วิตามิน เอ, วิตามิน อี , วิตามิน
บี 12, สังกะสี และ ซิลิเนียม จากอาหารเพื่อประกอบการวิเคราะห์ข้อมูล ในโครงการ " สภาวะจุลสารอาหารในผู้ใหญ่ที่ติดเชื้อเอชไอวี โรงพยาบาลมหาราช จังหวัดเชียงใหม่" ซึ่งเป็นส่วนหนึ่งของการทำวิทยานิพนธ์ ระดับปริญญาโท(หลักสูตรนานาชาติ) สาขาวิทยาศาสตร์สุขภาพ บัณฑิตวิทยาลัย มหาวิทยาลัยเชียงใหม่ คำตอบที่ได้จะไม่มีผลเสียต่อตัวท่านแต่อย่างใด ผลการศึกษาที่ได้จะใช้เป็นข้อมูลด้านการสนับสนุนการวิเคราะห์ผลการตรวจหาปริมาณจุลสารอาหารในเลือดของอาสาสมัคร เพื่อเป็นประโยชน์ด้านข้อมูลพื้นฐานในการช่วยเหลือ ให้คำแนะนำ ด้านโภชนาการแก่ผู้ติดเชื้อเอชไอวีต่อไป

แบบสอบถามชุดนี้ประกอบด้วย 2 ส่วน ดังนี้

ส่วนที่ 1 ข้อมูลส่วนตัว จำนวน 14 ข้อ
ส่วนที่ 2 การบริโภคอาหารในกลุ่มที่ให้ วิตามิน เอ, อี, บี12, สังกะสี และ ซิลิเนียม จำนวน 56 ข้อ

ส่วนที่ 1 แบบสอบถามข้อมูลส่วนตัว

code……

1. อายุ……ปี

2. เพศ

❑ ชาย ❑ หญิง

3. สถานภาพสมรส

❑ โสด ❑ คู่ ❑ หม้าย (หย่า/แยกกันอยู่/เสียชีวิต)

4. น้ำหนัก..... กิโลกรัม ส่วนสูง ..... เซนติเมตร

5. ท่านทราบว่าตนเองติดเชื้อเอชไอวี มานาน …….. ปี …… เดือน

6. ก่อนที่ท่านจะมารับยาต้านไวรัสที่โรงพยาบาลมหาราชนครเชียงใหม่ ท่านเคยรับต้านไวรัสมาก่อนหรือไม่ ถ้าเคยโปรดระบุชื่อยาและระยะเวลา

❑ เคย ชื่อยา.....ระยะเวลาที่รับยา .....ปี .....เดือน

❑ ไม่เคย

7. ท่านได้รับยาต้านไวรัสที่โรงพยาบาลมหาราชนครเชียงใหม่ มาเป็นระยะเวลานาน ...ปี ..เดือน

8. ตั้งแต่ท่านทราบว่าติดเชื้อเอชไอวี ท่านเคยรักษาโดยวิธีใช้สมุนไพรมาบ้างหรือไม่ ถ้าเคยโปรดระบุชนิดของสมุนไพรและระยะเวลา

❑ เคย ชื่อสมุนไพร.....ระยะเวลาที่ใช้.....ปี .....เดือน

❑ ไม่เคย

9. ระดับการศึกษา จบชั้น .....

❑ ไม่ได้เรียนหนังสือ

❑ ประถมศึกษาตอนต้น(ป.1-ป.4)

❑ ประถมศึกษาตอนปลาย (ป.5-ป.6)

❑ มัธยมศึกษาตอนต้น(ม.1-ม.3)

❑ มัธยมศึกษาตอนปลาย/ปวช.
❑ ปวส./อนุปริญญา
❑ ปริญญาตรี/สูงกว่าปริญญา

10. รายได้ ของครอบครัวโดยประมาณ.....
❑ น้อยกว่า 1,000 บาท/เดือน
❑ 1,001-3,000 บาท/เดือน
❑ 3,001-6,000 บาท/เดือน
❑ 6,001-10,000 บาท/เดือน
❑ มากกว่า 10,000 บาท/เดือน

11. ท่านเคยได้รับคำแนะนำเกี่ยวกับการดูแลสุขภาพและการรับประทานอาหารที่เหมาะสมบ้างหรือไม่
❑ เคย ❑ ไม่เคย

12. ท่านมีความรู้ ความเข้าใจเกี่ยวกับอาหารกับสุขภาพมากน้อยเพียงใด
❑ ไม่มี
❑ น้อย
❑ ปานกลาง
❑ มาก
❑ มากที่สุด

13. ท่านออกกำลังกายเป็นประจำหรือไม่
❑ ไม่เคยออกกำลังกายเลย
❑ ออกกำลังกาย 1-2 ครั้ง/สัปดาห์
❑ ออกกำลังกาย 3-5 ครั้ง/สัปดาห์
❑ ออกกำลังกายทุกวัน

14. ถ้าท่านออกกำลังกาย ท่านใช้ระยะเวลาในการออกกำลังกายแต่ละครั้งนานเท่าใด
❑ น้อยกว่า 20 นาที/ครั้ง ❑ มากกว่า 30 นาที/ครั้ง
❑ 20-30 นาที/ครั้ง

ส่วนที่ 2 รูปแบบการบริโภคอาหาร

จงบอกชนิดและความถี่ของการบริโภคอาหารต่างๆ ใน 1 สัปดาห์ ที่ผ่านมา โดยเรียงลำดับความถี่ตามนิยาม ดังต่อไปนี้

ไม่ได้บริโภค = 0 บริโภค 11-15 มื้อ/สัปดาห์ = 3

บริโภค 1- 5 มื้อ/สัปดาห์ = 1 บริโภค 16-21 มื้อ/สัปดาห์ = 4

บริโภค 6-10 มื้อ/สัปดาห์ = 2

ชนิดของอาหาร	ความถี่ของการบริโภค (มื้อ/สัปดาห์)				
	16-21	**11-15**	**6-10**	**1-5**	**0**
อาหารประเภทเสริมสร้างและให้พลังงาน					
หมู					
เนื้อ					
ไก่					
ตับ					
ปลาดุก					
ปลาทู					
ปลาเล็กปลาน้อย(รวมก้าง)					
ปลาร้า/กะปิ					
ไข่					
ถั่วลิสง					
ถั่วเขียว					
ถั่วแดง					
ถั่วเหลือง/ผลิตภัณฑ์จากถั่วเหลือง เช่น เต้าหู้, ถั่วเน่า					
โปรตีนเกษตร					
งาขาว					
งาดำ					
ข้าวกล้อง					
ขนมปังโอลวีท					
อาหารประเภทผักและผลไม้					
ยอดกระถิน					

ชนิดของอาหาร	ความถี่ของการบริโภค (มื้อ/สัปดาห์)				
	16-21	11-15	6-10	1-5	0
ยอดแค					
ชะอม					
ยอดสะเดา					
ยอดมันเทศ					
ยอดมะระ					
ตำลึง					
ใบกระเพา					
ผักแพว(ผักไผ่)					
สาระแหน่					
ผักชี/ต้นหอม					
ผักคราด(ผักเผ็ด)					
ผักหวานป่า					
ผักกระเฉด					
ผักขม					
ผักปลัง					
ผักบุ้งจีน					
ผักกาดกวางตุ้ง					
ผักกาดเขียว					
ยอดฟักทอง					
คะน้า					
แครอท					
บล็อคโคลี่					
เมล็ดฟักทอง					
มะละกอสุก					
มะม่วงดิบ					
ประเภทอาหารเสริม					
นมผง					
นมกล่อง(นมวัว)					
นมถั่วเหลือง เช่น แลตตาซอย					

ชนิดของอาหาร	ความถี่ของการบริโภค (มื้อ/สัปดาห์)				
	16-21	11-15	6-10	1-5	0
นมเปรี้ยว					
โยเกริต					
นมข้นหวาน					
เครื่องดื่มธัญพืชสำเร็จรูป เช่น เนสวีต้า					
อาหารเสริมอื่นๆ ระบุ.....					
ท่านบริโภคอาหารที่ปรุงจากน้ำมันพืช เช่น น้ำมัน ถั่วเหลือง					
ท่านบริโภคอาหารที่ปรุงจากน้ำมันสัตว์ เช่น น้ำมันหมู					
อื่น ๆ					
ความถี่ที่ท่านดื่มเครื่องดื่มที่มีแอลกอฮอล์ เช่น เหล้า เบียร์					
ความถี่ที่ท่านสูบบุหรี่(มวน/วัน)					

หมายเหตุ: ท่านรับประทานอาหารมื้อสุดท้ายเมื่อ วันที่.....เดือน.....พ.ศ.....

เวลา ..... น.

