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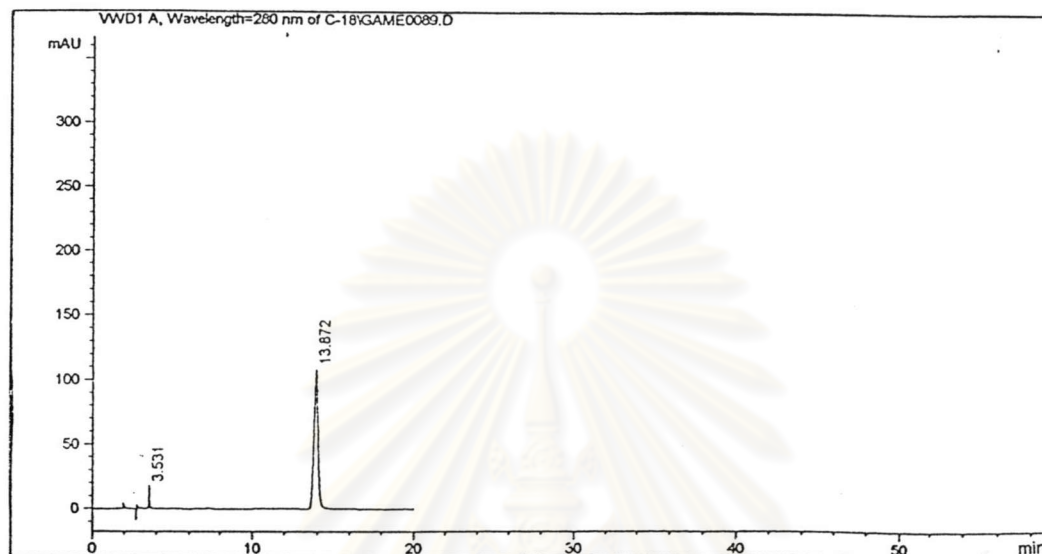


APPENDICES

ศูนย์วิทยทรัพยากร
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Appendix A

1. Chromatogram and standard curve of Naringin



Area Percent Report

Sorted by Retention Time
Multiplier : 1.000000
Dilution : 1.000000

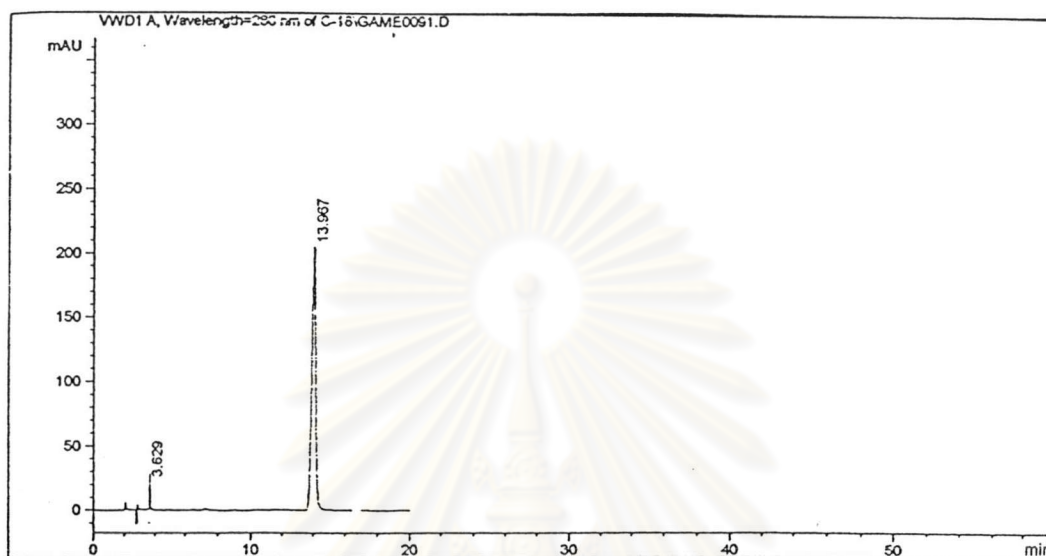
Signal 1: VWD1 A, Wavelength=280 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	3.531	1	BB	57.60295	17.12222	2.9146
2	13.872	1	BB	1918.73706	108.96300	97.0854

Totals : 1976.33997 126.08521

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Figure A.1 Chromatogram of standard naringin at 100 ppm



Area Percent Report.

Sorted by Retention Time

Multiplier : 1.000000
 Dilution : 1.000000

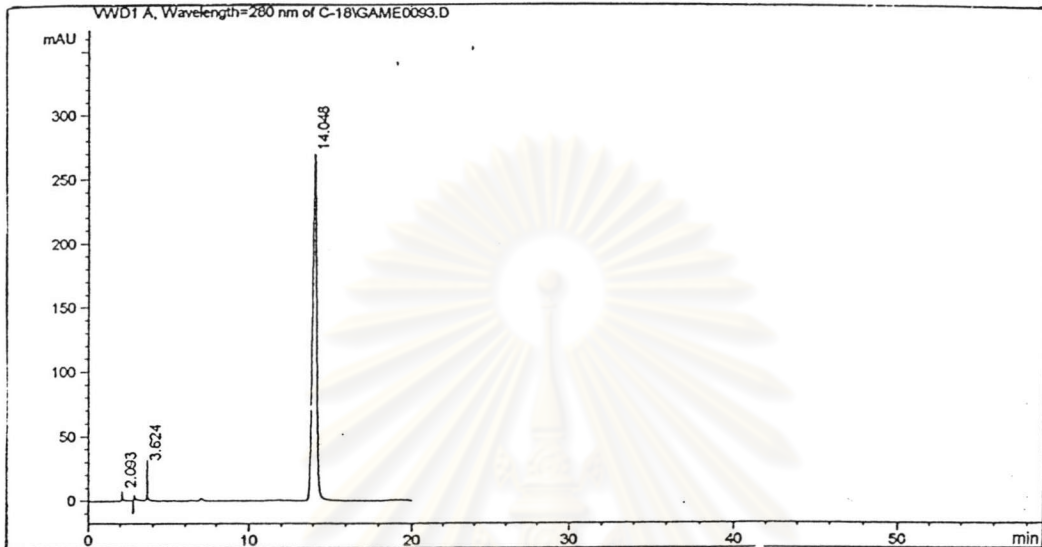
Signal 1: WVD1 A, Wavelength=280 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	3.629	1	BB	88.26590	22.38808	2.3988
2	13.967	1	BB	3591.30396	204.42575	97.6012

