

Appendix

Chemical substance of electrophoresis preparation

1. Extraction buffer

Tris-buffer 0.2 M pH 8.4 100 ml.

Preparation method

Stock A : 0.2 M Tris(hydroxymethyl) aminomethane (tris 2.42 g. in 100 ml. of water)

Stock B : 0.2 M HCl (1.7ml. of HCl 37% concentration in 100 ml. of filter water)

Extraction buffer was mixed together between 50 ml. of stock A and 16.5 ml. of stock B in 133.5 ml. of distil water total volume was 200 ml. and adjust pH was 8.4 by 0.2 M HCl 1N. NaOH, after that put this solution in dark brown color bottle and kept in refrigerator

2. Gel composite

2.1 Acrylamide / Bis (30% T, 2.67% C)

Preparation

A: 29.2 g of Acrylamide in 50 ml. of distil water

B: N, N- Methylene – bis acrylamide 0.8 g.

Mixed together between A and B in refrigerator

2.2 Tris – HCl (1M) and pH 8.8

Preparation

A: Tris – HCl (1M) (18.15 g. of Tris in 100 ml. of distil water)

B: 1M HCl (8.35ml of HCl (37%) in 100ml. of water

Mixed together between 50 ml. of A and 8 ml. of B and adjust pH was 8.8 by 1.0M HCl put this solution in dark brown color bottle and kept in refrigerator

3. Electrode buffer (pH 8.3)

Tris 3 g

Glycine 14.4 g

Preparation

Mixed together in 500 ml. of distil water

4. Plant sample preparation

4.1 The leaf was clean and put in plastic bag kept in low temperature

4.2 Freeze mortar

4.3 The weight of leaf sample was 1g

4.4 The leaf was fine grain add 0.05 g. of PVPP(Polyvinyl – polypyrrolidon) /sample

4.5 Solution of leaf was contained in close plastic tube for centrifuge and kept it in low temperature

Centrifuge solution of leaf

- Turn on centrifuge machine 30 minute and set temperature 2C with 3000 rpm.
- After put solution of leaf in centrifuge machine set 14,000 rpm at 2C for 30 minute

- Separate transparent solution portion on sediment by suction method be careful contact sediment, and kept in low temperature (don't shake)

5. 7.5% gel preparation method (2 gel)

- 5.1 Acrylamide 5 ml
- 5.2 Distil water 9.7ml.
- 5.3 Tris – HCl pH 8.8 (1M) 5ml
- 5.4 10% ammonium persulfate 200 ml.(0.1/1ml. of water)
- 5.5 TEMED 10 μ l

6. Extract sample + Marker dye.

- 6.1 Marker dye 10 μ l
 - 6.2 Extract sample 90 μ l
- Two solution mixed together and kept in low temperature

7. Enzyme dyestuffs preparation

7.1 Peroxidase

A: 0.042g of 3-amino-9-ethylcabazole was dissolve by acetone 10 ml (dark condition)

B: 0.29g. of β - naphthol was dissolve by acetone 10 ml.(dark condition)

C: Tris- buffer 0.1 M pH 4.0 80 ml.

D:H₂O₂ 3% 100 ml.

Preparation method: A+B+C mixed together and mixed with D when dye gel.(dark condition)

7.2 Esterase

A: phosphate – buffer pH 6.0 100 ml.

B: Fast blue β -salt 0.15 g.

C: naphthyl acetate 0.003g. dissolve in 3ml. of absolute ethanol

Preparation method: A+B mixed together after that filed it before dye 30 minute.(dark condition)

7.3 Acid-phosphatase

A: Acetate buffer pH 5.0 5 ml.

B:Fast ganet GBC disodium salt 50 ml.

C: Disodium naphthyl pospate 25 mg.

