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APPENDICES

APPENDIX A

The chromatograms and graphical data from analysis of green tea polyphenols using HPLC

Figure 51. HPLC Chromatograme of EGC Reference Standard
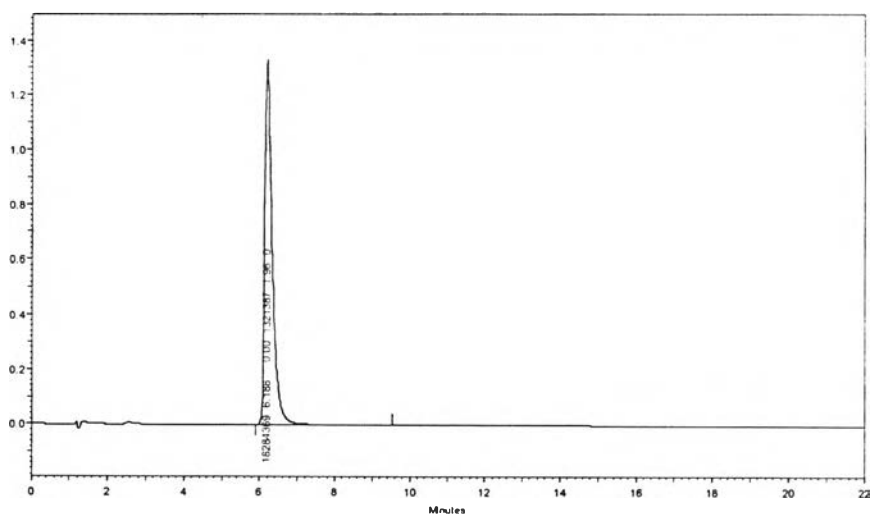
Figure 52. HPLC Chromatogram of Caffeine Reference Standard
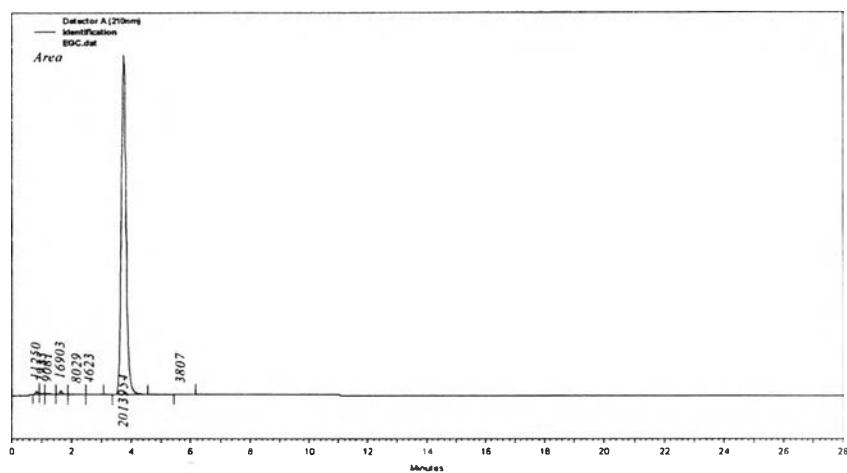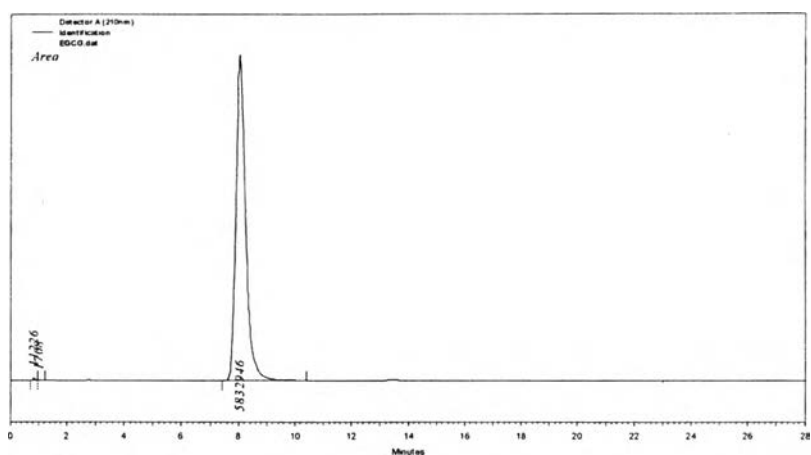
Figure 53. HPLC Chromatograme of EGCG Reference Standard
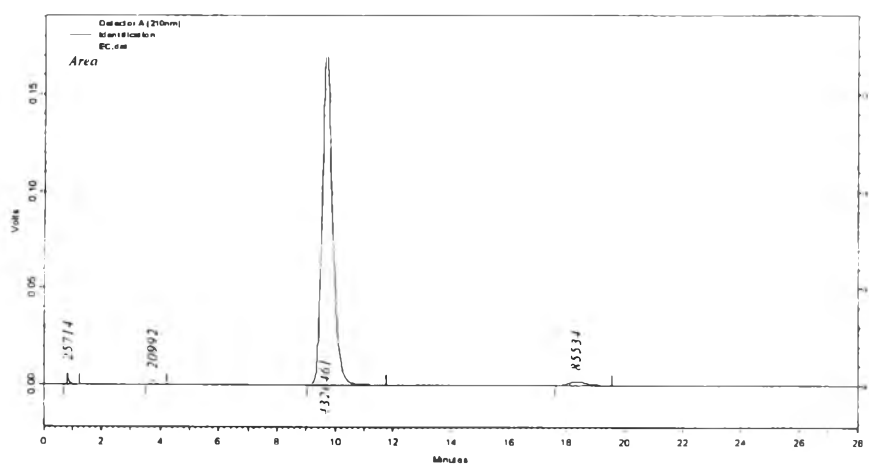
Figure 54. HPLC Chromatograme of EC Reference Standard
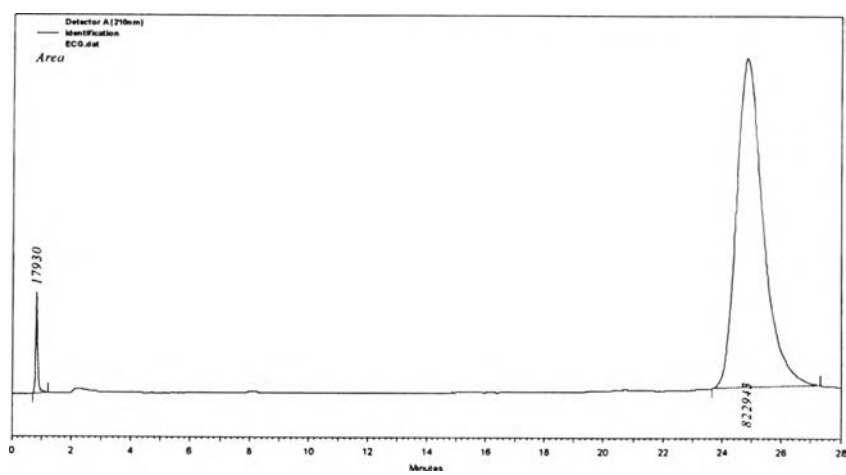
Figure 55. HPLC Chromatogram of ECG Reference Standard
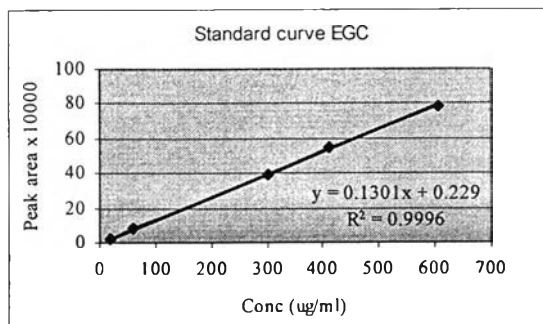
Figure 56. The calibration curve of the standard EGC
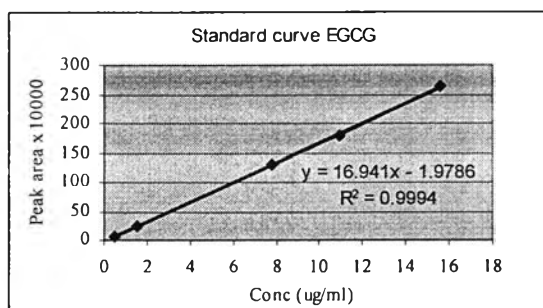
Figure 57. The calibration curve of the standard EGCG
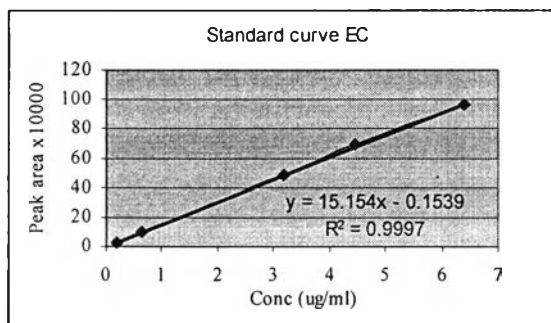
Figure 58. The calibration curve of the standard EC
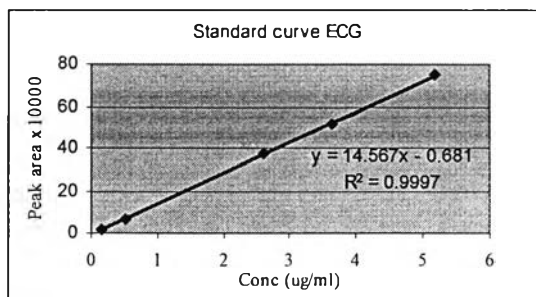
Figure 59. The calibration curve of the standard ECG