Totals : 3679.56982¹ 226.81384

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Figure A 2 Chromatogram of standard naringin at 200 ppm



Area Percent Report

Sorted by Retention Time
Multiplier : 1.000000
Dilution : 1.000000

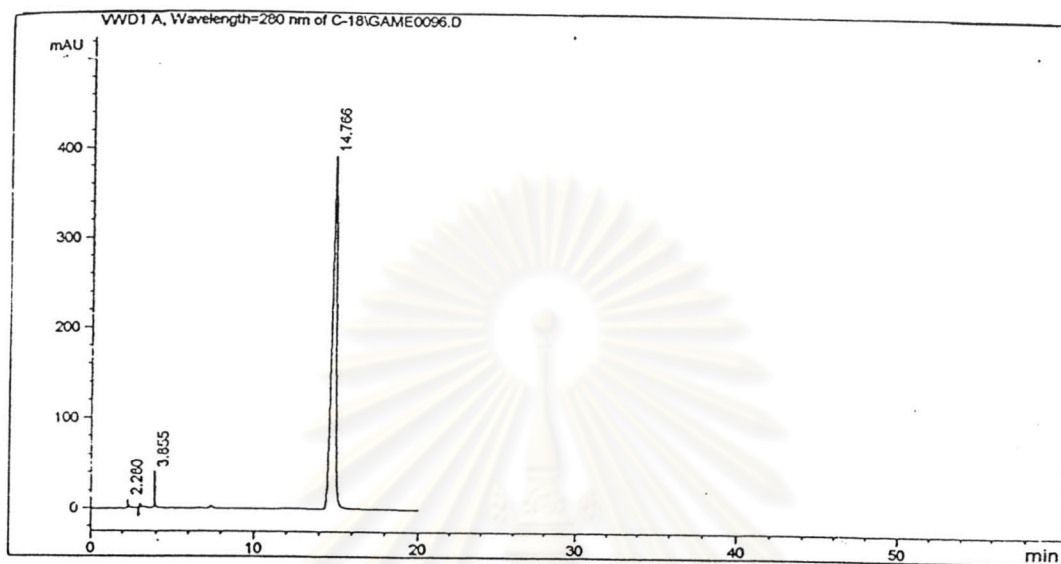
Signal 1: VWD1 A, Wavelength=280 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	2.093	1	BB	30.20816	6.91043	0.5969
2	3.624	1	BB	100.92299	27.74170	1.9941
3	14.048	1	BB	4929.87598	269.27469	97.4090

Totals : 5061.00732 303.92682

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Figure A 3 Chromatogram of standard naringin at 300 ppm



Area Percent Report

Sorted by Retention Time

Multiplier : 1.000000
 Dilution : 1.000000

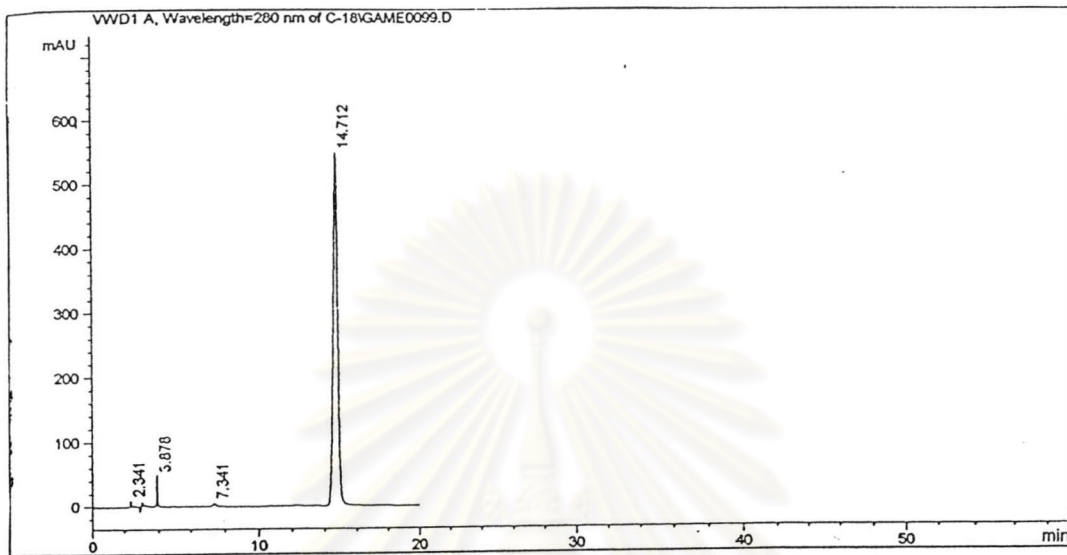
Signal 1: VWD1 A, Wavelength=280 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	2.280	1	BB	38.95936	8.97296	0.5097
2	3.855	1	BB	132.29030	39.62015	1.7308
3	14.766	1	BB	7472.08643	391.76331	97.7595