Preparation method: A+B+C mixed together and filed it in dark condition, maculate gel in this solution (2-12 hr. in dark condition)

8. Fixing solution

After dye gel until brand appear and clean gel with water after that fixing brand by fixing solution (10% glycerin+7% acetic acid)



สถานบริการวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยเชียงใหม่ (สวท-มช.)
 ชั้น 7 อาคาร 30 ปี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ เชียงใหม่ 50200
 โทรศัพท์ : 053-943397, 053-941971 โทรสาร : 053-892275 E-mail : stsc@science.cmu.ac.th

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 7th Floor, 30th year Science Building, Faculty of Science, Chiang Mai University, Chiang Mai 50200

รายงานผลการวิเคราะห์

เลขที่รับงาน : 003/006

วันที่รายงานผล : 25 เมษายน 2549

วันที่รับตัวอย่าง : 3 มีนาคม 2549

ตัวอย่าง : สารสกัดจากหางไหล

ชื่อของลูกค้า/หน่วยงาน : นายอรุณ โสติกกุล

ที่อยู่ : สถาบันวิจัยและฝึกอบรมการเกษตร ลำปาง

โทรศัพท์ : 04-6081039

โทรสาร : 054-342550

ผลการวิเคราะห์ตัวอย่างดังกล่าวเอกสารแนบ

ผลการตรวจสอบ/วิเคราะห์ตามเอกสารข้างต้นนี้ รับรองเฉพาะตัวอย่างที่ได้ตรวจวิเคราะห์
 เท่านั้น ไม่รับรองวัตถุหรือสินค้าที่ใช้เครื่องหมายเดียวกับตัวอย่างนี้ และห้ามใช้รายงานฉบับนี้ในการ
 ประกาศ หรือย่อตัดทอน

อนุมัติผลโดย

Handwritten signature

(รองศาสตราจารย์ ดร.นวลศรี รักอริยะธรรม)

ผู้อำนวยการ

สถานบริการวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยเชียงใหม่

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ผลการวิเคราะห์ตัวอย่างโดยใช้ GC-MS

เลขที่เอกสารจาก สวท-มช สร 0515(28)/0137 ลงวันที่ 6 มีนาคม 2549

จำนวนตัวอย่าง : 2 (003/006)

1. ข้อมูลของตัวอย่าง ประกอบด้วย

1.1 TIC (Total Ion Chromatogram)

1.2 Area Percent Report

1.3 Summary Library Search Report

1.3 ข้อมูลแมสสเปกตรัมของแต่ละพีคและการเปรียบเทียบกับแมสสเปกตรัมมาตรฐาน

2. เครื่องมือ/สภาวะในการวิเคราะห์

2.1 GC 6890 Agilent Technologies

Inlet : 270 °C

ปริมาณในการฉีด 0.5 µL split ratio 40 : 1

Oven : 80 °C---10 °C/min ---> 260 °C(42 min)