Detector A (210nm)						
Pk #	Name	Retention Time	Area	Height	Resolution	Asymmetry (10%)	Integration Codes
1	EGC	3.775	555490	49146	0.00	1.21	BB
2	EGCG	8.237	1684342	71015	9.79	1.22	BB
3	EC	9.866	156502	6709	2.64	1.29	BB
4	ECG	24.983	400582	6318	13.30	1.28	BB
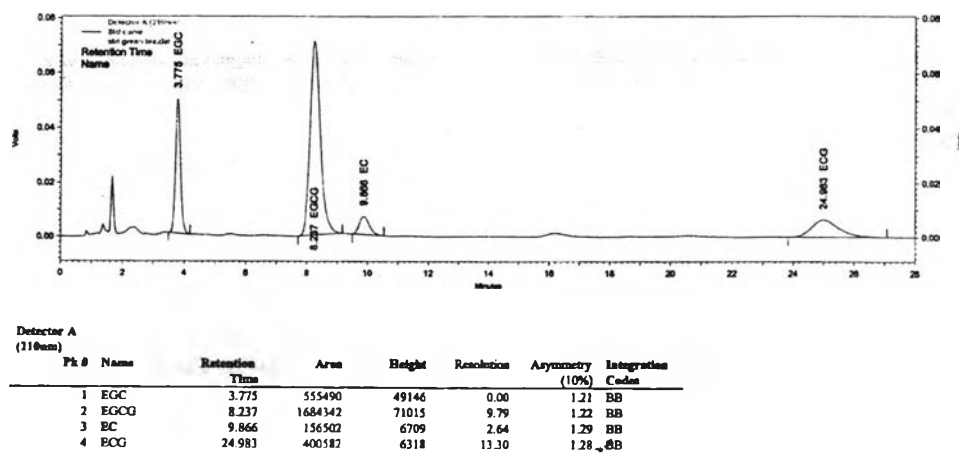
Figure 60. HPLC Chromatogram of Green tea extract