Totals : 7643.33594 440.35641

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 จุฬาลงกรณ์มหาวิทยาลัย

Figure A 4 Chromatogram of standard naringin at 400 ppm



Area Percent Report

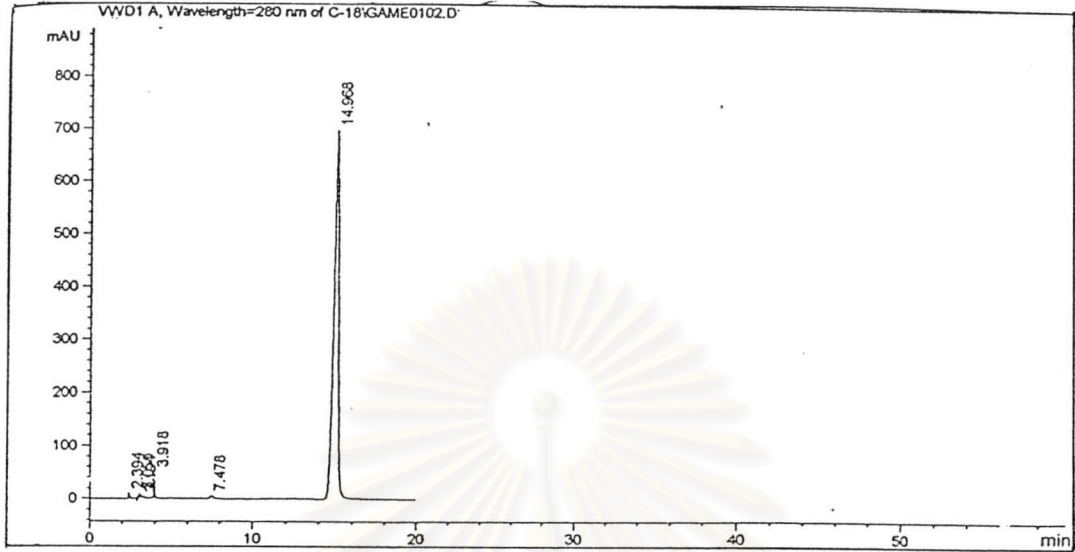
Sorted by Retention Time
Multiplier : 1.000000
Dilution : 1.000000

Signal 1: VWD1 A, Wavelength=280 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	2.341	1	BB	163.29909	10.77860	1.4817
2	3.878	1	BB	158.91805	46.81314	1.4419
3	7.341	1	BB	61.21502	4.69194	0.5554
4	14.712	1	BB	10637.63770	548.50897	96.5209

Totals : 11021.07031 610.79266

Figure A 5 Chromatogram of standard naringin at 500 ppm



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 Area Percent Report
 =====

Sorted by Retention Time
 Multiplier : 1.000000
 Dilution : 1.000000

Signal 1: VWD1 A, Wavelength=280 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	2.394	1	BV	97.89934	11.31219	0.6641
2	3.050	1	PV	82.89169	10.07291	0.5623
3	3.194	1	VV	92.68774	5.58462	0.6287
4	3.918	1	VB	223.46051	51.44086	1.5158
5	7.478	1	EB	81.22449	5.98798	0.5510
6	14.968	1	BE	14163.93457	700.14758	96.0781
Totals :				14742.09863	784.54614	

Figure A 6 Chromatogram of standard naringin at 600 ppm

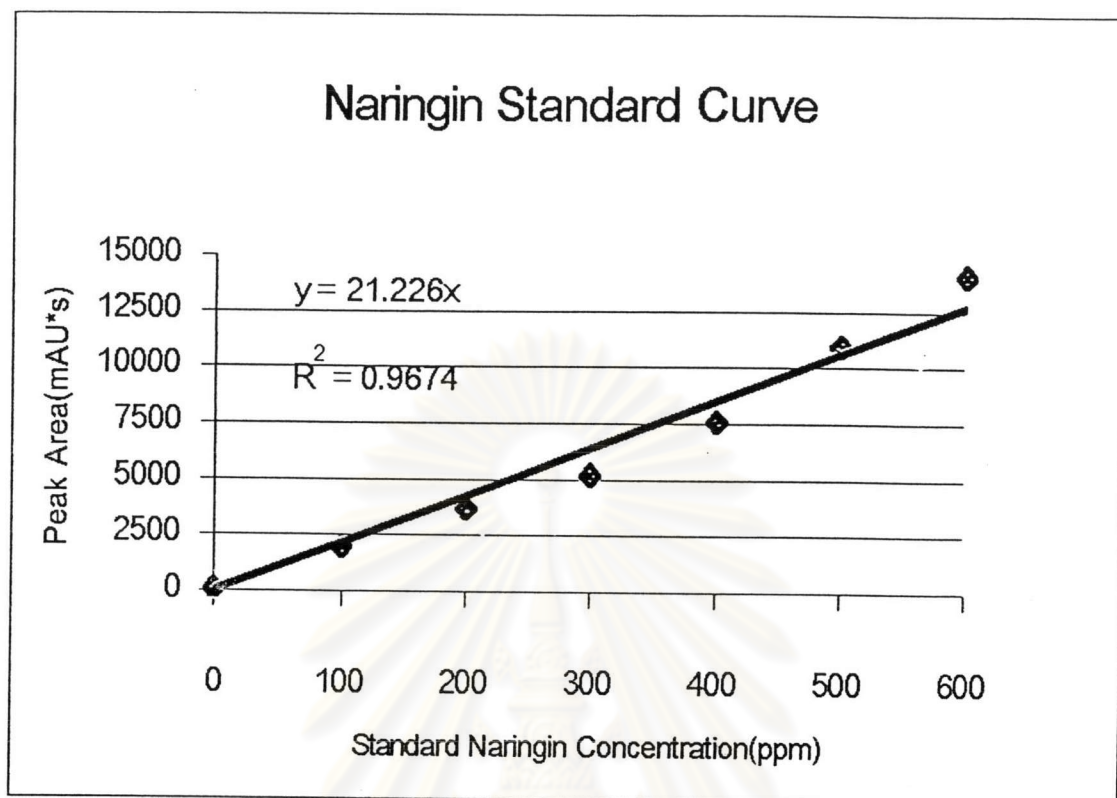
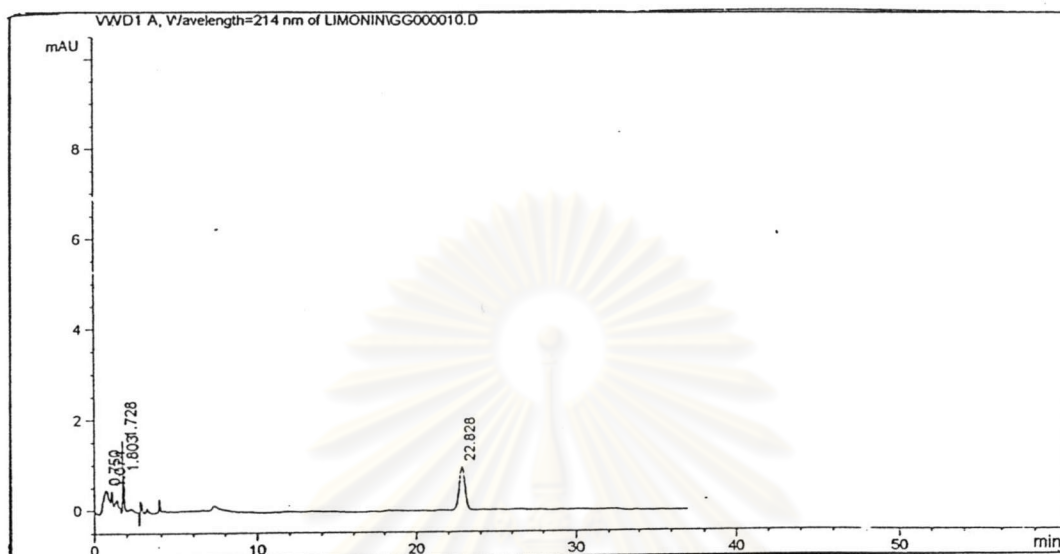