Carrier : Helium 1.0 ml/min

Column : HP-5MS 30 m x 0.25 mm ID x 0.25 µm film thickness

2.2 MSD 5973(EI) Hewlett Packard

MS Quadrupole : 150 °C

MS Source : 230 °C

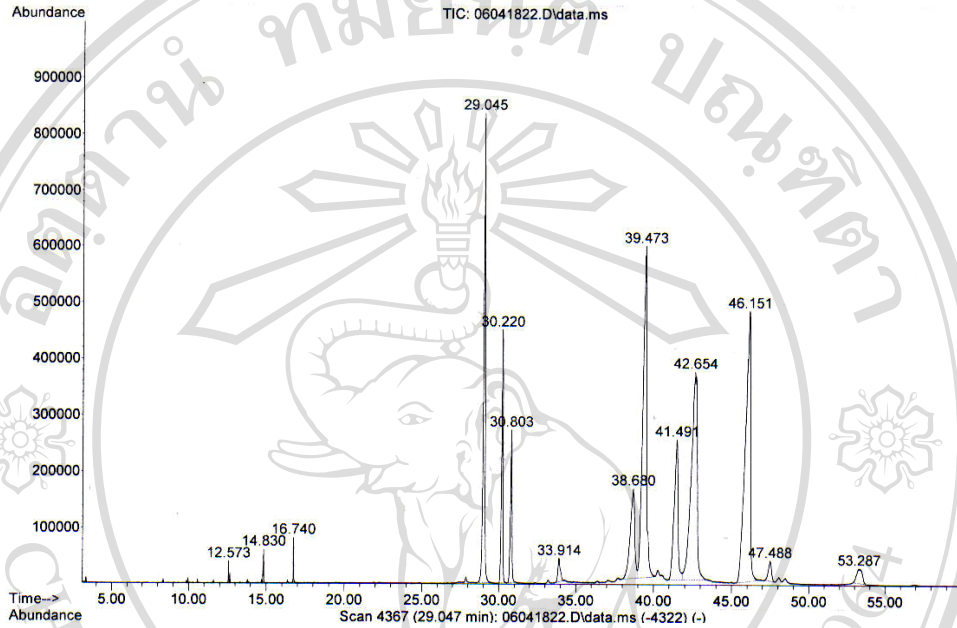
3. วันส่งผลการวิเคราะห์ถึง สวท-มช : 25 เมษายน 2549

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File : C:\msdchem\1\DATA\PK06\06041822.D
 Operator : Pisan
 Acquired : 18 Apr 2006 13:02 using AcqMethod S0030062.M
 Instrument : Instrument #1
 Sample Name: STSC 003/006 W
 Misc Info :
 Vial Number: 2



Chemical components in sediment powder of derris root extracted by water

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Library Search Report

Data Path : C:\msdchem\1\DATA\PK06\
 Data File : 06041822.D
 Acq On : 18 Apr 2006 13:02
 Operator : Pisan
 Sample : STSC 003/006 W
 Misc :
 ALS Vial : 2 Sample Multiplier: 1

Search Libraries: C:\Database\wiley7n.1 Minimum Quality: 60
 C:\Database\NIST05.L Minimum Quality: 0

Unknown Spectrum: Apex
 Integration Events: Chemstation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	12.570	0.12	C:\Database\wiley7n.1 Oxacyclopentadec-6-en-2-one, (Z)- (CAS) \$\$ (Z)-5-TETRADECEN-14-OLID	151483	063958-52-1	92
			Oxacyclopentadec-6-en-2-one, (Z)- 1H-Indene, octahydro-, cis- \$\$ cis-Hexahydroindan	151484 21521	063958-52-1 004551-51-3	92 64
2	14.830	0.28	unknown			
3	16.739	0.30	unknown			
4	29.047	11.55	unknown			
5	30.218	5.63	unknown			
6	30.801	3.59	unknown			
7	33.916	0.86	unknown			
8	38.679	6.20	unknown			
9	39.476	19.80	C:\Database\wiley7n.1 Rotenone \$\$ [1]Benzopyrano[3,4-b]f uro[2,3-h][1]benzopyran-6(6aH)-one , 1,2,12,12a-tetrahydro-8,9-dimeth oxy-2-(1-methylethenyl)-, [2R-(2.a lpha., 6a.alpha., 12a.alpha.)]- \$\$ C ube-Pulver \$\$ Dactinol \$\$ Deril \$\$ Derrin \$\$ Derris \$\$ Derris (insec ticide) \$\$ Dri-ki Rotenone Deguelin	328974 328973 328971	000083-79-4 000083-79-4 000522-17-8	98 87 78
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11	42.651	19.12	C:\Database\wiley7n.1 3.ALPHA., 5-CYCLO-ERGOSTA-7,22-DIEN -6-ONE \$\$ 3,5-Cycloergosta-7,22-di en-6-one, (3.beta., 5.alpha., 22E)- (CAS) \$\$ 3.alpha., 5-Cyclo-5.alpha. -ergosta-7,22-dien-6-one (CAS) \$\$ Cyclopenta[a]cyclopropa[2,3]cyclop enta[1,2-f]naphthalene, 3,5-cyclo ergosta-7,22-dien-	329244	003037-46-5	91

