APPENDICES

APPENDIX A

Table A.1 Calibration curve of NAM in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20.

[NAM]	Absorbance (λ_{max} = 262 nm)				SD
mM	1st	2nd	3rd	Average	
0.0625	0.1814	0.1815	0.1822	0.1817	0.0004
0.125	0.3628	0.3539	0.3752	0.3640	0.0107
0.25	0.7254	0.7345	0.7374	0.7324	0.0063
0.50	1.4512	1.4663	1.4820	1.4665	0.0154
0.75	2.1768	2.1757	2.1752	2.1759	0.0008

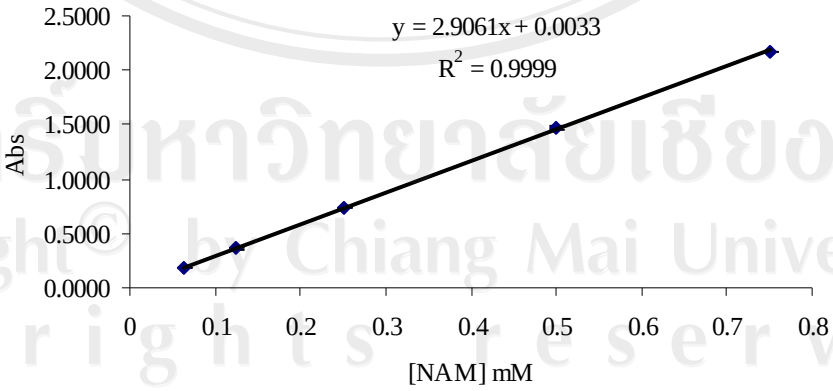

Figure A.1 Calibration curve of NAM in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20.

Table A.2 Calibration curve of NVP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20.

[NVP]	Absorbance (λ_{max} = 281 nm)				SD
mM	1st	2nd	3rd	Average	
0.025	0.1557	0.1538	0.1822	0.1639	0.0159
0.05	0.3092	0.3046	0.3752	0.3297	0.0395
0.10	0.6201	0.6228	0.7374	0.6601	0.0670
0.20	1.2455	1.2417	1.4820	1.3231	0.1377
0.25	1.5651	1.5494	2.1752	1.7632	0.3569

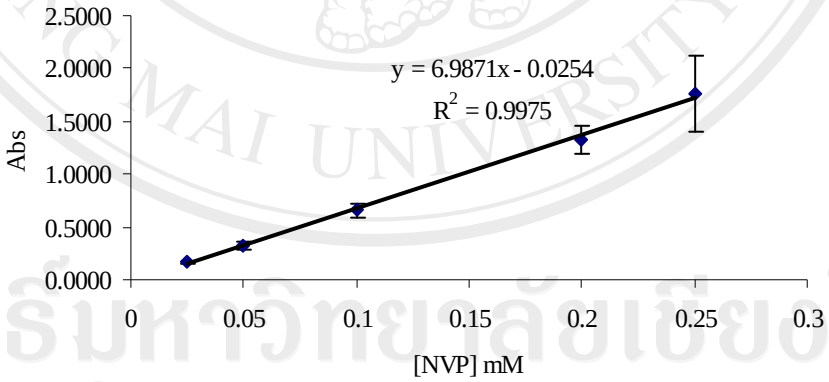

Figure A.2 Calibration curve of NVP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20.

Table A.3 Calibration curve of BZM in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20.

[BZM]	Absorbance (λ_{max} = 258 nm)				SD
mM	1st	2nd	3rd	Average	
0.125	0.0814	0.0743	0.0739	0.0765	0.0042
0.25	0.1638	0.1585	0.1488	0.1570	0.0076
0.50	0.3213	0.3156	0.3165	0.3178	0.0031
0.75	0.4434	0.4448	0.4456	0.4446	0.0011
1.00	0.6343	0.6352	0.6357	0.6351	0.0007

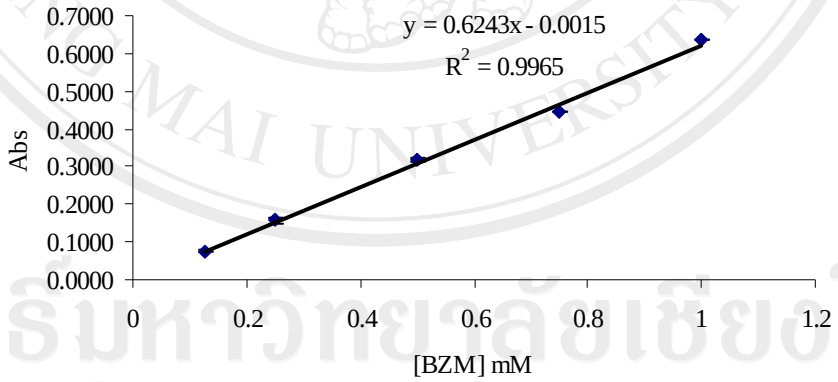

Figure A.3 Calibration curve of BZM in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20.

Table A.4 Calibration curve of BZP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20.