APPENDIX E

Pattern of frequency food intake in HIV-infected and healthy subjects

Vitamin A consumption patterns of HIV-infected subjects

Food sources	Frequency dietary of vitamin A (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
ตับ	65	53.7	53	43.8	3	2.5	0	0.0	0	0.0	59.0	12.2
ไข่	11	9.1	75	62.0	30	24.8	3	2.5	2	1.7	152.0	31.4
ยอดกระถิน	66	54.6	52	43.0	2	1.7	1	0.8	0	0.0	59.0	12.2
ยอดแค	91	75.2	29	24.0	1	0.8	0	0.0	0	0.0	31.0	6.4
ชะอม	48	39.7	71	58.7	1	0.8	1	0.8	0	0.0	76.0	15.7
ยอดสะเดา	113	93.4	8	6.6	0	0.0	0	0.0	0	0.0	8.0	1.7
ยอดมันเทศ	117	96.7	4	3.3	0	0.0	0	0.0	0	0.0	4.0	0.8
ยอดมะระ	95	78.5	26	21.5	0	0.0	0	0.0	0	0.0	26.0	5.4
ตำลึง	55	45.5	66	54.6	0	0.0	0	0.0	0	0.0	66.0	13.6
ใบกระเพา	35	28.9	83	68.6	2	1.7	1	0.8	0	0.0	90.0	18.6
ผักไผ่	58	47.9	62	51.2	1	0.8	0	0.0	0	0.0	64.0	13.2
สาระแหน่	55	45.5	64	52.9	2	1.7	0	0.0	0	0.0	68.0	14.1
ผักชี/ต้นหอม	12	9.9	56	46.3	27	22.3	17	14.1	9	7.4	197.0	40.7
ผักคราด	98	81.0	22	18.2	1	0.8	0	0.0	0	0.0	24.0	5.0
ผักหวานป่า	98	81.0	22	18.2	1	0.8	0	0.0	0	0.0	24.0	5.0
ผักกระเฉด	93	76.9	28	23.1	0	0.0	0	0.0	0	0.0	28.0	5.8
ผักขม	109	90.1	10	8.3	2	1.7	0	0.0	0	0.0	14.0	2.9
ผักปลัง	97	80.2	24	19.8	0	0.0	0	0.0	0	0.0	24.0	5.0

Table (continued)

Food sources	Frequency dietary of vitamin A (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
ผักบุ้งจีน	46	38.0	74	61.2	1	0.8	0	0.0	0	0.0	76.0	15.7
ผักกวางตุ้ง	47	38.8	71	58.7	3	2.5	0	0.0	0	0.0	77.0	15.9
ผักกาดเขียว	65	53.7	55	45.5	1	0.8	0	0.0	0	0.0	57.0	11.8
ยอดฟักทอง	91	75.2	30	24.8	0	0.0	0	0.0	0	0.0	30.0	6.2
คะน้า	51	42.2	68	56.2	2	1.7	0	0.0	0	0.0	72.0	14.9
แครอท	69	57.0	46	38.0	4	3.3	2	1.7	0	0.0	60.0	12.4
นมกล่อง	78	64.5	31	25.6	9	7.4	3	2.5	0	0.0	58.0	12.0
นมข้นหวาน	98	81.0	14	11.6	9	7.4	0	0.0	0	0.0	32.0	6.6
Average	**72**	**59.2**	**44.0**	**36.4**	**4**	**3.2**	**1**	**0.9**	**1**	**0.3**	**56.8**	**11.7**

Vitamin A consumption patterns of healthy subjects.

Food sources	Frequency dietary of vitamin A (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
ตับ	28	45.9	30	49.2	2	3.3	1	1.6	0	0.0	37.0	15.2
ไข่	4	6.6	41	67.2	13	21.3	3	4.9	0	0.0	76.0	31.2
ยอดกระถิน	25	41.0	30	49.2	5	8.2	0	0.0	1	1.6	44.0	18.0
ยอดแค	47	77.1	14	23.0	0	0.0	0	0.0	0	0.0	14.0	5.7
ชะอม	25	41.0	32	52.5	4	6.6	0	0.0	0	0.0	40.0	16.4
ยอดสะเดา	60	98.4	1	1.6	0	0.0	0	0.0	0	0.0	1.0	0.4
ยอดมันเทศ	57	93.4	4	6.6	0	0.0	0	0.0	0	0.0	4.0	1.6
ยอดมะระ	50	82.0	11	18.0	0	0.0	0	0.0	0	0.0	11.0	4.5
ตำลึง	28	45.9	32	52.5	1	1.6	0	0.0	0	0.0	34.0	13.9
ใบกระเพา	23	37.7	35	57.4	2	3.3	1	1.6	0	0.0	42.0	17.2
ผักไผ่	27	44.3	34	55.7	0	0.0	0	0.0	0	0.0	34.0	13.9
สาระแหน่	25	41.0	36	59.0	0	0.0	0	0.0	0	0.0	36.0	14.8
ผักชี/ต้นหอม	3	4.9	24	39.3	19	31.2	7	11.5	8	13.1	115.0	47.1
ผักคราด	43	70.5	17	27.9	1	1.6	0	0.0	0	0.0	19.0	7.8
ผักหวานป่า	52	85.3	9	14.8	0	0.0	0	0.0	0	0.0	9.0	3.7
ผักกระเฉด	52	85.3	9	14.8	0	0.0	0	0.0	0	0.0	9.0	3.7
ผักขม	52	85.3	9	14.8	0	0.0	0	0.0	0	0.0	9.0	3.7
ผักปลัง	46	75.4	14	23.0	1	1.6	0	0.0	0	0.0	16.0	6.6
ผักบุ้งจีน	26	42.6	31	50.8	4	6.6	0	0.0	0	0.0	39.0	16.0
ผักกวางตุ้ง	26	42.6	34	55.7	1	1.6	0	0.0	0	0.0	36.0	14.8
ผักกาดเขียว	33	54.1	25	41.0	2	3.3	1	1.6	0	0.0	32.0	13.1
ยอดฟักทอง	42	68.9	18	29.5	1	1.6	0	0.0	0	0.0	20.0	8.2
คะน้า	30	49.2	29	47.5	2	3.3	0	0.0	0	0.0	33.0	13.5

Table (continued)

Food sources	Frequency dietary of vitamin A (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
แครอท	37	60.7	23	37.7	1	1.6	0	0.0	0	0.0	25.0	10.3
นมกล่อง	34	55.7	16	26.2	8	13.1	3	4.9	0	0.0	41.0	16.8
นมข้นหวาน	45	73.8	11	18.0	5	8.2	0	0.0	0	0.0	21.0	8.6
Average	**35**	**58.0**	**22**	**35.9**	**3**	**4.5**	**1**	**1.0**	**0**	**0.6**	**30.7**	**12.6**

Vitamin E consumption patterns of HIV-infected subjects

Food source	Frequency dietary of vitamin E (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
ถั่วลิสง	70	57.9	49	40.5	2	1.7	0	0.0	0	0.0	53	11.0
ผักขม	109	90.1	10	8.3	2	1.7	0	0.0	0	0.0	14	2.9
บล็อคโคลี่	103	85.1	17	14.1	1	0.8	0	0.0	0	0.0	19	3.9
มะม่วงดิบ	27	22.3	69	57.0	21	17.4	2	1.7	1	0.8	121	25.0
น้ำมันพืช เช่น น้ำมันถั่วเหลือง	5	4.1	35	28.9	44	36.4	19	15.7	17	14.1	248	51.2
Average	**63**	**51.9**	**36**	**29.8**	**14**	**11.6**	**4**	**3.5**	**4**	**3.0**	**91**	**18.8**

Vitamin E consumption patterns of healthy subjects

Food sources	Frequency dietary of vitamin E (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
ถั่วลิสง	24	39.3	34	55.7	3	4.9	0	0.0	0	0.0	40	16.4
ผักขม	52	85.3	9	14.8	0	0.0	0	0.0	0	0.0	9	3.7
บล็อคโคลี่	54	88.5	7	11.5	0	0.0	0	0.0	0	0.0	7	2.9
มะม่วงดิบ	25	41.0	31	50.8	5	8.2	0	0.0	0	0.0	41	16.8
น้ำมันพืช เช่น น้ำมันถั่วเหลือง	3	4.9	15	24.6	22	36.1	14	23.0	7	11.5	129	52.9
Average	**32**	**51.8**	**19**	**31.5**	**6**	**9.8**	**3**	**4.6**	**1**	**2.3**	**45.2**	**18.5**

Vitamin B12 consumption patterns of HIV-infected subjects

Food sources	Frequency dietary of vitamin B12 (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
หมู	3	2.5	31	25.6	32	26.5	32	26.5	23	19.0	283	58.5
เนื้อ	72	59.5	41	33.9	7	5.8	1	0.8	0	0.0	58	12.0
ไก่	33	27.3	72	59.5	14	11.6	2	1.7	0	0.0	106	21.9
ตับ	65	53.7	53	43.8	3	2.5	0	0.0	0	0.0	59	12.2
ปลาดุก	87	71.9	33	27.3	0	0.0	1	0.8	0	0.0	36	7.4
ไข่	11	9.1	75	62.0	30	24.8	3	2.5	2	1.7	152	31.4
นมผง	115	95.0	3	2.5	2	1.7	0	0.0	0	0.0	7	1.5
นมกล่อง(นมวัว)	78	64.5	31	25.6	9	7.4	3	2.5	0	0.0	58	12.0
โยเกริต	93	76.9	25	20.7	3	2.5	0	0.0	0	0.0	31	6.4
เครื่องดื่มธัญพืชสำเร็จรูป เช่น เนสวีต้า	97	80.2	15	12.4	8	6.6	1	0.8	0	0.0	34	7.0
Average	**65**	**54.1**	**38**	**31.3**	**11**	**8.9**	**4**	**3.6**	**3**	**2**	**82.4**	**17.0**

Vitamin B12 consumption patterns of healthy subjects

Food sources	Frequency dietary of vitamin B12 (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
หมู	0	0.0	19	31.2	18	29.5	8	13.1	16	26.2	143	58.6
เนื้อ	31	50.8	26	42.6	4	6.6	0	0.0	0	0.0	34	13.9
ไก่	9	14.8	44	72.1	8	13.1	0	0.0	0	0.0	60	24.6
ตับ	28	45.9	30	49.2	2	3.3	1	1.6	0	0.0	37	15.2
ปลาดุก	44	72.1	16	26.2	1	1.6	0	0.0	0	0.0	18	7.4
ไข่	4	6.6	41	67.2	13	21.3	3	4.9	0	0.0	76	31.2
นมผง	58	95.1	3	4.9	0	0.0	0	0.0	0	0.0	3	1.2
นมกล่อง(นมวัว)	34	55.7	16	26.2	8	13.1	3	4.9	0	0.0	41	16.8
โยเกริต	48	78.7	11	18.0	2	3.3	0	0.0	0	0.0	15	6.2
เครื่องดื่มธัญพืชสำเร็จรูป เช่น เนสวีต้า	48	78.7	8	13.1	3	4.9	1	1.6	0	0.0	17	7.0
Average	**30**	**49.8**	**21**	**35.1**	**6**	**9.7**	**2**	**2.6**	**2**	**2.6**	**44.4**	**18.2**

