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APPENDIX

Appendix I

Composition of plant tissue culture media

1.1 Murashige and Skoog media (1962)

Micronutrients	mg/l	Iron	mg/l
NH_4NO_3	1650	Sodium EDTA	37.25
KNO_3	1900	$FeSO_4.7H_2O$	27.85
$CaCl_2.2H_2O$	440		
$MgSO_4.7H_2O$	370	Organic compounds	mg/l
KH_2PO_4	170	Glycine	2.0
		Nicotinic acid	0.5
Micronutrients	mg/l	Pyridoxine-HCl	0.5
H_3BO_3	6.2	Thiamine-HCl	0.1
$MnSO_4.H_2O$	6.9	Myoinositol	100
$ZnSO_4.H_2O$	6.14		
KI	0.83	Sucrose	20 g/l
$Na_2MoO_4.H_2O$	0.25		
$CuSO_4.5H_2O$	0.025	pH 5.7	
$CoCl_2.6H_2O$	0.025		

1.2 Linsmaier and Skoog media (1965)

Micronutrients	mg/l	Iron	mg/l
NH_4NO_3	1690	Na_2EDTA	37.25
KNO_3	1900	$FeSO_4.7H_2O$	27.85
$CaCl_2.2H_2O$	440		
$MgSO_4.7H_2O$	370	Organic compounds	mg/l
KH_2PO_4	170	Thiamine-HCl	0.4
Micronutrients	mg/l	Myoinositol	100
H_3BO_3	6.2		
$MnSO_4.4H_2O$	22.3	Sucrose	20 g/l
$ZnSO_4.4H_2O$	8.6		
KI	0.83	pH 5.7	
$Na_2MoO_4.H_2O$	0.25		
$CuSO_4.5H_2O$	0.025		
$CoCl_2.6H_2O$	0.025		

1.3 Gamborg media (1970)

Micronutrients	mg/l
KNO_3	2500
$MgSO_4.7H_2O$	250
$NaH_2PO_4.H_2O$	150
$CaCl_2.2H_2O$	150
$(NH_4)_2SO_4$	134

Micronutrients	mg/l
H_3BO_3	3.0
$MnSO_4.4H_2O$	10.0
$ZnSO_4.7H_2O$	2.0
KI	0.75
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4$	0.025
$CoCl_2.6H_2O$	0.025

Iron	mg/l
NaFeEDTA	28.0

Organic compounds	mg/l
Myoinositol	100.0
Nicotinic acid	1.0
Thiamine-HCl	10.0
Pyridoxine-HCl	1.0

Sucrose 20.0 g/l

pH 5.7

1.4 Chu media (1966)

Micronutrients	mg/l
KNO_3	2830
$MgSO_4.7H_2O$	185
KH_2PO_4	400
$CaCl_2.2H_2O$	166
$(NH_4)_2SO_4$	134

Micronutrients	mg/l
H_3BO_3	1.6
$MnSO_4.4H_2O$	4.4
$ZnSO_4.7H_2O$	1.5
KI	0.8

Iron	mg/l
NaEDTA	37.25
$FeSO_4.7H_2O$	27.85

Organic components	mg/l
Glycine	2.0
Nicotinic acid	0.5
Thiamine-HCl	1.0
Pyridoxine-HCl	0.5

Sucrose 20.0 g/l

pH 5.7

1.5 Hildebrandt media (1962)

Micronutrients	mg/l
KNO_3	160
$MgSO_4.7H_2O$	720
$NaH_2PO_4.H_2O$	132
$Ca(NO_3)_2.4H_2O$	800
KCl	130
Na_2SO_4	100

Micronutrients	mg/l
H_3BO_3	2.7
$MnSO_4.4H_2O$	4.5
$ZnSO_4.7H_2O$	3.0
KI	0.375

Iron	mg/l
$Fe_2(C_4H_4O_6)_3.H_2O$	5.0

Organic components	mg/l
Glycine	12.0
Pyridoxine-HCl	0.8
Thiamine-HCl	0.1

Sucrose 20.0 g/l

pH 5.7

Appendix II

Preparation of stock solution of media

2.1 Preparation of stock solution of MS media

Stock solution		g/1000ml
stock I	NH_4NO_3	165
	KNO_3	190
	used 10 ml/l media	
stock II	$MgSO_4.7H_2O$	37
	$MnSO_4.H_2O$	2.23
	$ZnSO_4.7H_2O$	0.86
	$CuSO_4.7H_2O$	0.0025
	used 10 ml/l media	
stock III	$CaCl_2.2H_2O$	44
	KI	0.083
	$CoCl_2.6H_2O$	0.0025
	used 10 ml/l media	
stock IV	KH_2PO_4	17
	H_3BO_3	0.62
	$Na_2MoO_4.2H_2O$	0.025
	used 10 ml/l media	
stock V	$FeSO_4.7H_2O$	2.78
	Na_2EDTA	3.73
	used 10 ml/l media	
stock Vitamin	myoinositol	6.0
(g/300ml.)	nicotinic acid	0.03
	pyridoxine-HCl	0.03
	thiamine-HCl	0.03
	glycine	0.12
	used 5 ml/l media	