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 uro[2,3-h][1]benzopyran-6(6aH)-one
 , 1,2,12,12a-tetrahydro-8,9-dimeth
 oxy-2-(1-methylethenyl)-, [2R-(2.a
 lpha.,6a.alpha.,12a.alpha.)]- \$\$ C
 ube-Pulver \$\$ Dactinol \$\$ Deril \$\$
 Derrin \$\$ Derris \$\$ Derris (insec
 ticide) \$\$ Dri-ki
 Isorotenone 328976 000083-79-4 90
 Rotenone \$\$ [1]Benzopyrano[3,4-b]f 328977 000083-79-4 87
 uro[2,3-h][1]benzopyran-6(6aH)-one
 , 1,2,12,12a-tetrahydro-8,9-dimeth
 oxy-2-(1-methylethenyl)-, [2R-(2.a
 lpha.,6a.alpha.,12a.alpha.)]- (CAS
) \$\$ Deril \$\$ Ronone \$\$ Derris \$\$
 Derrin \$\$ Rotenon \$\$ Noxfish \$\$ Da
 ctinol \$\$ Rotocid

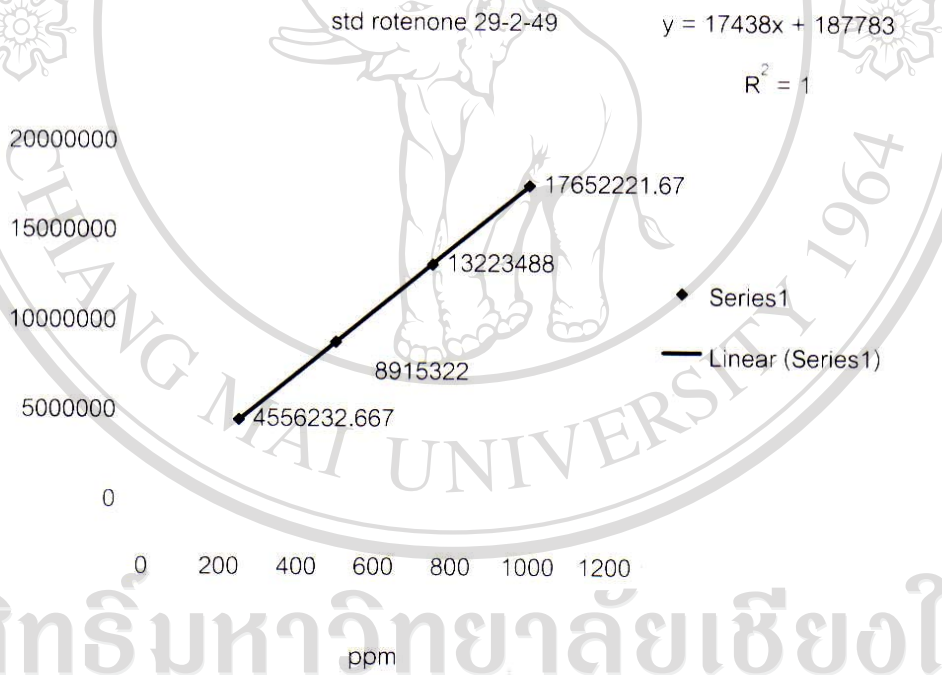
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 -7(7aH)-one, 13,13a-dihydro-9,10-d
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]benzopyrano[3,4-
 Rotenone \$\$ [1]Benzopyrano[3,4-b]f 328977 000083-79-4 76
 uro[2,3-h][1]benzopyran-6(6aH)-one
 , 1,2,12,12a-tetrahydro-8,9-dimeth
 oxy-2-(1-methylethenyl)-, [2R-(2.a
 lpha.,6a.alpha.,12a.alpha.)]- (CAS
) \$\$ Deril \$\$ Ronone \$\$ Derris \$\$
 Derrin \$\$ Rotenon \$\$ Noxfish \$\$ Da
 ctinol \$\$ Rotocid

