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APPENDICES

Appendix A

Table 5-1 ANOVA test of % drug loading of rifampicin-DL-PLGA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	901.276	2	450.638	14.740	.005
Within Groups	183.429	6	30.571		
Total	1084.705	8			

% drug loading

Scheffe

% polymer content	N	Subset for alpha = .05	
		1	2
40% DL-PLGA	3	45.8596	
30% DL-PLGA	3		62.8593
20% DL-PLGA	3		69.6531
Sig.		1.000	.383

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-2 ANOVA test of % EE of rifampicin-DL-PLGA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	299.200	2	149.600	2.184	.194
Within Groups	410.945	6	68.491		
Total	710.145	8			

% EE

Scheffe

% polymer content	N	Subset for alpha = .05
		1
40% DL-PLGA	3	76.4327
20% DL-PLGA	3	87.0664
30% DL-PLGA	3	89.7990
Sig.		.222

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-3 ANOVA test of $D_{50\%}$ of rifampicin-DL-PLGA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1246.628	2	623.314	746.000	.000
Within Groups	5.013	6	.836		
Total	1251.641	8			

$D_{50\%}$

Scheffe

% polymer content	N	Subset for alpha = .05		
		1	2	3
40% PLGA	3	18.6773		
20% PLGA	3		27.0047	
30% PLGA	3			46.7430
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-4 ANOVA test of % drug loading of rifampicin-DL-PLA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	827.205	2	413.603	14.325	.005
Within Groups	173.237	6	28.873		
Total	1000.442	8			

% drug loading

Scheffe

% polymer content	N	Subset for alpha = .05	
		1	2
40% DL-PLA	3	50.0238	
30% DL-PLA	3	56.3640	
20% DL-PLA	3		72.7759
Sig.		.408	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-5 ANOVA test of % EE of rifampicin-DL-PLA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	175.052	2	87.526	1.480	.300
Within Groups	354.934	6	59.156		
Total	529.986	8			

%EE

Scheffe

DLPLA	N	Subset for alpha = .05
		1
30% DL-PLA	3	80.5200
40% DL-PLA	3	83.3730
20% DL-PLA	3	90.9699
Sig.		.320

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-6 ANOVA test of $D_{50\%}$ of rifampicin-DL-PLA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	635.157	2	317.578	532.411	.000
Within Groups	3.579	6	.596		
Total	638.736	8			

$D_{50\%}$

Scheffe

% polymer content	N	Subset for alpha = .05	
		1	2
30% DL-PLA	3	27.4940	
40% DL-PLA	3	27.5990	
20% DL-PLA	3		45.3670
Sig.		.986	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-7 ANOVA test of % drug loading of rifampicin-L-PLA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15618.246	7	2231.178	529.537	.000
Within Groups	67.415	16	4.213		
Total	15685.661	23			

% Drug loading

Scheffe

% polymer content	N	Subset for alpha = .05					
		1	2	3	4	5	6
90% L-PLA	3	3.3598					
80% L-PLA	3	8.1281	8.1281				
70% L-PLA	3		16.3278	16.3278			
60% L-PLA	3			23.3010			
50% L-PLA	3				41.5153		
40% L-PLA	3					54.9678	
30% L-PLA	3					64.2119	64.2119
20% L-PLA	3						66.4174
Sig.		.721	.140	.288	1.000	.071	.994

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table 5-8 ANOVA test of % EE of rifampicin-L-PLA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11328.715	7	1618.388	25.782	.000
Within Groups	1004.348	16	62.772		
Total	12333.063	23			

% EE

Scheffe

% polymer content	N	Subset for alpha = .05		
		1	2	3
90% L-PLA	3	33.5980		
80% L-PLA	3	40.6406		
70% L-PLA	3	54.4261		
60% L-PLA	3	58.2524	58.2524	
20% L-PLA	3		83.0218	83.0218
50% L-PLA	3		83.0306	83.0306
40% L-PLA	3			91.6130
30% L-PLA	3			91.7313
Sig.		.107	.104	.961

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table 5-9 ANOVA test of $D_{50\%}$ of rifampicin-L-PLA microparticles

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2145.049	8	268.131	951.029	.000
Within Groups	5.075	18	.282		
Total	2150.124	26			

$D_{50\%}$

Scheffe

% polymer content	N	Subset for alpha = .05			
		1	2	3	4
100% L-PLA	3	3.2643			
80% L-PLA	3	3.3730			
70% L-PLA	3	3.3953			
90% L-PLA	3	3.6470			
60% L-PLA	3	4.0680			
40% L-PLA	3		6.6023		
50% L-PLA	3		8.0487		
20% L-PLA	3			18.7847	
30% L-PLA	3				30.5343
Sig.		.888	.265	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-10 ANOVA test of $D_{50\%}$ of rifampicin-L-PLA microparticles at various temperatures.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.399	2	3.699	358.347	.000
Within Groups	.062	6	.010		
Total	7.461	8			

$D_{50\%}$

Scheffe

Temperature	N	Subset for alpha = .05	
		1	2
33 °C	3	3.2707	
40 °C	3	3.3953	
50 °C	3		5.2533
Sig.		.384	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-11 ANOVA test of $D_{50\%}$ of rifampicin-L-PLA microparticles at various operating pressures.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.452	2	.226	4.333	.068
Within Groups	.313	6	.052		
Total	.765	8			

$D_{50\%}$

Scheffe

pressure	N	Subset for alpha = .05
		1
2500 psi	3	3.3953
3000 psi	3	3.4837
2000 psi	3	3.9087
Sig.		.086

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-12 ANOVA test of $D_{50\%}$ of rifampicin-L-PLA microparticles at various concentration of solutions.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.720	2	1.360	71.600	.000
Within Groups	.114	6	.019		
Total	2.834	8			

$D_{50\%}$

Scheffe

concentration of solution	N	Subset for alpha = .05	
		1	2
1% w/v	3	3.2773	
2% w/v	3	3.3953	
3% w/v	3		4.4980
Sig.		.604	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-13 ANOVA test of $D_{50\%}$ of rifampicin-L-PLA microparticles at various solution feed rate.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.196	2	.098	8.723	.017
Within Groups	.067	6	.011		
Total	.263	8			

$D_{50\%}$

Scheffe

solution feed rate	N	Subset for alpha = .05	
		1	2
0.5 ml/min	3	3.3953	
0.4 ml/min	3	3.4937	3.4937
0.6 ml/min	3		3.7457
Sig.		.557	.071

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-14 ANOVA test of $D_{50\%}$ of rifampicin-L-PLA microparticles produced at three consecutive batchs.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.023	2	.012	.705	.531
Within Groups	.099	6	.017		
Total	.123	8			

$D_{50\%}$

Scheffe

Batch No.	N	Subset for alpha = .05
		1
3	3	3.3933
2	3	3.3953
1	3	3.5023
Sig.		.609

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-15 Percent drug release of unprocessed rifampicin, processed rifampicin and rifampicin L-PLA microparticles at different polymer content.

Time (hr)	% Drug release average (SD)*				
	Unprocessed rifampicin	processed rifampicin	60% L-PLA	70 % L-PLA	80 % L-PLA
0	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
0.25	4.51(1.93)	7.81(1.02)	6.22(0.62)	1.48(0.29)	1.93(0.20)
0.5	14.05(2.05)	23.42(2.13)	22.09(3.47)	3.34(0.86)	3.89(0.09)
0.75	32.47(1.68)	43.05(6.82)	34.43(4.37)	5.77(1.97)	5.51(0.11)
1	46.97(2.71)	58.33(6.45)	44.19(4.54)	8.14(3.60)	6.93(0.17)
2	68.41(2.84)	79.74(3.83)	62.62(3.15)	19.55(7.69)	8.51(0.48)
3	78.83(3.83)	88.84(2.20)	76.88(2.48)	29.15(9.21)	10.06(0.87)
4	84.07(3.01)	92.64(2.48)	80.99(1.36)	38.55(8.49)	10.92(1.26)
6	89.32(3.01)	95.12(2.07)	83.62(1.48)	47.08(5.05)	12.36(1.28)
8	91.90(2.75)	95.91(1.59)	84.95(1.97)	52.74(4.19)	13.55(1.37)
10	93.64(2.11)	96.18(1.79)	85.60(2.00)	55.78(3.60)	15.64(1.87)
12	95.04(2.53)	97.27(2.04)	84.97(2.38)	58.00(3.99)	18.07(2.99)
16	96.53(3.68)	99.11(2.04)	86.64(1.72)	61.07(4.06)	23.09(5.81)
20	97.97(2.81)	100.50(2.33)	87.06(2.12)	64.69(6.09)	28.83(3.88)
24	99.92(3.15)	102.71(2.48)	88.13(1.98)	67.83(7.27)	31.85(3.95)

*Average and SD from three determinations.

Table 5-16 Percent drug release of rifampicin L-PLA microparticles of three consecutive batches.

Time (hr)	% Drug release average (SD)*		
	Batch I	Batch II	Batch III
0	0.00(0.00)	0.00(0.00)	0.00(0.00)
0.25	1.48(0.29)	2.10(1.61)	1.34(0.74)
0.5	3.34(0.86)	7.21(3.68)	8.67(0.21)
0.75	5.77(1.97)	11.66(4.52)	14.79(1.80)
1	8.14(3.60)	16.10(6.48)	19.45(2.06)
2	19.55(7.69)	27.18(7.46)	28.87(2.39)
3	29.15(9.21)	35.31(6.78)	35.12(3.68)
4	38.55(8.49)	42.10(6.50)	40.16(3.83)
6	47.08(5.05)	49.56(4.37)	46.46(3.27)
8	52.74(4.19)	53.24(2.99)	50.73(3.54)
10	55.78(3.60)	55.51(2.34)	53.42(3.57)
12	58.00(3.99)	58.37(1.15)	55.92(3.89)
16	61.07(4.06)	61.28(1.52)	59.87(3.20)
20	64.69(6.09)	62.38(1.61)	61.51(3.55)
24	67.83(7.27)	65.58(2.03)	66.62(2.57)

*Average and SD from three determinations

Table 5-17 Percent drug deposition on Anderson Cascade Impactor.