APPENDIX B

Validation data of green tea analytical method

Table 16. Accuracy data of EGC assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	%Recovery	Average (Range)	%RSD
1A	6.286	6.364	101.24	100.93	
1B	6.098	6.110	100.20	(100.20-101.35)	0.63
1C	6.173	6.256	101.35		
2A	6.926	7.001	101.09	100.90	
2B	6.888	6.987	101.44	(100.19-101.44)	0.64
2C	7.001	7.014	100.19		
3A	8.055	8.170	101.43	100.42	
3B	7.829	7.826	99.96	(99.87-101.43)	0.87
3C	7.904	7.894	99.87		
4A	8.318	8.272	99.44	99.11	
4B	8.469	8.364	98.76	(98.76-99.44)	0.34
4C	8.394	8.320	99.12		
5A	9.147	9.084	99.32	99.06	
5B	9.372	9.254	98.74	(98.74-99.32)	0.30
5C	9.297	9.216	99.13		

Table 17. Accuracy data of EGCG assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	%Recovery	Average (Range)	%RSD
1A	8.861	8.942	100.91	100.76	
1B	8.596	8.676	100.93	(100.44-100.93)	0.28
1C	8.702	8.740	100.44		
2A	9.763	9.796	100.34	100.32	
2B	9.710	9.806	100.99	(99.64-100.99)	0.67
2C	9.869	9.834	99.64		
3A	11.355	11.294	99.46	99.33	
3B	11.036	10.896	98.73	(98.73-99.80)	0.55
3C	11.143	11.120	99.80		
4A	11.726	11.668	99.50	99.38	
4B	11.939	11.884	99.54	(99.10-99.54)	0.25
4C	11.832	11.726	99.10		
5A	12.894	12.944	100.39	100.50	
5B	13.212	13.250	100.29	(100.29-100.83)	0.28
5C	13.106	13.214	100.83		

Table 18. Accuracy data of EC assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	%Recovery	Average (Range)	%RSD
1A	0.959	0.966	100.77	101.14	
1B	0.930	0.944	101.52	(100.77-101.52)	0.37
1C	0.941	0.952	101.13		
2A	1.056	1.066	100.93	100.94	
2B	1.050	1.064	101.29	(100.60-101.29)	0.35
2C	1.068	1.074	100.60		
3A	1.228	1.242	101.11	100.33	
3B	1.194	1.194	100.01	(99.88-101.11)	0.67
3C	1.205	1.204	99.88		
4A	1.269	1.266	99.80	99.63	
4B	1.292	1.284	99.42	(98.42-99.69)	0.20
4C	1.280	1.276	99.69		
5A	1.395	1.396	100.08	99.51	
5B	1.429	1.424	99.63	(98.82-100.08)	0.65
5C	1.418	1.401	98.82		

Table 19. Accuracy data of ECG assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	%Recovery	Average (Range)	%RSD
1A	2.381	2.386	100.19	100.71	
1B	2.310	2.338	101.21	(100.19-101.21)	0.50
1C	2.339	2.356	100.74		
2A	2.624	2.632	100.31	100.94	
2B	2.610	2.640	101.17	(100.31-101.34)	0.55
2C	2.652	2.688	101.34		
3A	3.052	3.082	100.99	100.12	
3B	2.966	2.944	99.26	(99.26-100.99)	0.87
3C	2.995	2.998	100.11		
4A	3.151	3.114	98.81	98.98	
4B	3.209	3.168	98.74	(98.74-99.40)	0.37
4C	3.180	3.161	99.40		
5A	3.465	3.446	99.45	99.18	
5B	3.551	3.514	98.97	(98.97-99.45)	0.25
5C	3.522	3.492	99.14		