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 Isorotenone 328976 000083-79-4 91
 Rotenone \$\$ [1]Benzopyrano[3,4-b]f 328974 000083-79-4 90
 uro[2,3-h][1]benzopyran-6(6aH)-one
 , 1,2,12,12a-tetrahydro-8,9-dimeth
 oxy-2-(1-methylethenyl)-, [2R-(2.a
 lpha.,6a.alpha.,12a.alpha.)]- \$\$ C
 ube-Pulver \$\$ Dactinol \$\$ Deril \$\$
 Derrin \$\$ Derris \$\$ Derris (insec
 ticide) \$\$ Dri-ki

Relation of peak area and standard rotenone concentration

std rotenone 230249

conc.	Peak area1	Peak area2	Peak area3	average
50	1282760	1315023	1216919	1271567.3
150	1841974	1773243	1780642	1798619.7
250	4461647	4575230	4631821	4556232.7
500	8863458	8865347	9017161	8915322
750	13080353	13275010	13315101	13223488
1000	17498814	17714854	17742997	17652222



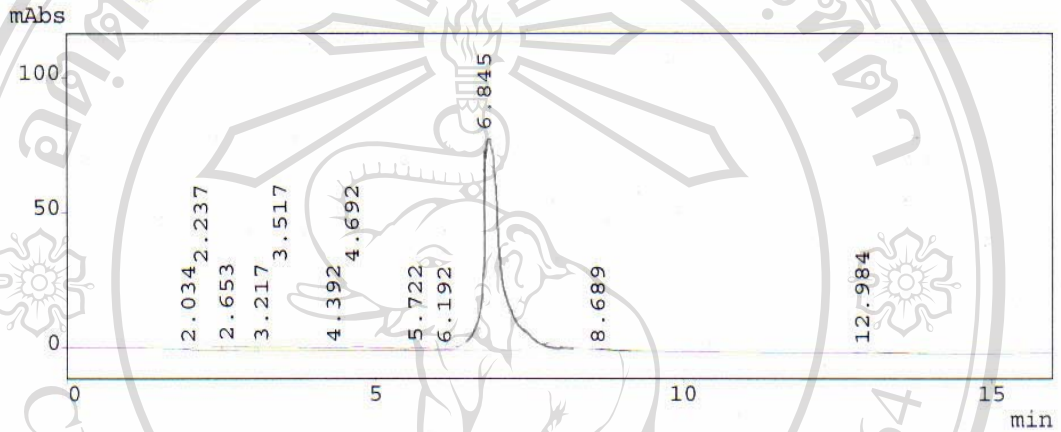
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Regression equation between standard rotenone concentration and peak area of HPLC

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CLASS-LC10 Ver.=1.63 REPORT.NO=19 DATA=230249.D04 06/02/23 13:42:48
 Sample : std rotenone 230249
 ID : 150ppm
 Type : Unknown
 Detector : SPD-10A Single
 Operator : Nut
 Method Name : 230249.M04

*** Chromatogram *** Filename:230249.C04



*** Peak Report ***

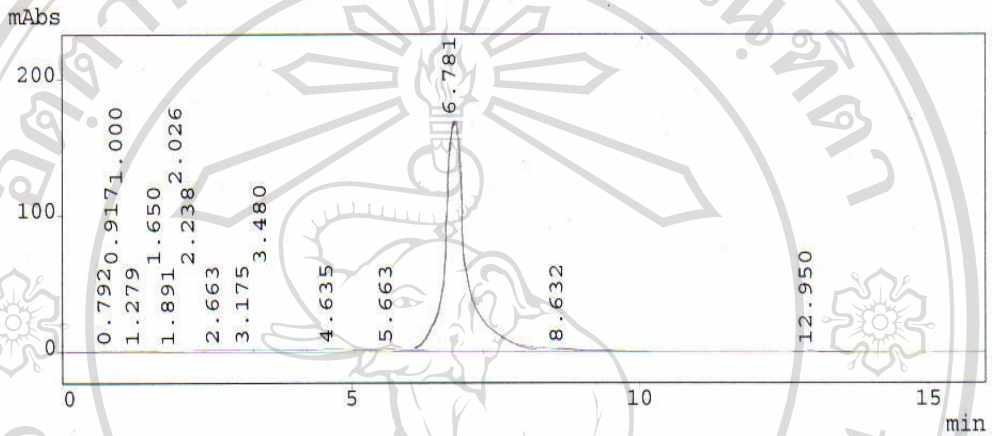
PKNO	TIME	AREA	IDNO	MK
1	2.034	6512		
2	2.237	33508		
3	2.653	53955		V
4	3.217	23593		V
5	3.517	62077		V
6	4.392	15857		V
7	4.692	68962		V
8	5.722	34419		V
9	6.192	7459		V
10	6.845	1841974		SV
11	8.689	3059		T
12	12.984	23852		