2.2 Preparation of stock solution of B_6 media

Stock solution		g/500ml
stock I	KNO_3	150
	used 20 ml/l media	
stock II	$MgSO_4.H_2O$	50
	$MnSO_4.H_2O$	1.0
	$ZnSO_4.7H_2O$	0.2
	$CuSO_4.7H_2O$	0.0025
	$(NH_4)_2SO_4$	13.4
	used 10 ml/l media	
stock III	$CaCl_2.2H_2O$	15
	KI	0.075
	$CoCl_2.6H_2O$	0.0025
	used 10 ml/l media	
stock IV	$NaH_2PO_4.H_2O$	15
	H_3BO_3	0.3
	$Na_2MoO_4.2H_2O$	0.025
	used 10 ml/l media	
stock V	$FeSO_4.7H_2O$	2.78
	Na_2EDTA	3.73
	used 10 ml/l media	
stock Vitamin	myoinositol	10.0
	nicotinic acid	0.1
	pyridoxine-HCl	0.1
	thiamine-HCl	0.1
	used 10 ml/l media	

2.3 Preparation of stock solution of N_6 media

Stock solution		g/500 ml
stock I	KNO_3	70.75
	used 40 ml/l media	
stock II	$MgSO_4.7H_2O$	9.25
	$MnSO_4.4H_2O$	0.22
	$ZnSO_4.7H_2O$	0.075
	$(NH_4)_2SO_4$	23.15
	used 20 ml/l media	
stock III	$CaCl_2.2H_2O$	16.6
	KI	0.08
	used 10 ml/l media	
stock IV	KH_2PO_4	40
	H_3BO_3	0.16
	used 10 ml/l media	
stock V	$FeSO_4.7H_2O$	2.78
	Na_2EDTA	3.73
	used 10 ml/l media	
stock Vitamin	glycine	0.2
	nicotinic acid	0.05
	pyridoxine-HCl	0.05
	thiamine-HCl	0.1
	used 10 ml/l media	

2.4 Preparation of stock solution of HM media

Stock solution		g/500 ml.
stock I	KNO_3	8
	$Ca(NO_3)_2.4H_2O$	40
	used 20 ml/l media	
stock II	$MgSO_4.7H_2O$	72
	$MnSO_4.4H_2O$	0.45
	$ZnSO_4.7H_2O$	0.3
	Na_2SO_4	10
	used 10 ml/l media	
stock III	KI	0.0375
	KCl	13
	used 10 ml/l media	
stock IV	$NaH_2PO_4.H_2O$	13.2
	H_3BO_3	0.27
	used 10 ml/l media	
stock V	$Fe_2(C_4H_4O_6)_3.H_2O$	0.5
	used 10 ml/l media	
stock Vitamin	glycine	1.2
	pyridoxine-HCl	0.08
	thiamine-HCl	0.01
	used 10 ml/l media	

2.5 Preparation of stock solution of LS media

Stock solution		g/500ml
stock I	NH_4NO_3	84.5
	KNO_3	95
	used 20 ml/l media	
stock II	$MgSO_4.7H_2O$	37
	$MnSO_4.4H_2O$	2.23
	$ZnSO_4.7H_2O$	0.86
	$CuSO_4.7H_2O$	0.0025
	used 10 ml/l media	
stock III	$CaCl_2.2H_2O$	44
	KI	0.083
	$CoCl_2.6H_2O$	0.0025
	used 10 ml/l media	
stock IV	H_3BO_3	0.62
	$Na_2MoO_4.2H_2O$	0.025
	used 10 ml/l media	
stock V	$FeSO_4.7H_2O$	2.78
	Na_2EDTA	3.73
	used 10 ml/l media	
stock Vitamin	myoinositol	10
	thiamine-HCl	0.04
	used 10 ml/l media	

Appendix III

Preparation of stock solution (100 ppm or 100 mg/l) of auxins, cytokinins and gibberellic acid

Auxins	mg/500ml.
NAA	100
IAA	100
IBA	100
2,4-D	100

Cytokinins	mg/500ml.
BA	100
Kn	100

Gibberellic acid	mg/500ml.
GA_3	100

Appendix IV

Medium preparation

The culture media were prepared by mixing the stock solution and sucrose into the distilled water. The pH each medium was adjusted to its desired value with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. The media were sterilized by autoclaving at 121 ^{o}C (15 lb/in^{2}) for 15 minutes.