Stage	Formulations	
	70% L-PLA +Lactose <45μm average (SD)*	70% L-PLA +Lactose 45-90 μm average (SD)*
device	3.09(1.26)	12.85(1.38)
throat	21.16(2.55)	26.76(5.45)
Preseparator	15.87(3.28)	10.96(2.71)
stage 0	10.02(0.78)	5.21(0.72)
stage 1	10.80(1.11)	7.86(0.35)
stage 2	7.59(1.09)	6.14(0.29)
stage 3	6.95(0.31)	5.90(0.49)
stage 4	3.53(1.05)	1.93(0.88)
stage 5	0.71(0.67)	0.53(0.70)
stage 6	0.64(0.71)	0.16(0.02)
stage 7	1.01(0.85)	0.44(0.03)
Total	78.25(5.74)	70.02(2.88)

* Average and SD from five determinations.

Table 5-18 Z-value and log size of the first experiment of Formula I for aerodynamic diameter.

Stage	Size cut off (μm)	log size	% deposition	% deposition (stage 0-7)	% Cumulative	P	Z-value
0	6.18	0.7910	10.732	26.59	26.59	0.2659	-0.6254
1	3.98	0.5999	10.802	26.76	53.34	0.5334	0.0839
2	3.23	0.5092	7.427	18.40	71.74	0.7174	0.5751
3	2.27	0.3560	6.982	17.29	89.03	0.8903	1.2283
4	1.44	0.1584	3.025	7.49	96.53	0.9653	1.8153
5	0.76	-0.1192	0.386	0.96	97.48	0.9748	1.9569
6	0.48	-0.3188	0.344	0.85	98.34	0.9834	2.1285
7	0.27	-0.5686	0.672	1.66	100.00	1.0000	

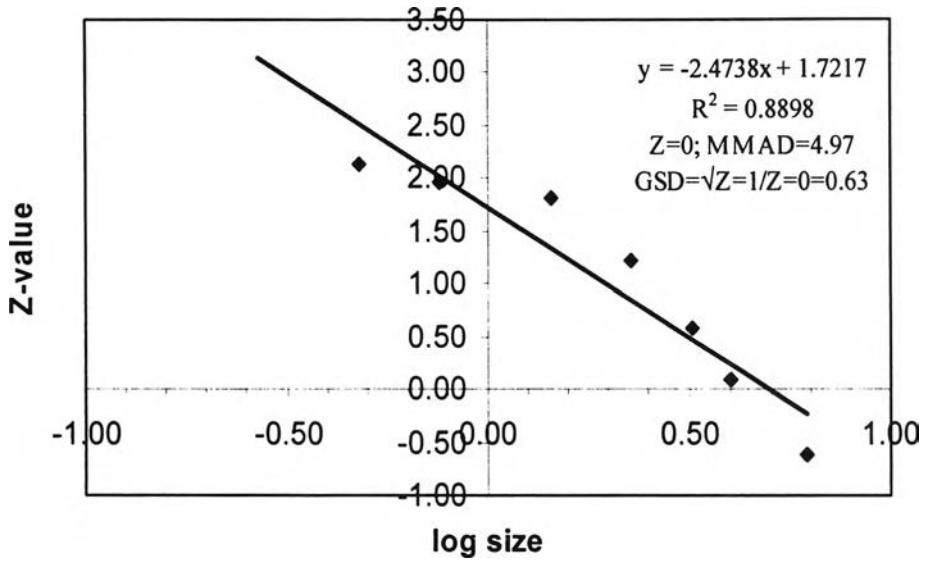
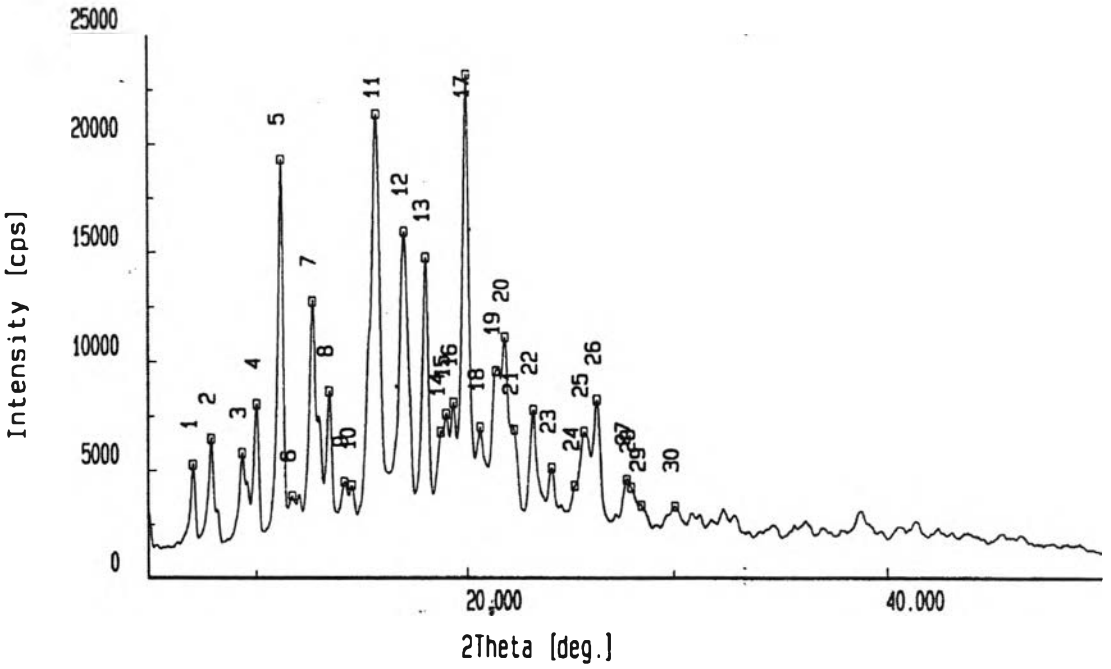


Figure 5-1 Curve between Z-value and log size of Formula I and the regression equation of the line.

Table 5-19 Independent samples test of mass medium aerodynamic diameter of L-PLA rifampicin loaded microparticles.

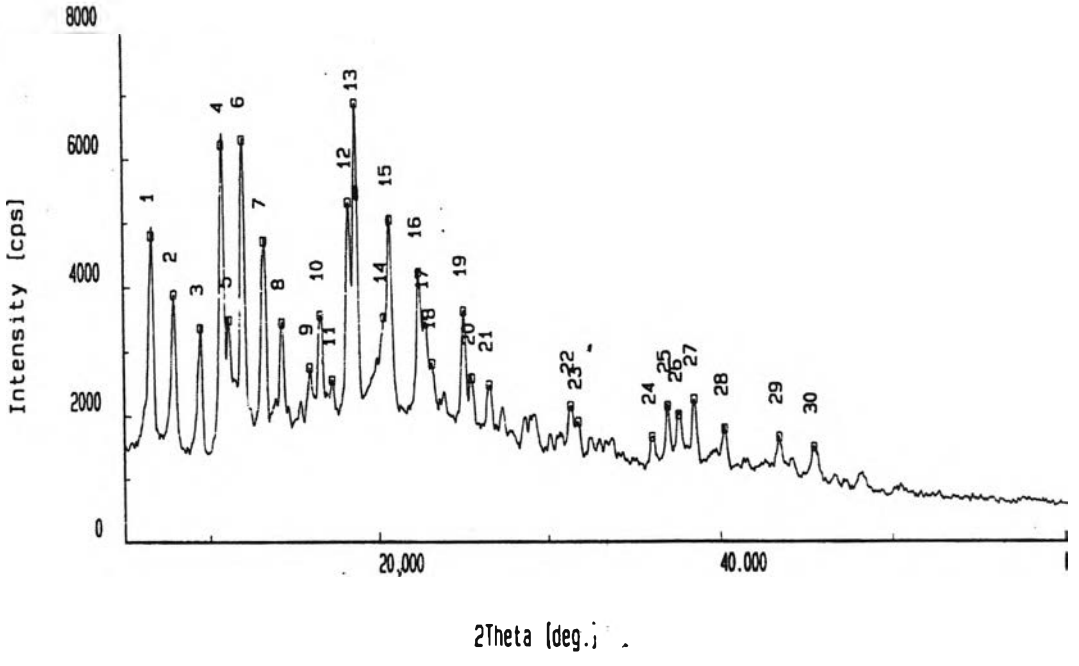
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
MMAD	Equal variances assumed	1.984	.197	3.349	8	.010	.57000	.17020	.17752	.96248
	Equal variances not assumed			3.349	7.065	.012	.57000	.17020	.16829	.97171

Table 5-20 XRD data of unprocessed rifampicin.



