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APPENDICES

APPENDIX A

Analysis of glucosamine hydrochloride and HPLC method validation

1.1 Spectrophotometric analysis of glucosamine hydrochloride

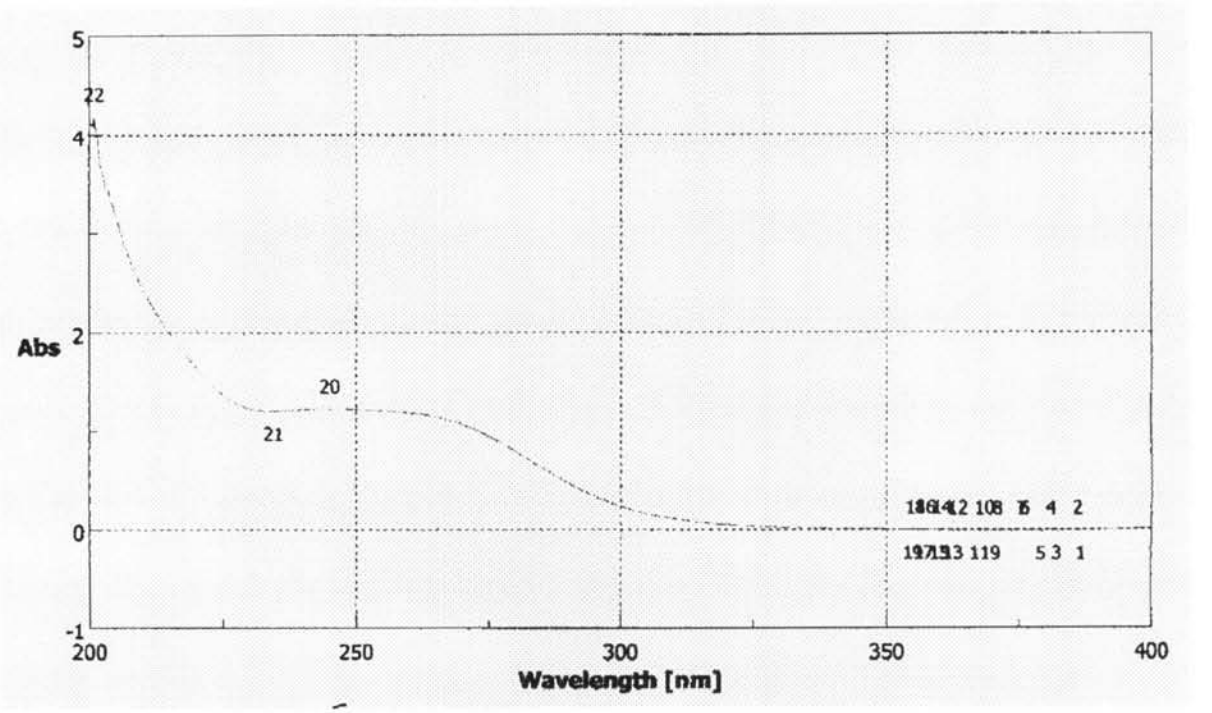


Figure 1A The UV spectra from UV spectrophotometer of standard glucosamine hydrochloride solution

From scanning UV-spectra of GS HCl at wavelength 200-400 nm, showed the maximum absorption wavelength at 245 nm and the UV spectra are shown in Figure 1A.

1.2 High-performance liquid chromatographic technique for drug analysis

GS HCl was analyzed by HPLC technique by pre-column PITC derivatization and the design chromatographic condition were previously mentioned. Analysis method validation parameter of GS HCl summarized in Table 1A. It can be determined with acceptable specificity, linearity, precision and accuracy.