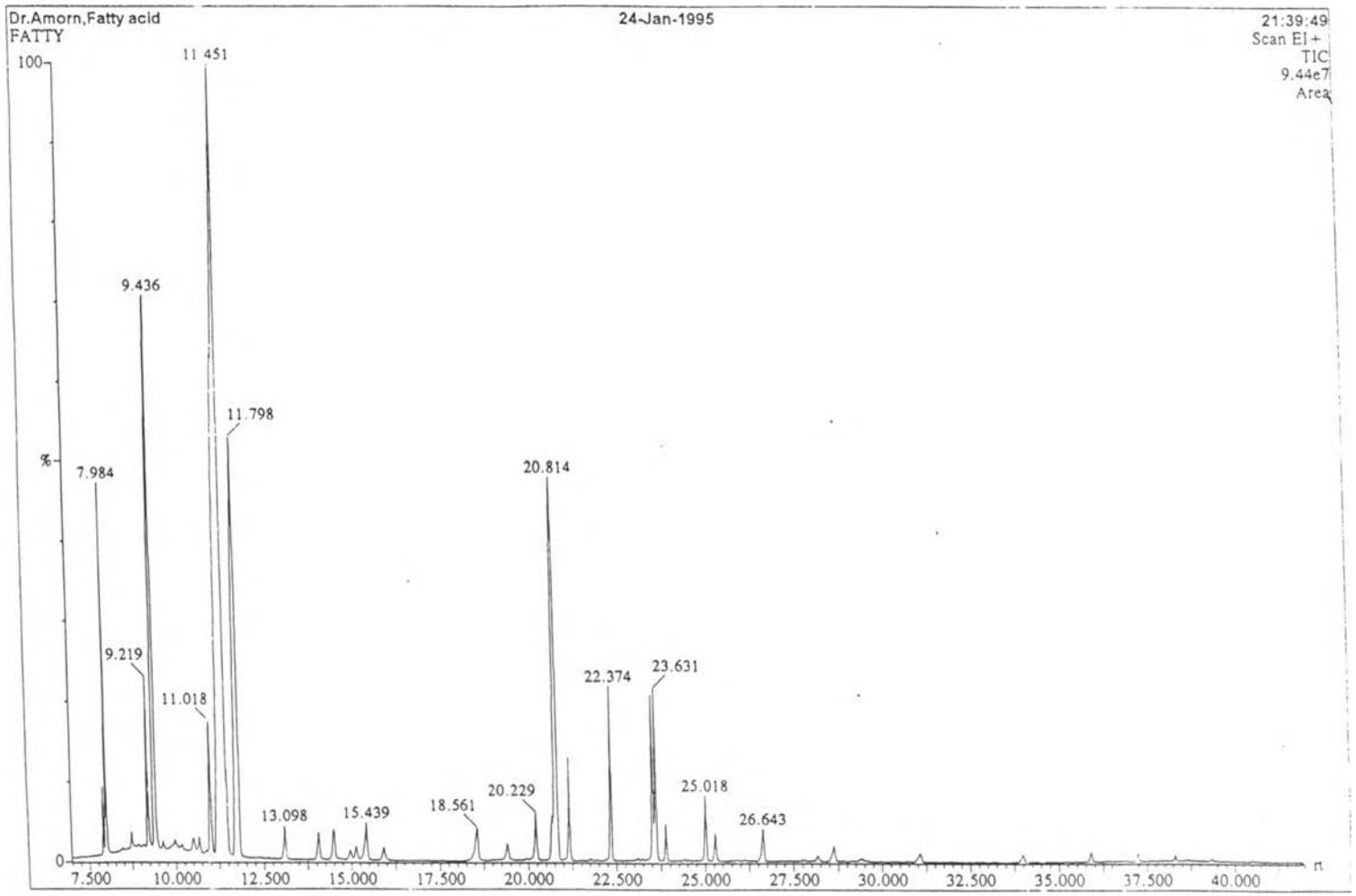

Figure. 5.1 The gas chromatogram of standard fatty acid mixture detected by Gas chromatography/Mass spectroscopy (GC/MS).