[BZP]	Absorbance (λ_{max} = 258 nm)				SD
mM	1st	2nd	3rd	Average	
0.025	0.2117	0.1993	0.2017	0.2042	0.0066
0.05	0.4211	0.4262	0.4211	0.4228	0.0029
0.10	0.8374	0.6521	0.8434	0.7776	0.1088
0.20	1.6836	1.6939	1.6754	1.6843	0.0093
0.25	2.0981	2.1119	2.1152	2.1084	0.0091

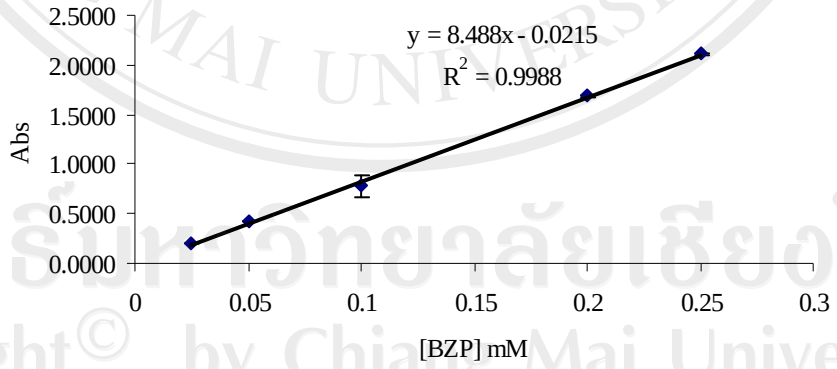

Figure A.4 Calibration curve of BZP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20.

Table A.5 The binding study of 0.2 mM NVP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 with 5 mg of P(NVP) and NIP.

Polymers	**Absorbance (λ_{max} = 281 nm)**				**%Bound**	**SD**
	1st	**2nd**	**3rd**	**Average**		
P(NVP)	0.0106	0.0376	0.0439	0.0307	97.5333	1.4224
NIP	0.7697	0.7686	0.7656	0.7680	38.3402	0.1689

Table A.6 The binding study of 0.2 mM NAM in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 with 5 mg of P(NAM) and NIP.

Polymers	**Absorbance (λ_{max} = 262 nm)**				**%Bound**	**SD**
	1st	**2nd**	**3rd**	**Average**		
P(NAM)	0.46705	0.45077	0.46803	0.46195	20.4193	1.6701
NIP	0.54597	0.434	0.38657	0.455513	21.5282	14.1002

Table A.7 The binding study of 0.2 mM BZM in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 with 5 mg of P(BZM) and NIP.

Polymers	**Absorbance (λ_{max} = 272 nm)**				**%Bound**	**SD**
	1st	**2nd**	**3rd**	**Average**		
P(BZM)	0.056831	0.072153	0.059526	0.062837	51.6715	6.2913
NIP	0.052913	0.045233	0.059424	0.052523	59.6037	5.4634

Table A.8 The binding study of 0.2 mM BZP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 with 5 mg of P(BZP) and NIP.

Polymers	**Absorbance (λ_{max} = 258 nm)**				**%Bound**	**SD**
	1st	**2nd**	**3rd**	**Average**		
P(BZP)	0.0035	-0.0703	-1.4348	-0.5005	114.7403	2.16
NIP	-0.3240	-0.3242	-0.2743	0.7680	115.1452	1.64

Table A.9 The binding study of 0.2 mM NVP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 with 5 mg of P(NVP), P(NAM), P(BZM) and P(BZP).

Polymers	**Absorbance (λ_{max} = 281 nm)**				**%Bound**	**SD**
	1st	**2nd**	**3rd**	**Average**		
P(NVP)	0.0106	0.0376	0.0439	0.0307	97.5333	1.4224
P(NAM)	0.0295	0.0301	0.0150	0.0249	98.0034	0.6897
P(BZM)	0.2188	0.2648	0.2189	0.2342	81.1976	2.1282
P(BZP)	0.0035	-0.0703	-1.4348	-0.5005	105.6900	0.9200
NIP	0.7697	0.7686	0.7656	0.7680	38.3402	0.1689

Table A.10 The binding study of 0.2 mM NAM in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 with P(NAM).

Amount of Polymers	Absorbance (λ_{max} = 262 nm)								%Bound		SD	
	1st		2nd		3rd		Average					
	P(NAM)	NIP	P(NAM)	NIP	P(NAM)	NIP	P(NAM)	NIP	P(NAM)	NIP	P(NAM)	NIP
5 mg/ml	0.494	0.516	0.494	0.460	0.491	0.411	0.493	0.462	19.970	24.971	0.281	8.538
10 mg/ml	0.457	0.524	0.414	0.530	0.482	0.529	0.451	0.528	26.790	14.310	5.584	0.524
20 mg/ml	0.360	0.492	0.417	0.508	0.369	0.502	0.382	0.501	37.990	18.736	4.975	1.317

Table A.11 The competitive binding study of mix solution of NAM and NVP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 with 5 mg of P(NAM).

NAM:NVP	Area (mAU)								%Bound		SD	
	1st		2nd		3rd		Average					
	NAM	NVP	NAM	NVP	NAM	NVP	NAM	NVP	NAM	NVP	NAM	NVP
1:01	345.55	166.91	397.64	201.03	342.67	170.42	361.95	179.45	28.64	86.21	6.10	1.44
5:01	1958.41	224.79	2042.84	277.13	2021.08	261.39	2007.44	254.44	2.32	82.20	2.13	1.88
10:01	4548.81	377.75	4417.50	436.67	4439.29	416.62	4468.53	410.35	9.83	73.78	1.42	1.91

Table A.12 Calibration curve of NVP in plasma sample.