Table 20. Precision data of EGC assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	%Recovery	Average (Range)	%RSD
3A	8.055	8.170	101.43	100.89	0.75
3B	7.829	7.826	99.96		
3C	7.641	7.748	101.40		
3D	7.791	7.902	101.42		
3E	7.904	7.894	99.87	(99.87-101.43)	
3 F	7.716	7.812	101.24		

Table 21. Precision of EGCG assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	%Recovery	Average (Range)	%RSD
3A	11.355	11.294	99.46	100.19	1.05
3B	11.036	10.896	98.73		
3C	10.771	10.824	100.49		
3D	10.983	11.138	101.41		
3E	11.143	11.122	99.82	(98.73-101.41)	
3 F	10.877	11.014	101.26		

Table 22. Precision data of EC assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	%Recovery	Average (Range)	%RSD
3A	1.228	1.246	101.44	100.33	0.95
3B	1.194	1.194	100.01		
3C	1.165	1.178	101.10		
3D	1.188	1.198	100.83		
3E	1.205	1.202	99.72	(98.92-101.44)	
3 F	1.177	1.164	98.92		

Table 23. Precision data of ECG assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	%Recovery	Average (Range)	%RSD
3A	3.052	3.082	100.99	100.16	0.69
3B	2.966	2.944	99.26		
3C	2.895	2.906	100.39		
3D	2.952	2.974	100.75		
3E	2.995	2.998	100.11	(99.26-100.99)	
3 F	2.923	2.908	99.48		

Table 24. Linearity data of EGC assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Average Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	Average Observed Conc. (mcg/ml)
1A	6.29		6.36	
1B	6.10	6.186	6.11	6.243
1C	6.17		6.26	
2A	6.93		7.00	
2B	6.89	6.938	7.02	7.012
2C	7.00		7.01	
3A	8.05		8.17	
3B	7.83	7.929	7.83	7.963
3C	7.90		7.89	
4A	8.32		8.27	
4B	8.47	8.394	8.36	8.319
4C	8.39		8.32	
5A	9.15		9.08	
5B	9.37	9.272	9.25	9.185
5C	9.30		9.22	

Figure 61. The representative linearity curve for EGC

Table 25. Linearity data of EGCG assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Average Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	Average Observed Conc. (mcg/ml)
1A	8.86		8.94	
1B	8.60	8.720	8.68	8.786
1C	8.70		8.74	
2A	9.76		9.80	
2B	9.71	9.781	9.81	9.812
2C	9.87		9.83	
3A	11.35		11.29	
3B	11.04	11.178	10.90	11.103
3C	11.14		11.12	
4A	11.73		11.67	
4B	11.94	11.832	11.88	11.759
4C	11.83		11.73	
5A	12.89		12.94	
5B	13.21	13.070	13.25	13.136
5C	13.11		13.21	

Linearity of EGCG
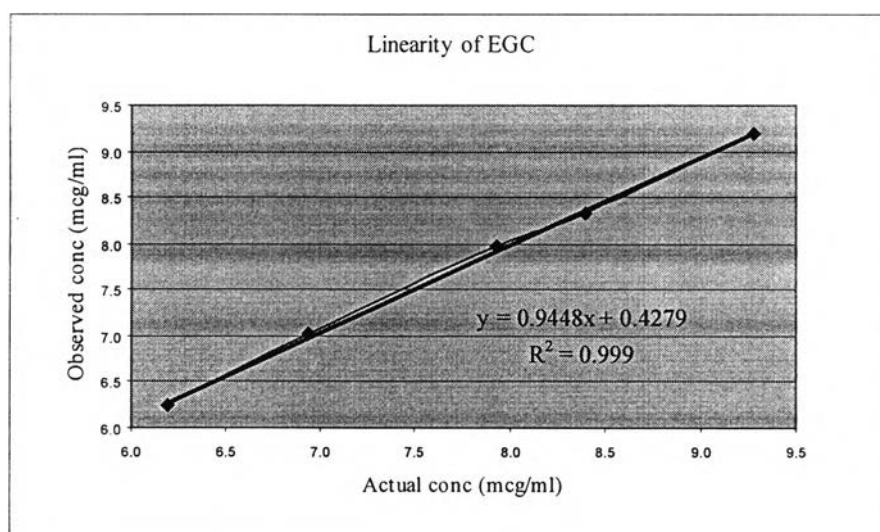
$y = 0.9893x + 0.1196$
$R^2 = 0.9983$