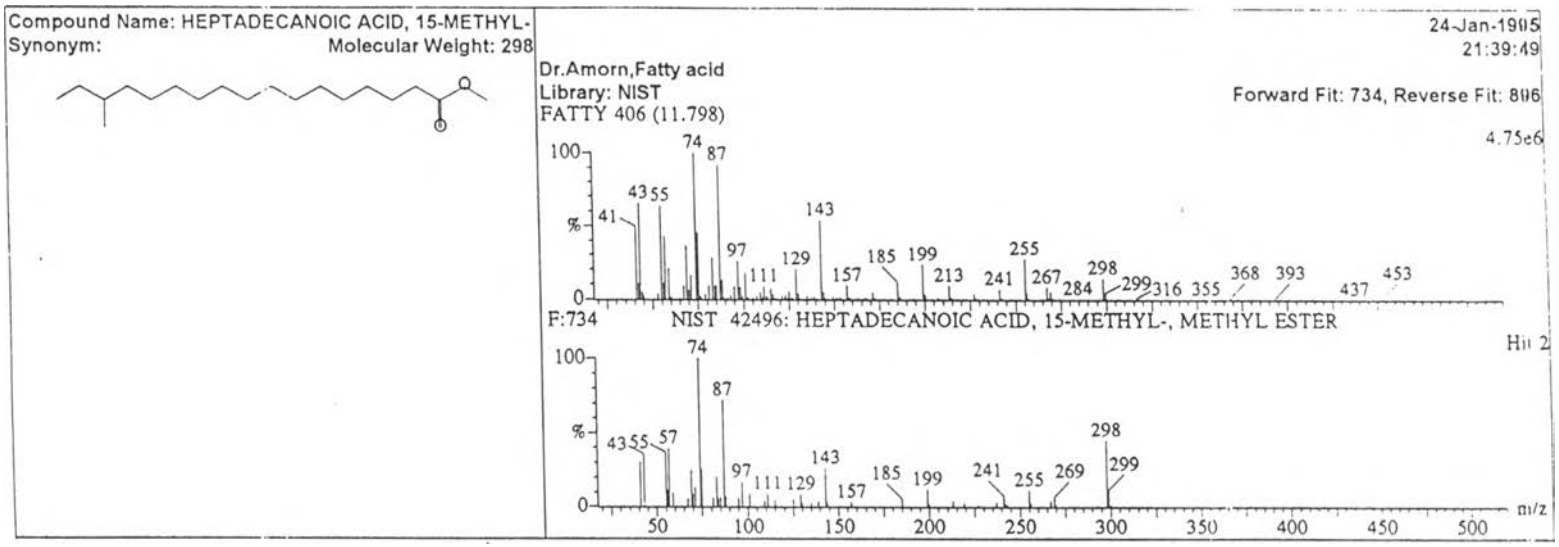

Figure. 5.2 Mass spectrum of stearic acid (C18:0) in standard fatty acid mixture detected by GC/MS.

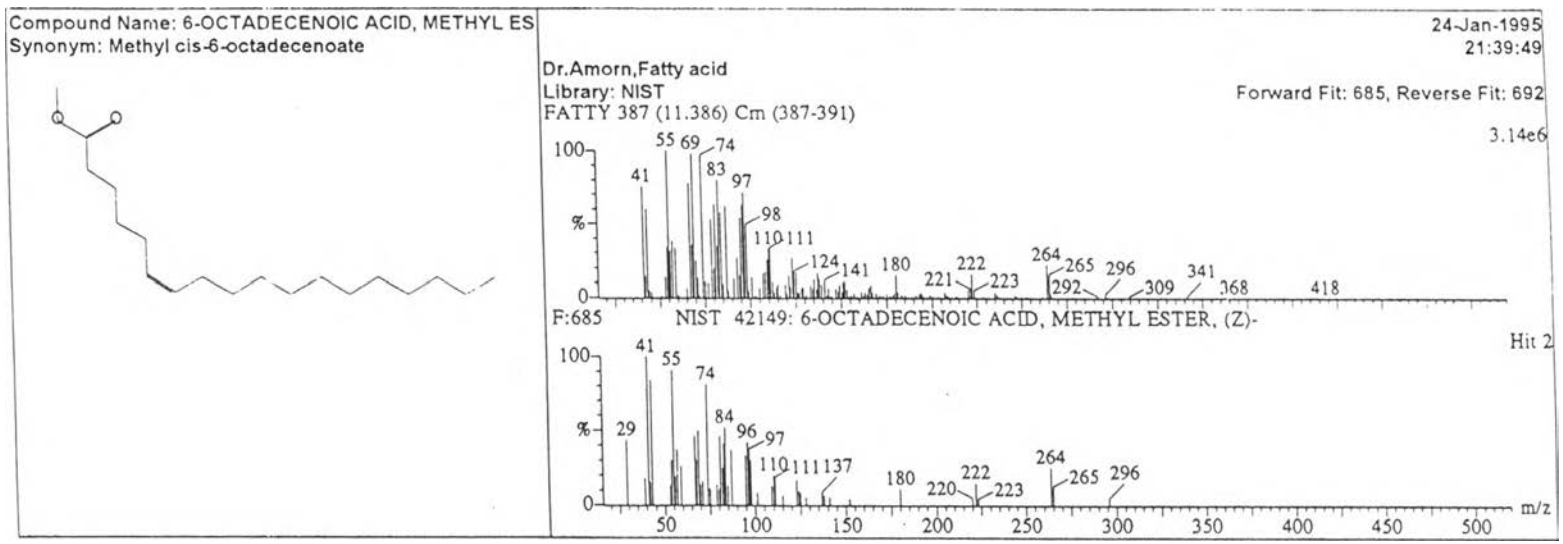

Figure. 5.3 Mass spectrum of oleic acid (C18:1) in standard fatty acid mixture detected by GC/MS