Zinc consumption patterns of HIV-infected subjects

Food sources	Frequency dietary of zinc(Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
หมู	3	2.5	31	25.6	32	26.5	32	26.5	23	19.0	283	58.5
เนื้อ	72	59.5	41	33.9	7	5.8	1	0.8	0	0.0	58	12.0
ไก่	33	27.3	72	59.5	14	11.6	2	1.7	0	0.0	106	21.9
ตับ	65	53.7	53	43.8	3	2.5	0	0.0	0	0.0	59	12.2
ปลาดุก	87	71.9	33	27.3	0	0.0	1	0.8	0	0.0	36	7.4
ปลาทู	52	43.0	68	56.2	0	0.0	1	0.8	0	0.0	71	14.7
ปลาร้า/กะปิ	16	13.2	33	27.3	22	18.2	28	23.1	22	18.2	249	51.5
ไข่	11	9.1	75	62.0	30	24.8	3	2.5	2	1.7	152	31.4
ถั่วลิสง	70	57.9	49	40.5	2	1.7	0	0.0	0	0.0	53	11.0
ถั่วเขียว	104	86.0	16	13.2	0	0.0	0	0.0	0	0.0	16	3.3
ถั่วแดง	115	95.0	6	5.0	0	0.0	0	0.0	0	0.0	6	1.2
ถั่วเหลือง/ ผลิตภัณฑ์จาก ถั่วเหลือง เช่น เต้าหู้, ถั่วเน่า	41	33.9	61	50.4	18	14.9	1	0.8	0	0.0	100	20.7
งาขาว	107	88.4	12	9.9	1	0.8	1	0.8	0	0.0	17	3.5
งาดำ	106	87.6	11	9.1	4	3.3	0	0.0	0	0.0	19	3.9
เมล็ดฟักทอง	101	83.5	20	16.5	0	0.0	0	0.0	0	0.0	20	4.1
นมกล่อง(นมวัว)	78	64.5	31	25.6	9	7.4	3	2.5	0	0.0	58	12.0
เครื่องดื่มธัญพืช สำเร็จรูป เช่น เนสวีต้า	97	80.2	15	12.4	8	6.6	1	0.8	0	0.0	34	7.0
Average	**68**	**56.3**	**37**	**30.5**	**9**	**7.3**	**4**	**3.6**	**3**	**2.3**	**78.7**	**16.3**

Zinc consumption patterns of healthy subjects

Food sources	Frequency dietary of zinc (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
หมู	0	0.0	19	31.2	18	29.5	8	13.1	16	26.2	143	58.6
เนื้อ	31	50.8	26	42.6	4	6.6	0	0.0	0	0.0	34	13.9
ไก่	9	14.8	44	72.1	8	13.1	0	0.0	0	0.0	60	24.6
ตับ	28	45.9	30	49.2	2	3.3	1	1.6	0	0.0	37	15.2
ปลาดุก	44	72.1	16	26.2	1	1.6	0	0.0	0	0.0	18	7.4
ปลาทู	27	44.3	32	52.5	1	1.6	1	1.6	0	0.0	37	15.2
ปลาร้า/กะปิ	9	14.8	18	29.5	15	24.6	9	14.8	10	16.4	115	47.1
ไข่	4	6.6	41	67.2	13	21.3	3	4.9	0	0.0	76	31.2
ถั่วลิสง	24	39.3	34	55.7	3	4.9	0	0.0	0	0.0	40	16.4
ถั่วเขียว	51	83.6	10	16.4	0	0.0	0	0.0	0	0.0	10	4.1
ถั่วแดง	51	83.6	10	16.4	0	0.0	0	0.0	0	0.0	10	4.1
ถั่วเหลือง/ ผลิตภัณฑ์จาก ถั่วเหลือง เช่น เต้าหู้, ถั่วเน่า	18	29.5	31	50.8	7	11.5	4	6.6	1	1.6	61	25.0
งาขาว	39	63.9	22	36.1	0	0.0	0	0.0	0	0.0	22	9.0
งาดำ	50	82.0	9	14.8	2	3.3	0	0.0	0	0.0	13	5.3
เมล็ดฟักทอง	52	85.3	9	14.8	0	0.0	0	0.0	0	0.0	9	3.7
นมกล่อง(นมวัว)	34	55.7	16	26.2	8	13.1	3	4.9	0	0.0	41	16.8
เครื่องดื่มธัญพืช สำเร็จรูป เช่น เนสวีต้า	48	78.7	8	13.1	3	4.9	1	1.6	0	0.0	17	7.0
Average	**30**	**50.1**	**22**	**36.2**	**5**	**8.2**	**2**	**2.9**	**2**	**2.6**	**43.7**	**17.9**

Selenium consumption patterns of HIV-infected subjects

Food sources	Frequency dietary of selenium (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
หมู	3	2.5	31	25.6	32	26.5	32	26.5	23	19.0	283	58.5
เนื้อ	72	59.5	41	33.9	7	5.8	1	0.8	0	0.0	58	12.0
ไก่	33	27.3	72	59.5	14	11.6	2	1.7	0	0.0	106	21.9
ปลาดุก	87	71.9	33	27.3	0	0.0	1	0.8	0	0.0	36	7.4
ไข่	11	9.1	75	62.0	30	24.8	3	2.5	2	1.7	152	31.4
ถั่วลิสง	70	57.9	49	40.5	2	1.7	0	0.0	0	0.0	53	11.0
บล็อคโคลี่	103	85.1	17	14.1	1	0.8	0	0.0	0	0.0	19	3.9
นมกล่อง(นมวัว)	78	64.5	31	25.6	9	7.4	3	2.5	0	0.0	58	12.0
เครื่องดื่มธัญพืชสำเร็จรูป เช่น เนสวีต้า	97	80.2	15	12.4	8	6.6	1	0.8	0	0.0	34	7.0
Average	**67**	**55.4**	**38**	**31.7**	**10**	**7.8**	**4**	**3.2**	**2**	**1.9**	**78.1**	**16.1**

Selenium consumption patterns of healthy subjects

Food sources	Frequency dietary of selenium (Times/week)										Total score	% of total score
	0		1 - 5		6 - 10		11 - 15		16 - 21			
	n	%	n	%	n	%	n	%	n	%		
หมู	0	0.0	19	31.2	18	29.5	8	13.1	16	26.2	143	58.6
เนื้อ	31	50.8	26	42.6	4	6.6	0	0.0	0	0.0	34	13.9
ไก่	44	72.1	16	26.2	1	1.6	0	0.0	0	0.0	18	7.4
ปลาดุก	9	14.8	44	72.1	8	13.1	0	0.0	0	0.0	60	24.6
ไข่	4	6.6	41	67.2	13	21.3	3	4.9	0	0.0	76	31.2
ถั่วลิสง	24	39.3	34	55.7	3	4.9	0	0.0	0	0.0	40	16.4
บล็อคโคลี่	54	88.5	7	11.5	0	0.0	0	0.0	0	0.0	7	2.9
นมกล่อง(นมวัว)	34	55.7	16	26.2	8	13.1	3	4.9	0	0.0	41	16.8
เครื่องดื่มธัญพืชสำเร็จรูป เช่น เนสวีต้า	48	78.7	8	13.1	3	4.9	1	1.6	0	0.0	17	7.0
Average	**28**	**45.2**	**23**	**38.4**	**6**	**10.6**	**2**	**2.7**	**2**	**2.9**	**48.4**	**19.9**

APPENDIX F

Principle of High Performance Liquid Chromatograph (HPLC)

Chromatography is a general technique that separates a mixture into its individual components. The chemical compounds of interest to the analyst. High-performance liquid chromatography (HPLC) is one such method, it is used to analyze liquid samples or the liquid extract of a sample. The fundamental basis for HPLC consists of passing a sample (analyte mixture) in a high pressure solvent (called the mobile phase) through a column, it packed with sorbents (called stationary phase). As the analytes pass through the column they interact between the two phases, mobile and stationary at difference in rates is primarily due to different polarities for the analytes. As the analytes exit the column, they can be detected by various means. The detector measures a signal peak as each analyte leaves the column by comparing the time it takes for the peak to show up (called the retention time) with the retention times for a mixture of known compounds, the components of unknown sample mixtures can be identified. The results of your sample run can then be interpreted and printed in a variety of report formats. The components of HPLC system are shown in the simple diagram below.

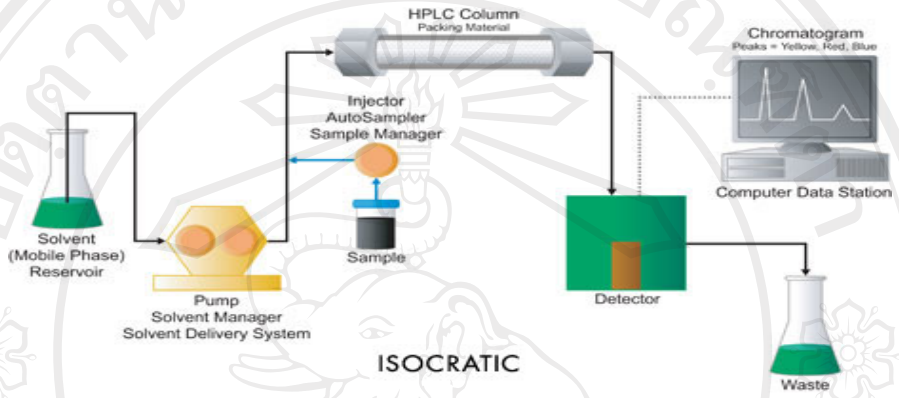


Figure showed HPLC System Diagram

APPENDIX G

Validation of HPLC method for the determination of serum vitamin A and vitamin E concentration

1. Apparatus and reagents

- **Apparatus**
  - HPLC Machine, Waters 501 Pump, Waters 490E Multi-wavelength detector.
  - Analytical column (Supelco Discovery® C18)
  - Beaker
  - Cylender
  - Test tubes
  - Autopipette

- **Reagents**
  - all - trans retinol (Sigma, USA)
  - Retinol acetate (Sigma, USA)
  - dl- α- tocopherol(Sigma, USA)
  - Tocopherol acetate(Sigma, USA)
  - n- hexane (HPLC grade, J.T Baker)
  - n-heptane (HPLC grade, J.T Baker)

- Methanol (HPLC grade, J.T Baker)
- Absolute ethanol (J.T Baker)
- Sodium sodecyl sulfate (SDS)
- Butylated hydroxytoluene (BHT)
- Ultra pure water

2. Chromatography condition

all - trans- retinol analysis;

Mobile phases: methanol: water (95:5 v/v)

Flow rate: 1.3 mL/min.