The results of validation process are as in the following :

Table 1A Analytical method validation parameter of HPLC for glucosamine hydrochloride [a]

Parameter	Result value	Limited of acceptability
1.Specificity	No other peak interfere	No other peak interfere major peak
2.Linearity -the correlation coefficient(r^2)	0.9999	>0.9995
3.Precision -RSD (%)	2.0	≤2
4.Accuracy -recovery (%)	102.91	98-105

[a] From the European Agency for the Evaluation of Medicinal Products *Human Medicines Evaluation Unit*, 1996

1.2.1 Specificity

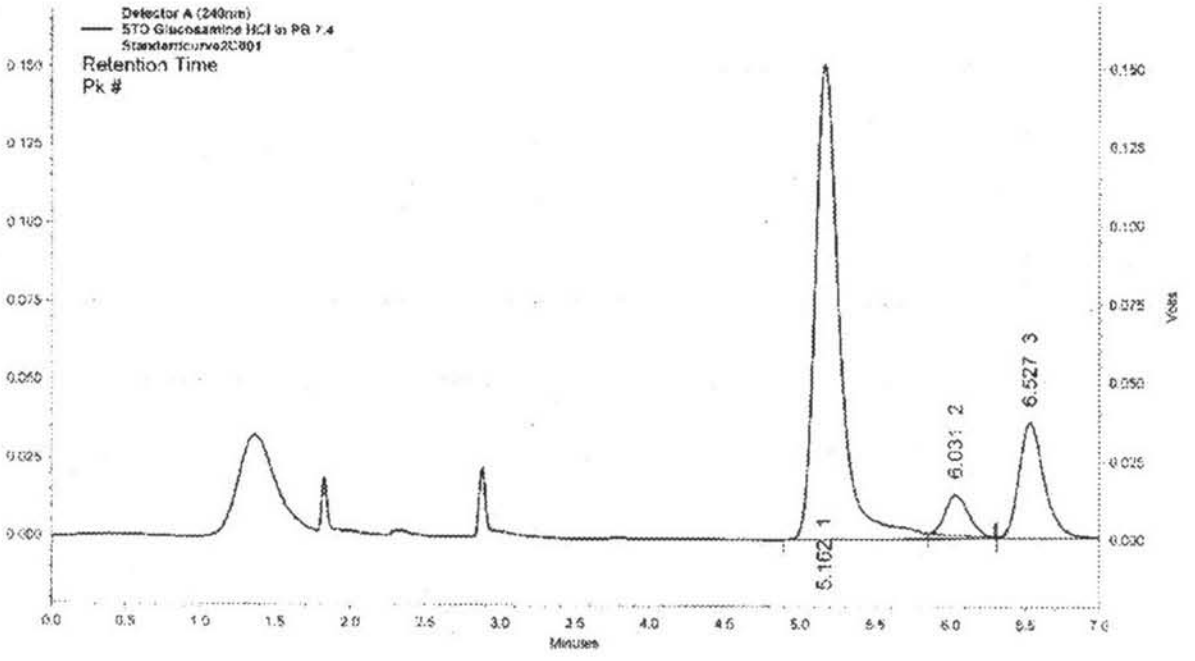


Figure 2A HPLC chromatogram of glucosamine hydrochloride and paracetamol (internal standard) having good resolution, with retention time of 5.162 and 6.527 minute, respectively