Appendix VI

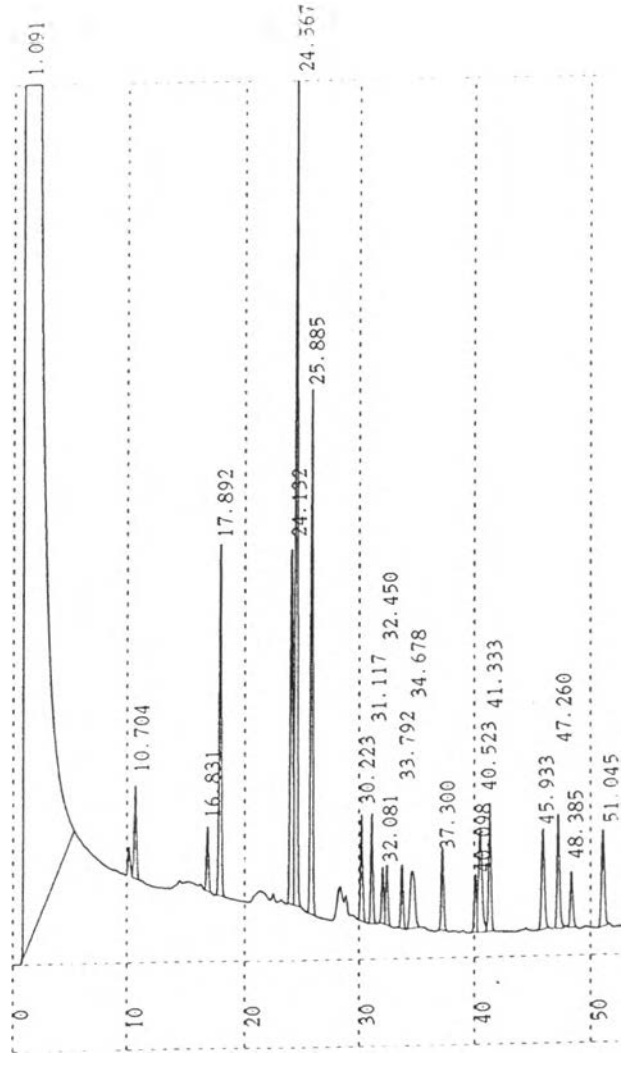

Figure. 6 The gas chromatogram of standard fatty acid mixture detected by Shimadzu GC-15A Gas chromatography.

Appendix VII

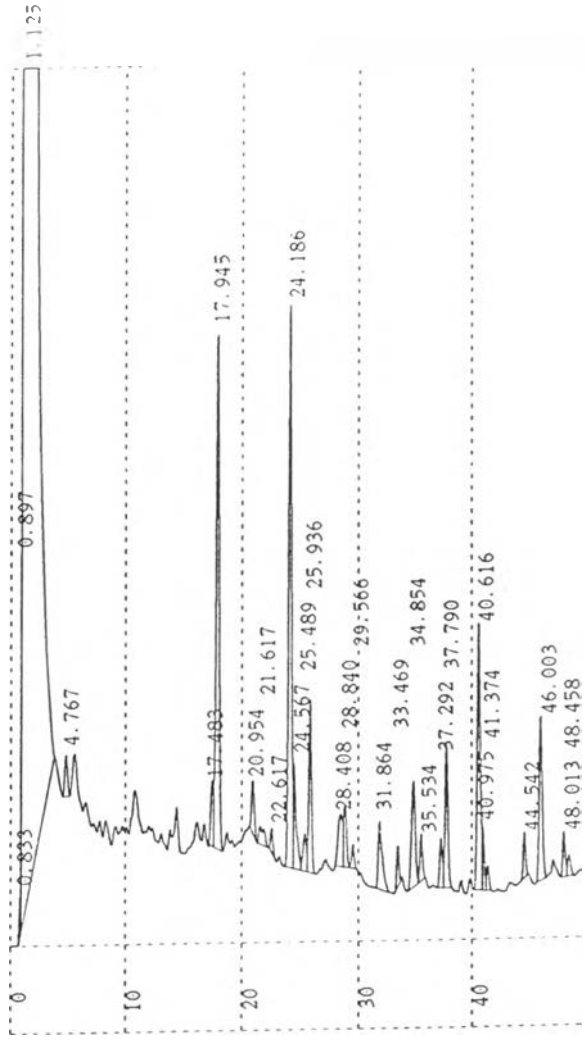

Figure. 7 The gas chromatogram of fatty acid methyl ester of the sample lipid extracts, detected by Shimadzu GC-15A Gas chromatography.