UV detector: 325 nm.

α - tocopherol analysis,

Mobile phases: 100% methanol

Flow rate: 1.0 mL/min.

UV detector: 292 nm.

3. Standard preparation for vitamin A analysis

.

▪ **Stock standard vitamin A (all-*trans* retinol)**

25 mg of all - *trans* retinol standard were dissolved in 100 mL absolute ethanol. The actual concentration of stock standard was determined by dilution stock standard in absolute ethanol (1: 50 v/v) and measured OD at wavelength of

325 nm ($\varepsilon_{1\ cm}^{1\%}$ = 1780) by spectrophotometer. The actual concentration was calculated according to the following formula;

$$\text{Concentration (g/100 mL)} = \frac{\text{Abs} \times \text{dilution factor}}{\text{extinction coefficient}}$$

$$= \frac{0.4038 \times 50}{1780}$$

$$= 0.01134 \text{ g/100 mL}$$

$$= 113.40 \text{ µg/mL}$$

Therefore, the final concentration of stock all-trans retinol was 113.40µg/mL. The stock standard was stored at - 20 °C, wrapped with aluminum foil in order to protect them from light.

The intermediately concentration of 11.34 µg/mL all-trans retinol was prepared by preparing 2.5 mL of stock all-trans retinol into 25 mL volumetric flask and made up to 25 mL with absolute ethanol.

- **Stock retinol acetate (Internal standard)**

Retinol acetate was used as internal standard for retinol. 1.0 g of retinol acetate was dissolved and made up to 100 ml with absolute ethanol in volumemetric flask. The actual concentration of stock of retinol acetate was determined by dilution stock retinol acetate in absolute ethanol (1: 100 v/v) and

measured OD at wavelength of 326 nm ($\varepsilon_{1\ cm}^{1\%}$ = 1550) by spectrophotometer. The actual concentration was calculated according to the following formula;

$$\text{Concentration (g/100 mL)} = \frac{\text{Abs} \times \text{dilution factor}}{\text{extinction coefficient}}$$

$$= \frac{0.6254 \times 100}{1550}$$

$$= 0.04035 \text{ g/100 mL}$$

$$= 0.40 \text{ mg/mL}$$

Therefore, the final concentration of stock retinol acetate was 0.40 mg/mL. The stock standard was stored at - 20 °C, covered with aluminum foil in order to protect them from light. 40 µg/mL retinol acetate intermediately concentration was prepared by preparing 2.5 mL of stock retinol acetate into 25 mL volumetric flask and made up to 25 mL with absolute ethanol.

4. Serum preparation for vitamin A analysis

Serum samples were extracted in duplicates. 200 µL of serum were added with 300 µL of 0.75 µg/ml retinol acetate in absolute ethanol, followed by 100 µL of absolute ethanol in order to precipitate protein by shaking for 5 minutes and added 200 µL of distillation water. Then the mixture was extracted with 500 µL of hexane by vigorously vortex for 10 minutes and centrifuged at 2,000 rpm at 20°C

for 10 minutes. 400 µL of supernatant were transferred to another tube. The extraction was two repeated with each 300 µL of hexane. The second and third extractions were pooled together. 1.0 mL supernatants were evaporated to dryness under nitrogen gas and the residue was reconstituted in 300 µL of mobile phase. Samples extracting under dimmed natural lighting, excluding fluorescent light at all times. 20 µL of the extract were injected into HPLC system.

5. Quantification of vitamin A in serum samples using internal standard method

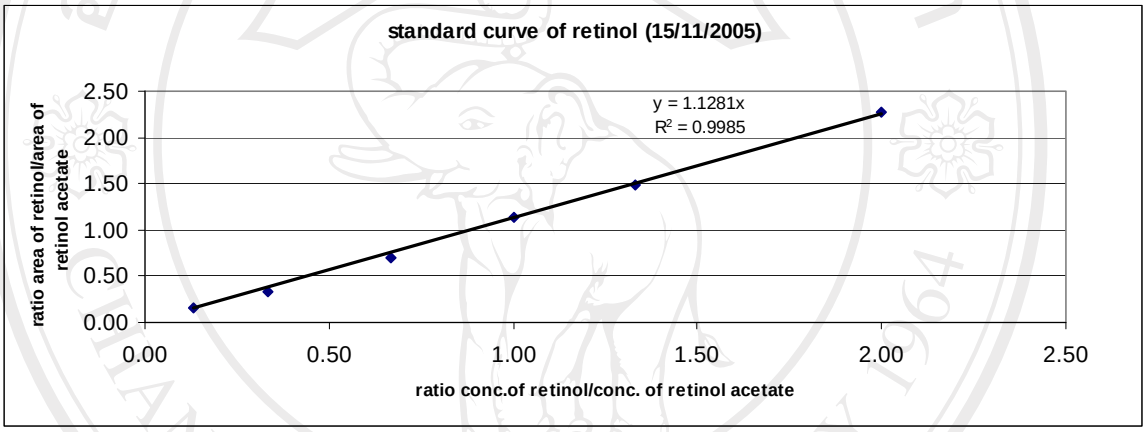
5.1 Sensitivity of the method

The limit of quantitation (LOQ) was defined as lowest of the concentration of all- *trans* retinol in serum sample which can be determined with acceptable precision and accuracy under the stated experiment condition. LOQ of all-trans retinol concentration performed a signal of peak height equal or more than the baseline noise value plus 10SD.

The procedure was used for measuring LOQ which prepared serial dilution of serum control. After serum controls were extracted and the residues were dissolved in mobile phase. They were double diluted by mobile phase until the diluted solution with give peak height at least equal or more than the baseline noise value plus 10SD. Finally, this solution was analyzed 10 independent repeats and calculated mean and SD. The LOQ of this method was 0.03µg/mL

5.2 Linearity

Calibration curves were obtained by least-squares linear regression analysis. Linearity of these standard curves was established with correlation coefficients averaging 0.9985. The standard curve was realized injecting standard in the range 0.10 – 2.0 µg/mL for all- *trans* retinol standard and 0.75 µg/mL of retinol acetate (internal standard) . The standard curve was showed as following;



For this method, the working standard was selected one point for daily analysis which was mixtures of 0.75 µg/mL all – *trans* retinol/0.75 µg/mL retinol acetate. The response factor (RF) value, the response ratio between all-trans retinol standard and retinol acetate (internal standard) was calculated for determination of all-*trans* retinol in unknown sample. The formula as followed;

$$RF\ (i) = \left\{ \frac{C(i)}{Area\ (i)} \right\} \left\{ \frac{Area(is)}{C\ (is)} \right\}$$

Where:

RF (i) = The response factor for all-*trans* retinol.

C (i) = The concentration of all-*trans* retinol standard.

Area (i) = The peak area of all-*trans* retinol standard solution.

Area (is) = The peak area of internal standard solution.

C (is) = The concentration of internal standard.

Analysis for all-*trans* retinol in serum samples, formula as followed;

$$\text{Conc. (i)} = \left\{ \frac{\text{IS}}{\text{SA}} \right\} \left\{ \frac{(\text{RF i})(\text{Area s})}{(\text{RF is})(\text{Area is})} \right\} (\text{XF})$$

Where:

Conc. (i) = The concentration of all-*trans* retinol in serum sample.

IS = The concentration of internal standard added to serum sample.

SA = The amount of sample material measured.

RF (i) = The response factor for all-*trans* retinol determined by standard solution.

Area (s) = The peak area of all-*trans* retinol in serum sample.

Area (is) = The peak area of internal standard in serum sample.

XF = A scaling factor (multiplier) that may be used as a conversion factor.

5.2.1 Preparation of working standard 0.75 µg/mL of all-*trans* retinol and 0.75 µg/mL of retinol acetate.

1,653 µL of 11.34 µg/mL intermediately standard retinol were mixed to 470 µL of 40 µg/mL intermediately retinol acetate standard in methanol and made up to 25 mL in volumetric flask. 20 µL of standard mixtures were injected into HPLC system.

The quality control employed to monitor the precision of RF value. The precision was determined through the repeated analysis both intra and inter assay precision by using working standard 0.75µg/mL of all-*trans*-retinol and 0.75 µg/mL of retinol acetate. Intra- assay variation was determined regularly by analyzing the standard mixtures twenty times on the same day. The mean ± SD showed 0.881 ± 0.029 (%CV =3.31). For inter-assay variation, one measurement of the standard mixtures per day were carried out on 13 days, mean ± SD was 0.887 ± 0.009 (%CV = 1.07). The detail of results showed as following;

5.2.1.1 Intra -and Inter batch of response factor (RF)

(1) Intra-batch precision of RF

To determine mixtures standard solution of 0.75 µg/mL all-trans retinol/ 0.75 µg/mL retinol acetate on the same day, n = 20.

$$RF = \frac{\text{conc. of retinol std.}}{\text{Area of retinol std.}} \times \frac{\text{Area of retinol acetate}}{\text{conc. Of retinol acetate}}$$

no.	retinol standard		retinol acetate		RF
	RT	Area	RT	Area	
1	3.17	509593	4.39	450931	0.885
2	3.17	510815	4.39	455762	0.892
3	3.17	514563	4.39	455035	0.884
4	3.17	518724	4.39	481261	0.928
5	3.16	510520	4.37	438983	0.860
6	3.17	506492	4.39	435982	0.861
7	3.18	509119	4.39	440026	0.864
8	3.18	499952	4.40	439026	0.878
9	3.17	522641	4.39	440641	0.843
10	3.17	491668	4.40	442654	0.900
11	3.18	507275	4.39	435480	0.858
12	3.17	504429	4.40	460885	0.914
13	3.17	502007	4.39	440497	0.877
14	3.17	509919	4.40	468118	0.918
15	3.18	514204	4.41	442762	0.861
16	3.18	507889	4.40	425666	0.838
17	3.18	510719	4.41	429390	0.841
18	3.18	511755	4.41	442854	0.865
19	3.18	489420	4.41	442444	0.904
20	3.18	509948	4.41	478335	0.938
mean		**508083**	**4.40**	**447337**	**0.881**
SD		**7901**	**0.01**	**15048**	**0.029**
%CV		**1.56**	**0.24**	**3.36**	**3.31**

