APPENDICES

APPENDIX A

Table A.1 Calibration curve of chromone (I) in ACN for binding studies

[I] (mM)	Absorbance (λ_{max} = 294 nm)				SD
	1st	2nd	3rd	Average	
0.068	0.479	0.463	0.469	0.470	0.008
0.137	0.908	0.934	0.954	0.932	0.023
0.205	1.381	1.427	1.397	1.402	0.023
0.274	1.862	1.856	1.880	1.866	0.012
0.342	2.315	2.259	2.289	2.288	0.028

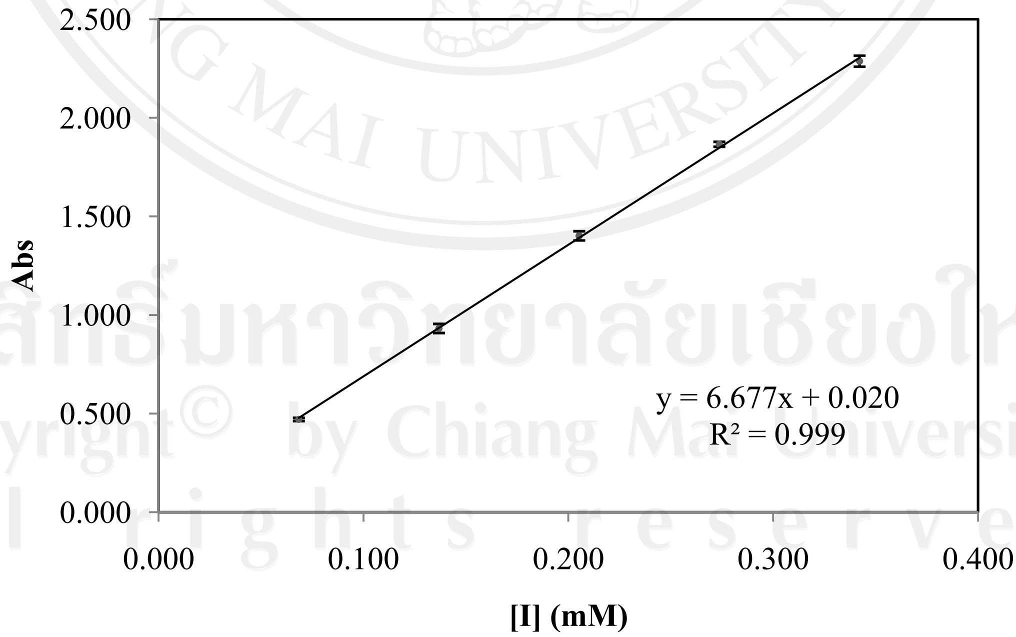


Figure A.1 Calibration curve of chromone (I) in ACN for binding studies

Table A.2 Calibration curve of 4-hydroxyphenylacetic acid (III) in ACN for binding studies

[III] (mM)	Absorbance (λ_{max} = 277 nm)				SD
	1st	2nd	3rd	Average	
0.066	0.107	0.098	0.103	0.103	0.005
0.131	0.214	0.200	0.208	0.207	0.007
0.263	0.424	0.403	0.416	0.414	0.011
0.394	0.640	0.624	0.623	0.629	0.010
0.526	0.864	0.839	0.834	0.834	0.016
0.657	1.076	1.043	1.039	1.053	0.020

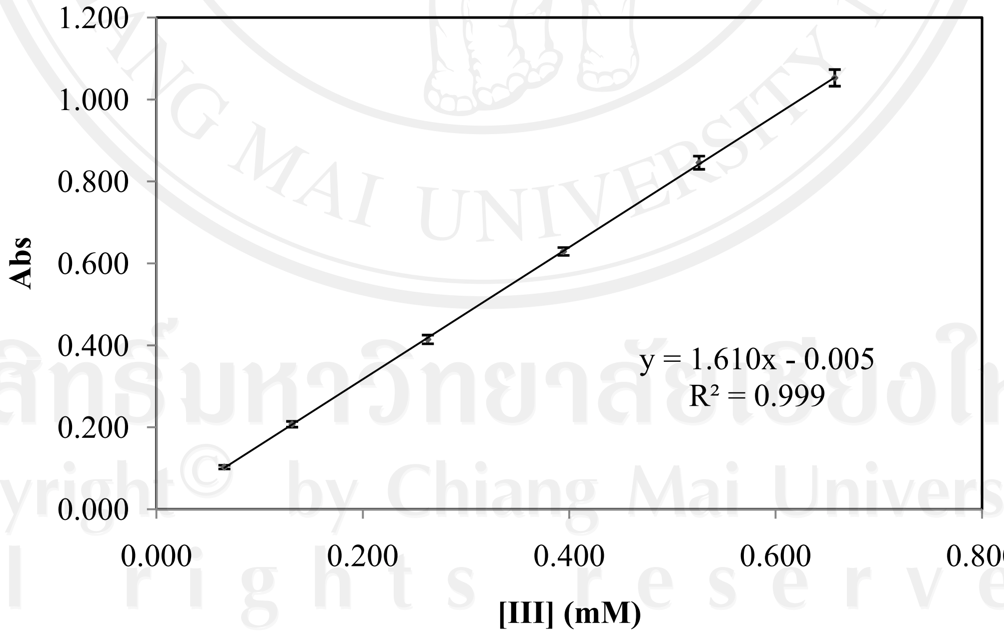


Figure A.2 Calibration curve of 4-hydroxyphenylacetic (III) acid in ACN for binding studies

Table A.3 Calibration curve of genistein in ACN for binding studies

[Genistein] (ppm)	Absorbance (λ_{max} = 260 nm)				SD
	1st	2nd	3rd	Average	
0.005	0.256	0.216	0.225	0.232	0.021
0.010	0.407	0.413	0.438	0.419	0.016
0.020	0.811	0.833	0.842	0.829	0.016
0.040	1.650	1.656	1.639	1.648	0.009
0.060	2.347	2.388	2.344	2.360	0.025