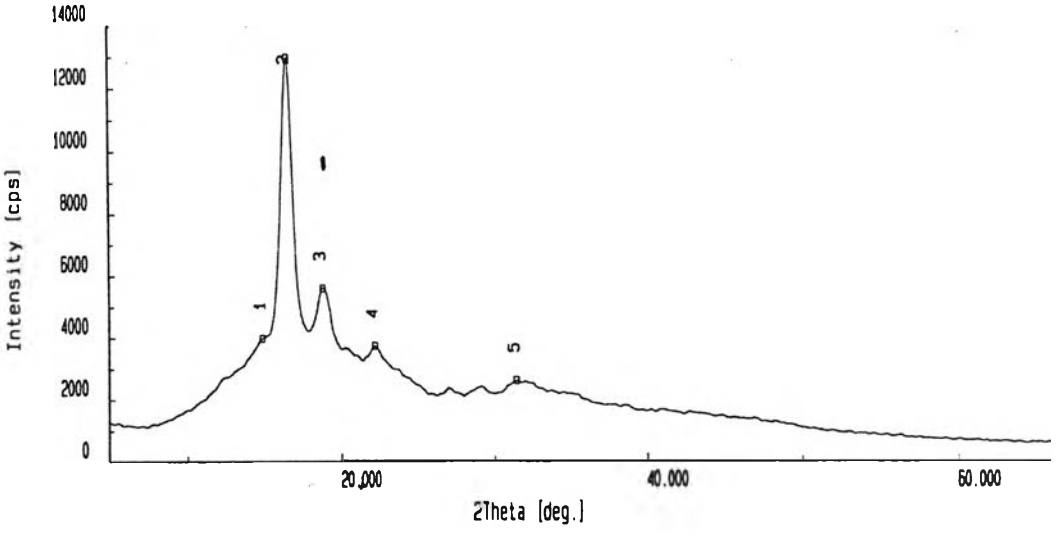
Peak No.	2Theta	FWHM	d-value	Intensity	I/Io	Peak No.	2Theta	FWHM	d-value	Intensity	I/Io
1	7.060	0.259	12.5104	5264	24	16	19.340	0.212	4.5857	8129	36
2	7.900	0.259	11.1820	6459	28	17	19.960	0.329	4.4447	23194	100
3	9.340	0.259	9.4610	5795	26	18	20.620	0.259	4.3039	6995	32
4	10.020	0.282	8.8204	8057	36	19	21.380	0.376	4.1526	9567	42
5	11.180	0.306	7.9077	19299	84	20	21.820	0.259	4.0698	11120	48
6	11.700	0.235	7.5574	3809	16	21	22.260	0.353	3.9904	6874	30
7	12.680	0.306	6.9754	12744	56	22	23.180	0.353	3.8340	7801	34
8	13.480	0.282	6.5632	8634	38	23	24.080	0.329	3.6927	5114	24
9	14.180	0.282	6.2407	4481	20	24	25.200	0.212	3.5311	4291	20
10	14.540	0.235	6.0870	4320	20	25	25.640	0.471	3.4715	6766	30
11	15.720	0.376	5.6326	21377	94	26	26.260	0.353	3.3909	8254	36
12	17.020	0.424	5.2052	15962	70	27	27.700	0.400	3.2178	4576	20
13	18.040	0.329	4.9132	14756	64	28	27.920	0.212	3.1929	4200	20
14	18.740	0.235	4.7312	6769	30	29	28.420	0.541	3.1379	3355	16
15	18.980	0.212	4.6719	7619	34	30	30.040	0.447	2.9723	3324	16

Table 5-21 XRD data of processed rifampicin produced by SAS technique.



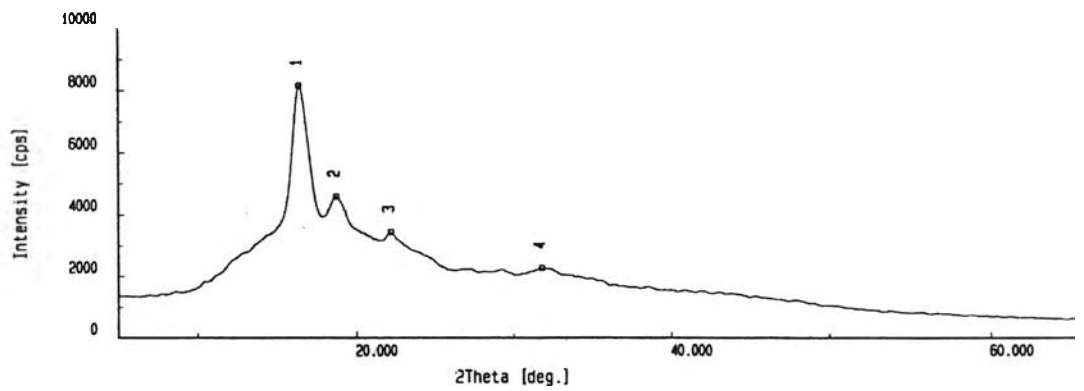
Peak No.	2Theta	FWHM	d-value	Intensity	I/Io	Peak No.	2Theta	FWHM	d-value	Intensity	I/Io
1	6.600	0.353	13.3813	4810	70	16	22.440	0.282	3.9588	4231	62
2	7.900	0.306	11.1820	3893	58	17	22.820	0.235	3.8937	3441	50
3	9.460	0.329	9.3412	3375	50	18	23.200	0.259	3.8308	2817	42
4	10.800	0.329	8.1850	6225	92	19	25.060	0.329	3.5505	3635	54
5	11.180	0.212	7.9077	3498	52	20	25.520	0.282	3.4875	2593	38
6	12.040	0.329	7.3447	6313	92	21	26.560	0.400	3.3533	2489	38
7	13.280	0.400	6.6616	4724	70	22	31.340	0.329	2.8519	2153	32
8	14.320	0.329	6.1800	3458	52	23	31.800	0.306	2.8117	1908	28
9	15.960	0.306	5.5485	2762	42	24	36.080	0.376	2.4873	1663	26
10	16.600	0.353	5.3360	3570	52	25	36.980	0.306	2.4288	2155	32
11	17.280	0.235	5.1275	2561	38	26	37.620	0.376	2.3890	2009	30
12	18.280	0.282	4.8492	5326	78	27	38.500	0.329	2.3364	2256	34
13	18.720	0.306	4.7362	6882	100	28	40.280	0.353	2.2371	1791	28
14	20.340	0.235	4.3625	3536	52	29	43.400	0.353	2.0833	1666	26
15	20.720	0.329	4.2833	5049	74	30	45.440	0.518	1.9944	1503	22

Table 5-22 XRD data of pure L-PLA.



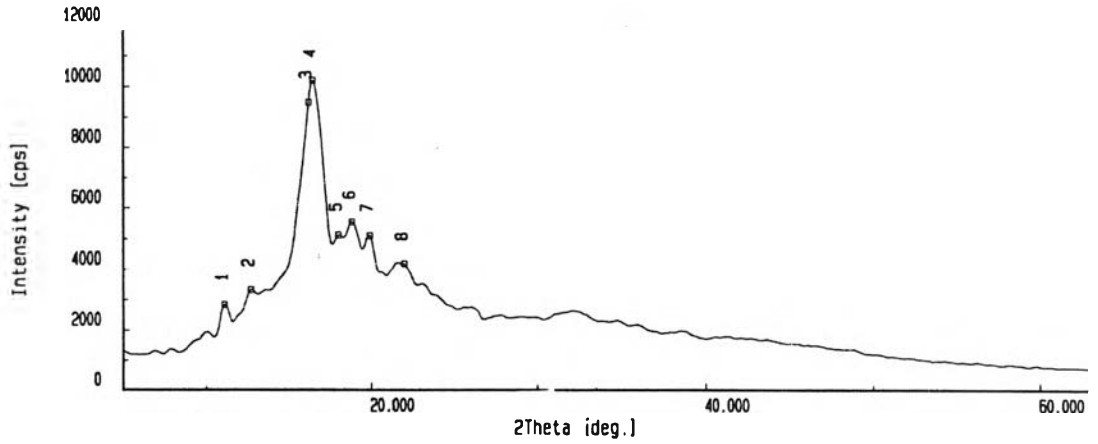
Peak No.	2Theta	FWHM	d-value	Intensity	I/Io
1	14.880	0.471	5.9487	3996	32
2	16.500	0.776	5.3681	12989	100
3	18.800	0.941	4.7162	5615	44
4	22.200	0.706	4.0010	3758	30
5	31.420	*****	2.8448	2645	22

Table 5-23 XRD data of processed 70% L-PLA-rifampicin microparticles.



Peak No.	2Theta	FWHM	d-value	Intensity	I/Io
1	16.340	0.941	5.4203	8160	100
2	18.720	1.224	4.7362	4594	58
3	22.200	*****	4.0010	3451	44
4	31.820	*****	2.8099	2285	28

Table 5-24 XRD data of physical mixture L-PLA and rifampicin.



Peak No.	2Theta	FWHM	d-value	Intensity	I/Io
1	11.100	0.682	7.9645	2849	28
2	12.700	0.635	6.9645	3338	34
3	16.160	0.259	5.4803	9486	94
4	16.420	1.459	5.3941	10200	100
5	18.000	0.447	4.9240	5132	52
6	18.820	0.753	4.7113	5542	56
7	19.900	0.635	4.4579	5114	52
8	22.020	0.706	4.0333	4187	42

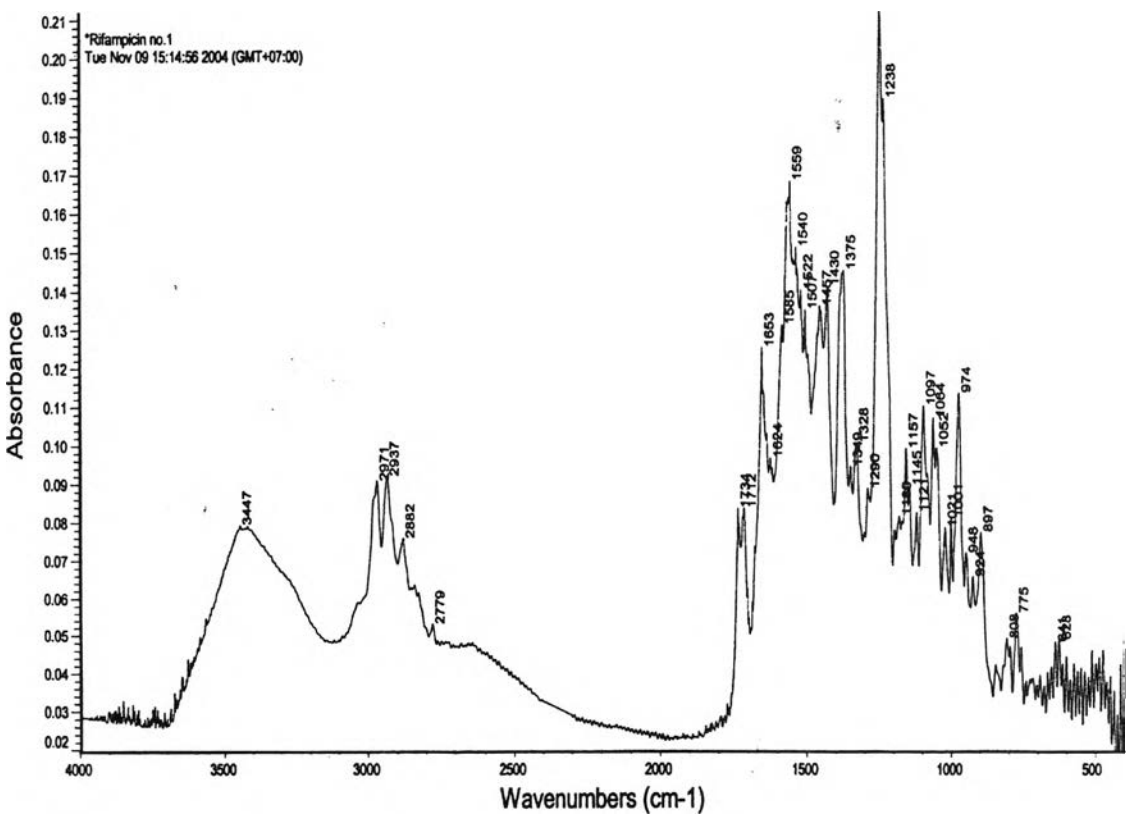


Figure 5-2 FTIR spectra of unprocessed rifampicin.