(2) Inter-batch precision of RF

To determine mixtures standard solution of 0.75 µg/mL all-trans retinol/ 0.75 µg/mL retinol acetate, one measurement of mixtures standard per day were carried out on 13 days.

no.	Area		RF
	all-trans retinol	retinol acetate	
1			0.877
2	506732	452663	0.893
3	501198	440581	0.879
4	492033	447300	0.909
5	498928	436938	0.876
6	510831	454675	0.890
7	498800	438382	0.880
8	499758	447601	0.896
9	497258	440829	0.887
10	499772	444106	0.889
11	493599	438881	0.889
12	510765	454256	0.889
13	505331	417541	0.876
mean	**501250**	**442813**	**0.887**
SD	**6062**	**10138**	**0.009**
%CV	**1.21**	**2.29**	**1.07**

5.3 Accuracy

Accuracy is ability of a measuring instrument to give responses close to a true value. The accuracy expressed as % recovery. This experiment was carried out using pool serum control. The mean ± SD was 0.58 ± 0.03 µg/mL, in-house control. The procedure was used for measuring of recovery that the serum controls were unfortified and fortified with the standard concentration at a range of standard curve (added 0.25 µg/mL, 0.50 µg/mL and 1.0 µg/mL of all- *trans* retinol standard solution, respectively). At each concentration, it was measured 3 repeats. The recoveries were calculated according to the following formula;

$$\% \text{ Recovery} = \frac{(C1 - C2)}{C3} \times 100$$

Where

C1 = concentration determined in fortified sample.

C2 = concentration determined in unfortified sample.

C3 = concentration of fortification.

The recoveries data from this method were a range 91-97 %. These results were recommended to be performed at the 80-110% of label claim as The AOAC manual for the Peer Verified Methods program with estimated analyte concentration at ppm (part per million) level. Detail of results show as following;

		all – trans retinol		% recovery
		Area	Conc. (µg/mL)	
Pool serum only	1	248959	0.378	
	2	261664	0.397	
	3	257574	0.391	
	mean	**256066**	**0.389**	
	SD	**6485**	**0.010**	
	%CV	**2.53**	**2.53**	
Pool serum was added	1	414907	0.630	96.24
with 0.25 µg/mL all – trans	2	429163	0.651	104.89
retinol	3	404641	0.614	90.01
	mean	**416237**	**0.632**	**97.05**
	SD	**12315**	**0.019**	**7.47**
	%CV	**2.96**	**2.96**	**7.70**
Pool serum was added	1	552081	0.838	89.75
with 0.50 µg/mL all – trans	2	568427	0.863	94.71
retinol	3	550874	0.836	89.39
	mean	**557127**	**0.845**	**91.28**
	SD	**9804**	**0.015**	**2.98**
	%CV	**1.76**	**1.76**	**3.26**
Pool serum was added	1	915361	1.389	100.00
with 1.00µg/mL all – trans	2	854729	1.297	90.80
retinol	3	905964	1.375	98.58
	mean	**880347**	**1.336**	**96.46**
	SD	**32633**	**0.050**	**4.952**
	%CV	**3.71**	**3.71**	**5.13**

5.4 Precision

The precision was determined through the repeated analysis of the pool serum, both intra and inter assay precision. The coefficients of variation (%CV) were calculated by replicating extraction and analysis of these control serum pools. Intra- assay variation was determined regularly by analyzing the pool serum ten times on the same day. For inter-assay variation, two injections of the pool serum control per day were carried out on day. The results showed as following;

(1) Intra – batch precision

To determine all-trans retinol concentration in pool human serum on the same day, n = 10.

No.	Peak area		Correct conc. of retinol (µg/mL)
	all-trans retinol	retinol acetate	
	Area	Area	
1	246765	456809	0.550
2	256080	442425	0.580
3	255496	446739	0.580
4	253836	448930	0.570
5	253722	463345	0.550
6	247119	433516	0.580
7	262881	422497	0.630
8	266160	428222	0.630
9	255406	452732	0.570
10	256976	463584	0.560
mean	255444	445880	0.580
SD	5991	14214	0.03
%CV	2.35	3.19	4.87

(2) Inter-batch precision

To determine all-trans retinol concentration in pool human serum, two measurements of each analyte signal per day were carried out on 13 days.

No.	Pool serum + 75 µg/ml of retinol acetate				correct conc. of all-trans retinol (µg/mL)		
	all-trans retinol Peak area		retinol acetate Peak area				
	1	2	1	2	1	2	Average
1	266575	268605	419179	433711	0.629	0.612	0.621
2	251636	248383	447250	428139	0.584	0.566	0.575
3	275490	282656	459860	478574	0.592	0.584	0.588
4	271328	271380	461093	462430	0.602	0.600	0.601
5	271205	260734	442271	414814	0.604	0.619	0.612
6	257077	259671	437454	416153	0.588	0.631	0.610
7	262263	257851	442266	439242	0.587	0.581	0.584
8	245244	246184	395153	391946	0.625	0.633	0.629
9	233782	229475	412797	408591	0.565	0.560	0.563
10	295921	290610	512325	496708	0.576	0.584	0.580
11	247902	245332	401023	409053	0.618	0.600	0.609
12	316702	211295	447987	312262	0.707	0.677	0.692
13	263721	253278	403746	406916	0.644	0.613	0.629
mean	**266065**	**255804**	**437108**	**422965**	**0.609**	**0.605**	**0.607**
SD	**21820**	**21056**	**31771**	**45169**	**0.037**	**0.032**	**0.033**
%CV	**8.20**	**8.23**	**7.27**	**10.68**	**6.09**	**5.23**	**5.43**

6. Standard preparation for vitamin E analysis

Stock standard vitamin E (dl-α-tocopherol)

1.0 g of dl-α-tocopherol was dissolved in hexane/BHT and made up to 100 ml with hexane/BHT in volume metric flask. The actual concentration of stock standard was determined by evaporating 500 µL stock standard under N_2 gas, reconstituted with 50 mL (dilution 1:100) absolute ethanol and measured OD at wavelength of 294 nm ($\varepsilon_{1\ cm}^{1\%}$ = 71) by spectrophotometer. The actual concentration dl -α-tocopherol was calculated according to the following formula;

$$\text{Concentration (g/100 mL)} = \frac{\text{Abs} \times \text{dilution factor}}{\text{extinction coefficient}}$$

$$= \frac{0.736 \times 100}{71}$$

$$= 1.037 \text{ g/100 mL}$$

$$= 10.37 \text{ mg/mL}$$

Therefore, the actual concentration of stock dl-α-tocopherol was 10.37 mg/mL. The stock standard was stored at – 20 °C, covered with aluminum foil in order to protect them from light.

1.04 µg/mL dl-α-tocopherol intermediately concentration was prepared by preparing 2.5 mL of stock dl-α-tocopherol into 25 mL volumetric flask and made up to 25 mL with hexane.

Stock standard tocopherol acetate (Internal standard)

1.0 g of tocopherol acetated was dissolved in hexane and made up to 100 ml with hexane in volume metric flask. The actual concentration of stock standard was determined by evaporating 500 µL stock standard under N2 gas, reconstituted with 50 mL (dilution 1:100) absolute ethanol and measured OD at wavelength of 284 nm ($\varepsilon_{1\ cm}^{1\%}$ = 43.6) by spectrophotometer. The actual concentration dl-α-tocopherol was calculated according to the following formula;

$$\text{Concentration (g/100 mL)} = \frac{\text{Abs} \times \text{dilution factor}}{\text{extinction coefficient}}$$

$$= \frac{0.467 \times 100}{43.6}$$

$$= 1.071 \text{ g/100 mL}$$

$$= 10.71 \text{ mg/mL}$$

Thus, the actual concentration of stock tocopherol acetated was 10.71 mg/mL. The stock standard was stored at – 20 °C, covered with aluminum foil in order to protect them from light.

The intermediately concentration of 1.07 µg/mL tocopherol acetated was prepared daily by preparing 2.5 mL of stock tocopherol acetated into 25 mL volumetric flask and made up to 25 mL with hexane. The working concentration of 280 µg/mL of tocopherol acetated was prepared daily by preparing 2.617 mL of the

intermediately concentration of tocopherol acetated and made up to 10 mL with hexane.

7. Serum preparation for vitamin E analysis

Serum samples were extracted in duplicates. 200 µL of serum were added with 50 µL of 280 µg/ml tocopherol acetate in hexane, followed by 400 µL of absolute ethanol in order to precipitate protein. The mixture was vortexed vigorously for 5 minutes and added 200 µL of water. After that, the mixture was extracted with 500 µL of hexane by vigorously vortex for 10 minutes and centrifuged at 2,000 rpm at 20°C for 10 minutes. 400 µL of supernatant were transferred to another tube. The extraction was two repeated with each 300 µL of hexane. The second and third extractions were pooled together. 1.0 mL supernatants were evaporated to dryness under nitrogen gas and and the residue was reconstituted in 200 µL of mobile phase. Samples extracting under dimmed natural lighting, excluding fluorescent light at all times. 20 µL of the extract were injected into HPLC system.