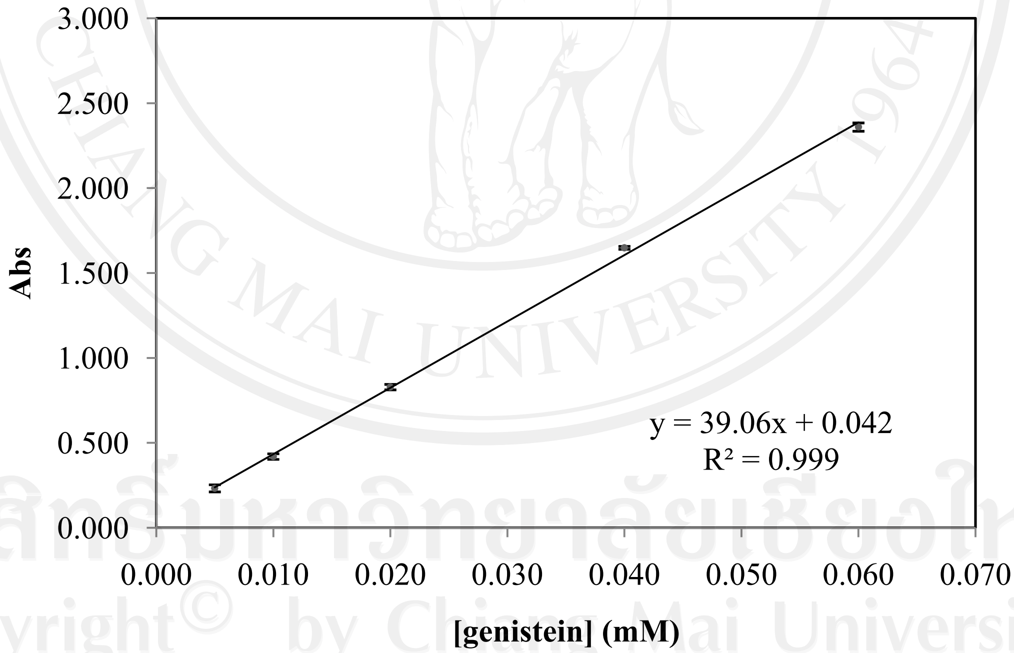


Figure A.3 Calibration curve of genistein in ACN for binding studies

Table A.4 The binding study of 0.30 mM chromone in ACN with 50 mg of PI-AA and its NIP

Polymers	Absorbance (λ_{max} = 294 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-AA	1.538	1.429	1.511	1.493	18.12	3.11
NIP	1.486	1.582	1.472	1.513	16.99	3.28

Table A.5 The binding study of 0.30 mM chromone in ACN with 50 mg of PI-MAA and its NIP

Polymers	Absorbance (λ_{max} = 294 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-MAA	1.526	1.488	1.497	1.504	17.52	1.09
NIP	1.514	1.478	1.462	1.485	18.56	1.46

Table A.6 The binding study of 0.30 mM chromone in ACN with 50 mg of PI-VP and its NIP

Polymers	Absorbance (λ_{max} = 294 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-VP	1.584	1.451	1.562	1.532	15.94	3.91
NIP	1.488	1.549	1.445	1.494	18.05	2.87

Table A.7 The binding study of 0.30 mM 4-hydroxyphenylacetic acid in ACN with 50 mg of PIII-AA and its NIP

Polymers	Absorbance (λ_{max} = 277 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-AA	0.370	0.343	0.368	0.360	24.14	3.17
NIP	0.276	0.299	0.301	0.292	38.53	2.92

Table A.8 The binding study of 0.30 mM 4-hydroxyphenylacetic acid in ACN with 50 mg of PIII-MAA and its NIP

Polymers	Absorbance (λ_{max} = 277 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-MAA	0.363	0.379	0.385	0.376	20.91	2.39
NIP	0.406	0.372	0.369	0.382	22.00	0.45

Table A.9 The binding study of 0.30 mM 4-hydroxyphenylacetic acid in ACN with 50 mg of PIII-VP and its NIP

Polymers	Absorbance (λ_{max} = 277 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-VP	0.174	0.128	0.156	0.153	65.92	5.17
NIP	0.278	0.299	0.289	0.289	35.57	2.34

Table A.10 The binding study of 0.10 mM genistein in ACN with 50 mg of PI-AA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-AA	1.620	1.640	1.682	1.647	22.19	1.49
NIP	1.629	1.603	1.683	1.638	22.61	1.93

Table A.11 The binding study of 0.10 mM genistein in ACN with 50 mg of PI-MAA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-MAA	1.816	1.780	1.835	1.810	14.49	1.32
NIP	1.798	1.670	1.727	1.732	18.20	3.03

Table A.12 The binding study of 0.10 mM genistein in ACN with 50 mg of PI-VP and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-VP	1.471	1.438	1.481	1.463	30.88	1.06
NIP	1.468	1.458	1.448	1.458	31.13	0.47

Table A.13 The binding study of 0.10 mM genistein in DCM with 50 mg of PI-AA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-AA	0.842	0.809	0.803	0.818	50.21	1.28
NIP	0.608	0.832	0.691	0.710	56.77	6.89

Table A.14 The binding study of 0.10 mM genistein in DCM with 50 mg of PI-MAA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-MAA	1.225	1.116	1.149	1.163	29.19	3.40
NIP	1.142	1.085	1.099	1.109	32.52	1.81

Table A.15 The binding study of 0.10 mM genistein in DCM with 50 mg of PI-VP and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PI-VP	0.786	0.757	0.737	0.760	53.74	1.50
NIP	0.782	0.757	0.785	0.775	52.85	0.94

Table A.16 The binding study of 0.10 mM genistein in ACN with 50 mg of PII-AA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PII-AA	1.663	1.646	1.704	1.671	21.07	1.41
NIP	1.675	1.651	1.686	1.671	21.08	0.85

Table A.17 The binding study of 0.10 mM genistein in ACN with 50 mg of PII-MAA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PII-MAA	1.791	1.830	1.796	1.806	14.71	1.00
NIP	1.720	1.809	1.751	1.760	16.86	2.13

Table A.18 The binding study of 0.10 mM genistein in ACN with 50 mg of PII-VP and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PII-VP	1.746	1.707	1.696	1.716	18.93	1.24
NIP	1.586	1.578	1.569	1.578	25.48	0.40