Appendix VIII

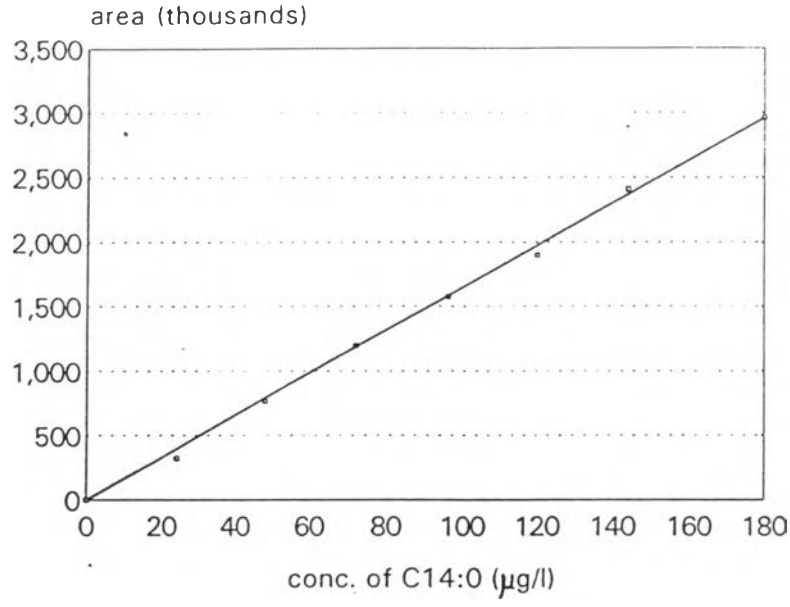

Figure. 8.1 Standard calibration curve of myristic acid (C14:0)

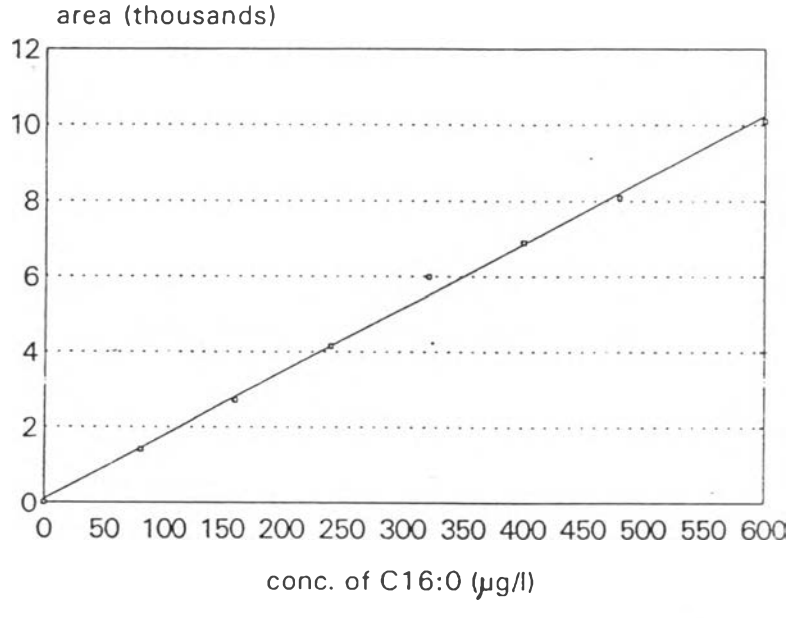

Figure. 8.2 Standard calibration curve of palmitic acid (C16:0)