Figure A 7 Standard curve of naringin

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2. Chromatogram and standard curve of Limonin



Area Percent Report

Sorted by Retention Time
Multiplier : 1.000000
Dilution : 1.000000

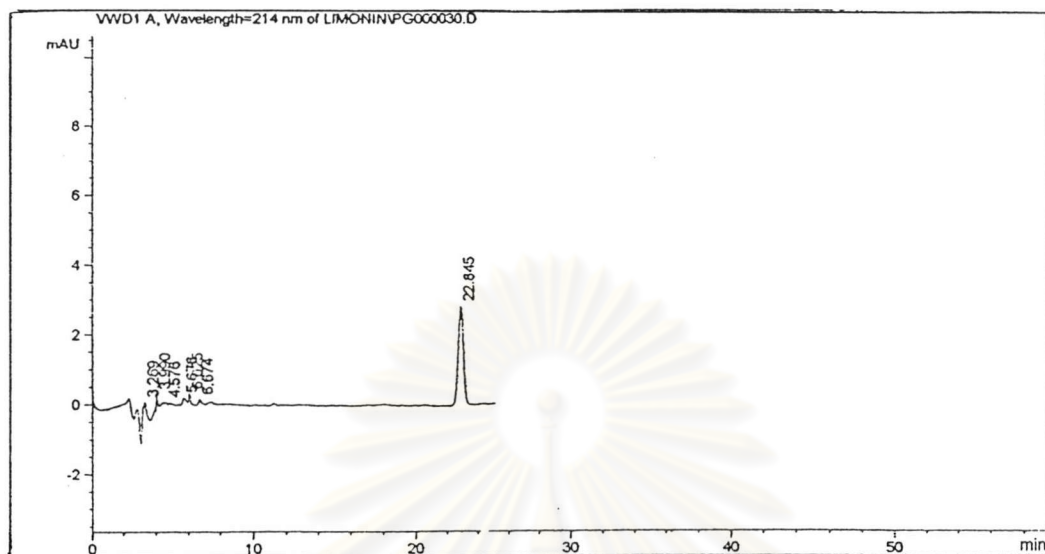
Signal 1: VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	0.750	1	BV	12.33592	5.32909e-1	25.9961
2	1.074	1	VV	3.88499	4.95025e-1	8.1870
3	1.728	1	PV	4.08904	1.56178	8.6170
4	1.803	1	VB	2.60237	8.02164e-1	5.4841
5	22.828	1	BB	24.54071	9.74385e-1	51.7158

Totals : 47.45303 4.36626

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Figure A 8 Chromatogram of standard limonin at 2.5 ppm



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 Area Percent Report
 =====

Sorted by Retention Time
 Multiplier : 1.000000
 Dilution : 1.600000

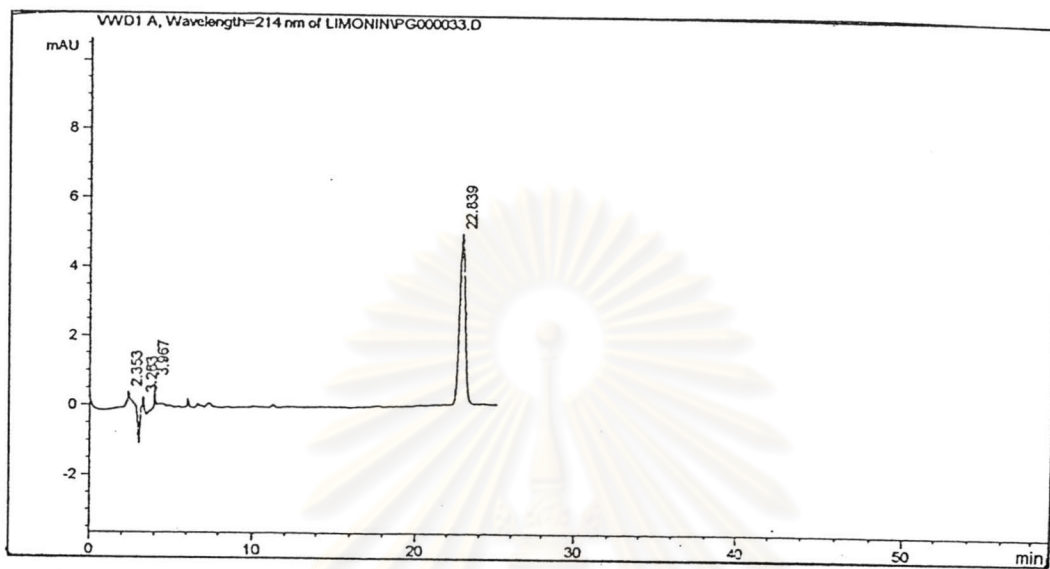
Signal 1: VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	3.269	1	PV	24.57869	1.13674	12.6048
2	3.990	1	VV	23.25431	1.16168	11.9256
3	4.576	1	VV	52.09393	7.47240e-1	26.7155
4	5.676	1	VV	12.20702	5.75039e-1	6.2602
5	6.025	1	VV	9.38037	5.84603e-1	4.8106
6	6.674	1	VV	4.98501	2.52671e-1	2.5565
7	22.845	1	BB	68.49609	2.83985	35.1270