Table A.19 The binding study of 0.10 mM genistein in DCM with 50 mg of PII-AA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PII-AA	0.849	0.798	0.701	0.783	52.36	4.58
NIP	0.968	0.816	0.941	0.908	44.71	4.94

Table A.20 The binding study of 0.10 mM genistein in DCM with 50 mg of PII-MAA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PII-MAA	1.142	1.109	1.099	1.117	32.05	1.37
NIP	1.161	1.080	1.138	1.126	31.45	2.54

Table A.21 The binding study of 0.10 mM genistein in DCM with 50 mg of PII-VP and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PII-VP	1.066	1.062	1.038	1.055	35.77	0.92
NIP	0.792	0.790	0.785	0.789	51.98	0.22

Table A.22 The binding study of 0.10 mM genistein in ACN with 50 mg of PIII-AA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-AA	1.885	1.893	1.857	1.878	11.27	0.89
NIP	1.629	1.603	1.683	1.638	22.61	1.93

Table A.23 The binding study of 0.10 mM genistein in ACN with 50 mg of PIII-MAA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-MAA	1.812	1.814	1.836	1.821	14.00	0.63
NIP	1.720	1.809	1.751	1.760	16.86	2.13

Table A.24 The binding study of 0.10 mM genistein in ACN with 50 mg of PIII-VP and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-VP	1.277	1.342	1.376	1.332	37.10	2.38
NIP	1.468	1.458	1.448	1.458	31.13	0.47

Table A.25 The binding study of 0.10 mM genistein in DCM with 50 mg of PIII-AA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-AA	1.074	1.147	1.160	1.127	31.41	2.82
NIP	0.608	0.832	0.691	0.710	56.77	6.89

Table A.26 The binding study of 0.10 mM genistein in DCM with 50 mg of PIII-MAA and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-MAA	1.012	1.067	1.089	1.056	35.73	2.41
NIP	1.161	1.080	1.138	1.126	31.45	2.54

Table A.27 The binding study of 0.10 mM genistein in DCM with 50 mg of PIII-VP and its NIP

Polymers	Absorbance (λ_{max} = 260 nm)				% Bound	SD
	1st	2nd	3rd	Average		
PIII-VP	0.560	0.588	0.543	0.564	65.69	1.38
NIP	0.782	0.757	0.785	0.775	52.85	0.94

Table A.28 Calibration curve of genistein in ACN using 2-napthol internal standard for MISPE from genistein standard

[genistein] (ppm)	Area ratio (λ_{max} = 260 nm)				SD
	1st	2nd	3rd	Average	
4	0.799	0.800	0.674	0.757	0.073
12	1.750	1.865	1.844	1.820	0.061
20	2.884	2.965	3.045	2.965	0.081
30	4.482	4.238	4.192	4.304	0.156
40	5.408	5.480	5.514	5.468	0.054

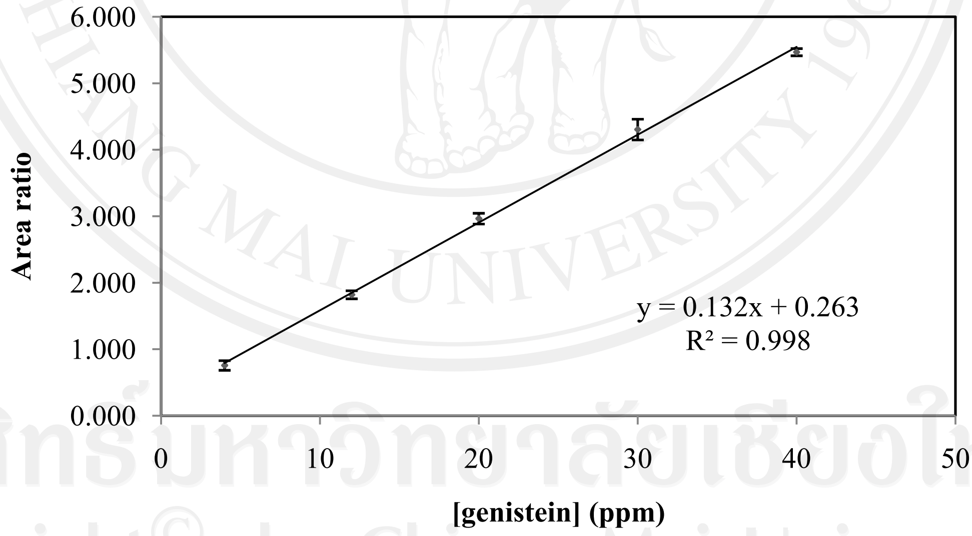


Figure A.4 Calibration curve of genistein in ACN using 2-napthol internal standard for MISPE from genistein standard

Table A.29 Calibration curve of quercetin in ACN using 2-napthol internal standard for MISPE from quercetin standard

[quercetin] (ppm)	Area ratio (λ_{max} = 260 nm)				SD
	1st	2nd	3rd	Average	
2	0.081	0.095	0.081	0.086	0.008
6	0.242	0.244	0.271	0.252	0.016
10	0.430	0.406	0.404	0.413	0.015
15	0.613	0.636	0.654	0.634	0.020
20	0.813	0.860	0.807	0.827	0.029