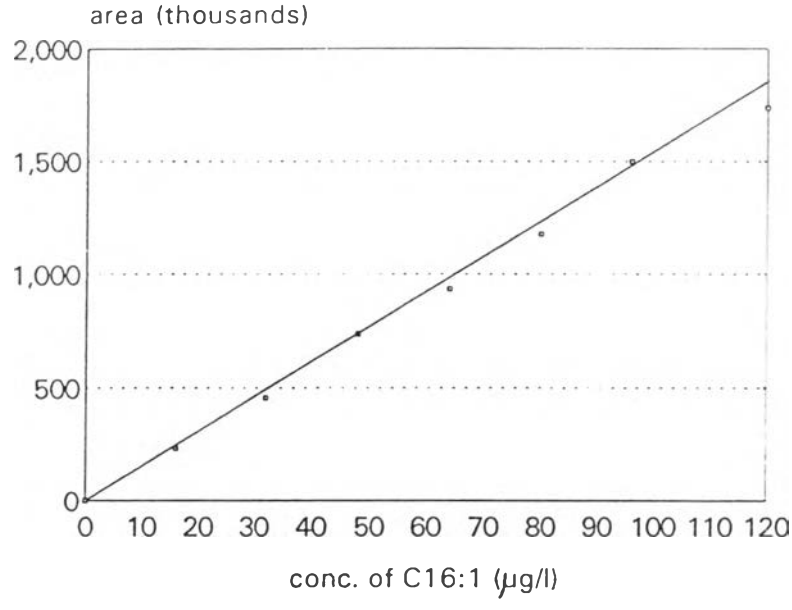

Figure. 8.3 Standard calibration curve of palmitoleic acid (C16:1)

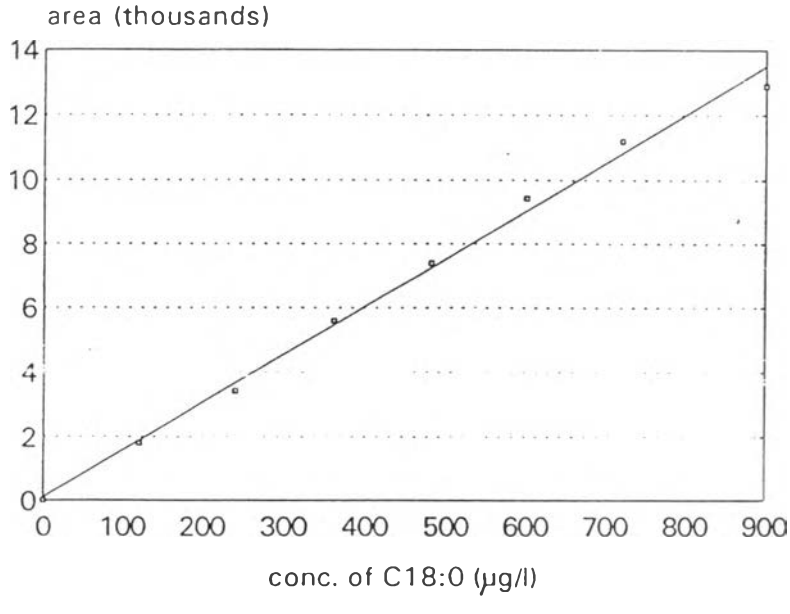

Figure. 8.4 Standard calibration curve of stearic acid (C18:0)

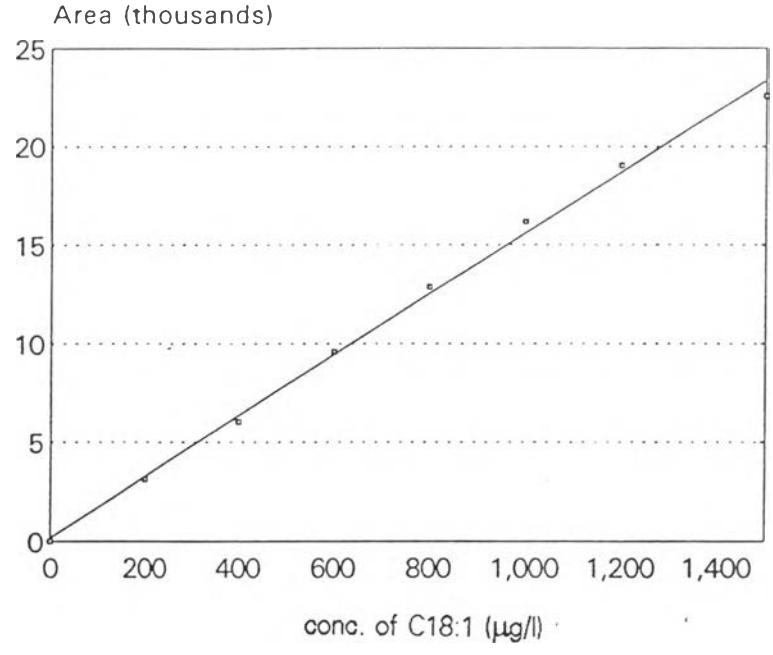

Figure. 8.5 Standard calibration curve of oleic acid (C18:1)