Totals : 194.99541 7.29782

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Figure A 9 Chromatogram of standard limonin at 7.5 ppm



Area Percent Report

Sorted by Retention Time

Multiplier : 1.000000

Dilution : 1.000000

Signal 1: WWD1 A, Wavelength=214 nm.

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	2.353	1	BV	37.01061	8.76983e-1	20.0332
2	3.263	1	PV	17.40175	1.07395	9.4193
3	3.967	1	VB	10.40275	8.27413e-1	5.6308
4	22.839	1	BB	119.93149	4.93012	64.9168

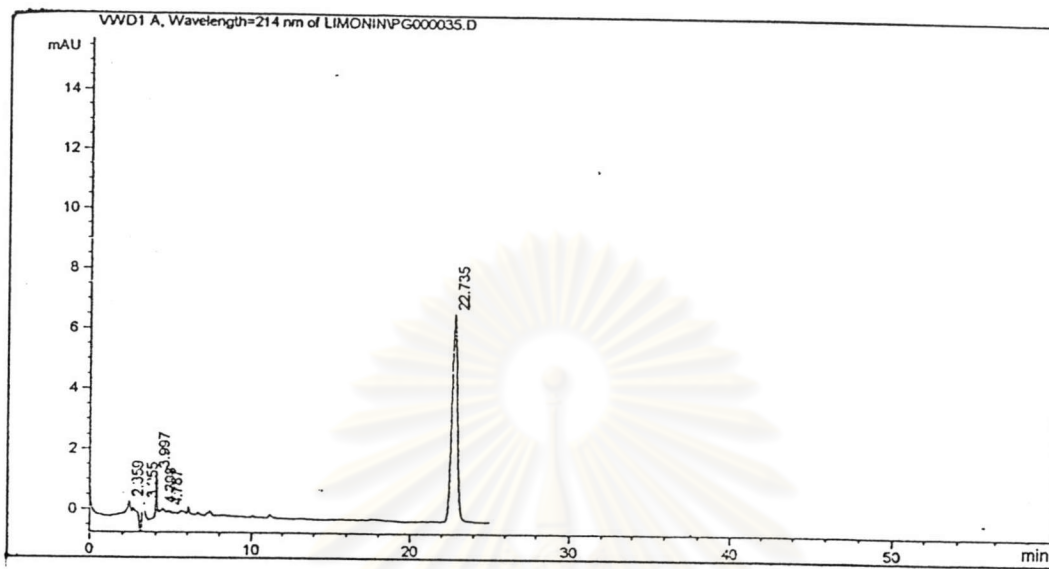
Totals :

134.74660

7.70846

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Figure A 10 Chromatogram of standard limonin at 12.5 ppm



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 Area Percent Report
 =====

Sorted by Retention Time
 Multiplier : 1.000000
 Dilution : 1.000000

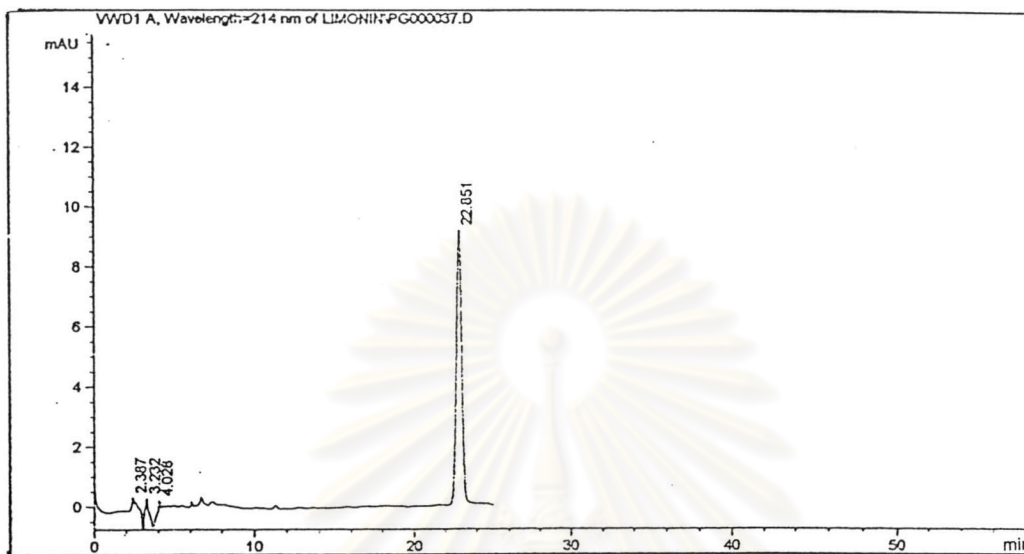
Signal 1: VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	2.359	1	BV	23.70636	9.24706e-1	9.5802
2	3.255	1	PV	19.45647	1.14044	7.8627
3	3.997	1	VV	26.18627	1.83197	10.5824
4	4.398	1	VV	9.26292	4.85230e-1	3.7433
5	4.787	1	VB	4.27760	2.51383e-1	1.7237
6	22.735	1	BB	164.56172	6.87803	66.5027