Figure 62. The representative linearity curve for EGCG

Table 26. Linearity data of EC assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Average Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	Average Observed Conc. (mcg/ml)
1A	0.959		0.966	
1B	0.930	0.943	0.944	0.954
1C	0.941		0.952	
2A	1.056		1.066	
2B	1.050	1.058	1.064	1.068
2C	1.068		1.074	
3A	1.228		1.25	
3B	1.194	1.209	1.194	1.216
3C	1.205		1.204	
4A	1.269		1.266	
4B	1.292	1.280	1.284	1.275
4C	1.280		1.276	
5A	1.395		1.396	
5B	1.429	1.414	1.424	1.407
5C	1.418		1.401	

Linearity of EC
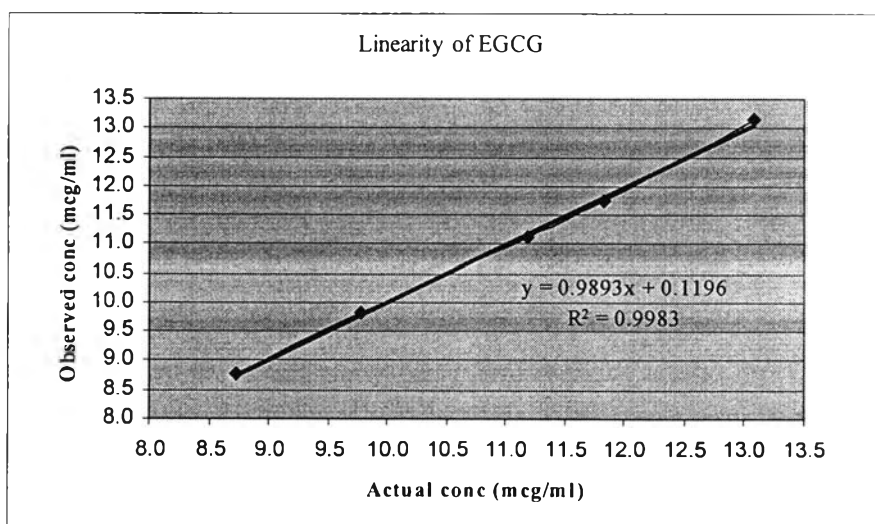
$y = 0.9577x + 0.0531$
$R^2 = 0.9996$

Figure 63.. The representative linearity curve for EC

Table 27. Linearity data of ECG assayed by the HPLC method

Samples	Actual Conc. (mcg/ml)	Average Actual Conc. (mcg/ml)	Observed Conc. (mcg/ml)	Average Observed Conc. (mcg/ml)
1A	2.381		2.386	
1B	2.310	2.343	2.338	2.360
1C	2.339		2.356	
2A	2.624		2.632	
2B	2.610	2.629	2.64	2.653
2C	2.652		2.688	
3A	3.052		3.082	
3B	2.966	3.004	2.944	3.008
3C	2.995		2.998	
4A	3.151		3.114	
4B	3.209	3.180	3.168	3.148
4C	3.180		3.161	
5A	3.465		3.446	
5B	3.551	3.513	3.514	3.484
5C	3.522		3.492	

Figure 64.. The representative linearity curve for ECG

Table 28. Conclusion data of green tea analytical method validation

Criteria	EGC	EGCG	EC	ECG
Accuracy % Recovery (98-102) % RSD (< 2)	98.74 - 101.44 0.30 - 0.87	98.73 -100.93 0.25 - 0.67	98.82 -101.52 0.20 - 0.67	98.74 - 101.4 0.25 - 0.87
Precision % RSD (< 2)	0.75	1.05	0.95	0.69
Linearity Equation Intercept R^2 (> 0.995)	y=1.9448x + 0.4279 0.4279 0.999	y =0.9893x + 0.1196 0.1196 0.998	y=0.9577x + 0.0531 0.0531 0.999	y=0.996x 0 0.997
Range (meg/ml)	6.186 - 9.272	8.720 - 13.070	0.943 -1.414	2.343 -3.513
Specificity	peak not overlap			