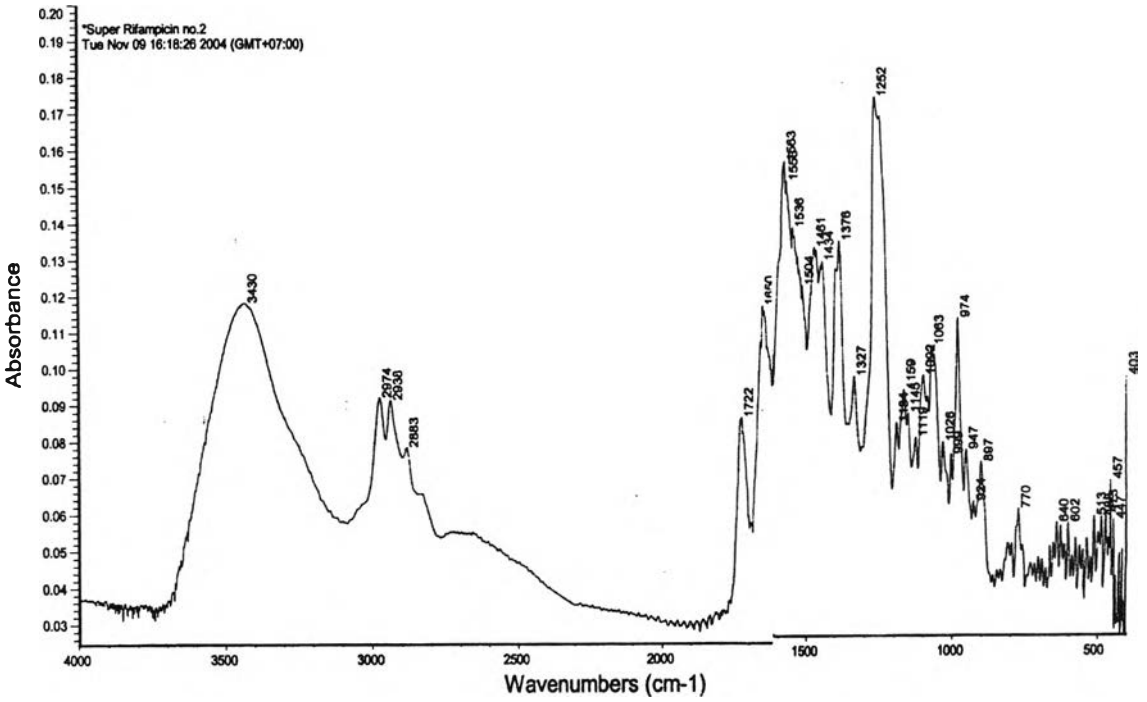


Figure 5-3 FTIR spectra of processed rifampicin by SAS technique.

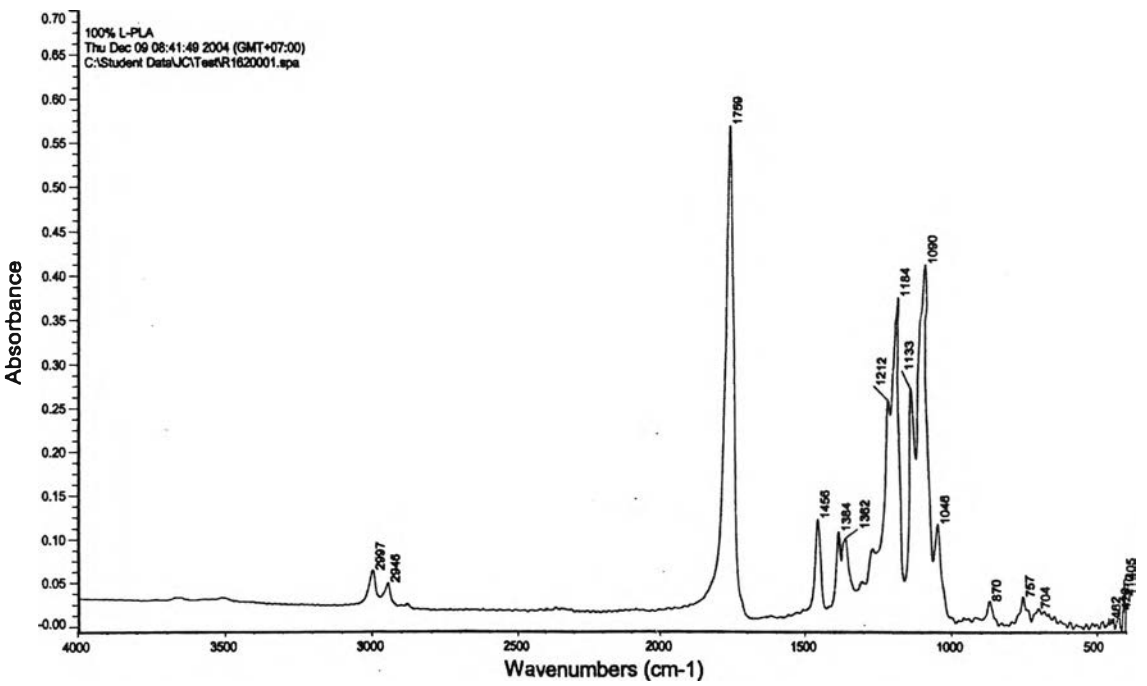


Figure 5-4 FTIR spectra of pure L-PLA.

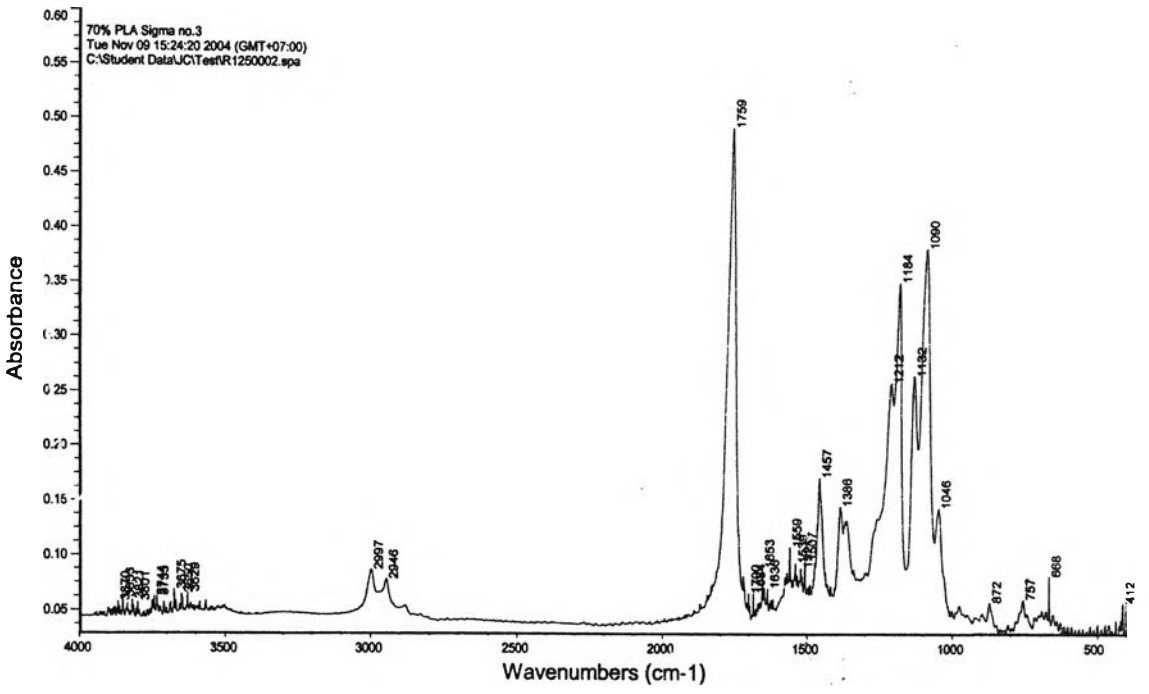


Figure 5-5 FTIR spectra of processed 70% L-PLA rifampicin microparticles by SAS technique.