[NVP]	Area (mAU) (λ_{max} = 281 nm)				SD
µg/ml	1st	2nd	3rd	Average	
0.5	49.310	38.018	36.416	41.248	7.03
1	32.239	30.678	31.462	31.460	0.78
10	226.033	261.764	280.515	256.104	27.68
20	525.280	527.140	583.005	545.142	32.80
100	4237.165	4033.410	3966.520	4079.032	140.97

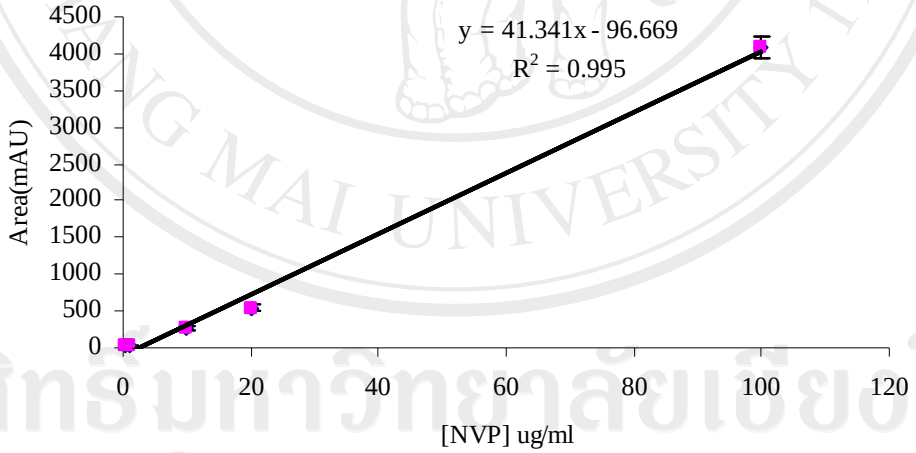

Figure A.5 Calibration curve of NVP in plasma sample.

Table A.13 Percentage of NVP retained in MISPE cartridge after washing step in various washing conditions.

Washing conditions*	Area (mAU) (λ_{max} = 281 nm)				%Retained	SD
	1st	2nd	3rd	Average		
a	24.32	27.18	27.14	26.21	98.69	0.08
b	70.53	60.02	68.75	66.43	95.77	0.30
c	39.15	42.84	42.66	41.55	97.42	0.12
d	25.92	15.95	16.07	19.31	99.03	0.29
e	127.04	122.99	146.50	132.18	93.23	0.62
f	1939.65	1839.10	1911.40	1896.72	4.93	2.60
g	150.46	129.32	144.35	141.38	92.35	0.57
h	2.64	2.94	2.75	2.78	99.86	0.01
i	1356.58	1375.23	1371.81	1367.87	31.43	0.50

*Washing conditions;

(a) 0.01 M phosphate buffer pH 4 with 0.05% Tween 20

(b) 0.01 M phosphate buffer pH 7 with 0.05% Tween 20

(c) 0.01 M phosphate buffer pH 10.5 with 0.05% Tween 20

(d) 5% MeOH in (b)

(e) 10% MeOH in (b)

(f) 20% MeOH in (b)

(g) 5% acetonitrile in (b)

(h) 10% acetonitrile in (b)

(i) 20% acetonitrile in (b)

Table A.14 Percentage of NVP recovery after MISPE process in various eluting conditions.

Eluting conditions*	Area (mAU) (λ_{max} = 281 nm)				%Recovery	SD
	1st	2nd	3rd	Average		
a	2154.06	1979.33	1798.75	1977.38	98.58	8.86
b	1878.52	1710.59	1513.72	1700.94	84.80	9.10
c	2077.03	2189.07	2120.90	2129.00	106.14	2.81
d	2006.43	2065.76	2013.46	2028.55	101.13	1.62

* Eluting conditions;

(a) Acetonitrile

(b) 1% TEA in acetonitrile

(c) 1% formic acid in acetonitrile

(d) 1% acetic acid in acetonitrile

Table A.15 The effect of NAM to recovery of NVP in plasma sample after MISPE process.

NAM:NVP	Area (mAU) (λ_{max} = 254 nm)				%Recovery of NVP	SD
	1st	2nd	3rd	Average		
1:01	2154.06	1979.33	1798.75	1977.38	98.58	8.86
6:01	1326.39	1282.19	1326.15	1311.58	98.47	1.91
12:01	1354.41	1388.25	1387.43	1376.70	89.67	1.26

Table A.16 The precision study.

[NVP] µg/ml	Area (mAU) (λ_{max} = 281 nm)				SD	%RA	%RSD
	1st	2nd	3rd	Average			
1	32.2385	30.6779	31.4623	31.4596	0.78	100.04	2.48
10	226.0325	261.7635	280.5145	256.1035	27.68	100.81	10.81
100	4237.1650	4033.4100	3966.5200	4079.0317	140.97	100.08	3.46

Table A.17 The accuracy of the recovery of 10 μg/ml NVP in plasma sample after MISPE process.

Times	Area (mAU)	[NVP]	%Relative Accuracy
1st	224.05	7.31	74.87
2nd	322.50	10.52	107.78
3rd	271.83	8.87	90.84
4th	373.86	12.20	124.94
5th	300.18	9.79	100.32
6th	259.40	8.46	86.69
7th	296.54	9.67	99.10
8th	297.55	9.71	99.44
9th	345.21	11.26	115.37
10th	301.22	9.83	100.67