Totals : 247.45134 11.51176

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Figure A 11 Chromatogram of standard limonin at 17.5 ppm



Area Percent Report

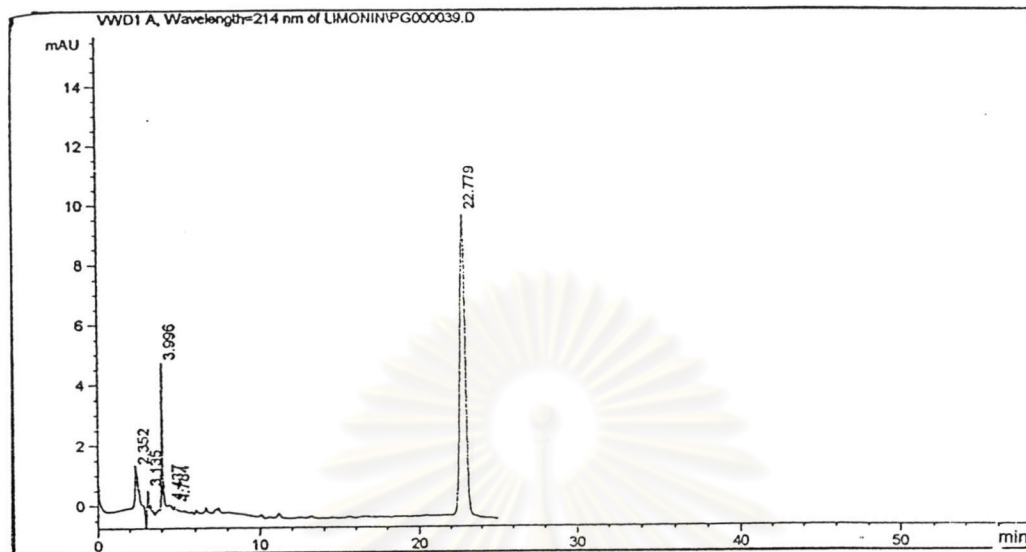
Sorted by Retention Time
Multiplier : 1.000000
Dilution : 1.000000

Signal 1: VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	2.387	1	BV	29.11998	7.09822e-1	10.5821
2	3.232	1	PV	22.03480	1.20191	8.0074
3	4.028	1	PV	2.97331	3.29972e-1	1.0807
4	22.851	1	BB	221.05237	9.12147	80.3298
Totals :				275.18097	11.36324	

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Figure A 12 Chromatogram of standard limonin at 22.5 ppm



Area Percent Report

Sorted by Retention Time

Multiplier : 1.000000
Dilution : 1.000000

Signal 1: WWD1 A, Wavelength=214 nm

Peak #	RT [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	2.352	1	BV	41.76279	1.57809	11.2229
2	3.135	1	PV	22.50402	1.34438	6.0475
3	3.996	1	VV	45.89631	5.23853	12.3337
4	4.437	1	VV	8.18678	4.46076e-1	2.2000
5	4.784	1	VV	4.02694	2.85019e-1	1.0822
6	22.779	1	EE	249.74289	10.05616	67.1136

Totals : 372.11975 18.94826

Figure A 13 Chromatogram of standard limonin at 25.0 ppm

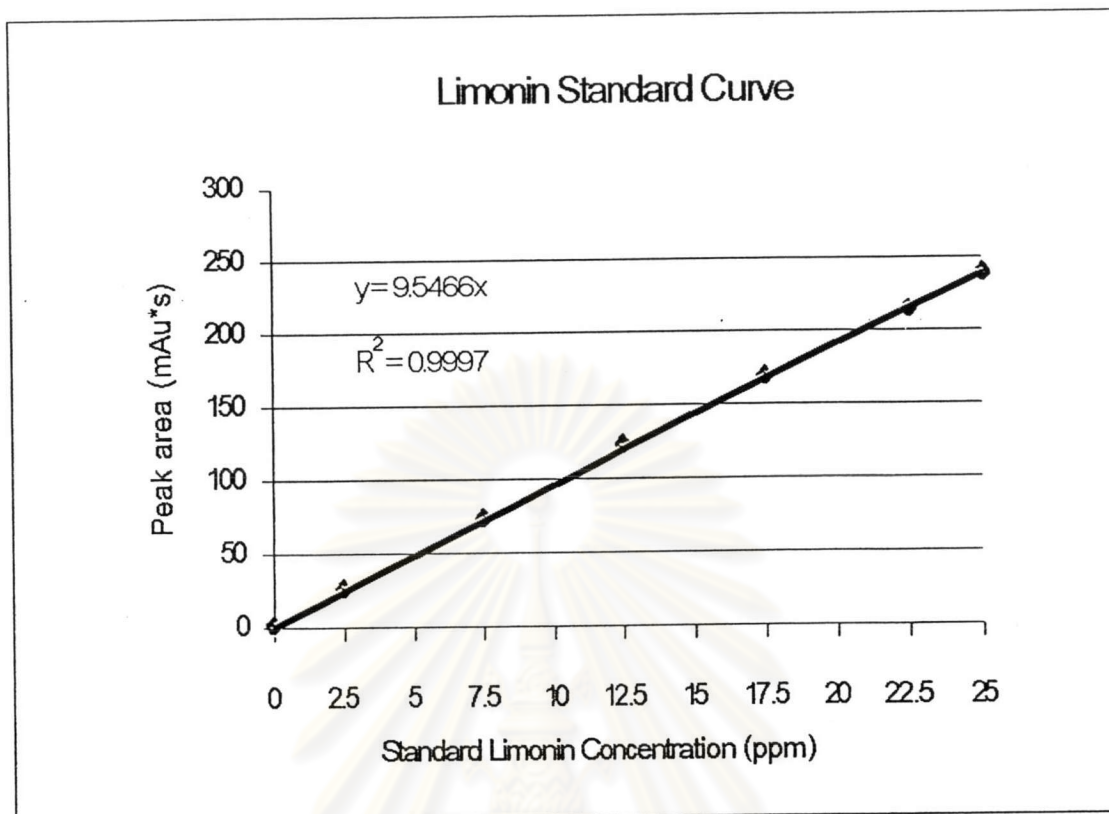


Figure A 14 Standard curve of limonin

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Table A 1 %Recoveries of naringin of initial and reused Extra Sep C-18 cartridge