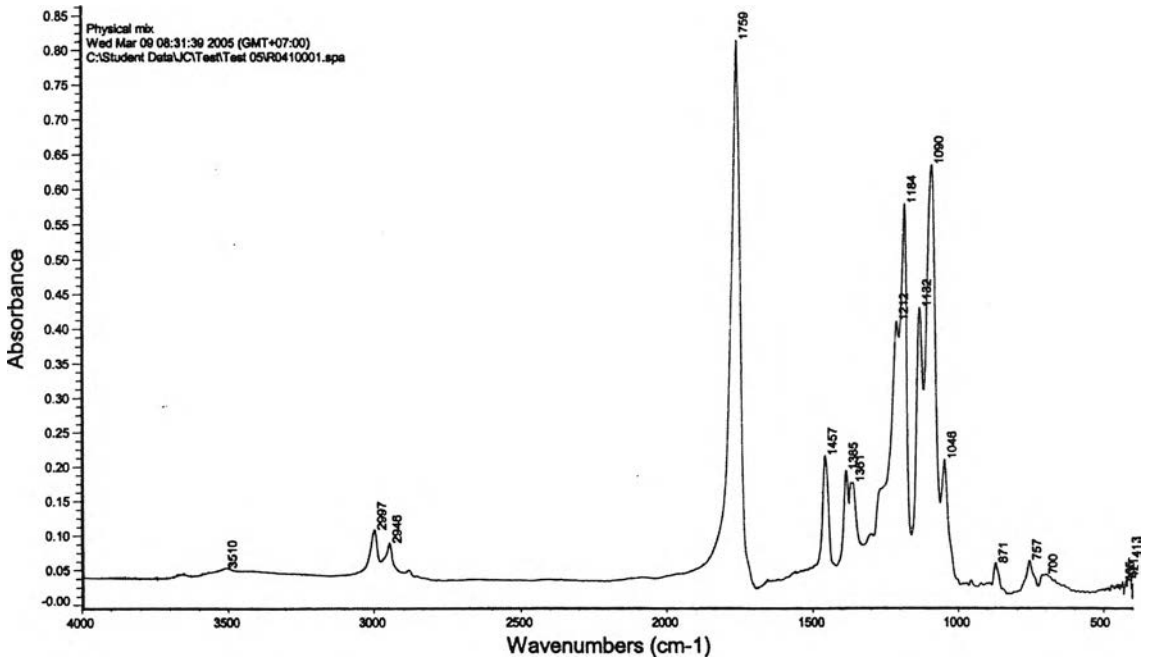


Figure 5-6 FTIR spectra of physical mixture L-PLA and rifampicin.

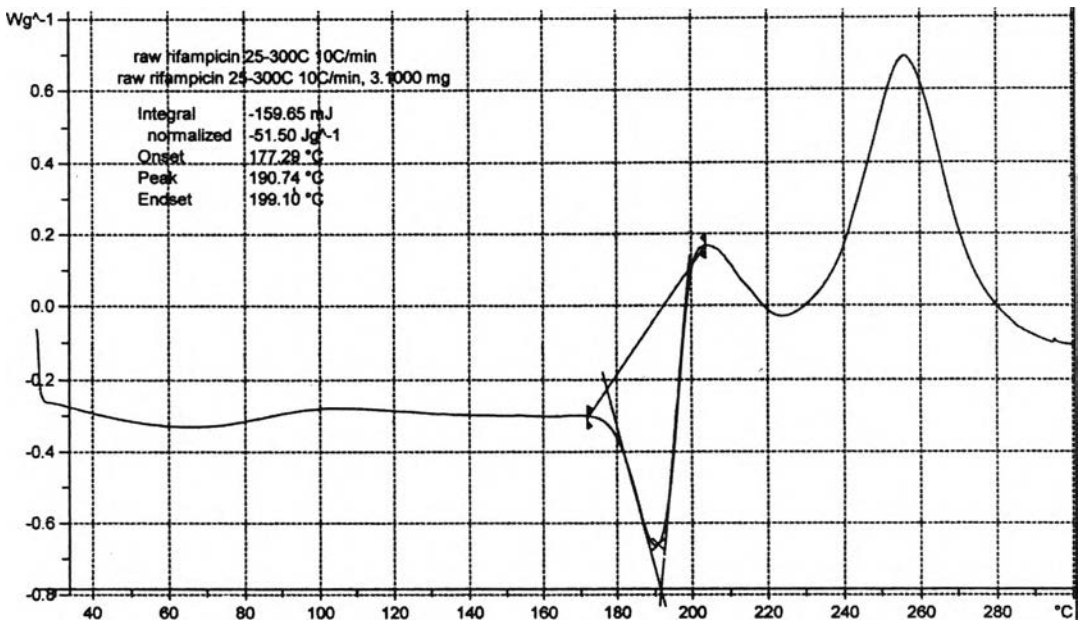


Figure 5-7 DSC thermogram of unprocessed rifampicin.

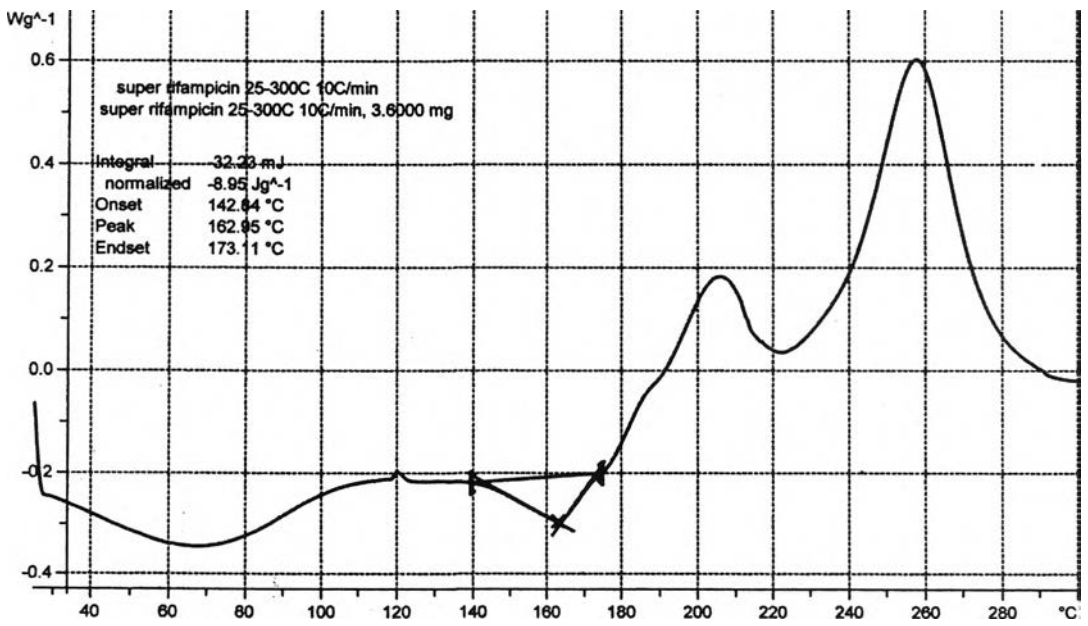


Figure 5-8 DSC thermogram of processed rifampicin by SAS technique.

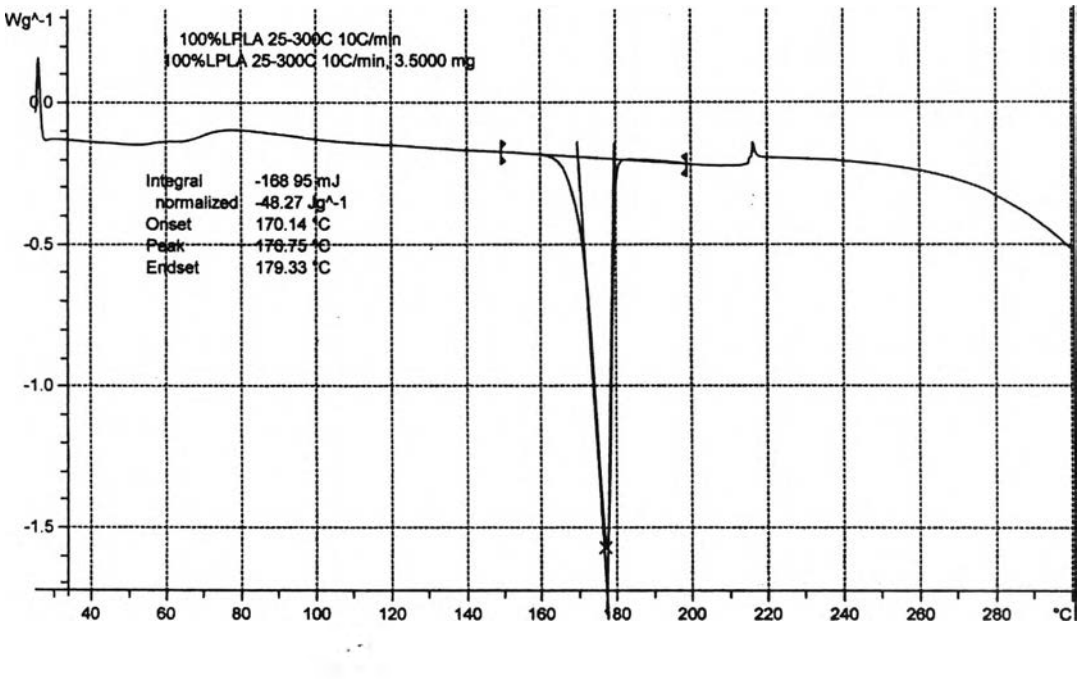


Figure 5-9 DSC thermogram of pure L-PLA.

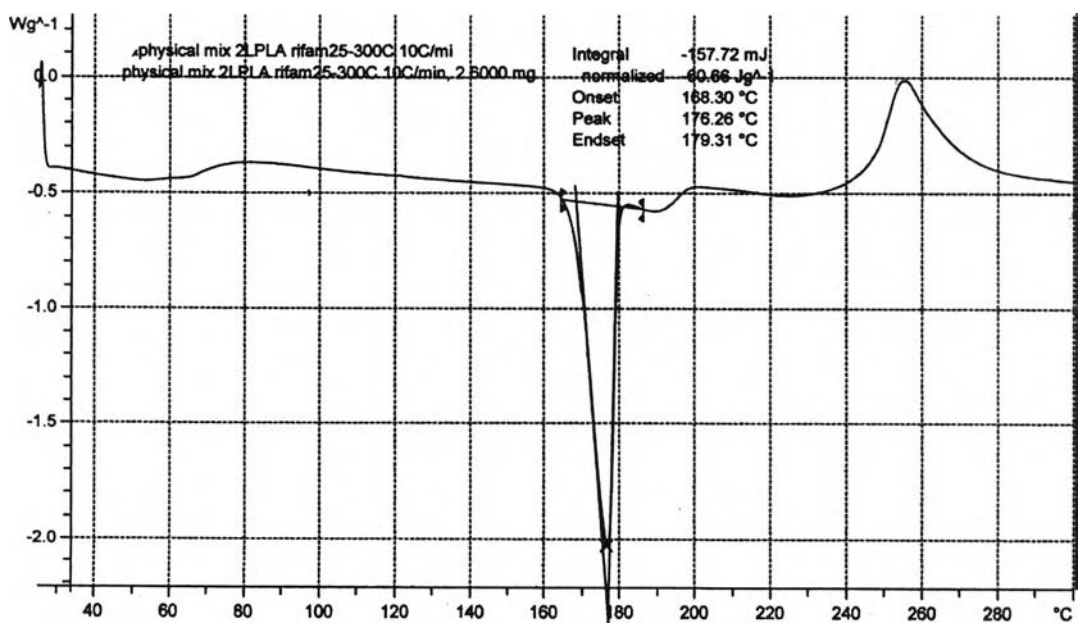


Figure 5-10 DSC thermogram of physical mixture of L-PLA and rifampicin.