APPENDIX B

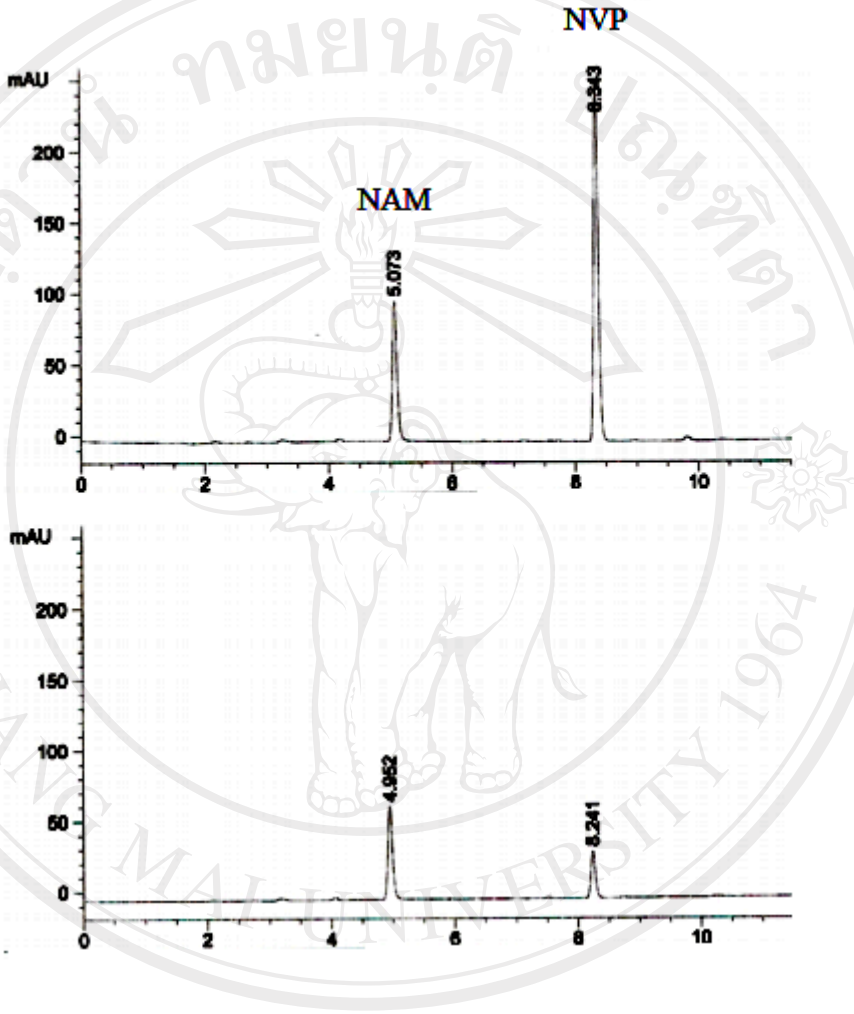

Figure B.1 The HPLC chromatogram of competitive binding study of P(NAM) in mix solution of NAM:NVP (1:1) in 0.01 M phosphate buffer pH7 containing 0.05% Tween 20 ;

(a) mix solution before binding

(b) mix solution after binding with P(NAM)

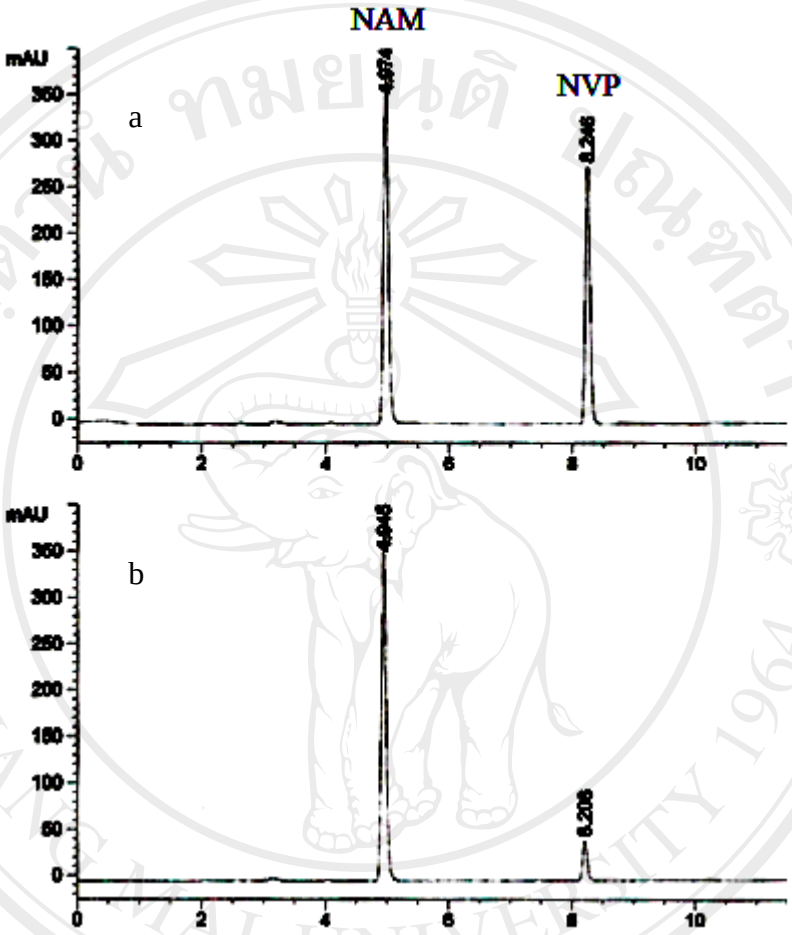

Figure B.2 The HPLC chromatogram of competitive binding study of P(NAM) in mix solution of NAM:NVP (5:1) in 0.01 M phosphate buffer pH7 containing 0.05% Tween 20.