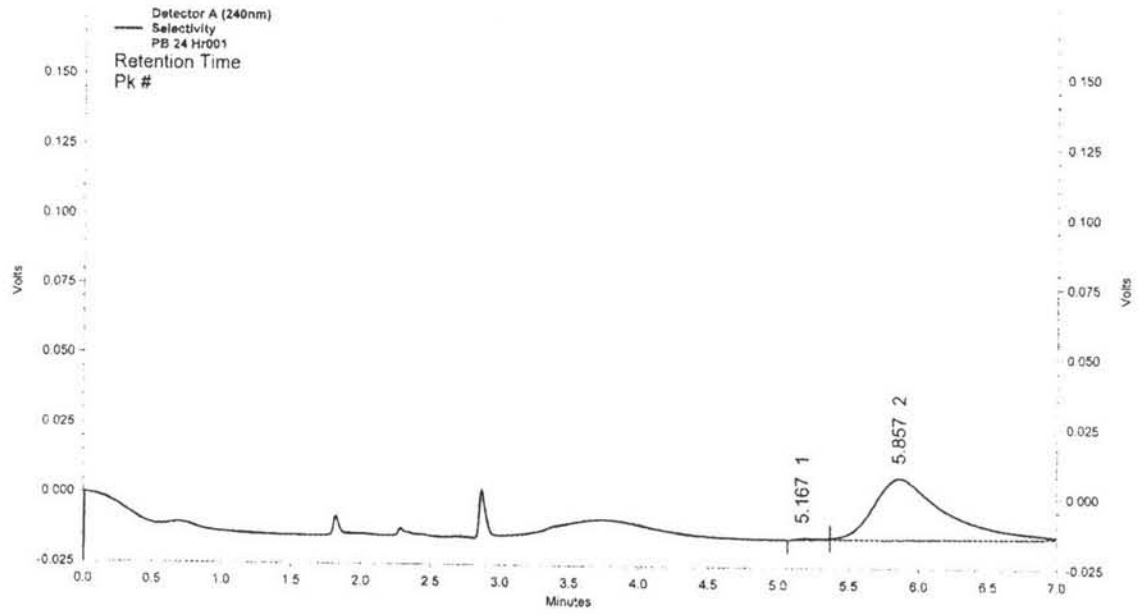


Figure 3A HPLC chromatogram of the receptor solution taken from diffusion cell with non-drug containing microemulsion system having no other peak interfere

1.2.2 Linearity

Table 2A Linearity of glucosamine hydrochloride

GS HCl concentration (mcg/ml)	Peak area ratio				
	Set no.1	Set no.2	Set no 3.	average	%CV
1.25	0.07	0.07	0.07	0.07	3.27
2.5	0.09	0.10	0.10	0.10	5.88
12.5	0.45	0.46	0.43	0.45	3.99
25	0.88	0.91	0.92	0.91	2.30
125	4.71	4.52	4.58	4.60	2.07
250	9.19	8.99	9.06	9.06	1.13