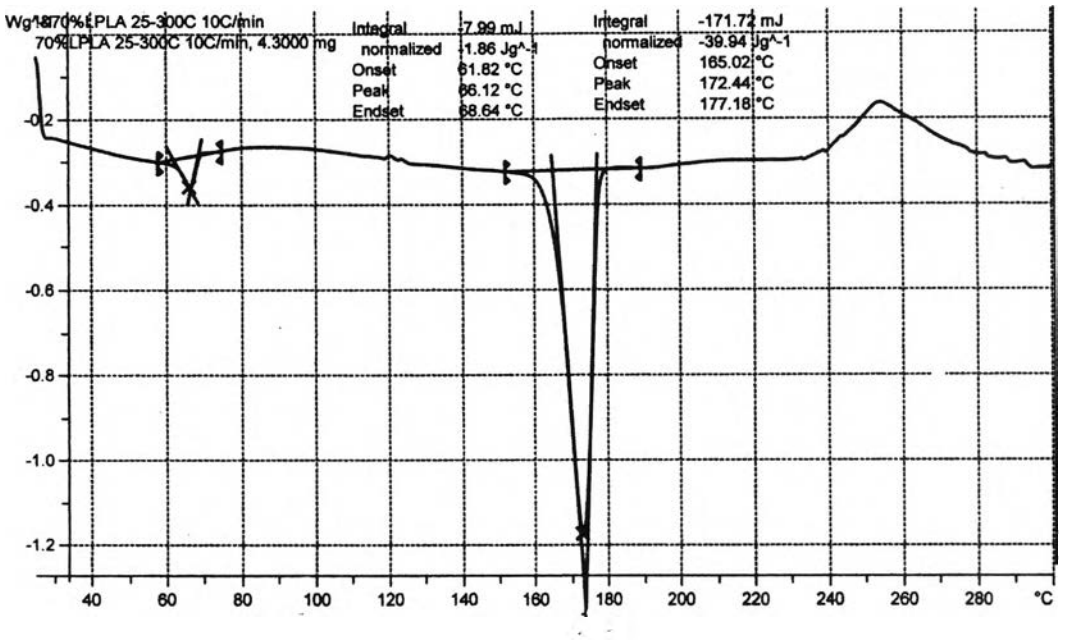


Figure 5-11 DSC thermogram of processed 70% L-PLA rifampicin microparticles prepared by SAS technique.

Table 5-25 Independent samples test of % rifampicin content of unprocessed and processed rifampicin.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
% rifampicin content	Equal variances assumed	6.907	.058	1.325	4	.256	.27000	.20380	-.29583	.83583
	Equal variances not assumed			1.325	2.358	.299	.27000	.20380	-.49098	1.03098

Table 5-26 ANOVA test of $D_{50\%}$ of rifampicin-L-PLA microparticles at various polymer molecular weight.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	128969.926	3	42989.975	10938.488	.000
Within Groups	31.441	8	3.930		
Total	129001.367	11			

$D_{50\%}$

Scheffe

MW	N	Subset for alpha = .05	
		1	2
70% L-PLA Sigma	3	3.5023	
70% L-PLA M2	3	3.7327	
70% L-PLA M1	3	3.9903	
70% L-PLA M3	3		243.1573
Sig.		.992	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table 5-27 Percent Drug release of rifampicin L-PLA microparticles of different polymer molecular weight.

Time (hr)	% Drug release average (SD)*		
	70% L-PLA M1	70% L-PLA M2	70% L-PLA Sigma
0	0.00(0.00)	0.00(0.00)	0.00(0.00)
0.25	1.40(1.19)	0.65(0.58)	1.48(0.29)
0.5	8.37(1.08)	3.15(1.05)	3.34(0.86)
0.75	13.95(0.44)	5.09(1.69)	5.77(1.97)
1	18.57(2.88)	6.98(1.39)	8.14(3.60)
2	28.05(5.87)	10.52(0.97)	19.55(7.69)
3	36.67(6.46)	13.24(0.98)	29.15(9.21)
4	41.74(8.30)	15.33(1.02)	38.55(8.49)
6	47.13(9.95)	18.51(2.38)	47.08(5.05)
8	50.79(10.61)	20.92(2.46)	52.74(4.19)
10	54.02(10.58)	23.37(3.22)	55.78(3.60)
12	56.63(9.86)	25.40(3.28)	58.00(3.99)
16	58.15(8.79)	28.64(2.76)	61.07(4.06)
20	60.78(7.91)	31.76(4.27)	64.69(6.09)
24	63.07(6.81)	34.27(4.84)	67.83(7.27)

Table 5-28 Physical properties of biodegradable polymers

Polymer Type	Inherent viscosity (dL/g)	Melting Point (°C)	Glass Transition Temperature (°C)	Degradation time (months)
L-PLA	0.90-1.2	170-200	55-65	>24
DL-PLA	0.55-0.75	Amorphous	50-60	12-16
50:50 DL-PLGA	0.55-0.75	Amorphous	40-50	1-2

Appendix B

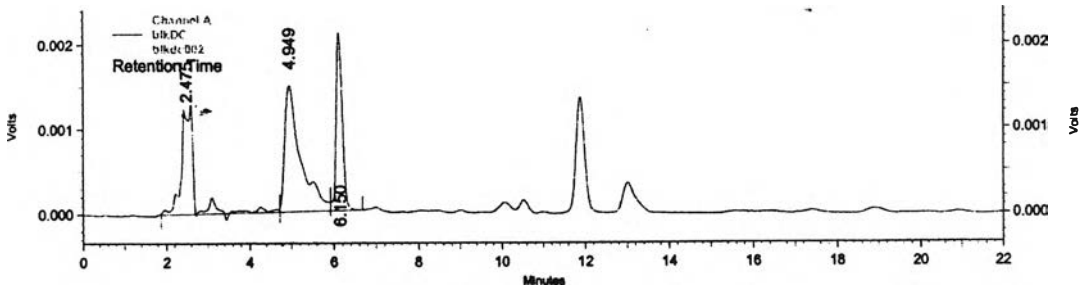
Validation of HPLC and UV Spectrophotometric Method

1. Validation of High Performance Liquid Chromatographic Technique for Drug Analysis

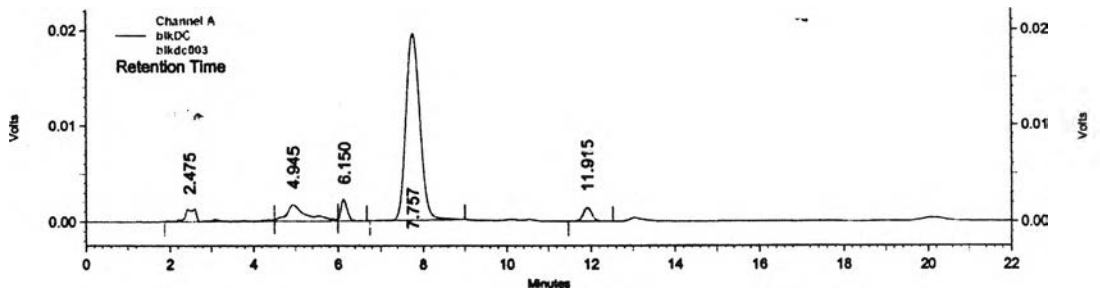
The HPLC method for the quantitative determination of rifampicin was validated as follows:

1.1 Specificity

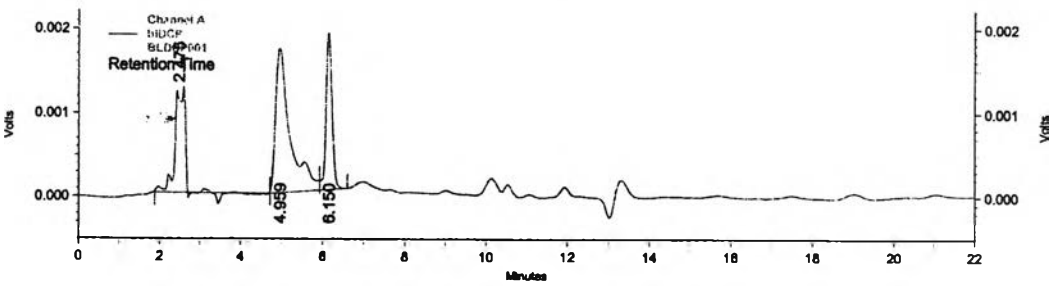
The HPLC chromatogram of blank solution, standard rifampicin solution, unloaded L-PLA polymer and standard rifampicin with unloaded L-PLA polymer are illustrated in Figure 5-11. The retention time of rifampicin is approximately 7.7 min. The peak of other component in the sample did not interfere with the peak of rifampicin. It was shown that this HPLC method had specificity to measure rifampicin.



(A)



(B)



(C)

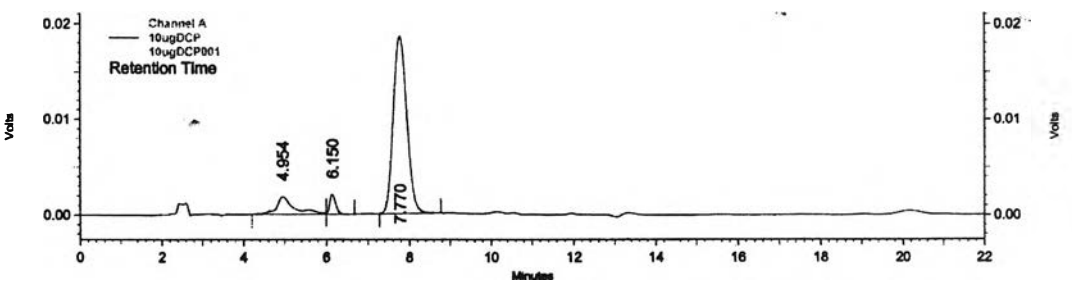


Figure 5-12 HPLC chromatogram of (A) blank solution (B) standard rifampicin solution (C) unloaded L-PLA polymer and (D) standard rifampicin with unloaded L-PLA polymer.