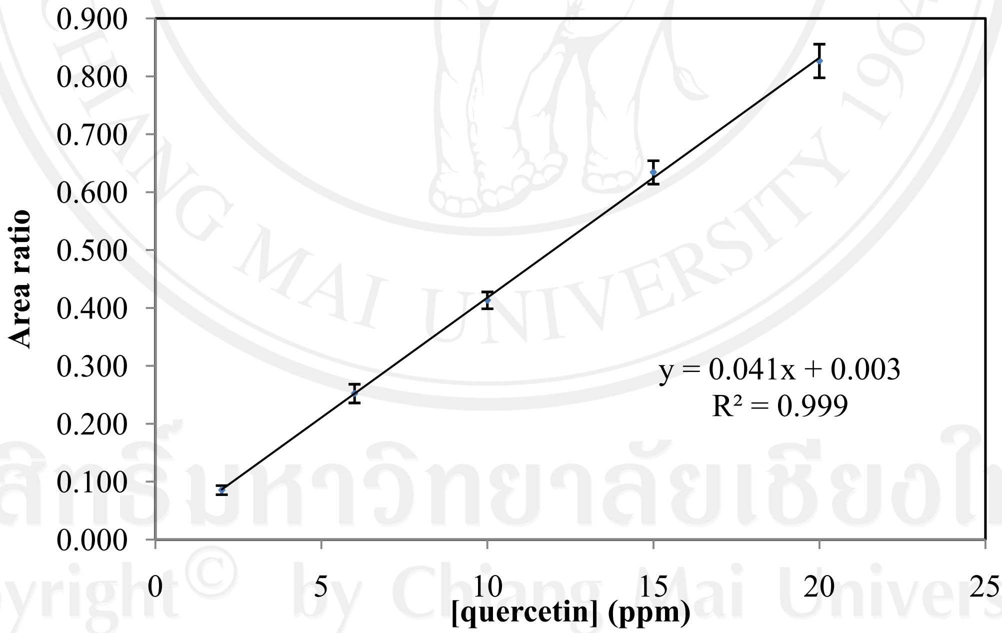


Figure A.5 Calibration curve of quercetin in ACN using 2-napthol internal standard for MISPE from quercetin standard

Table A.30 Percentage of genistein recovery from PIII-VP and its NIP after MISPE process in various using washing conditions

Washing Conditions*	Polymers	Area ratio (λ_{max} = 260 nm)				% Recovery	SD
		1st	2nd	3rd	Avg.		
1	PIII-VP	1.894	1.924	1.868	1.895	63.91	0.95
	NIP	1.840	1.915	1.933	1.896	63.96	1.66
2	PIII-VP	1.740	1.761	1.722	1.741	58.74	0.87
	NIP	1.886	1.918	1.853	1.886	63.61	1.54
3	PIII-VP	2.550	2.588	2.514	2.551	86.03	1.24
	NIP	2.638	2.601	2.674	2.638	88.96	1.22
4	PIII-VP	1.402	1.370	1.399	1.390	46.71	0.78
	NIP	1.550	1.482	1.493	1.508	50.97	1.40

*Washing conditions;

(1) 40% MeOH in water

(2) 20% ACN in water

(3) 0.1% FA in 20% ACN in water

(4) 0.1% FA in 30% ACN in water

Table A.31 Percentage of genistein recovery from PIII-VP after MISPE process in various eluting conditions

Eluting Conditions*	Area ratio (λ_{max} = 260 nm)				%Recovery	SD
	1st	2nd	3rd	Average		
1	1.346	1.393	1.465	1.401	47.27	1.64
2	2.395	2.588	2.514	2.499	84.29	2.66
3	2.176	2.123	2.221	2.173	73.29	1.34

*Eluting conditions;

(1) ACN

(2) 1% FA in ACN

(3) 1% TEA in ACN

Table A.32 Calibration curve of genistein in ACN using 2-napthol as internal standard for MISPE of genistein from soybean extracts

[genistein] (ppm)	Area ratio (λ_{max} = 260 nm)				SD
	1st	2nd	3rd	Average	
4	0.776	0.782	0.676	0.745	0.06
12	1.740	1.783	1.897	1.807	0.08
20	2.955	2.785	2.912	2.884	0.09
30	4.212	4.384	4.230	4.275	0.09
40	5.686	5.924	5.803	5.805	0.12

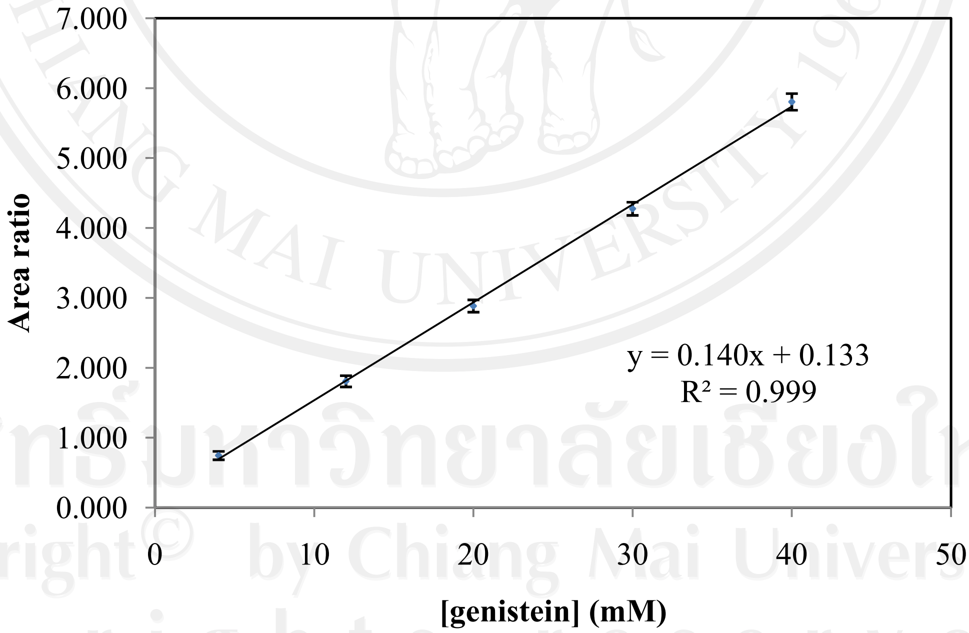


Figure A.6 Calibration curve of genistein in ACN using 2-napthol internal standard for MISPE of genistein from soybean extracts

Table A.33 The percentage of genistein recovery from MISPE process of PIII-VP in soybean extracts

Sample	Area ratio (λ_{max} = 260 nm)				% Recovery	SD
	1st	2nd	3rd	Avg		
MIP	2.919	2.567	2.219	2.568	73.08	9.95
NIP	2.654	2.655	2.328	2.545	72.42	5.37

Table A.34 The percentage of genistein recovery from MISPE process of PI-II-III in soybean extracts

Sample	Area ratio (λ_{max} = 260 nm)				% Recovery	SD
	1st	2nd	3rd	Avg		
MIP	2.463	2.757	2.739	2.653	74.85	4.30
NIP	2.538	2.856	2.741	2.712	76.52	4.53