(a) mix solution before binding

(b) mix solution after binding with P(NAM)

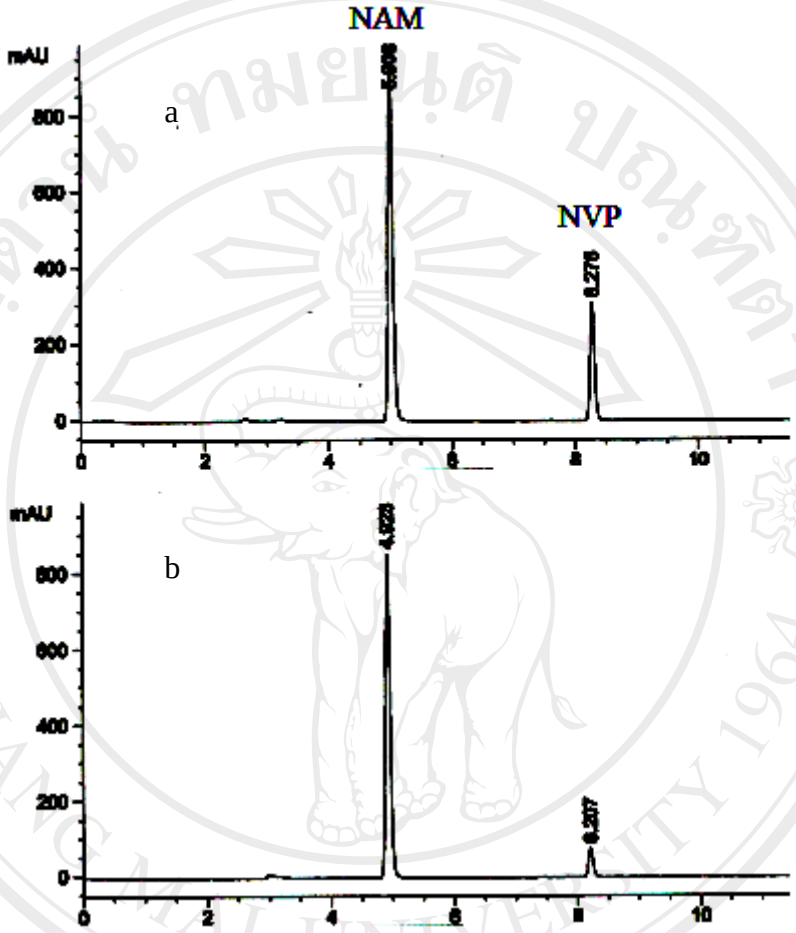

Figure B.3 The HPLC chromatogram of competitive binding study of P(NAM) in mix solution of NAM:NVP (10:1) in 0.01 M phosphate buffer pH7 containing 0.05% Tween 20.

(a) mix solution before binding

(b) mix solution after binding with P(NAM)

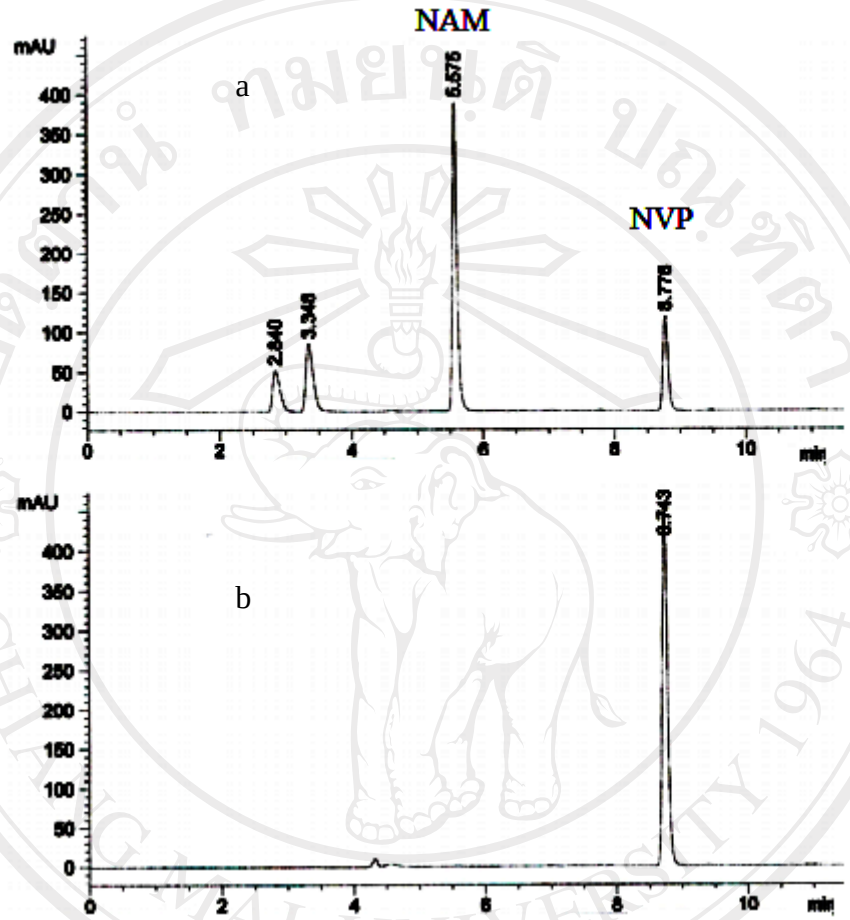

Figure B.4 The HPLC chromatogram of the study of MISPE with mix solution of NAM:NVP (6:1) in 0.01 M phosphate buffer pH7 containing 0.05% Tween 20.