APPENDIX C

The optical density data

Table 29. The optical density data of melanin standard at different wave lengths

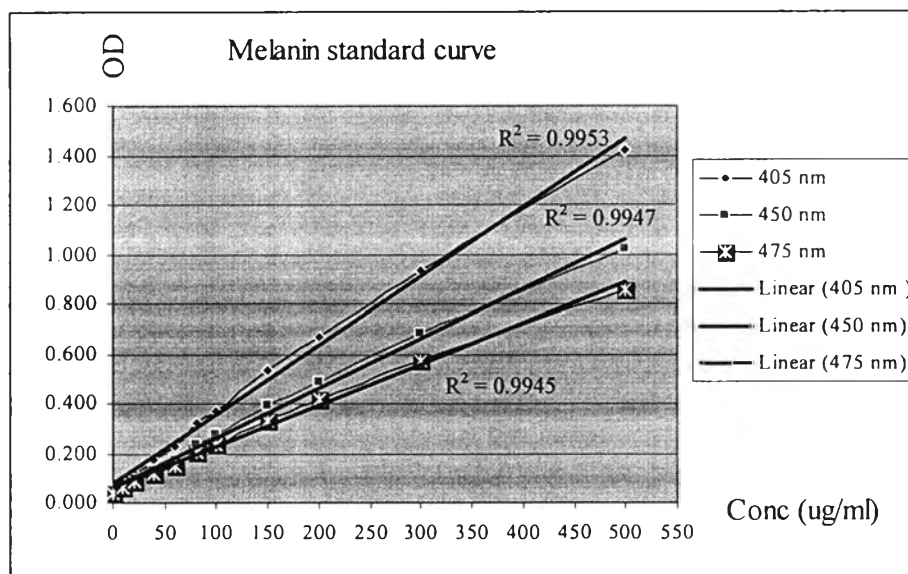| Melanin	Average OD		
conc (ug/ml)	405 nm	450 nm	475 nm
0	0.048	0.043	0.041
10	0.077	0.065	0.059
20	0.114	0.091	0.083
40	0.175	0.131	0.118
60	0.227	0.173	0.149
80	0.320	0.239	0.207
100	0.368	0.274	0.234
150	0.531	0.391	0.331
200	0.668	0.490	0.413
300	0.935	0.679	0.573
500	1.419	1.023	0.856

Figure 65. The calibration curve of the melanin standard at different wave lengths
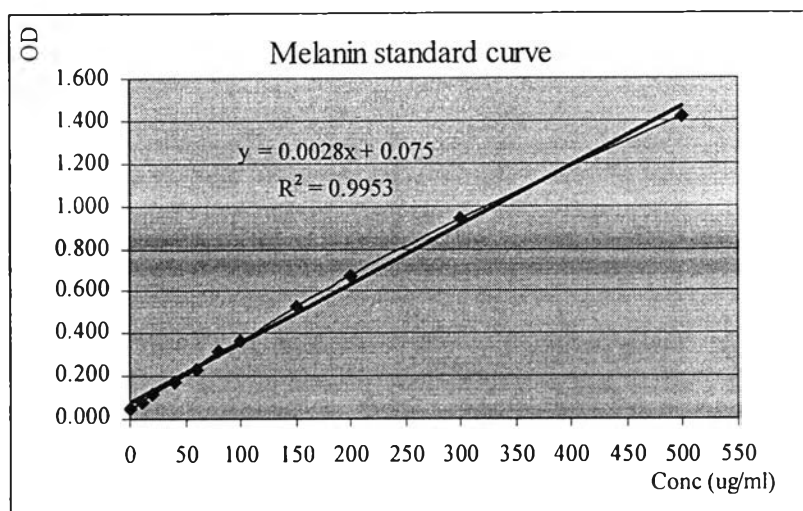
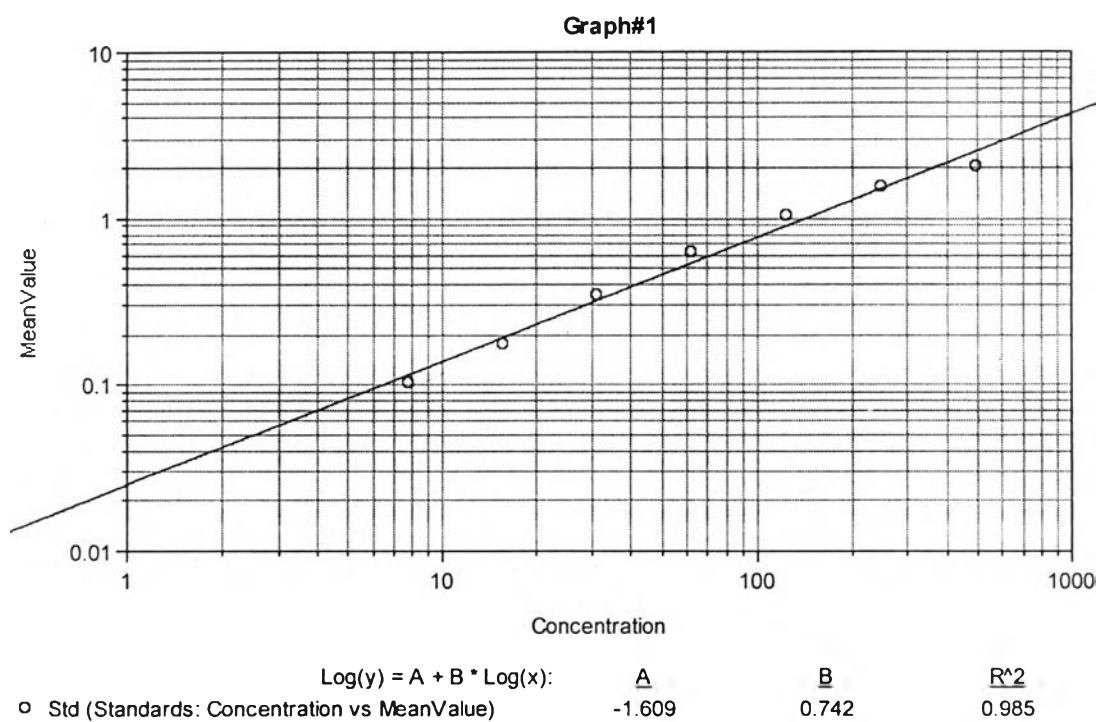Figure 66. The calibration curve of the melanin standard at 405 nm