APPENDIX B

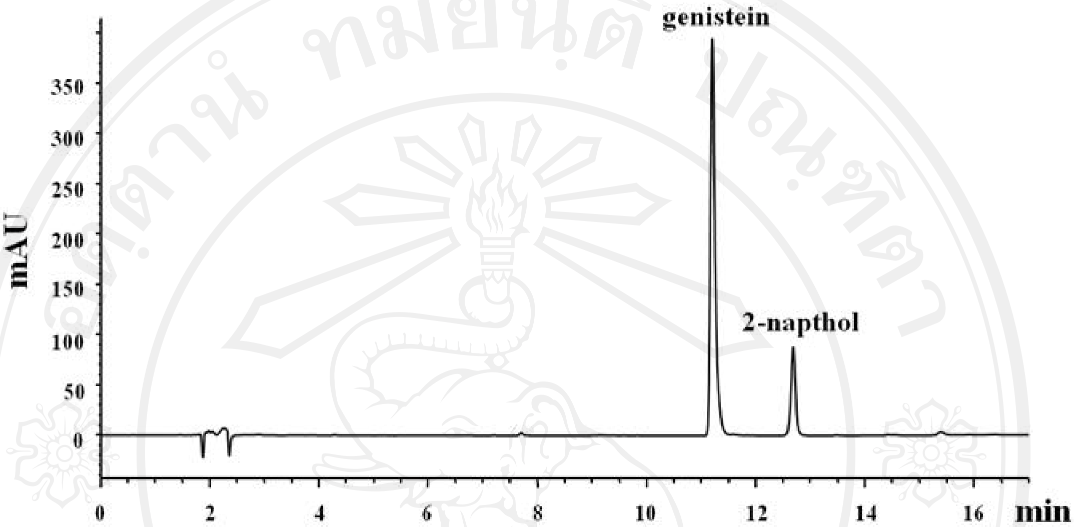


Figure B.1 Representative HPLC chromatogram of genistein in optimization of MISPE process

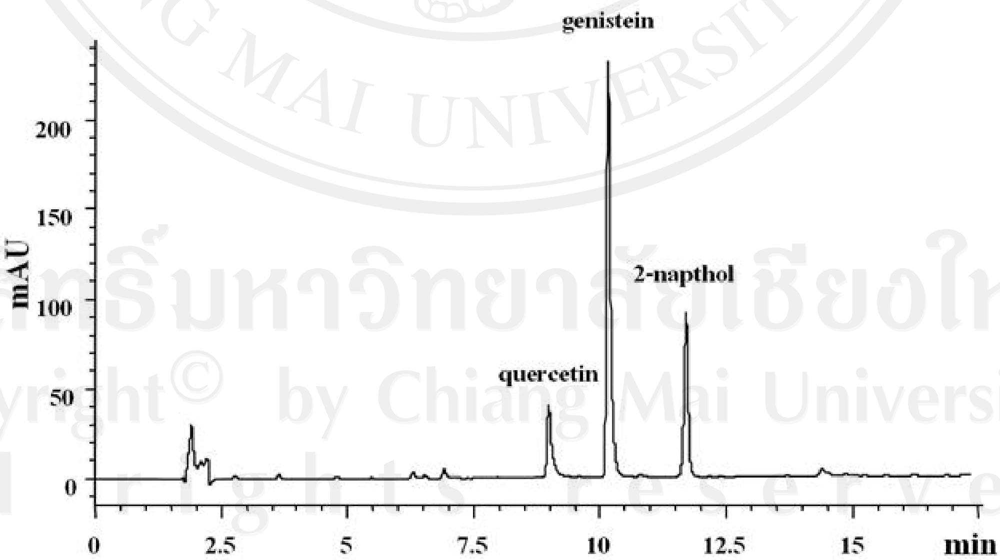
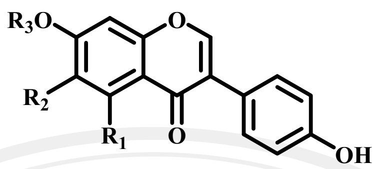


Figure B.2 Representative HPLC chromatogram of genistein and quercetin in the study of MISPE selectivity



Name	$\mathbf{R_1}$	$\mathbf{R_2}$	$\mathbf{R_3}$	**MW**
Daidzein	H	H	H	254
Glycitein	H	OCH_3	H	284
Genistein	OH	H	H	270
Daidzin	H	H	Glu	416
Glycitin	H	OCH_3	Glu	446
Genistin	OH	H	Glu	432
Acetyldaidzin	H	H	Glu-$COCH_3$	458
Acetylglycitin	H	OCH_3	Glu-$COCH_3$	488
Acetylgenistein	OH	H	Glu-$COCH_3$	474
Malonyldaidzin	H	H	Glu-$COCH_2COOH$	502
Malonylglycitin	H	OCH_3	Glu-$COCH_2COOH$	532
Malonylgenistein	OH	H	Glu-$COCH_2COOH$	518

Figure B.3 Structural formula and molecular weight of the main isoflavones detected in soybean[29,72]