1.2 Precision

The precision of the analysis of rifampicin by HPLC method were determined for both within-run and between run as illustrated in Table 5-28 and Table 5-29, respectively. The percent coefficients of variation (%CV) of all determinations were ranging from 0.26-1.68 %. The accepted criteria of % CV are not more than 2%. It was indicated that HPLC method could be used to determine the amount of rifampicin over the range of studies.

Table 5-29 Data of within-run precision of rifampicin assayed by HPLC method.

Rifampicin concentration (μg/ml)	Calculated concentration from calibration curve. (μg/ml)	Mean	%CV
10	10.22 10.06 9.88	10.05	1.68
20	19.74 19.89 19.88	19.84	0.43
30	30.12 29.84 29.78	29.91	0.61
40	40.48 40.26 40.29	40.34	0.30
50	49.85 50.04 49.79	49.89	0.26

Table 5-30 Data of between-run precision of rifampicin assayed by HPLC method.

Rifampicin concentration (μg/ml)	Day	Calculated concentration from calibration curve. (μg/ml)	Mean	%CV
10	1	10.05	10.09	0.44
	2	10.09		
	3	10.14		
20	1	19.84	19.78	1.41
	2	20.02		
	3	19.47		
30	1	29.77	29.90	0.40
	2	29.91		
	3	30.01		
40	1	39.89	40.09	0.58
	2	40.34		
	3	40.04		
50	1	49.89	50.04	0.28
	2	50.07		
	3	50.16		

1.3 Accuracy

The accuracy of the analysis of rifampicin by HPLC method was performed by analyzing percent recoveries of five sets of 10, 20, 30, 40 and 50 μg/ml of known rifampicin solutions. The known rifampicin samples were prepared from physical mixture of rifampicin and L-PLA. Percent recovery of each concentration is showed in Table 5-30. The percent recovery was ranging from 98.57-101.20 %. The percent coefficient of variation of percent recoveries was 0.83 %. It was indicated that the HPLC method could be used to determine the amount of rifampicin within the concentration range studied.

Table 5-31 The analytical recovery data of rifampicin assayed by HPLC method.

Known concentration of Rifampicin. (μg/ml)	Calculated concentration from calibration curve. (μg/ml)	% Recovery
10	9.90	98.97
	10.10	100.96
	9.97	99.74
	10.06	100.58
	9.87	98.67
20	19.72	98.61
	20.13	100.64
	20.14	100.70
	20.09	100.46
	19.80	99.02
30	29.73	99.11
	30.04	100.14
	29.95	99.82
	29.97	99.90
	29.88	99.60
40	40.48	101.20
	40.26	100.64
	39.43	98.58
	39.43	98.57
	39.54	98.85
50	49.82	99.63
	49.47	98.94
	49.53	99.07
	49.70	99.40
	49.62	99.23
	Mean	99.64
	SD	0.82
	% CV	0.83

1.4 Linearity

Table 5-31 shows the standard curve data of rifampicin assayed by HPLC method. A standard curve plotted between the area and concentration in μg/ml is shown in Figure 5-12.

The linear regression analysis was applied for fitting the data obtained. The straight line was provided with a coefficient of determination (R^2) of 0.9999. The regression equation of the line is

$$y = 41497x + 514.9$$

where y is the area of rifampicin peak and x is the concentration of rifampicin solution in μg/ml.

Table 5-32 The standard curve data of rifampicin assayed by HPLC method.

Rifampicin concentration (μg/ml)	Area	Mean	%CV
10	423,969 409,470 424,552 417,903 410,533	417,285.40	1.71
20	819,728 826,093 825,644 823,671 822,325	823,492.20	0.32
30	1,250,533 1,238,814 1,236,318 1,247,179 1,257,449	1,246,058.60	0.69
40	1,680,253 1,671,034 1,672,280 1,672,499 1,680,677	1,675,348.60	0.28
50	2,068,950 2,076,968 2,066,607 2,057,565 2,057,035	2,065,425.00	0.40

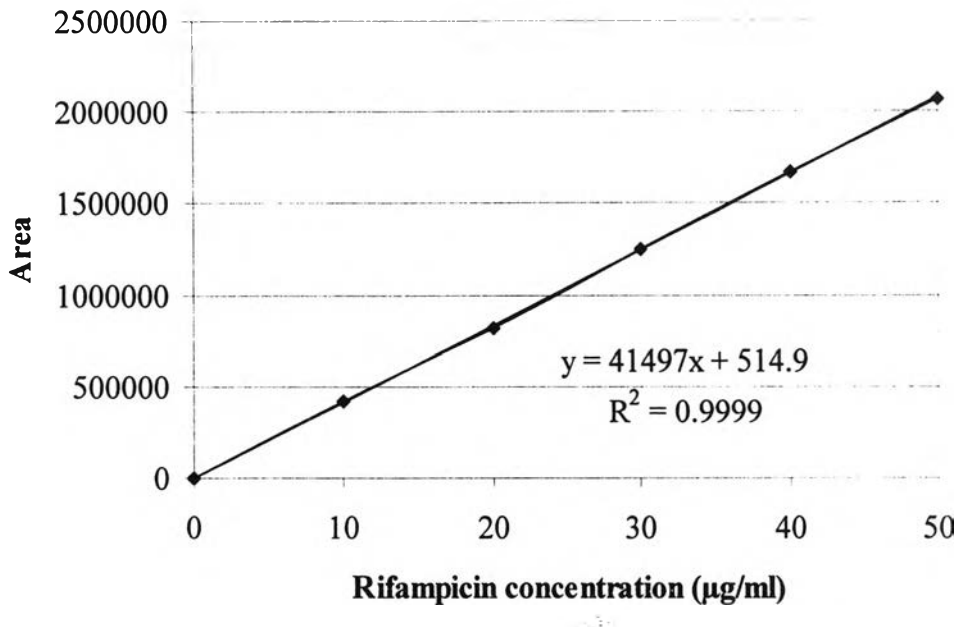


Figure 5-13 The standard curve of area against concentration of rifampicin.

2. Validation UV Spectrophotometric Method for Drug Analysis

The amount of rifampicin in dissolution medium could be determined by UV spectrophotometric method. The validation of UV spectrophotometric method used is presented as follow: (Gorog 1995).

2.1 Specificity

The absorption spectrum of rifampicin in phosphate buffer in saline (PBS) pH 7.4 is shown in Figure 5-13. The four maximum peaks of absorbance of rifampicin standard solution were 237, 255, 334 and 475 nm, respectively (Figure 5-13 A). The spectrum of the solution from rifampicin and L-PLA mixture did not be interfered and showed same maximum absorbance peak of drug (Figure 5-13 B). In this experiment, the rifampicin was assayed by measurement absorbance at 475 nm.

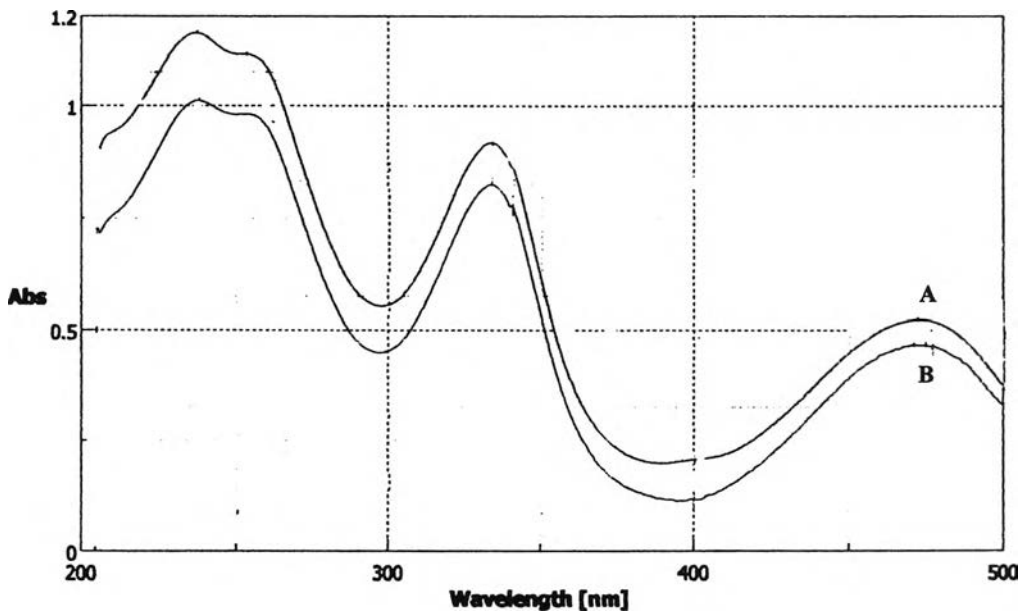


Figure 5-14 UV spectrum of rifampicin in phosphate buffer in saline pH 7.4 (A) rifampicin standard solution (B) rifampicin and L-PLA solution.

2.2 Precision

The precision of the analysis of rifampicin by UV spectrophotometric method at 475 nm of absorbance < 0.2 and absorbance > 0.2 were determined for both within-run and between run as illustrated in Table 5-32, Table 5-33, Table 5-34 and Table 5-35, respectively. The percent coefficients of variation (%CV) of all determinations were ranging from 0.13-1.62 %. The accepted criteria of % CV are not more than 2% (Gorog 1995). It was indicated that UV spectrophotometric method could be used to determine the amount of rifampicin over the range of studies.

Table 5-33 Data of within-run precision of rifampicin assayed by UV spectrophotometric method at 475 nm (absorbance <0.2).

Conc. (μg/ml)	Absorbance at 475 nm					
	Samp.1	Samp.2	Samp.3	Mean	SD	%CV
2	0.0385	0.0378	0.0379	0.0381	0.0004	0.99
4	0.0743	0.0733	0.0737	0.0738	0.0005	0.68
8	0.1479	0.1446	0.1442	0.1456	0.0020	1.39

Table 5-34 Data of between-run precision of rifampicin assayed by UV spectrophotometric method at 475 nm (absorbance <0.2).