8. Quantification of vitamin E in serum samples

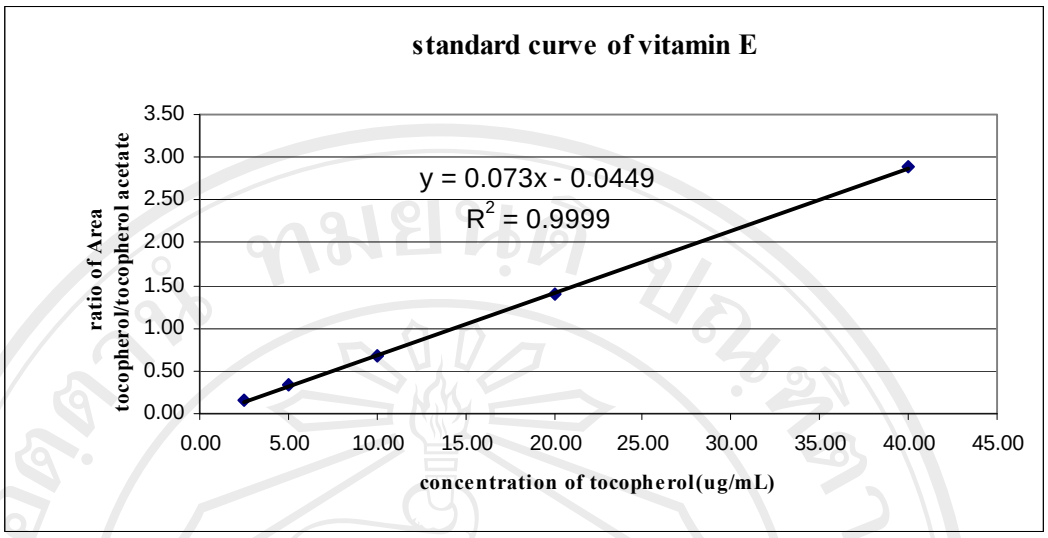
Sensitivity of the method

The limit of detection (LOQ) of this method was 0.90 µg/mL of α - tocopherol that the concentration could be determined with acceptable precision and accuracy under the stated experiment condition. The procedure was used for measuring LOQ

which prepared serial dilution of serum control. After serum controls were extracted and the residues were dissolved in mobile phase. They were double diluted by mobile phase until the diluted solution with give peak height at least equal or more than the baseline noise value plus 10SD. Finally, analyses 10 independent repeats of this solution and calculated mean and SD.

8.2 Linearity

Linearity range is the ability range of analytical method to determine α-tocopherol concentrations. This calibration curves were realized injecting standard in the range 2.5-40 μg/mL for α-tocopherol using tocopherol acetate as internal standard and measuring the ratio of the peak areas of α-tocopherol to area of tocopherol acetate plotting versus the concentration of each standard. The correlation coefficient was 0.9999. The standard curve was showed as following;



For this method, the working standard was selected one point for daily analysis which was mixtures of 10.0 µg/mL dl-α- tocopherol /70 µg/mL tocopherol acetate. The response factor (RF) value, the response ratio between the component and the internal standard, was calculated for determination of dl- α - tocopherol in unknown sample. The formula as followed;

$$RF(i) = \left\{ \frac{C(i)}{Area(i)} \right\} \left\{ \frac{Area(is)}{C(is)} \right\}$$

Where

RF (i) = The response factor for dl- α - tocopherol.

C (i) = The concentration of dl- α - tocopherol standard solution.

Area (i) = The peak area of dl- α - tocopherol standard solution.

Area (is) = The peak area of internal standard solution.

C (is) = The concentration of internal standard.

Analysis for dl- α - tocopherol in serum samples, formula as followed;

$$\text{Conc. (i)} = \left\{ \frac{\text{IS}}{\text{SA}} \right\} \left\{ \frac{(\text{RF i})(\text{Area s})}{(\text{RF is})(\text{Area is})} \right\} (\text{XF})$$

Where:

Conc. (i) = The concentration of dl- α - tocopherol in serum sample.

IS = The concentration of internal standard added to serum sample.

SA = The amount of sample material measured.

RF (i) = The response factor for dl- α - tocopherol determined by standard solution.

Area (s) = The peak area of dl- α - tocopherol in serum sample.

Area (is) = The peak area of internal standard in serum sample.

XF = A scaling factor (multiplier) that may be used as a conversion factor.

8.2.1 Preparation of working standard 10.0 µg/mL of α-tocopherol and 70.0 µg/mL of tocopherol acetate

240 µL of 1.04 µg/mL intermediately standard α-tocopherol were added to 6.25 mL of 280 µg/mL working standard of tocopherol acetate. After that they were evaporated under N_2 gas and were reconstituted with 25 mL of mobile phase and made up to 25 mL with mobile phase in volumetric flask. 20 µL of standard mixtures were injected into HPLC system.

The quality control employed to monitor the precision of RF value. The precision was determined through the repeated analysis of by using working standard 10.0µg/mL of α-tocopherol and 70 µg/mL of tocopherol acetate, both intra and inter assay precision. Intra- assay variation determined regularly by analyzing the standard mixtures twenty times on the same day, mean ± SD was 0.156 ± 0.003 (%CV = 1.73). For inter-assay variation, one measurement of the standard mixtures per day were carried out on 13 days, mean ± SD was 0.156 ± 0.003 (%CV = 1.73). The detail of results showed as following;

(1) Intra – batch precision of Response Factor (RF)

To determine mixtures standard solution of 10.0μg/mL dl-α-tocopherol/ 70 μg/mL tocopherol acetate on the same day, n = 20**.**

$$RF = \frac{\text{conc. of } \alpha\text{-tocopherol std.}}{\text{Area of } \alpha\text{-tocopherol std.}} \times \frac{\text{Area of } \alpha\text{-tocopherol acetate}}{\text{conc. of } \alpha\text{-tocopherol acetate}}$$

no.	Area		RF
	α -tocopherol	α -tocopherol acetate	
1	430634	478941	0.159
2	444801	474063	0.152
3	450374	482139	0.153
4	445919	482763	0.155
5	476780	521914	0.156
6	440729	477058	0.155
7	419819	455007	0.155
8	423123	444584	0.150
9	447613	488168	0.156
10	453850	495020	0.156
11	445179	483617	0.155
12	435966	488330	0.160
13	427528	481360	0.161
14	446334	483467	0.155
15	421744	460499	0.156
16	457322	502851	0.157
17	446378	484482	0.155
mean	**442005**	**481427**	**0.156**
SD	**14562**	**17624**	**0.003**
%CV	**3.29**	**3.66**	**1.73**

(2) Inter – batch precision of RF

To determine mixtures standard solution of 10.0μg/mL dl-α-tocopherol/ 70μg/mL tocopherol acetate, one measurement of mixtures standard per day were carried out on 17 days.

no.	Area		RF
	a-tocopherol	a-tocopherol acetate	
1	430634	478941	0.159
2	444801	474063	0.152
3	450374	482139	0.153
4	445919	482763	0.155
5	476780	521914	0.156
6	440729	477058	0.155
7	419819	455007	0.155
8	423123	444584	0.150
9	447613	488168	0.156
10	453850	495020	0.156
11	445179	483617	0.155
12	435966	488330	0.160
13	427528	481360	0.161
14	446334	483467	0.155
15	421744	460499	0.156
16	457322	502851	0.157
17	446378	484482	0.155
mean	**442005**	**481427**	**0.156**
SD	**14562**	**17624**	**0.003**
%CV	**3.29**	**3.66**	**1.73**

8.3 Accuracy

The quality control of this method employed to monitor accuracy by using pool serum control, in-house control. The procedure was used for measuring of recovery that the serum controls were unfortified and fortified with the standard concentration at a range of standard curve (added 2.5 µg/mL, 5.0 µg/mL and 10.0 µg/mL of standard α - tocopherol). At each level, it was measured 3 repeats. The recoveries were calculated according to the following formula;

$$\% \text{ Recovery} = \frac{(C1 - C2)}{C3} \times 100$$

Where

C1 = concentration determined in fortified sample.

C2 = concentration determined in unfortified sample.

C3 = concentration of fortification.

The recovery in this method was a range 97-101 %. These results were recommended to be performed at the 80-110% of label claim as The AOAC manual for the Peer Verified Methods program with estimated analyte concentration at part per million (ppm) unit. Detail of results showed as following;

		dl- α-tocopherol		% recovery
		Area	Conc. (µg/mL)	
Pool serum only	1	318136	7.454	
	2	317305	7.434	
	3	330626	7.746	
	mean	**322022**	7.545	
	SD	**6093**	**0.143**	
	%CV	**1.89**	**1.89**	
Pool serum was added	1	443182	10.28	109.41
with 2.5 µg/mLα-tocopherol	2	443214	10.29	109.60
	3	418773	9.81	90.68
	mean	**435056**	**10.13**	**103.23**
	SD	**14102**	**0.272**	
	%CV	**3.24**	**2.68**	
Pool serum was added	1	524236	12.28	94.76
with 5.0 µg/mL α-tocopherol	2	540828	12.67	102.52
	3	544911	12.77	104.44
	mean	**536658**	**12.57**	**100.57**
	SD	**10950**	**0.26**	
	%CV	**2.04**	**2.04**	
Pool serum was added	1	725477	17.00	94.52
with 10.00 µg/mL α-tocopherol	2	749045	17.55	100.05
	3	735036	17.22	96.76
	mean	**742041**	**17.26**	**97.11**
	SD	**11854**	**0.278**	
	%CV	**1.60**	**1.61**	

8.4 Precision

The precision was determined through the repeated analysis of the pool serum, both intra and inter assay precision. The coefficients of variation (%CV) were calculated by replicating extraction and analysis of these control serum pools. Intra-assay variation was determined regularly by analyzing the pool serum ten times on the same day. For inter-assay variation, two injections of the pool serum control in each assay. The results showed as following;

8.4.1 Intra-batch precision

To determine α - tocopherol concentration in pool human serum on the same day, n = 10.

No.	Pool serum + 70 ug/ml tocopherol acetate		correct conc.of α-tocopherol (µg/mL)
	α -tocopherol	tocopherol acetate	
	Peak area	Peak area	
1	375264	436916	9.584
2	354717	442170	8.951
3	351053	414799	9.443
4	386250	435567	9.895
5	337630	446239	8.442
6	351843	441449	8.893
7	349350	435909	8.942
8	332301	425586	8.712
9	381229	466680	9.115
10	371515	448421	9.244
mean	**357321**	**439374**	**9.12**
SD	**18585**	**14606**	**0.43**
%CV	**5.20**	**3.32**	**4.73**

8.4.2 Inter-batch precision

To determine α - tocopherol concentration in pool human serum, two measurements of each analyte signal per day were carried out on 17 day.