(a) mix solution before MISPE process

(b) mix solution after MISPE process

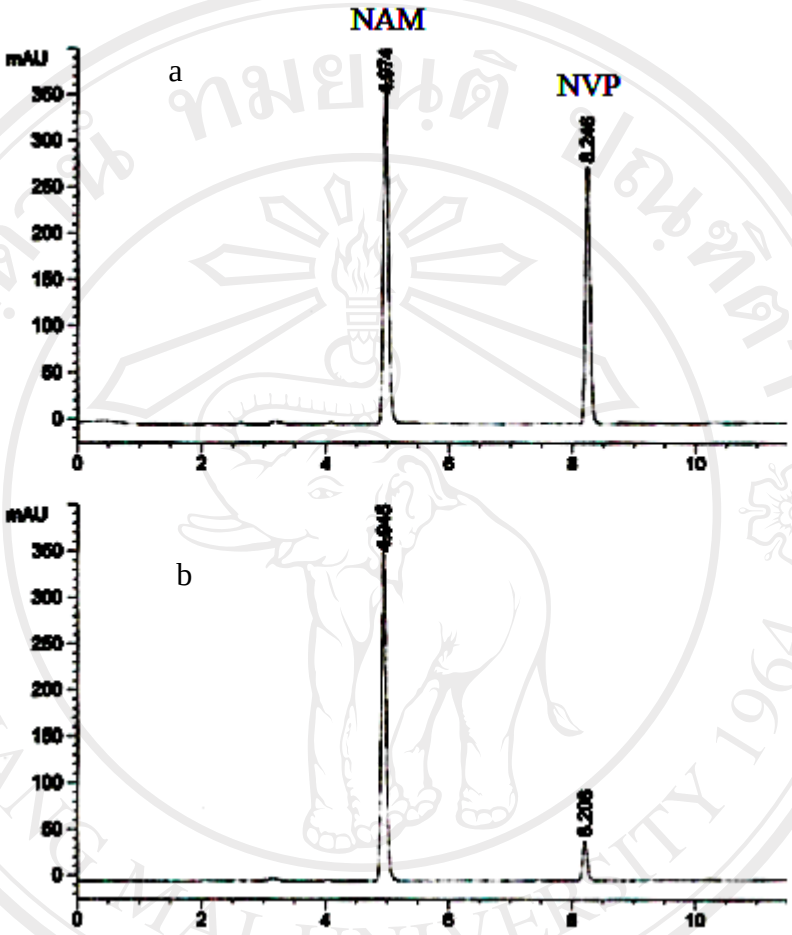

Figure B.5 The HPLC chromatogram of the study of MISPE with mix solution of NAM:NVP (1:1) in 0.01 M phosphate buffer pH7 containing 0.05% Tween 20.

(a) mix solution before MISPE process

(b) mix solution after MISPE process

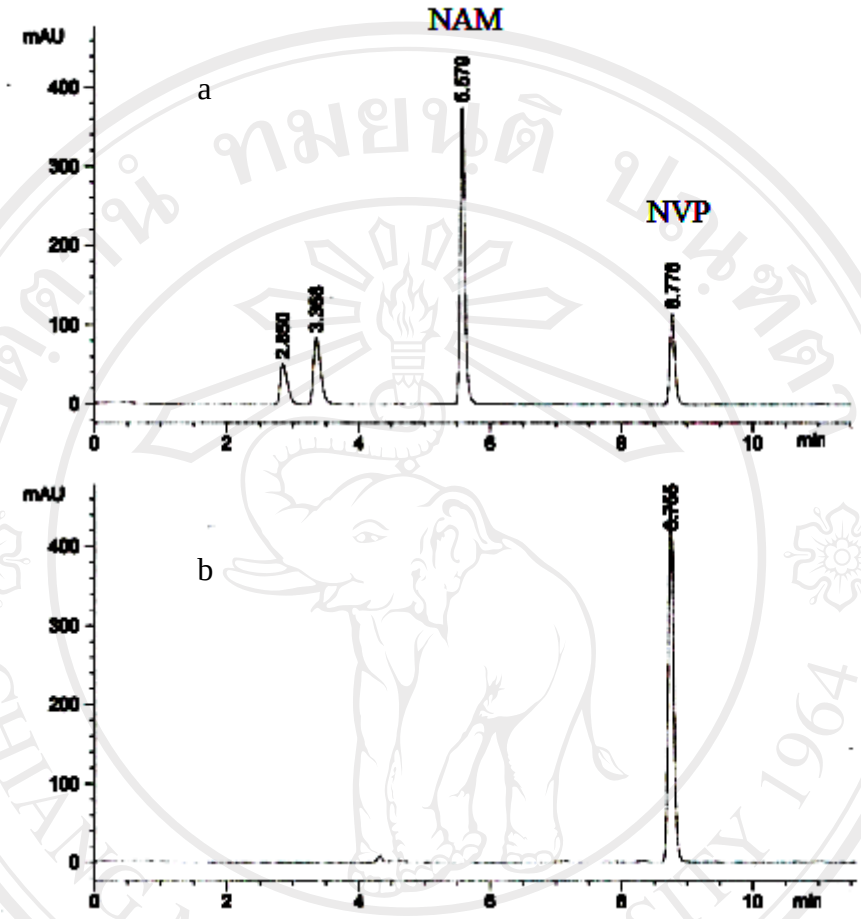

Figure B.6 The HPLC chromatogram of the study of MISPE with mix solution of NAM:NVP (12:1) in 0.01 M phosphate buffer pH7 containing 0.05% Tween 20.