2175228

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CLASS-LC10 Ver.=1.63 REPORT.NO=22 DATA=230249.D07 06/02/23 14:31:00
 Sample : std rotenone 230249
 ID : 250ppm
 Type : Unknown
 Detector : SPD-10A Single
 Operator : Nut
 Method Name : 230249.M07

*** Chromatogram *** Filename:230249.C07



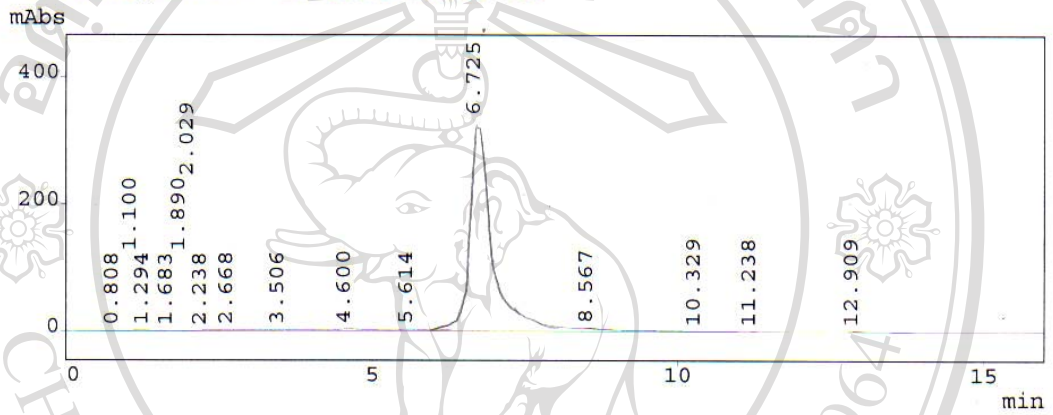
*** Peak Report ***

PKNO	TIME	AREA	IDNO	MK
1	0.792	22674		
2	0.917	3464		V
3	1.000	9701		V
4	1.279	21448		V
5	1.650	11828		V
6	1.891	4433		V
7	2.026	6858		V
8	2.238	31689		V
9	2.663	58248		V
10	3.175	41284		V
11	3.480	107959		V
12	4.635	122327		V
13	5.663	68330		V
14	6.781	4461647		SV
15	8.632	7288		T
16	12.950	26189		

5005366

CLASS-LC10 Ver.=1.63 REPORT.NO=20 DATA=230249.D10 06/02/23 15:19:08
 Sample : std rotenone 230249
 ID : 500ppm
 Type : Unknown
 Detector : SPD-10A Single
 Operator : Nut
 Method Name : 230249.M10