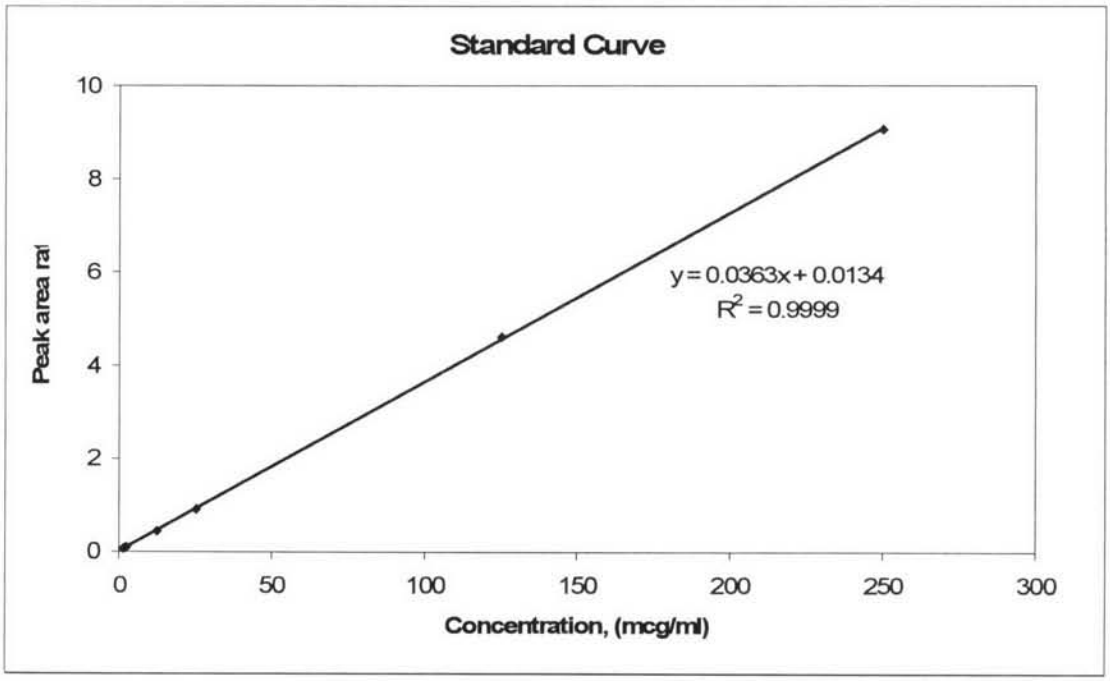


Figure 4A The calibration curve of glucosamine hydrochloride

1.2.3 Precision

Table 3A Within run precision

GS HCl Concentration (mcg/ml)	Calculated concentration from calibration curve(mcg/ml)							
	No.1	No.2	No.3	No.4	No.5	No.6	average	%CV
2.5	2.67	2.63	2.64	2.60	2.70	2.70	2.66	1.55
25	24.30	24.43	23.34	23.98	24.53	24.88	24.25	2.11
125	126.11	123.27	126.68	123.48	124.73	120.60	124.15	1.77

Table 4A Between run precision

Day	Calculated concentration of GS HCl from calibration curve (mcg/ml)							
	No.1	No.2	No.3	No.4	No.5	No.6	average	%CV
1	24.31	24.43	23.34	23.98	24.53	24.88	24.25	2.11
2	23.96	25.73	24.48	24.40	24.05	24.73	24.52	2.6
Average between day	24.4029							
%CV between day	2.35							

1.2.4 Accuracy

Table 5A The analytical recovery of glucosamine hydrochloride

Known concentration (mcg/ml)	Calculated concentration from calibration curve (mcg/ml)	% Recovery
12.43	13.09	105.27
	12.96	104.27
	13.04	104.93
24.86	24.53	98.68
	24.39	98.12
	24.41	98.17
248.56	262.38	105.56
	262.58	105.64
	262.43	105.58
Average		102.91
SD		0.29
%CV		0.28

APPENDIX B

Permeation of Glucosamine hydrochloride formulations across pig ear skin

Table 1B Permeation of glucosamine hydrochloride solution across pig ear skin (GS HCl concentration in donor = 0.5 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.12	0.55	0.97	4.44	9.12

$$Y = 0.0361X + 0.0444$$

$$R^2 = 0.9997$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	12.35	15.94	17.19	11.34	20.37	18.08	15.89	3.46
6	22.13	25.44	27.22	26.72	26.31	29.09	26.15	2.32
12	30.83	29.76	30.73	31.75	31.39	31.46	30.98	0.72
18	27.62	29.64	30.86	29.47	32.76	35.64	31.00	2.84
24	29.90	32.77	33.41	32.80	29.89	32.15	31.82	1.54

Table 2B Permeation of Glucosamine hydrochloride solution across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.17	0.51	0.95	4.76	9.44

$$Y = 0.0376X + 0.0451$$

$$R^2 = 1.0000$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	13.21	16.91	11.97	18.31	14.60	14.47	14.91	2.34
6	26.59	27.33	25.66	29.01	27.73	25.34	26.94	1.37
12	27.23	28.39	26.83	29.63	32.45	35.18	29.95	3.26
18	30.62	32.16	31.90	34.96	29.98	33.22	32.14	1.80
24	34.42	33.91	32.12	32.89	35.27	35.63	34.04	1.36

Table 3B Permeation of Glucosamine hydrochloride solution across pig ear skin (GS HCl concentration in donor = 10 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.11	0.43	0.96	4.26	8.54

$$Y = 0.0340X + 0.0423$$

$$R^2 = 0.9999$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	15.17	16.21	12.08	17.65	15.57	16.85	15.59	1.94
6	25.07	29.72	24.49	29.30	28.84	31.85	28.21	2.86
12	33.78	42.99	33.22	39.67	40.40	45.08	39.19	4.81
18	37.10	43.84	35.96	38.36	44.90	43.81	40.66	3.95
24	36.06	43.16	38.26	39.92	41.23	39.79	39.74	2.43

Table 4B Permeation of Glucosamine hydrochloride hydroalcoholic solution (10:90% ethanol:water) across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.13	0.67	1.00	4.72	9.14

$$Y = 0.0362X + 0.1318$$

$$R^2 = 0.9996$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	17.05	16.13	16.46	18.20	16.23	16.55	16.77	0.77
6	29.98	26.70	30.59	31.74	26.85	30.73	29.43	2.13
12	40.07	32.64	42.65	42.11	32.79	42.83	38.85	4.85
18	40.29	31.85	42.91	41.89	31.96	43.05	38.66	5.32
24	39.04	34.97	39.47	40.20	35.07	39.56	38.05	2.38