Figure 67. The calibration curve of the IL1α standard at 450 nm

APPENDIX D

The statistical data from analysis of catechin contents in green tea extracts using HPLC

Analysis of variance; ANOVA by SPSS program

Table 30. Analysis data of catechin contents using different extracting methods

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
EGC	pH_3	3	4.5367	.28676	.16556	3.8243	5.2490	4.27	4.84
	pH_3.5	3	4.7000	.24880	.14364	4.0820	5.3180	4.43	4.92
	pH_4.0	3	4.1000	.22539	.13013	3.5401	4.6599	3.96	4.36
	pH4.5	3	4.9167	.25697	.14836	4.2783	5.5550	4.62	5.07
	DI	3	7.8567	.40526	.23398	6.8500	8.8634	7.46	8.27
	Total	15	5.2220	1.41314	.36487	4.4394	6.0046	3.96	8.27
ECGG	pH_3	3	7.6867	.35572	.20537	6.8030	8.5703	7.28	7.94
	pH_3.5	3	7.3267	.29006	.16746	6.6061	8.0472	7.04	7.62
	pH_4.0	3	7.4333	.32036	.18496	6.6375	8.2292	7.17	7.79
	pH4.5	3	7.7533	.41429	.23919	6.7242	8.7825	7.28	8.05
	DI	3	2.0767	.16442	.09493	1.6682	2.4851	1.89	2.20
	Total	15	6.4553	2.28805	.59077	5.1883	7.7224	1.89	8.05
EC	pH_3	3	.7900	.09849	.05686	.5453	1.0347	.68	.87
	pH_3.5	3	.8233	.12055	.06960	.5239	1.1228	.71	.95
	pH_4.0	3	.9533	.09018	.05207	.7293	1.1774	.86	1.04
	pH4.5	3	.7567	.08021	.04631	.5574	.9559	.68	.84
	DI	3	1.4933	.12342	.07126	1.1867	1.7999	1.39	1.63
	Total	15	.9633	.29618	.07647	.7993	1.1274	.68	1.63
ECG	pH_3	3	2.3967	.14468	.08353	2.0373	2.7561	2.23	2.49
	pH_3.5	3	2.1233	.12702	.07333	1.8078	2.4389	2.05	2.27
	pH_4.0	3	2.4767	.15308	.08838	2.0964	2.8569	2.30	2.57
	pH4.5	3	2.2133	.11372	.06566	1.9308	2.4958	2.12	2.34
	DI	3	.2767	.05033	.02906	.1516	.4017	.23	.33
	Total	15	1.8973	.85521	.22081	1.4237	2.3709	.23	2.57

Table 31. Multiple Comparisons of different extracting methods

Scheffe

Dependent Variable	(I) SAMPLE	(J) SAMPLE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EGC	pH_3	pH_3.5	-.1633	.23810	.973	-1.0514	.7248
		pH_4.0	.4367	.23810	.530	-.4514	1.3248
		pH4.5	-.3800	.23810	.648	-1.2681	.5081
		DI	-3.3200*	.23810	.000	-4.2081	-2.4319
	pH_3.5	pH_3	.1633	.23810	.973	-.7248	1.0514
		pH_4.0	.6000	.23810	.252	-.2881	1.4881
		pH4.5	-.2167	.23810	.929	-1.1048	.6714
		DI	-3.1567*	.23810	.000	-4.0448	-2.2686
	pH_4.0	pH_3	-.4367	.23810	.530	-1.3248	.4514
		pH_3.5	-.6000	.23810	.252	-1.4881	.2881
		pH4.5	-.8167	.23810	.076	-1.7048	.0714
		DI	-3.7567*	.23810	.000	-4.6448	-2.8686
	pH4.5	pH_3	.3800	.23810	.648	-.5081	1.2681
		pH_3.5	.2167	.23810	.929	-.6714	1.1048
		pH_4.0	.8167	.23810	.076	-.0714	1.7048
		DI	-2.9400*	.23810	.000	-3.8281	-2.0519
	DI	pH_3	3.3200*	.23810	.000	2.4319	4.2081
		pH_3.5	3.1567*	.23810	.000	2.2686	4.0448
		pH_4.0	3.7567*	.23810	.000	2.8686	4.6448
		pH4.5	2.9400*	.23810	.000	2.0519	3.8281
ECGG	pH_3	pH_3.5	.3600	.26127	.754	-.6145	1.3345
		pH_4.0	.2533	.26127	.912	-.7212	1.2278
		pH4.5	-.0667	.26127	.999	-1.0412	.9078
		DI	5.6100*	.26127	.000	4.6355	6.5845
	pH_3.5	pH_3	-.3600	.26127	.754	-1.3345	.6145
		pH_4.0	-.1067	.26127	.996	-1.0812	.8678
		pH4.5	-.4267	.26127	.629	-1.4012	.5478
		DI	5.2500*	.26127	.000	4.2755	6.2245
	pH_4.0	pH_3	-.2533	.26127	.912	-1.2278	.7212
		pH_3.5	.1067	.26127	.996	-.8678	1.0812
		pH4.5	-.3200	.26127	.821	-1.2945	.6545
		DI	5.3567*	.26127	.000	4.3822	6.3312
	pH4.5	pH_3	.0667	.26127	.999	-.9078	1.0412
		pH_3.5	.4267	.26127	.629	-.5478	1.4012
		pH_4.0	.3200	.26127	.821	-.6545	1.2945
		DI	5.6767*	.26127	.000	4.7022	6.6512
	DI	pH_3	-5.6100*	.26127	.000	-6.5845	-4.6355
		pH_3.5	-5.2500*	.26127	.000	-6.2245	-4.2755
		pH_4.0	-5.3567*	.26127	.000	-6.3312	-4.3822
		pH4.5	-5.6767*	.26127	.000	-6.6512	-4.7022