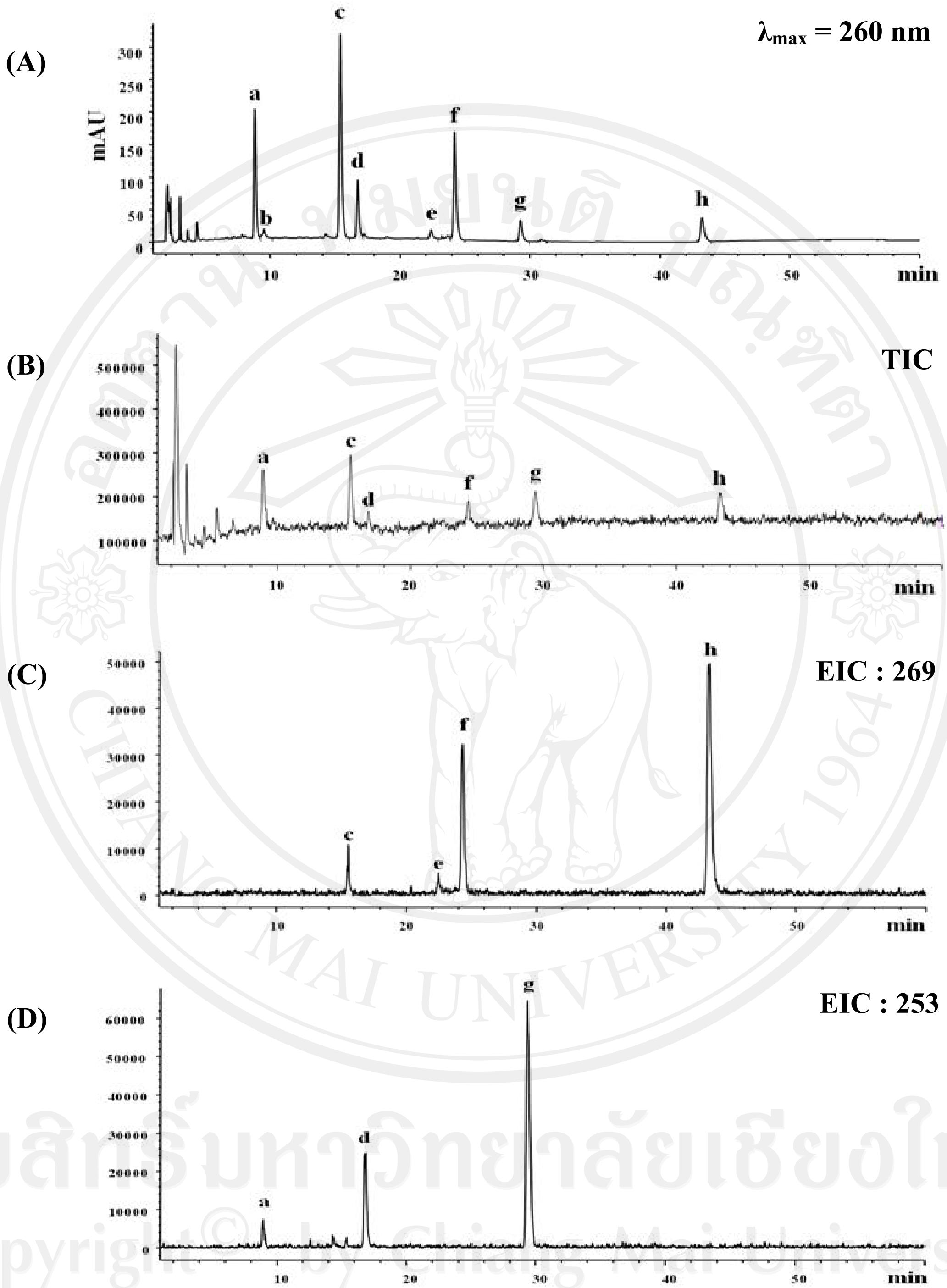


Figure B.4 The HPLC-UV **(A)** and MS **(B-D)** chromatograms of soybean extract. **(B)** Total ion chromatogram; **(C)** reconstructed ion chromatogram for genistein and its derivatives; **(D)** reconstructed ion chromatogram for daidzein and its derivatives.