(a) mix solution before MISPE process

(b) mix solution after MISPE process

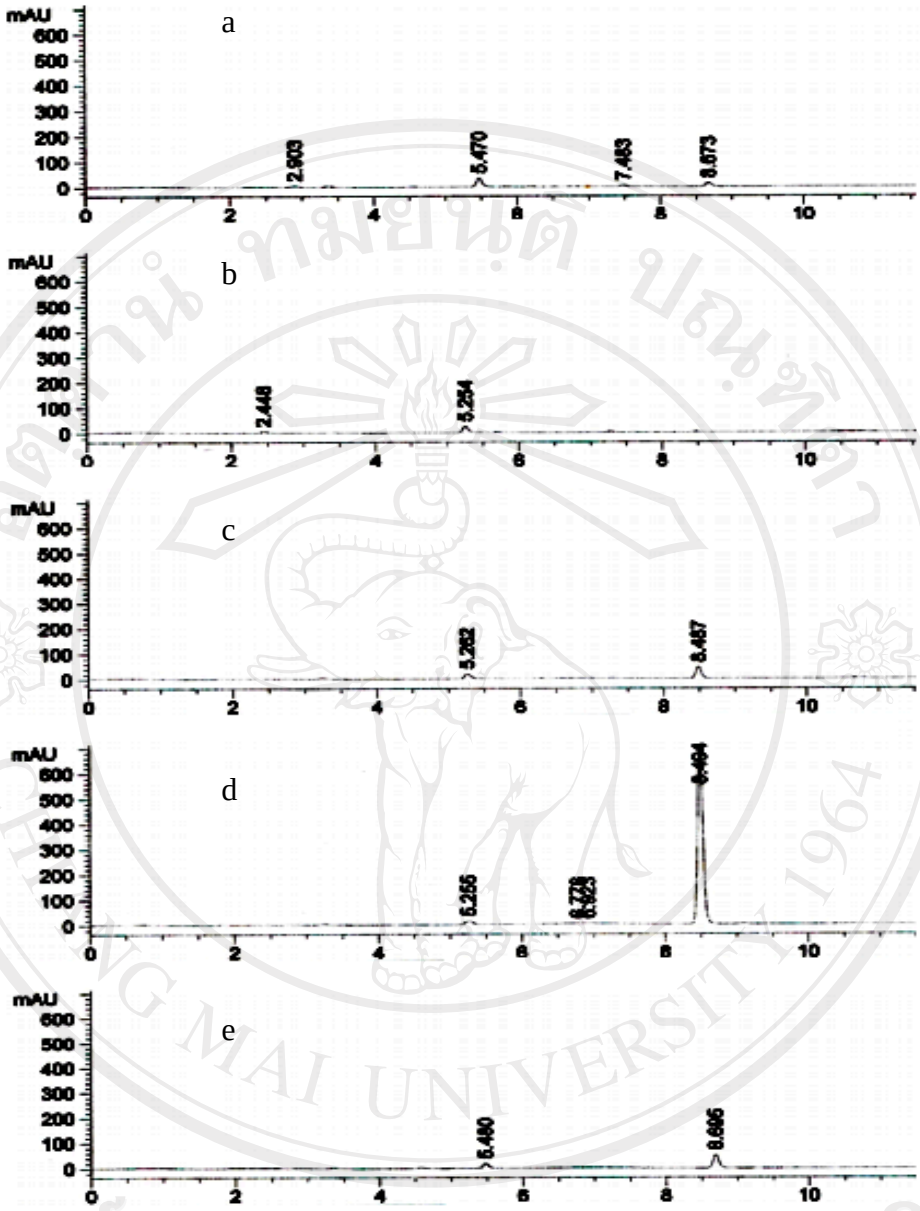

Figure B.7 The HPLC chromatograms of 0.2 mM NVP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 after washing step.

(a) washed with 0.01 M phosphate buffer pH 7 with 0.05% Tween 20

(b) washed with 5% MeOH in (a)

(c) washed with 10% MeOH in (a)

(d) washed with 20% MeOH in (a)

(e) washed with 5% acetonitrile in (a)