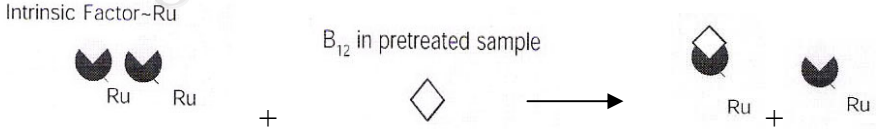
No.	Pool serum + 70 ug/ml of tocopherol acetate				correct conc. of alpha-tocopherol (µg/mL)		
	dl- α -tocopherol Peak area		tocopherol acetate Peak area				
	1	2	1	2	1	2	Average
1	359851	374361	443451	456947	9.025	9.112	9.069
2	360616	418662	433145	495438	8.913	9.046	8.980
3	338507	317105	435152	409790	8.328	8.284	8.306
4	348019	346704	421563	426371	8.938	8.803	8.871
5	342384	355455	458929	472063	8.147	8.243	8.195
6	447982	446229	530771	500303	9.136	9.654	9.395
7	364488	348887	449234	451478	8.794	8.375	8.585
8	344900	349641	435744	431550	8.317	8.513	8.415
9	346681	372195	418985	455466	9.024	8.912	8.968
10	320030	345180	364852	381429	9.567	9.871	9.719
11	357262	355178	418371	418124	9.277	9.228	9.253
12	356702	379628	403830	434055	9.894	9.797	9.846
13	340757	347236	407072	407902	9.428	9.588	9.508
14	352293	344053	402930	402777	9.471	9.253	9.362
15	367290	367817	459412	444392	8.752	9.061	8.907
16	350385	351192	454725	460236	8.473	8.390	8.432
17	324342	339469	393399	428646	8.922	8.570	8.746
mean	**354264**	**362294**	**431269**	**439822**	**8.965**	**8.982**	**8.974**
SD	**27370**	**30635**	**36156**	**32097**	**0.477**	**0.537**	**0.493**
%CV	**7.73**	**8.46**	**8.38**	**7.30**	**5.32**	**5.98**	**5.50**

APPENDIX H

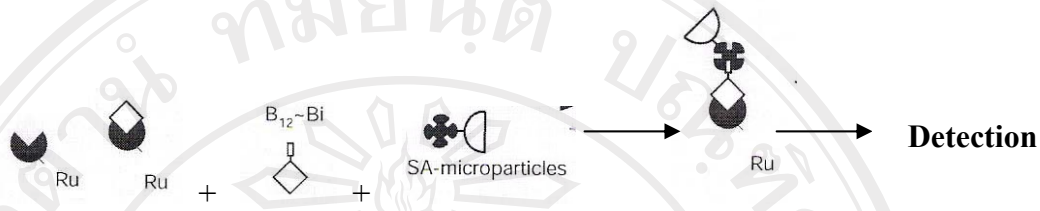
Principle of Elecsys® Vitamin B12 Immunoassay

Elecsys® Vitamin B12 Immunoassay is electrochemiluminescence immunoassay principle which employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 in the sample competes with added vitamin B12 labeled with biotin for binding sites on the ruthenium-labeled intrinsic-factor complex. Intrinsic factor is a glycoprotein and it has an important role in the absorption of vitamin B12 (cobalamin) in the intestine. Diagram of test principle is shown as following;

The first step, the sample is incubated with the reagents, releasing the bound vitamin B12 from endogenous intrinsic factor (IF).



The second step, the pretreated sample is incubated with ruthenium labeled IF, and vitamin B12- binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.



The third step, streptavidin-coated microparticles and vitamin B12 labeled with biotin are added, and the still-vacant sites of ruthenium labeled IF become occupied, with formation of a ruthenium labeled IF-vitamin B12 biotin complex.

Figure Diagram of Elecsys ® Vitamin B12 Immunoassay principle

The electrochemiluminescent label is a ruthenium complex [$Ru(bpy)^{2+}_3$]. In the electrochemical reaction $Ru(bpy)^{2+}_3$ is first oxidized at the electrode surface to give $Ru(bpy)^{3+}_3$. At the same time, tripropylamine (TPA, component of Procell), which is present in excess, is oxidized to a radical cation $TPA^{+\bullet}$, which spontaneously lose a proton. The strong oxidant $Ru(bpy)^{3+}_3$ reacts with the free radical $TPA^{\bullet}$ (a powerful reductant), the complex $Ru(bpy)^{2+}_3$ is produced in an electronically excited state. This returns to the ground state by emitting a photon at 620 nm, and is then again available for new light-generating reaction cycle (Figure 2).After that a photomultiplier measures the signal intensity in RLU [Relative Light Units].

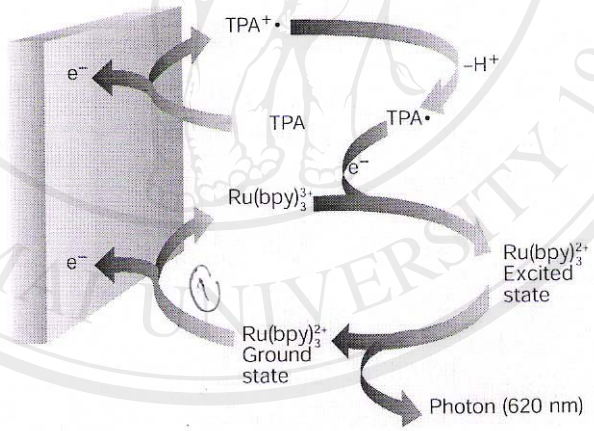


Figure Electrochemiluminescence detection; Reaction Scheme

APPENDIX I

Determination of serum vitamin B12 by Elecsys® vitamin B12 Immunoassay

1. Apparatus and reagents

- **Apparatus**
  - The Boehringer Mannheim Elecsys 2010 Immunoassay analyzer
  - Elecsys vials
  - Auto pipette, 200 μL

- **Reagents**
  - **Elecsys vitamin B12 reagent kit**

    PT1 = Pretreatment 1, dithiothreitol

    PT2 = Pretreatment 2, sodium hydroxide;sodium cyanide

    M = Streptavidin-coated micropaticles

    R1 = Instrinsic factor ~Ru(bpy)32+

    R2 = Vitamin B12 ~biotin

  - **Calibrator**

    Vitamin B12 in two concentration ranges: approximately 100 pg/mL (Cal 1) and 1500 pg/mL (Cal2) in human serum albumin matrix with sodium cyanide.

- **PreciControl Universal 1 and 2 controls consist of lyophillized human sera**
- **System reagent;** Elecsys Procell; Phosphate buffer, tripropylamine(TPA), Elecsys Cleancell; KOH and detergent

2. The performance of the assay

- Performed the calibration curve from measurement of the two calibator (Cal 1 and Cal2). Information encoded in the bar code label and on the bar code card is scanned into the analyzer automatically.
- Transferred the reconstituted PreciControl Universal 1 and 2 and 500 µL of serum samples into Elecsys vials. Avoid the formation of foam.
- Placed the vials of the PreciControl Universal 1 and 2 on first and second channel and followed sample vials on the reaction vessels of the analyzer, respectively and every tenth sample was prepared and analyzed a second time.
- Run system automatically.
- Print out data.

3. Quality controls for serum vitamin B12 analysis

The quality control of this method employed to monitor accuracy and precision by PreciControl Universal 1 lot 165476, mean was 1,010 pg/mL (range 707-1,313 pg/mL) and PreciControl Universal 2 lot 165474, mean was 492pg/mL (range 300-684 pg/mL). The precision was determined through the repeated analysis of PreciControl Universal 1 and 2, both intra and inter assay and calculating the %CV.

Intra- assay variation was determined regularly by analyzing each the controls ten times on the same day. For inter-assay variation, the PreciControl Universal 1 and 2 were analyzed in each assay. The results showed as followed;

1. Intra-batch precision for serum vitamin B12 analysis

This method employed to monitor accuracy and precision by PreciControl Universal 1 lot 165476, mean was 1,010 pg/mL (range 707-1,313 pg/mL) and PreciControl Universal 2 lot 165474, mean was 492pg/mL (range 300-684 pg/mL**)**

❖ **PreciControl Universal 1**

No.	PreciControl Universal 1 (pg/mL)	PreciControl Universal 2 (pg/mL)
1	1,014	491.9
2	1,003	503.3
3	1,028	484.4
4	1,026	473.5
5	1,038	499.0
6	1,020	507.9
7	1,017	494.4
8	1,004	490.8
9	1,041	481.5
10	1,028	516.5
Mean	**1,021.9**	**494.32**
SD	**12.84**	**12.88**
% CV	**1.25**	**2.60**

2. Inter-batch precision for serum vitamin B12 analysis.

No.	PreciControl Universal 1 (pg/mL)	PreciControl Universal 2 (pg/mL)
1	960.5	453.2
2	980.7	413.1
3	1030	584.4
4	1088	554.9
5	887.1	462.7
6	967.3	480.1
Mean	**985.6**	**491.4**
SD	**68.0**	**65.2**
% CV	**6.9**	**13.26**

APPENDIX J

Principle of Atomic Absorption Spectroscopy (AAS)

Atomic absorption (AA) spectroscopy in analytical chemistry is a technique for determining the concentration of a particular metal element within a sample. The technique uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. Three steps are involved in turning a liquid sample into an atomic gas. Firstly, the liquid solvent is evaporated, and the dry sample remains. Secondly, vaporization, the solid sample vaporizes to a gas. Finally, volatilizations, the compounds making up the sample are broken into free atoms. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption. The components of AA spectrometer instrumental system are shown in the diagram as follow;

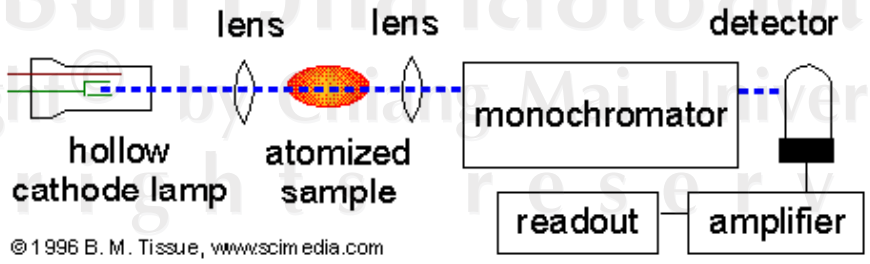


Figure AA- spectrometer diagram

Light source

The light source is usually a hollow-cathode lamp of the element that is being measured. Hollow-cathode lamps are a type of discharge lamp that produces narrow emission from atomic species. They get their name from the cup-shaped cathode, which is made from the element of interest. The electric discharge ionizes rare gas atoms, which are accelerated into the cathode and sputter metal atoms into the gas phase. Collisions with gas atoms or electrons excite the metal atoms to higher energy levels, which decay to lower levels by emitting light.