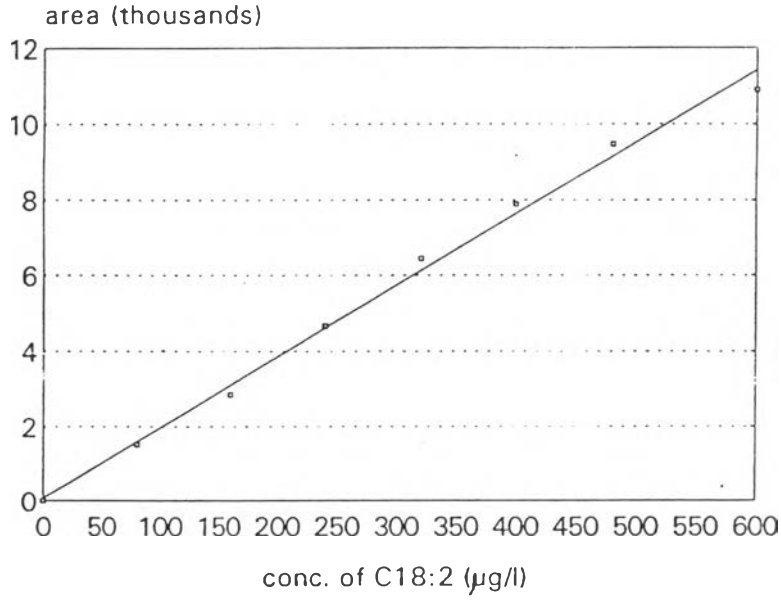

Figure. 8.6 Standard calibration curve of linoleic acid (C18:2)

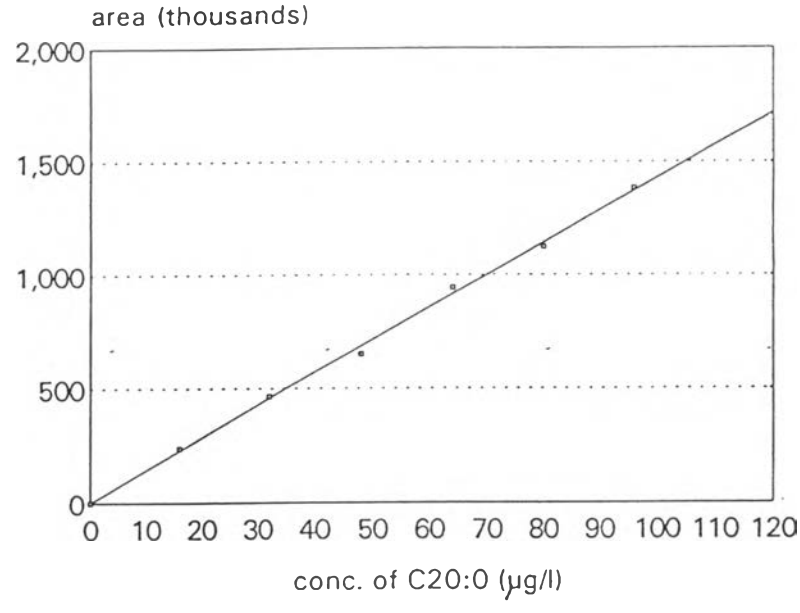

Figure. 8.7 Standard calibration curve of arachidic acid (C20:0)

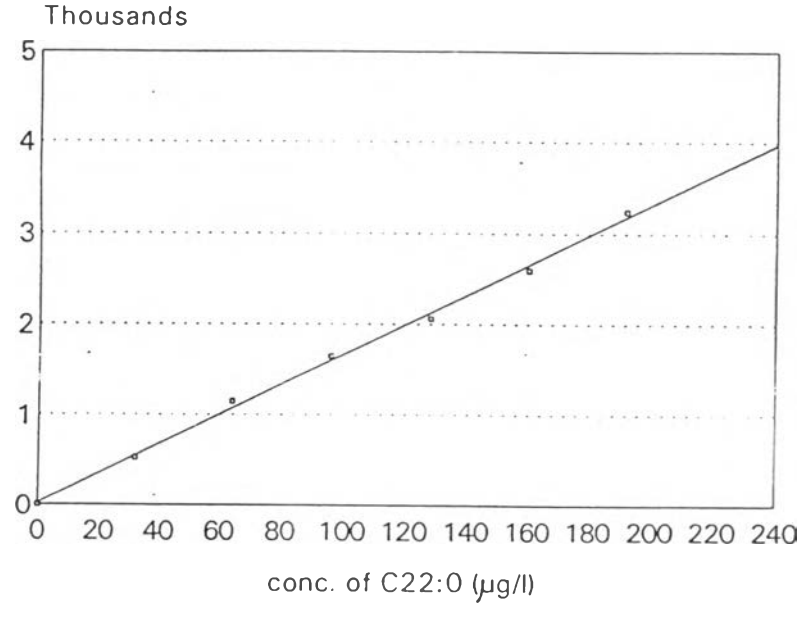

Figure. 8.8 Standard calibration curve of behenic acid (C22:0)

VITA

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