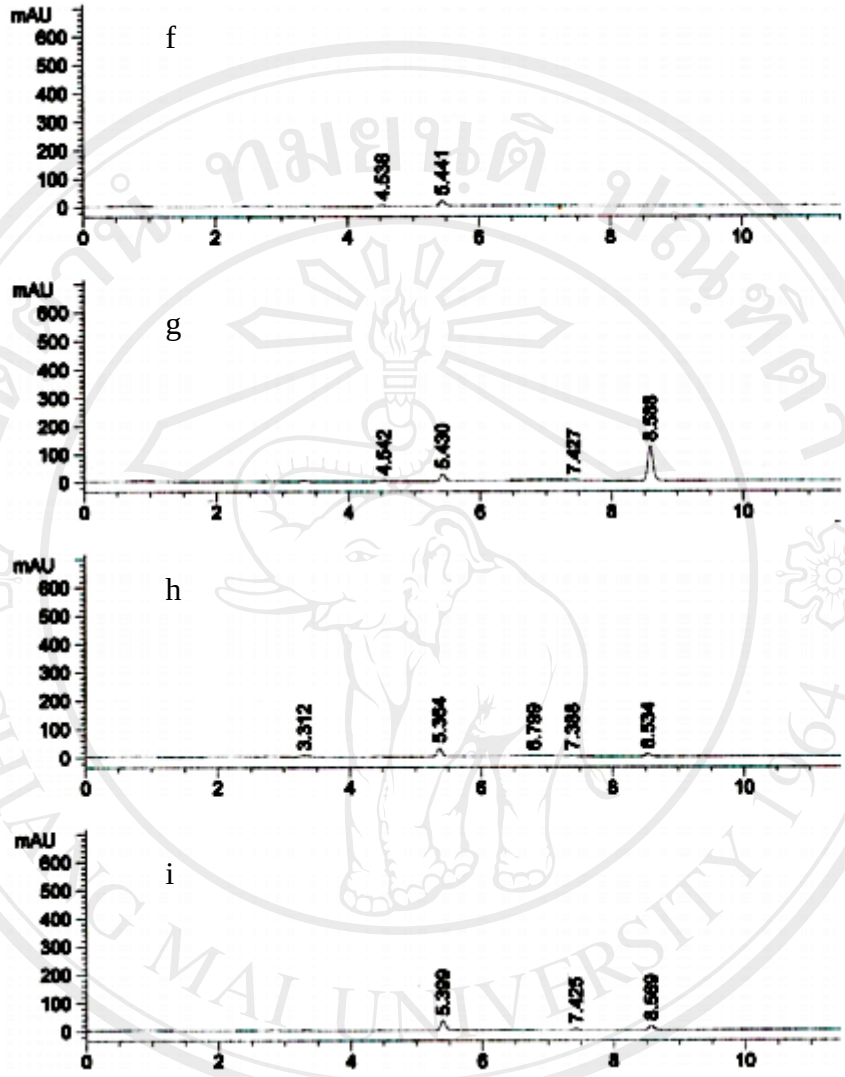

Figure B.7 (continue) The HPLC chromatograms of 0.2 mM NVP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 after washing step.

(f) washed with 10%acetonitrile in (a)

(g) washed with 20%acetonitrile in (a)

(h) washed with 0.01 M phosphate buffer pH 4 with 0.05% Tween 20

(i) washed with 0.01 M phosphate buffer pH 10.5 with 0.05% Tween 20

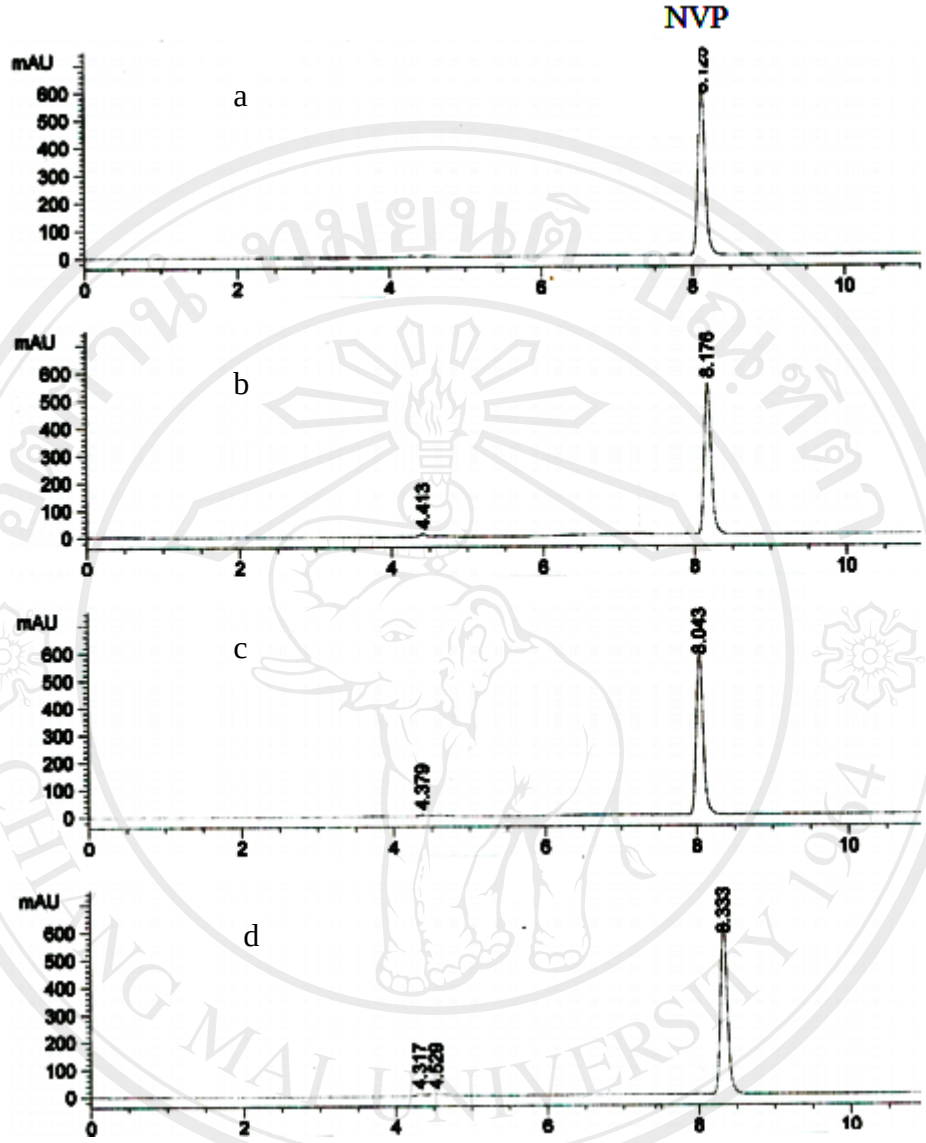

Figure B.8 The HPLC chromatograms of 0.2 mM NVP in 0.01 M phosphate buffer pH 7 containing 0.05% Tween 20 after eluting step.

(a) washed with pure acetonitrile

(b) washed with 1% TEA in acetonitrile

(c) washed with 1% formic acid in acetonitrile

(d) washed with 1% acetic acid in acetonitrile

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Poster Presentation

1. W.Karuehanon, S. Komkham and M. Pattarawarapan, Molecularly Imprinted Solid Phase Extraction of Nevirapine. The 3rd Colloqium on Postgraduate Research National Postgraduate Colloquim on Materials, Minerals and Polymers 2007, Penang, Malaysia. April 10-11 2007