Conc. (μg/ml)	Absorbance at 475 nm					
	Day 1	Day 2	Day 3	Mean	SD	%CV
2	0.0381	0.0371	0.0371	0.0374	0.0006	1.54
4	0.0738	0.0732	0.073	0.0733	0.0004	0.57
8	0.1456	0.1443	0.1489	0.1463	0.0024	1.62

Table 5-35 Data of within-run precision of rifampicin assayed by UV spectrophotometric method at 475 nm (absorbance >0.2).

Conc. (µg/ml)	Absorbance at 475 nm					
	Samp.1	Samp.2	Samp.3	Mean	SD	%CV
12	0.2193	0.2185	0.219	0.2189	0.0004	0.18
24	0.4339	0.4348	0.4362	0.4350	0.0012	0.27
32	0.5770	0.5760	0.5805	0.5778	0.0024	0.41

Table 5-36 Data of between-run precision of rifampicin assayed by UV spectrophotometric method at 475 nm (absorbance >0.2).

Conc. (µg/ml)	Absorbance at 475 nm					
	Day 1	Day 2	Day 3	Mean	SD	%CV
12	0.2189	0.216	0.2173	0.2174	0.0015	0.67
24	0.4350	0.4355	0.4361	0.4355	0.0006	0.13
32	0.5778	0.5763	0.5833	0.5791	0.0037	0.64

2.3 Accuracy

The accuracy of the analysis of rifampicin by UV spectrophotometric method at 475 nm was performed by analyzing percent recoveries of three sets of 2, 4, 8 μg/ml (absorbance <0.2) and 12, 24, 32 μg/ml (absorbance >0.2) of known rifampicin solutions. The known rifampicin samples were prepared from physical mixture of rifampicin and L-PLA. Percent recovery of each concentration is showed in Table 5-36 and Table 5-37. The percent recovery were ranging from 99.10-101.38 % and 99.24-100.78 % for absorbance <0.2 and absorbance >0.2, respectively. The accepted criteria of the percent recovery should not be less than 90 % and not more than 110 % (Gorog 1995). The percent coefficient of variation of percent recoveries were 0.86 % and 0.61 %. It was indicated that the UV spectrophotometric could be used to determine the amount of rifampicin within the concentration range studied.

Table 5-37 Accuracy data of rifampicin assayed by UV spectrophotometric method at 475 nm (absorbance <0.2)

Known concentration of Rifampicin. (μg/ml)	Calculated concentration from calibration curve. (μg/ml)	% Recovery
2	2.01	100.55
2	2.03	101.38
2	1.99	99.45
4	4.02	100.55
4	4.03	100.83
4	3.98	99.59
8	7.93	99.17
8	7.99	99.86
8	7.93	99.10
	Mean	100.15
	SD	0.87
	% CV	0.86

Table 5-38 Accuracy data of rifampicin assayed by UV spectrophotometric method at 475 nm (absorbance >0.2).

Known concentration of Rifampicin. (μg/ml)	Calculated concentration from calibration curve. (μg/ml)	% Recovery
12	12.09	100.78
12	12.02	100.14
12	11.93	99.45
24	23.72	98.83
24	23.82	99.24
24	24.05	100.21
32	31.82	99.45
32	31.77	99.27
32	31.78	99.33
	Mean	99.63
	SD	0.61
	% CV	0.61

2.4 Linearity

Table 5-38 and Table 39 show the standard curve data of rifampicin assayed by UV spectrophotometric at 475 nm for absorbance < 0.2 and absorbance > 0.2, respectively. A standard curve plotted between the absorbance which was less than 0.2 of rifampicin vs. concentration in μg/ml and absorbance which was more than 0.2 vs. the concentration in μg/ml at 475 nm are shown in Figure 5-13 and 5-14, respectively.

The linear regression analysis was applied for fitting the data obtained. The both of straight lines were provided with a coefficient of determination (R^2) of 0.9999. The regression equation of the line is

For absorbance <0.2:

$$y = 0.0181x + 0.001$$

For absorbance >0.2:

$$y = 0.0179x + 0.0026$$

where y is the absorbance of rifampicin and x is the concentration of rifampicin solution in μg/ml.

Table 5-39 Calibration curve data of rifampicin assayed by UV spectrophotometric at 475 nm for absorbance < 0.2.

Concentration (μg/ml)	Absorbance at 475 nm
0.5	0.0099
1	0.0199
2	0.0381
4	0.0738
6	0.1092
8	0.1456
10	0.1827

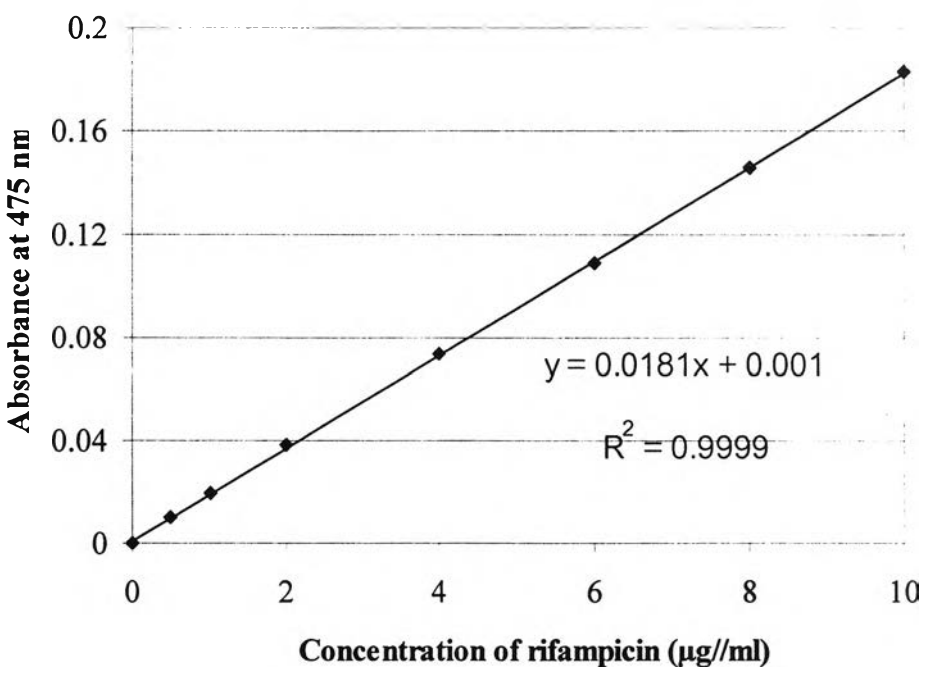


Figure 5-15 Calibration curve of rifampicin assayed by UV spectrophotometric at 475 nm (absorbance < 0.2).

Table 5-40 Calibration curve data of rifampicin assayed by UV spectrophotometric at 475 nm for absorbance > 0.2.

Concentration (μg/ml)	Absorbance at 475 nm
12	0.2189
16	0.2901
20	0.3634
24	0.435
32	0.5778
40	0.7176

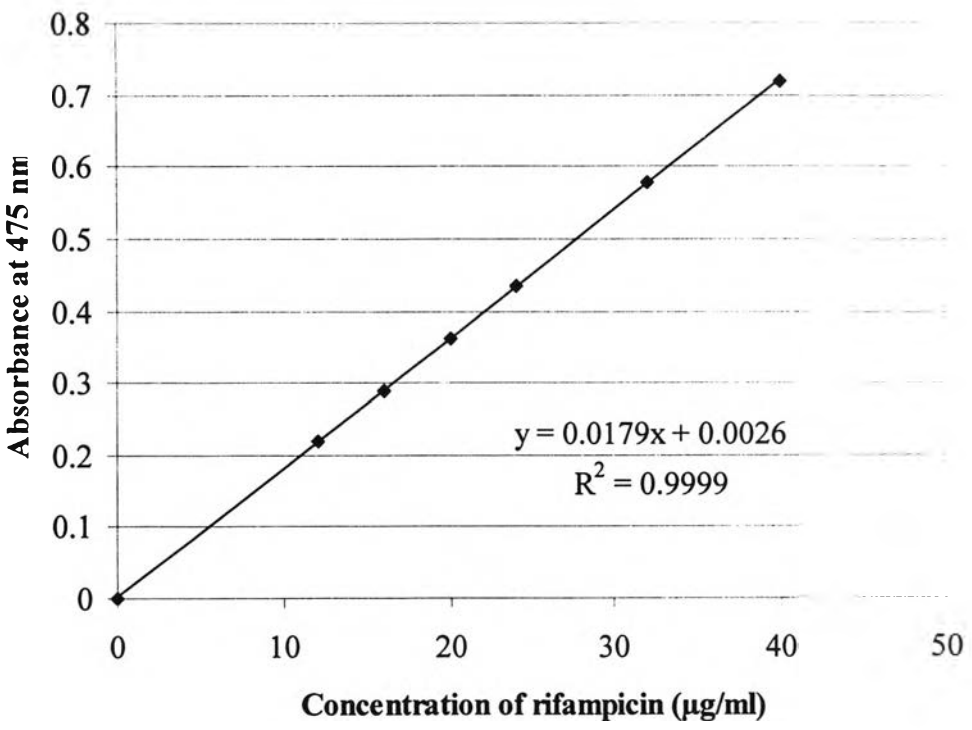


Figure 5-16 Calibration curve of rifampicin assayed by UV spectrophotometric at 475 nm (absorbance > 0.2).

VITA

Miss Vipaluk Patomchaiviwat was born on October 23, 1967 in Bangkok, Thailand. She got her bachelor degree in Pharmacy with first class honor from Faculty of Pharmacy, Mahidol University in 1989. After graduation from Mahidol University, she started to continue studying at Chulalongkorn University and got her Master degree in the field of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences in 1993. She started to work as a lecturer at Faculty of Pharmaceutical Sciences, Silpakorn University since 1993. Now she works as a lecturer at Faculty of Pharmaceutical Sciences, Silpakorn University.