*** Chromatogram *** Filename:230249.C10

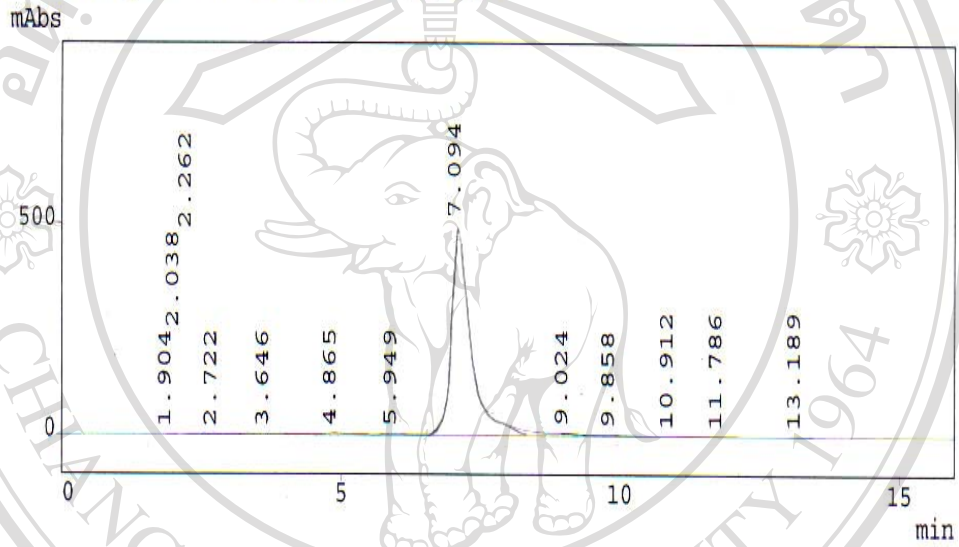


*** Peak Report ***

PKNO	TIME	AREA	IDNO	MK
1	0.808	28346		
2	1.100	7390		V
3	1.294	16570		V
4	1.683	12302		V
5	1.890	4299		V
6	2.029	6758		V
7	2.238	30181		
8	2.668	56398		V
9	3.506	183835		V
10	4.600	156811		V
11	5.614	97537		V
12	6.725	8863458		SV
13	8.567	14643		T
14	10.329	2010		T
15	11.238	1386		T
16	12.909	27093		

CLASS-LC10 Ver.=1.63 REPORT.NO=21 DATA=230249.D15 06/02/23 16:39:36
 Sample : std rotenone 230249
 ID : 750ppm
 Type : Unknown
 Detector : SPD-10A Single
 Operator : Nut
 Method Name : 230249.M15

*** Chromatogram *** Filename:230249.C15



*** Peak Report ***

PKNO	TIME	AREA	IDNO	MK
1	1.904	1489		
2	2.038	3257		V
3	2.262	30425		
4	2.722	59867		V
5	3.646	197055		V
6	4.865	167977		V
7	5.949	106001		V
8	7.094	13315101		SV
9	9.024	26883		T
10	9.858	2340		T
11	10.912	2289		T
12	11.786	2119		T
13	13.189	25070		