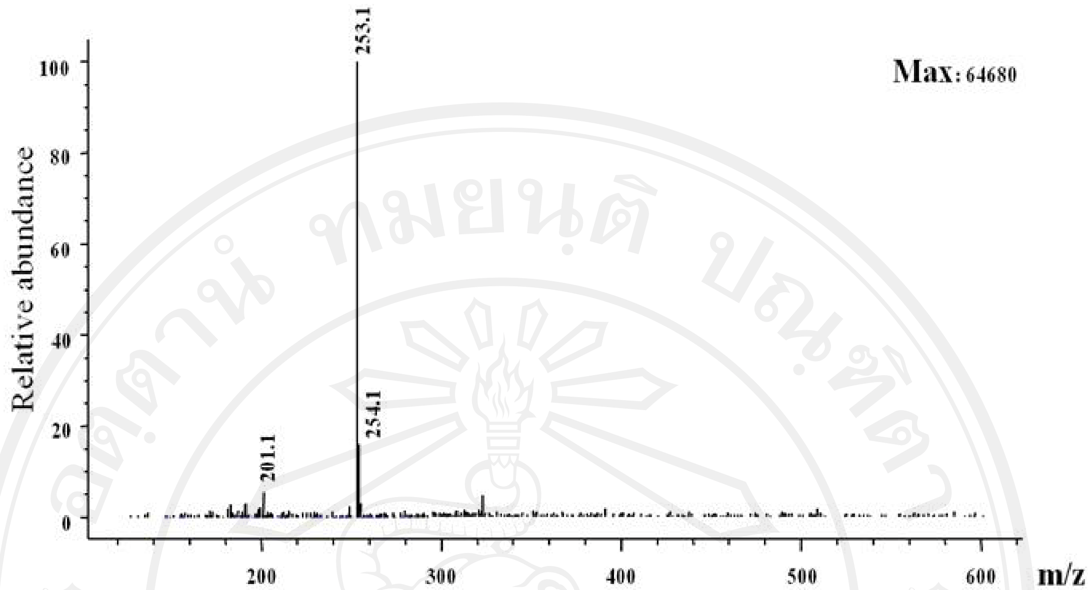


Figure B.5 Mass spectrum of diadzein (**g**) obtained from soybean extract

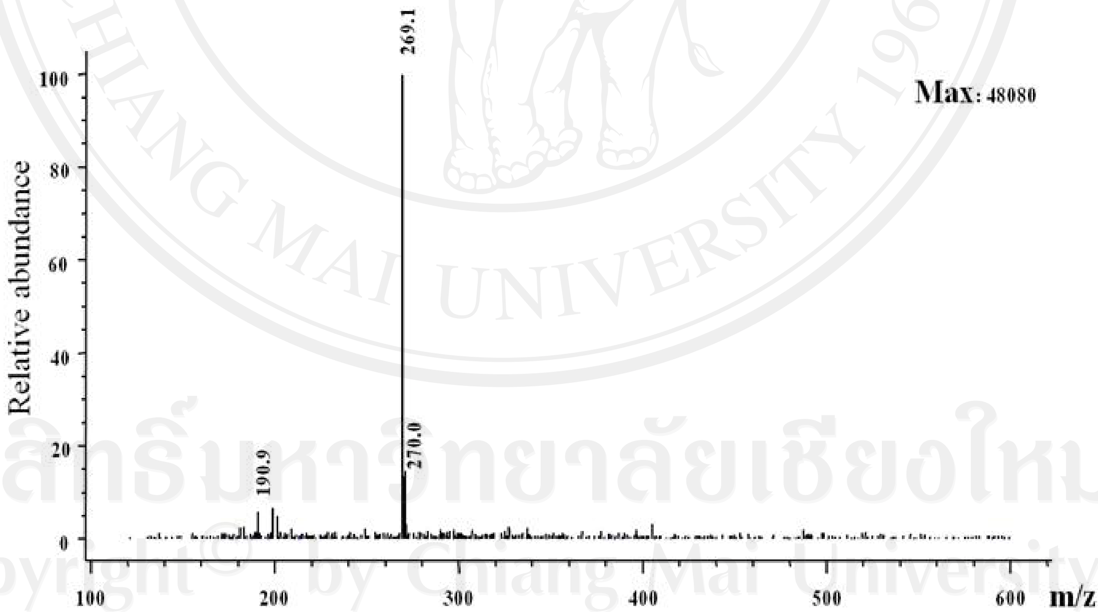


Figure B.6 Mass spectrum of genistein (**h**) obtained from soybean extract

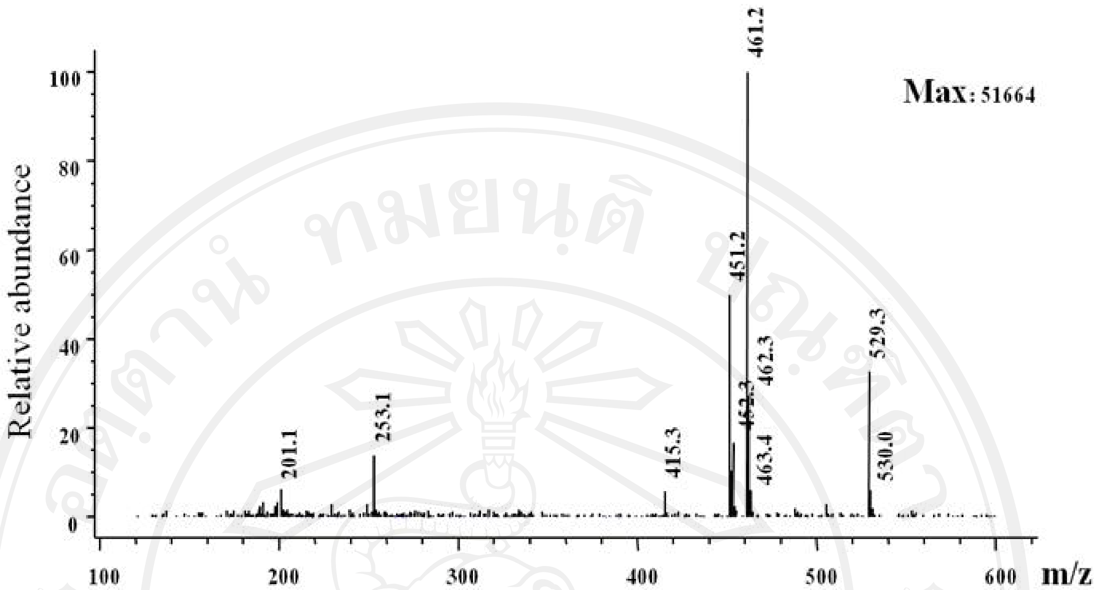


Figure B.7 Mass spectra of malonyl daidzin (**a**) obtained from soybean extract

Table B.1 Calculation of genistein relative content in soybean extract

Calculation of	**Equation**	
Area ratio (A_{ri})	$A_{ri} = \frac{A_i}{A_{IS}}$ Where A_i = peak area of peak i i = a, b, c …h	**(B.1)**
Total area ration (A_{Tr})	$A_{Tr} = A_{ra} + A_{rb} + A_{rc} + \cdots A_{rh}$	**(B.2)**
% Relative content	$\%\ \text{Relative content} = \frac{A_{ri}}{A_{Tr}} \times 100$	**(B.3)**