Replications	%Recoveries		
	Initial C-18 column	1 st Reused C-18 column	2 nd Reused C-18 column
1	75.60±0.17	78.20±0.62	71.60±0.95
2	85.47±2.05	66.70±1.57	67.50±1.06
3	82.90±2.38	84.97±0.55	87.60±0.98
Average	81.32±5.11	76.62±9.23	75.57±10.62
%C.V.	6.29	12.04	14.06

Note: a, b, c means with the same letter were not significant different at 5% level by Duncan's multiple range test with three replications.

Table A 2 % Recoveries of limonin of initial and reused Extra Sep C-18 column

Replications	%Recoveries		
	Initial C-18 column	1 st Reused C-18 column	2 nd Reused C-18 column
1	82.725±1.312	78.333±0.207	69.176±0.319
2	84.083±0.296	78.079±0.440	66.559±0.152
3	82.720±0.216	80.330±0.701	64.864±0.283
Average	83.176±0.785 ^a	78.914±1.232 ^b	66.866±2.172 ^c
%C.V.	0.944	1.562	3.248

Note: a, b, c means with the same letter were not significant different at 5% level by Duncan's multiple range test with three replications.

Table A 3 Limonin contents of each sample juice

Sample	Limonin content (ppm)			Average	%C.V
Fresh juice	3.950	3.978	3.991	3.973±0.021 ^a	0.53
Juice chilled at 6 °C for 24 hours	4.010	4.081	4.121	4.071±0.057 ^a	1.40
Pasteurized juice	4.526	4.579	4.703	4.602±0.091 ^b	1.97

Note: The samples were analyzed with three replications.

Table A 4 Limonin contents at each heating temperature

Column	Limonin(ppm)		
	Heated at 60 ^o C for 15 min	Heated at 70 ^o C for 15 min	Heated at 80 ^o C for 15 min
1	7.864	8.202	8.285
2	7.916	8.217	8.188
3	7.916	8.307	8.330
Average	7.899±0.03 ^a	8.242±0.057 ^b	8.268±0.072 ^b
%CV	0.376	0.693	0.878

Note: a, b, c means with the same letter were not significantly different at 5% level by Duncan's multiple range test (n= 3), when n represented the replication.

Table A 5 Limonin content in tangerine juice of each heating time

Replication	Limonin content (ppm)		
	Heating time (min)		
	5 min	10 min	15 min
1	9.1876	9.1453	9.3792
2	8.9978	9.2149	9.5625
3	8.9449	9.4138	9.8336
Average±SE	9.0451±0.13 ^a	9.2580±0.14 ^{ab}	9.5917±0.2286 ^b
%CV	1.44	1.51	2.38

Note: means with the same letter are not significant different at 5% level by Duncan's multiple range test analyzing with three replications.

Table A 6 Operating cost for β -CD polymer column (Laboratory scale)

Item	Unit	Quantity	Cost/unit (Bahts/unit)	Cost (Bahts)	(%)
a) Tangerine fruit	kg	1	10	10	0.826
b) β -CD polymer	g	3	400	1,200	99.07
c) NaOH	g	4	0.26	1.06	0.0857
d) Electricity	kw*h	0.004	4	0.016	0.0013
e) Water	m ³	0.02	10	0.2	0.017
Total cost (Bahts/column)				1,211.276	
Practical maximum capacity of column (mg limonin/g β -CD polymer)				0.8088	
Productivity (ml juice/column/hour)				21	

Note: The dimension of bed = 1.2 cm i.d. x 9 cm height of bed

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Appendix B

Calculation of limonin content in tangerine juice

Standard limonin equation : $Y = 9.5466 * X$

Slope (S) = 9.5466

When Y represents to Peak area (mAu*s) and

X represents to limonin concentration (ppm)

From HPLC analysis

Peak area of the limonin peak at 22 min = P

Thus limonin concentration of the extract = P/S ppm or $\mu\text{g/ml}$

The 3 ml of extract was prepared from 3 ml of sample juice

Finally, limonin content in the sample juice = $3*(P/S) \mu\text{g}$

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Appendix C

Statistical Analysis

Table C 1 ANOVA for the study of regeneration of Extra-Sep C-18 column for naringin determination

SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	Pr>F
Model	2	56.345755	28.172877	0.38	0.7012
Error	6	448.669200	74.778200		
Corrected total	8	505.014955			

Duncan's Multiple Range Test

Duncan Grouping	Mean	N	Between group
A	81.323	3	initial use
A	76.623	3	1 st reused
A	75.567	3	2 nd reused

R-square 0.11572

C.V. 11.10957

Root MSE 8.64743

Data Mean 77.8377

Table C 2 ANOVA for the study of regeneration of Extra-Sep C-18 column for limonin determination

SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	Pr>F
Model	4	24.0324220	6.00810551	162.61	0.0001
Error	22	0.81285021	0.03694774		
Corrected total	26	24.8452722			

Duncan's Multiple Range Test

Duncan Grouping	Mean	N	Between group
A	11.36434	9	1
B	10.78678	9	2
C	9.140000	9	3

R-square 0.967284

C.V. 1.842867

Root MSE 0.192217

Data Mean 10.43037

Table C 3 ANOVA for the study of limonin content in the fresh, chilled and pasteurized juice.

SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	Pr>F
Model	2	0.689	0.344	87.21	0.0001
Error	6	0.024	0.004		
Corrected total	8	0.712			

Duncan's Multiple Range Test

Duncan Grouping	Mean	N	Between group
A	4.602	3	3
B	4.071	3	2
B	3.973	3	1

R-square 0.966

C.V. 1.491

Root MSE 0.062

Data Mean 4.215

Table C 4 ANOVA for the effect of temperature on limonin content for juice preparation

SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	Pr>F
Model	2	2.14192689	1.07093644	40.65	0.0003
Error	5	0.15808333	0.02634722		
Corrected total	8	2.30001022			

Duncan's Multiple Range Test

Duncan Grouping	Mean	N	Between group
A	23.9763	3	3
A	23.9013	3	2
B	22.9060	3	1

R-square 0.931268

C.V. 0.687948

Root MSE 0.152318

Data Mean 23.59455

Table C 5 ANOVA for the effect of heating time on limonin content for juice preparation

SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	Pr>F
Model	2	4.121236522	2.03031811	7.82	0.0213
Error	6	1.58139467	0.26355578		
Corrected total	8	5.70263089			

Duncan's Multiple Range Test

Duncan Grouping	Mean	N	Between group
A	28.7750	3	3
B	27.7740	3	2
AB	27.1303	3	1

R-square 0.722690

C.V. 1.84055

Root MSE 0.51338

Data Mean 27.8931

Table C 6 ANOVA for the optimum of batch process for debittering

SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	Pr>F
Model	7	14862.9180722	2123.2740103	87.56	0.0001
Error	36	873.003555562	24.25009877		
Corrected total	43	15735.9216279			

Type I					
SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	Pr>F
Temp	1	670.03714	670.03714	27.63	0.0001
Conc	1	13642.84867	13642.84867	562.59	0.0001
Temp*Conc	1	435.33203	435.33503	17.95	0.0002
Time	1	64.442810	64.442821	2.66	0.1118
Temp*Time	1	5.3112817	5.3112817	0.22	0.6426
Conc*Time	1	22.549386	22.549386	0.93	0.3413
Temp*Conc*time1		22.396722	22.396722	0.92	0.3429

R-square 0.944522

C.V. 7.997730

Root MSE 4.924438

Data Mean 61.57295

Table C 7 ANOVA for the effect of rotor speed and mixing time on limonin reduction in tangerine juice by β -CD polymer

SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	Pr>F
Model	5	32.35433294	6.47286659	5.15	0.0093
Error	12	15.06118200	1.25509850		
Corrected total	17	47.42551494			
Time	2	18.77914211	9.38957105	7.48	0.0078
Speed	1	0.98607606	0.98607606	0.79	0.3928
Time*Speed	2	12.5991147	6.29955739	5.02	0.0261

Duncan's Multiple Range Test

Duncan Grouping	Mean	N	Time
A	71.7677	6	2
B	69.8240	6	3
B	69.4315	6	1
R-square	0.682424		
C.V.	1.592685		
Root MSE	1.120311		
Data Mean	70.34105		

Appendix D

1. Capacity of β -CD polymer in limonin adsorption for batch process at the optimum condition

Initial limonin content in juice load = 7.34 ppm

Juice load = 25 ml

Amount of β -CD polymer used = 0.75 g

Total limonin in 25 ml juice = 184 μ g

% limonin reduction of batch = 68 %

Thus Amount of limonin adsorbed by β -CD polymer = $0.68 \times 184 = 125 \mu\text{g}$

Capacity of β -CD polymer in limonin reduction = $125 / 0.75$

= 166 μ g limonin/g β -CD polymer

= 0.17 mg limonin/g β -CD polymer

2. Capacity of β -CD polymer column in limonin adsorption at the optimum condition

Initial limonin content in juice load = 8.70 ppm

Juice load = 100 ml

Amount of β -CD polymer used = 3 g

Total limonin in 100 ml juice = 870 μ g

% limonin reduction of batch = 94 %

Thus Amount of limonin adsorbed by β -CD polymer = $0.94 \times 870 = 818 \mu\text{g}$

Capacity of β -CD polymer in limonin reduction = $818 / 3$

$$= 273 \mu\text{g limonin/g } \beta\text{-CD polymer}$$

$$= 0.27 \text{ mg limonin/g } \beta\text{-CD polymer}$$

3. Capacity of β -CD polymer column in limonin adsorption at practical maximum load

Basis : 1 column operating at maximum load and the initial limonin content in juice was 10.11 ppm

Productivity of the laboratory scale

At maximum load, it produced 240 ml of debittered juice

Flow rate = 0.35 ml/minute

Process time for 240 ml load = 686 minutes or 11.43 hours / column

Productivity of laboratory scale (P) = 240 ml debittered juice / column / 1.43 hours

$$= 21 \text{ ml juice/column/hour}$$

Limonin adsorption capacity of the laboratory scale

$$= 10.11(\mu\text{g/ml juice}) \cdot 240 \text{ ml juice} / 3 \text{ g } \beta\text{-CD polymer}$$

$$= 808.8 \mu\text{g limonin /g } \beta\text{-CD polymer}$$

$$\approx 0.81 \text{ mg limonin /g } \beta\text{-CD polymer}$$

4. Capacity of XAD-16 in limonin adsorption at the optimum condition

Initial limonin content in juice load = 10.11 ppm

Juice load = 100 ml

Amount of XAD-16 resin used = 5.6 g

Total limonin in 100 ml juice = 1011 μ g

% limonin reduction of batch ~ 100 %

Thus Amount of limonin adsorbed by XAD-16 resin = $1 \times 1011 = 1011 \mu$ g

Capacity of β -CD polymer in limonin reduction = $1011/5.6$

= 180.53 μ g limonin/ g XAD-16 resin

= 0.18 mg limonin/ g XAD-16 resin

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BIOGRAPHY

Miss Piriya Rodart was born on October 3, 1977 in Bangkok. She graduated her Bachelor degree of Food Technology, Faculty of Science from Chulalongkorn University in 1997 and then enrolled the graduate program for M.Sc. in Biotechnology at Chulalongkorn University in 1998.



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