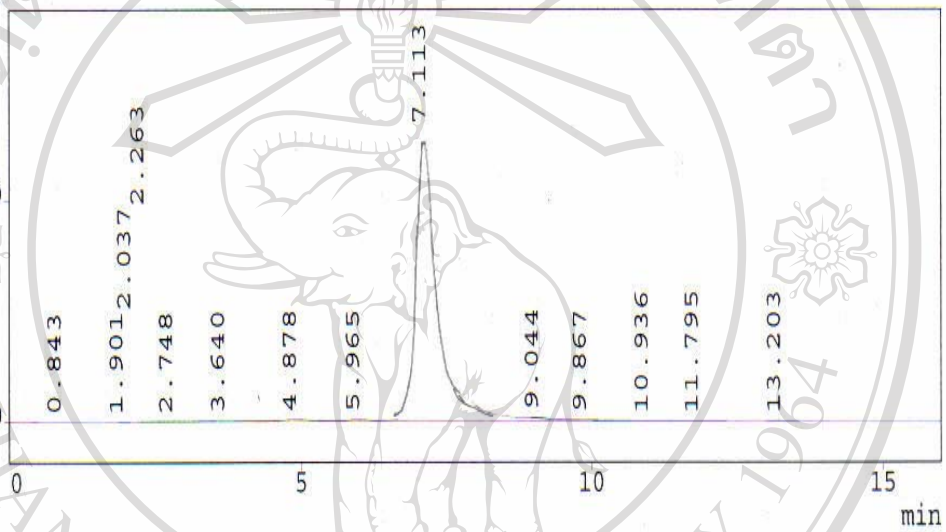
13939872

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CLASS-LC10 Ver.=1.63 REPORT.NO=18 DATA=230249.D16 06/02/23 16:55:38
 Sample : std rotenone 230249
 ID : 1000ppm
 Type : Unknown
 Detector : SPD-10A Single
 Operator : Nut
 Method Name : 230249.M16

*** Chromatogram *** Filename:230249.C16

mAbs



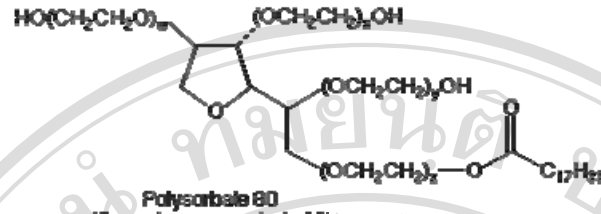
*** Peak Report ***

PKNO	TIME	AREA	IDNO	MK
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2	1.901	1736		
3	2.037	3468		V
4	2.263	31318		
5	2.748	61234		V
6	3.640	223409		V
7	4.878	198941		V
8	5.965	126060		V
9	7.113	17498814		SV
10	9.044	36294		T
11	9.867	2910		T
12	10.936	2908		T
13	11.795	2395		T
14	13.203	24849		

18217067

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Tween 80



Tween 80 (also known as Polysorbate 80) is used in the manufacture of protein solution formulations to help solubilize and stabilize the protein. It is one of a series of materials (including Tween 20, 40 and 60) which are fatty acid esters of sorbitan polyethoxylates. The various Tweens differ in the type of fatty acid present; Tween 80 is an oleate. Tween 80 is a nonionic surfactant and emulsifier derived from sorbitol which is obtained from various types of fruit. Polysorbate 80 is a water-soluble somewhat yellowish amber liquid that is used as a dispersing agent to mix oil and water and to solubilize fragrances and essential oils. It is also a great lubricant and has a pleasant, soothing effect on the skin. Polysorbates are nonirritating and readily biodegradable. Tween 20 - Polysorbate 20 is derived from coconut oil. Other polysorbates are derived from palm oil (Polysorbate 40 and 60) and olive oil (polysorbate 80).

CURRICULUM VITAE

NAME: Mr. Aroon Sottikul

MARRITAL STATUS: Married

DATE OF BIRTH: 31 October 1959

POSITION: Instructor / Head of Plant Protection Branch

ADDRESS : Lampang Agricultural Research and Training Center, P.O. Box 89
A. Muang, Lampang 52000

E-mail Sotikul@hotmail.com **Telephone** 013877327 **Fax** 054342550

EDUCATION:

Field of Study	Institute	Qualification	From	To
Entomology	Chiang Mai University, Chiang Mai, THAILAND	B.Sc.	1978	1981
Entomology	Kasetsart University, Bangkok, THAILAND	M.Sc.	1985	1988

WORK EXPERIENCE :

Research publication more than 20 publications such as

1. Determine of Rotenone Degradation after Sprayed Derris Extract by High Performance Liquid Chromatography (HPLC.) Method. (2005-2006)
2. Rotenone Quantity and Isozyme Patterns of *Derris malacensis* Prain and *Derris elliptica*. (2005-2006)
3. Formulation of derris extract to controlled Cabbage aphid (*Lipaphis erysimi* Kattenbach) (2005-2006)
4. Increasing effective of water extracted rotenone from derris root (2005-2006)

Teaching and Training (for student, farmers / agricultural and extension officers/secondary school teachers)

Training course: Important Insect Pest and their Control by Botanical Insecticide (1990-at present)