Atomizer

AA spectroscopy requires that the analyte atoms be in the gas phase. Ions or atoms in a sample must undergo desolation and vaporization in a high-temperature source such as a flame or graphite furnace. Flame-AA can only analyze solutions, while graphite furnace-AA can accept solutions, slurries, or solid samples.

Flame-AA uses a slot type burner to increase the path length, and therefore to increase the total absorbance (see Beer-Lambert law). Sample solutions are usually aspirated with the gas flow into a nebulizing/mixing chamber to form small droplets before entering the flame.

The graphite furnace has several advantages over a flame. It is a much more efficient atomizer than a flame and it can directly accept very small absolute quantities of sample. It also provides a reducing environment for easily oxidized elements. Samples are placed directly in the graphite furnace and the furnace is electrically heated in several steps to dry the sample, ash organic matter, and vaporize the analyte atoms.

Light separation and detection

AA spectrometers use monochromators and detectors for UV and visible light. The main purpose of the monochromator is to isolate the absorption line from background light due to interferences. Simple dedicated AA instruments often replace the monochromator with a band pass interference filter. Photomultiplier tubes are the most common detectors for AA spectroscopy.

APPENDIX K

Determination of serum zinc by Flame – AAS

1. Apparatus and Reagents

- **Apparatus**

  - Perkin Elmer, AA-3100 Atomic absorption spectrometer

    Air - C_2H_2 Flame

- **Reagents**

  - Zinc standard solution 1000 mg/L, Merck.
  - 20% w/v Trichlroacetic acid (TCA), Sigma.
  - 10% W/V Trichlroacetic acid (TCA), Sigma.
  - Lyphochek® Assay Chemistry Control, Level 1, lot 14131, Bio-rad.

2. Standard preparation for zinc analysis

Zinc standards of 0.10, 0.25, 0.50 and 1.0 μg/mL were used to establish the calibration by diluting stock standard solution of zinc (Commercial product of 1,000 mg/L zinc solution) before measurement. The procedure was used for diluting as followed; first, prepared 10 μg/mL intermediate by diluting 1.0 ml of stock standard

solution of zinc in100 mL of demineralized water. The working standard for a calibration curve was prepared as follows;

Working standard Conc. (µg/mL)	10 mg/L Intermidiate standard (mL)	10% TCA (mL)	Total Volume (mL)
0.000	0.000	10.00	10.00
0.100	0.100	9.90	10.00
0.250	0.250	9.75	10.00
0.500	0.500	9.50	10.00
1.000	1.000	9.00	10.00
QC, 0.500	0.500	9.50	10.00

3. Serum preparation for zinc analysis

Serum samples were extracted and analyzed in duplicates. 500 µL of serum were added to 500 µL of 20% w/v trichlroacetic acid (TCA) and then the mixtures were mixed and incubated at 90°C for 15 minutes. After that the mixtures were centrifuged at 3000 rpm at 4°C for 20 minutes and transferred the supernatants to clean test tube and analyses in flame-AAS system.

4. Parameter of flame - AAS system for serum zinc analysis

Wavelength	217.9 nm
Slit	0.7 high
Sensitivity check	0.5 mg/L
Integration time	2.0 sec

5. Quality controls for serum zinc analysis

Accuracy and precision was monitored by using Lyphochek® Assay Chemistry Control, the mean was 0.73 µg/mL, range 0.58 - 0.87µg/mL. The precision was determined through the repeated analysis of Lyphochek® Assay Chemistry Control, both intra and inter assay and calculating the %CV. Intra-assay variation was determined regularly by analyzing the Lyphochek® Assay Chemistry Control twenty times on the same day. For inter-assay variation, duplicates extraction of the Lyphochek®. The results show as following;

Intra- and Inter batch precision for serum zinc analysis by flame-AAS

No.	Zinc concentration (µg/mL)	
	Intra-batch	**Inter-batch**
1	0.79	0.86
2	0.82	0.83
3	0.82	0.86
4	0.81	0.82
5	0.79	0.89
6	0.82	0.87
7	0.82	0.83
8	0.86	0.88
9	0.80	0.85
10	0.80	0.87
11	0.80	0.92
12	0.77	0.87
13	0.83	0.85
14	0.87	0.85
15	0.80	0.81
16	0.88	-
17	0.87	-
18	0.82	-
19	0.86	-
20	0.84	-
mean	**0.82**	**0.86**
SD	**0.03**	**0.028**
%CV	**3.66**	**3.26**

Remarks; the precision of this method was monitor by using Lyphochek® Assay Chemistry Control, the mean was 0.73 µg/mL, range 0.58-0.87 µg/mL.

APPENDIX L

Determination of serum selenium by GF-AAS

1. Apparatus and reagents

- **Apparatus**

  - Hitachi-Z-8200, Polarized Zeeman Atomic absorption spectrometer with the tube type pyrocuvette
  - Vials
  - Volumetric flask, 100 mL
  - Beaker
  - Auto pipette 20 mL, 100 mL, 1000 mL
  - Microcentrifuge tube

- **Reagents**

  - Selenium standard solution 1000 mg/L, Merck
  - 500 ug/mL palladium nitrate for die graphitoten -AAS, Merck
  - 1 mg/mL albumin solution
  - Seronorm™ Trace elements serum, Level 1, lot MI0181

2. Standard preparation for selenium analysis

This method used a standard addition method which was carried out on serum sample to avoid incorrect measurements caused by matrix effects. Standard solutions for a calibration curve were prepared by diluting stock standard solution (1,000 mg/L).The procedure was used for diluting as follows;

Step 1; Preparation selenium concentration of 100.0 mg/L (Solution A).

mL of stock standard solution of selenium were added into volumetric flask and made up to 10 mL with 5% HNO_3.

Step 2; Preparation of selenium concentration of 10.0 mg/L (Solution B)**.**

1.0 mL of solution A were added into volumetric flask and made up to 10 mL with 5% HNO_3.

Step 3; Preparation of selenium concentration of 1.0 mg/L (Solution C)**.**

1.0 mL of solution B were added into volumetric flask and made up to 10 mL with 5% HNO_3.

Step 4; Preparation of working standard for a calibration curve as followed;

Working standard. Conc. (µg/L)	Se 1.0 mg/L (Solution C) (µl)	Solution mixture (µl)
0.00	0.00	1000
20.00	20.00	980
40.00	40.00	960
60.00	60.00	940

Remarks: solution mixture;

500 µg/mL of palladium nitrate: 0.1% albumin: seronorm (1:2:1)

3. Serum preparation for selenium analysis

After the samples had reached room temperature, they were homogenized for 30 seconds. 250 µL of the serum sample was diluted with 0.1% albumin and 500 µg/mL of palladium nitrate as showed as follows;

Sample	**Volume (µL)**
Serum sample	250
0.1% albumin	500
500 µg/mL of palladium nitrate	250
Total	**1000**

For analysis, each sample was duplicating injected into GF-AAS system and samples injection volume was 20 µL.

4. Parameter of GF - AAS system for serum selenium analysis

Wavelength	196.0	nm
PMT voltage	670	V
Slit Width	1.30	nm
Lamp Current	16.0	mA
Injection Volume	20	ul
Temp. Control	Optimal	
Time Constant	0.05 s.	
Measurement Mode	Simple STD Add	
Signal Mode	BKG Correct	
Curve Order/Renewal	1st/Renewal	
STD Replicates	2	
Sample Replicates	2	
Calculation	Peak Height	
STD Conc. Unit	ug/L	
Sample Conc.	Cup Position	
STD 1: 0.00	1	
STD 2: 20.00	31	
STD 3: 40.00	32	
STD 4: 60.00	33	

Temperature program:

Stage	Temp. (°C)		Time(s)		Gas Flow (mL/min)
	start	end	ramp	hold	
Dry	50	105	80		200
Dry	105	250	20		200
Ash	600	1400	30		200
Ash	1400	1400	10		200
Atom	2900	2900		4	
Clean	3000	3000		4	200
Cool				10	200

5. Quality controls for serum selenium analysis

Accuracy and precision of this method was monitor by using Seronorm™ Trace elements serum, mean was 81 µg/L (range 78-84 µg/L). The precision was determined through the inter assay precision of Seronorm™ Trace elements and calculating the %CV. Inter-assay variation, injection of the Seronorm™ Trace elements in each assay were made with a total of twenty - one assays (7 days), every tenth sample was analyzed to inserted with the control serum. The results show as following;

Inter batch precision for selenium analysis by GF –AAS

No.	Conc. from curve (µg/L)			Dilution factor	Correct conc. (µg/L)
	1	2	mean		
1	19.11	20.56	19.84	4	79.34
2	19.73	20.73	20.23	4	80.92
3	21.07	18.46	19.77	4	79.06
4	24.16	15.93	20.05	4	80.18
5	19.80	19.43	19.62	4	78.46
6	19.52	21.99	20.76	4	83.02
7	19.1	21.15	20.13	4	80.50
8	21.28	20.09	20.69	4	82.74
9	19.25	20.97	20.11	4	80.44
10	19.34	20.65	20.00	4	79.98
11	19.3	20.42	19.86	4	79.44
12	20.88	20.95	20.92	4	83.66
13	20.68	19.01	19.85	4	79.38
14	18.01	21.5	19.76	4	79.02
15	19.45	19.65	19.55	4	78.20
16	19.89	19.27	19.58	4	78.32
17	17.78	21.62	19.70	4	78.80
18	18.97	22.8	20.89	4	83.54
19	18.65	23.05	20.85	4	83.40
20	22.97	16.34	19.66	4	78.62
21	19.8	20.99	20.40	4	81.58
mean					**80.41**
SD					**1.86**
%CV					**2.31**

Remark; Seronorm™ Trace Element Serum, Level 1, Lot MI 0181;
mean = 81 ug/L; range 78 - 84 ug/L (lower level - upper level).

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