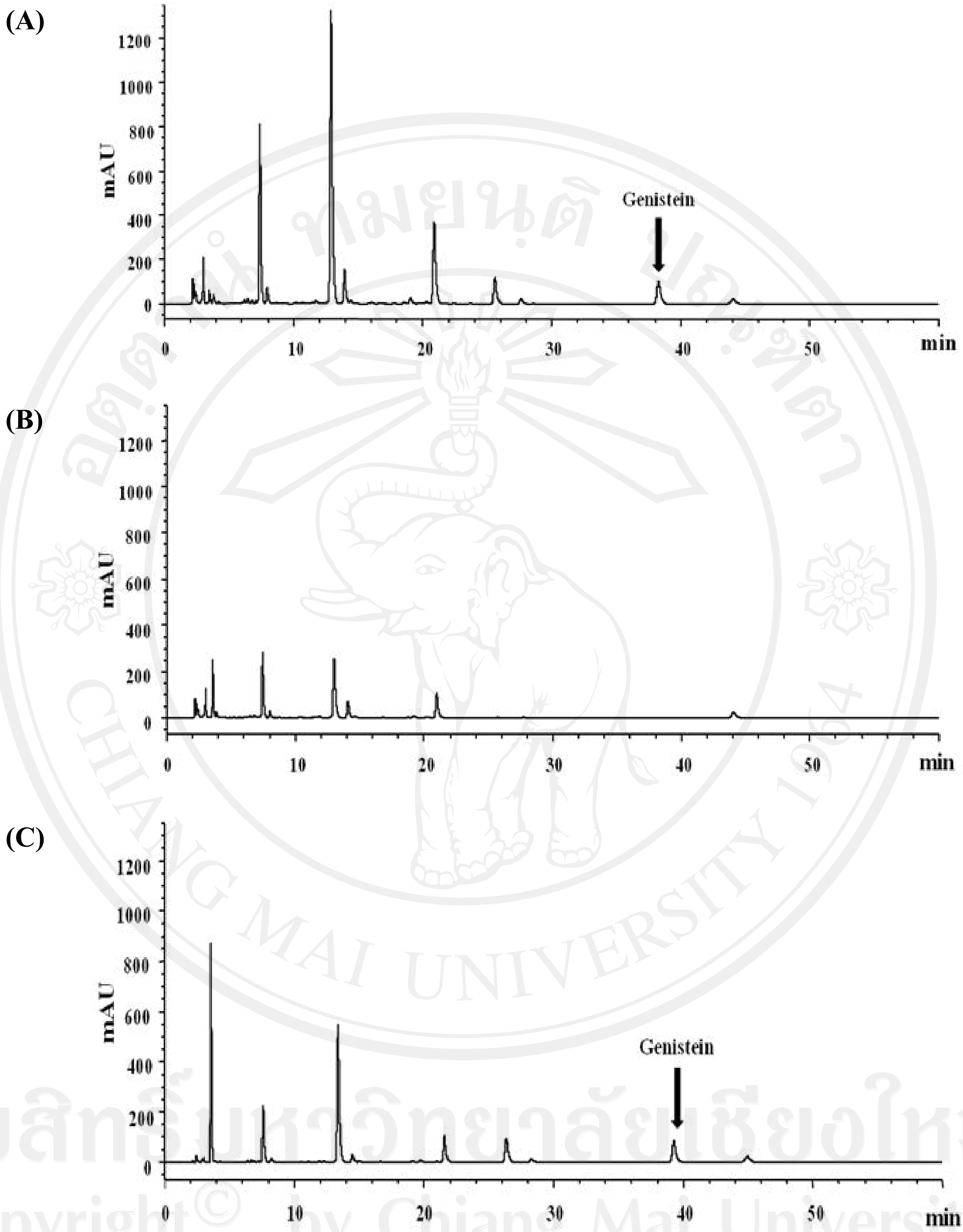


Figure B.8 Representative HPLC chromatogram of soybean extracts; **(A)** before MISPE process; **(B)** after loaded solution; **(C)** after MISPE process using PIII-VP

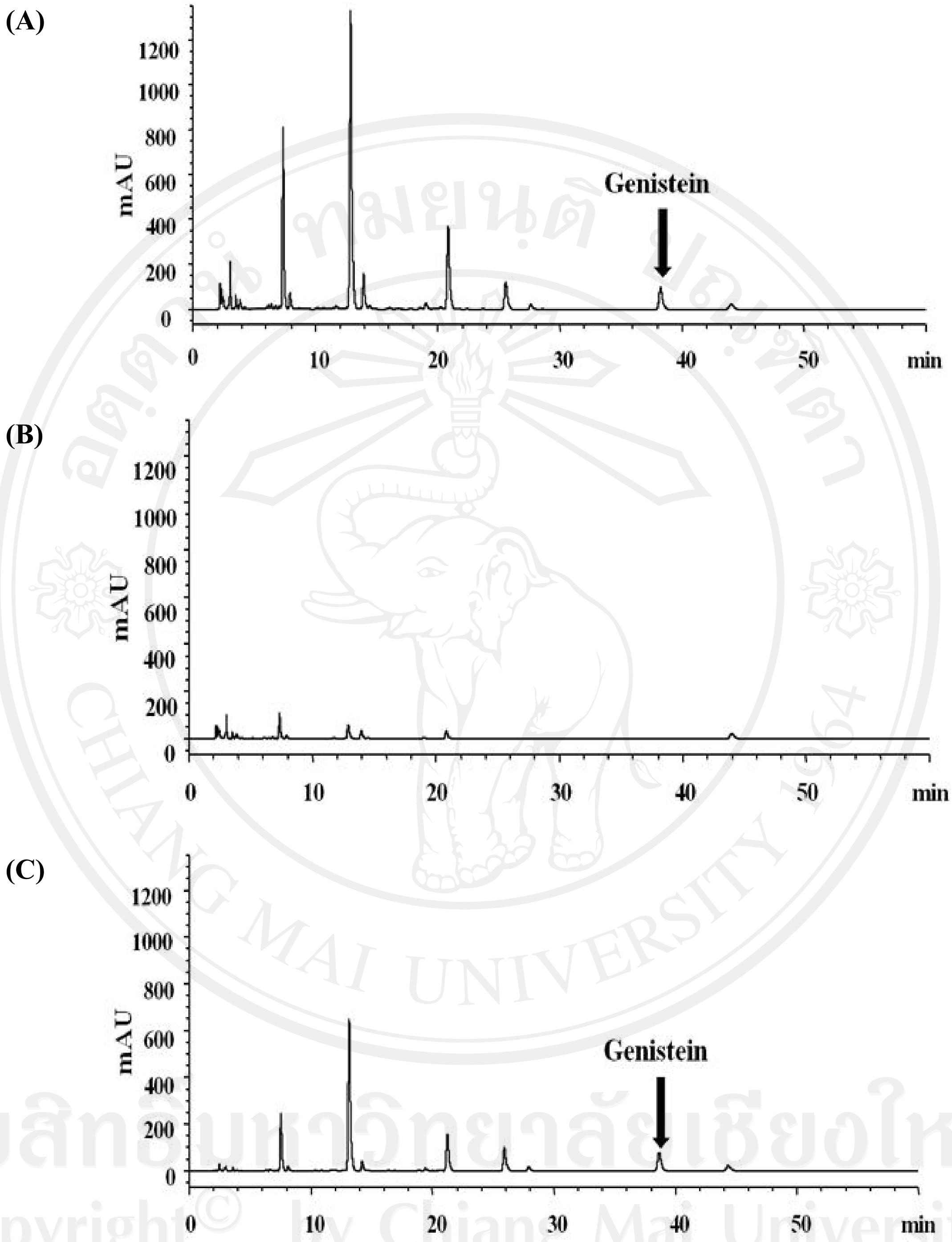


Figure B.9 Representative HPLC chromatogram of soybean extracts; **(A)** before MISPE process; **(B)** after loaded solution; **(C)** after MISPE process using PI-II-III

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List of conferences	Poster Presentation, "Synthesis of Molecularly Imprinted Polymer Selective to Some Flavonoids in Plant Extracts" at the 6th International Congress on Chemistry for Innovation (PERCH-CIC Congress VI), Pattaya, Chonburi, Thailand. May 3-6 2009.

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