Table 31. Multiple Comparisons of different extracting methods (continue)

Scheffe

Dependent Variable	(I) SAMPLE	(J) SAMPLE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EC	pH_3	pH_3.5	-.0333	.08488	.997	-.3499	.2833
		pH_4.0	-.1633	.08488	.487	-.4799	.1533
		pH4.5	.0333	.08488	.997	-.2833	.3499
		DI	-.7033*	.08488	.000	-1.0199	-.3867
	pH_3.5	pH_3	.0333	.08488	.997	-.2833	.3499
		pH_4.0	-.1300	.08488	.680	-.4466	.1866
		pH4.5	.0667	.08488	.957	-.2499	.3833
		DI	-.6700*	.08488	.000	-.9866	-.3534
	pH_4.0	pH_3	.1633	.08488	.487	-.1533	.4799
		pH_3.5	.1300	.08488	.680	-.1866	.4466
		pH4.5	.1967	.08488	.320	-.1199	.5133
		DI	-.5400*	.08488	.002	-.8566	-.2234
	pH4.5	pH_3	-.0333	.08488	.997	-.3499	.2833
		pH_3.5	-.0667	.08488	.957	-.3833	.2499
		pH_4.0	-.1967	.08488	.320	-.5133	.1199
		DI	-.7367*	.08488	.000	-1.0533	-.4201
	DI	pH_3	.7033*	.08488	.000	.3867	1.0199
		pH_3.5	.6700*	.08488	.000	.3534	.9866
		pH_4.0	.5400*	.08488	.002	.2234	.8566
		pH4.5	.7367*	.08488	.000	.4201	1.0533
ECG	pH_3	pH_3.5	.2733	.10064	.197	-.1021	.6487
		pH_4.0	-.0800	.10064	.955	-.4554	.2954
		pH4.5	.1833	.10064	.536	-.1921	.5587
		DI	2.1200*	.10064	.000	1.7446	2.4954
	pH_3.5	pH_3	-.2733	.10064	.197	-.6487	.1021
		pH_4.0	-.3533	.10064	.068	-.7287	.0221
		pH4.5	-.0900	.10064	.933	-.4654	.2854
		DI	1.8467*	.10064	.000	1.4713	2.2221
	pH_4.0	pH_3	.0800	.10064	.955	-.2954	.4554
		pH_3.5	.3533	.10064	.068	-.0221	.7287
		pH4.5	.2633	.10064	.223	-.1121	.6387
		DI	2.2000*	.10064	.000	1.8246	2.5754
	pH4.5	pH_3	-.1833	.10064	.536	-.5587	.1921
		pH_3.5	.0900	.10064	.933	-.2854	.4654
		pH_4.0	-.2633	.10064	.223	-.6387	.1121
		DI	1.9367*	.10064	.000	1.5613	2.3121
	DI	pH_3	-2.1200*	.10064	.000	-2.4954	-1.7446
		pH_3.5	-1.8467*	.10064	.000	-2.2221	-1.4713
		pH_4.0	-2.2000*	.10064	.000	-2.5754	-1.8246
		pH4.5	-1.9367*	.10064	.000	-2.3121	-1.5613

*. The mean difference is significant at the .05 level.

VITA

Miss Panpen Diawpanich was born on March 1, 1951 in Suratthanee,Thailand. She received her Bachelor Degree in Pharmaceutical Science from Faculty of Pharmaceutical Sciences, Chulalongkorn University in 1974 and Master Degree in Business Administration from Kasetsart University in 2000. After graduation, she worked as analytical chemist at JAWA Manufacturing Co., Ltd., Bangkok during 1974-1978, cosmetic researcher during 1978-1981, and plant manager during 1981-2004 at J.M.T. Laboratories Co.,Ltd., Bangkok, Thailand.