Table 5B Permeation of Glucosamine hydrochloride hydroalcoholic solution (60:40% ethanol:water) across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.17	0.51	0.95	4.76	9.44

$$Y = 0.0376X + 0.0451$$

$$R^2 = 1.0000$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	4.08	5.86	6.84	5.10	6.09	8.79	6.13	1.61
6	13.06	16.43	19.95	13.50	17.60	17.08	16.27	2.61
12	19.65	22.69	24.76	17.88	24.45	20.88	21.72	2.73
18	20.58	22.28	25.47	20.63	23.31	23.42	22.61	1.87
24	22.20	24.16	24.51	21.21	25.46	25.87	23.90	1.84

Table 6B Permeation of Glucosamine hydrochloride in HPMC gel across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.13	0.67	1.00	4.72	9.14

$$Y = 0.0362X + 0.1318$$

$$R^2 = 0.9996$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	15.68	10.08	11.33	16.73	10.15	11.39	12.56	2.90
6	25.77	20.09	28.34	27.27	20.20	28.48	25.03	3.90
12	26.89	24.82	29.74	28.13	24.94	29.86	27.40	2.24
18	26.47	26.51	29.00	27.40	26.61	29.08	27.51	1.23
24	27.30	25.32	29.80	28.08	25.39	29.87	27.63	2.02

Table 7B Permeation of Glucosamine hydrochloride in AOT microemulsion across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.10	0.42	0.85	4.11	8.48

$$Y = 0.0338X + 0.0092$$

$$R^2 = 0.9997$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1.82	1.47	1.28	1.57	3.09	1.48	1.79	0.66
6	5.05	3.20	3.29	3.61	5.92	3.73	4.14	1.10
12	18.65	13.39	20.54	16.60	20.23	18.90	18.05	2.68
18	22.41	21.50	22.36	18.00	13.80	17.64	19.29	3.42
24	21.05	19.08	21.68	19.16	19.12	19.12	19.87	1.18

Table 8B Permeation of Glucosamine hydrochloride in CTAB microemulsion across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.19	0.56	0.97	4.09	8.34

$$Y = 0.0327X + 0.1177$$

$$R^2 = 0.9996$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	4.97	7.04	8.05	6.25	7.26	10.56	7.36	1.89
6	15.03	15.98	16.97	15.11	12.99	19.94	16.00	2.34
12	21.52	21.17	20.79	14.64	19.12	21.17	19.73	2.64
18	20.59	20.11	18.26	19.69	20.36	23.74	20.46	1.81
24	20.62	18.73	21.28	18.62	19.61	21.83	20.11	1.34

Table 9B Permeation of Glucosamine hydrochloride in lecithin microemulsion across pig ear skin (GS HCl concentration in donor = 0.5 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.12	0.42	0.77	3.79	7.88

$$Y = 0.0313X + 0.0021$$

$$R^2 = 0.9995$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.12	0.35	0.21	0.11	0.09	0.12	0.17	0.10
6	0.16	0.21	0.19	0.15	0.16	0.17	0.17	0.02
12	0.14	0.17	0.16	0.13	0.11	0.58	0.22	0.18
18	0.17	0.22	0.20	0.18	0.16	0.28	0.20	0.04
24	0.19	0.26	0.22	0.13	0.19	0.27	0.21	0.05

Table 10B Permeation of Glucosamine hydrochloride in lecithin microemulsion across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.22	0.63	1.02	4.73	9.16

$$Y = 0.0362X + 0.1504$$

$$R^2 = 0.9999$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.01	0.62	0.77	2.44	0.13	0.64	0.77	0.87
6	0.50	0.58	0.67	1.10	0.15	2.25	0.88	0.74
12	1.03	1.33	1.40	0.57	0.02	0.49	0.81	0.54
18	0.70	0.91	0.95	1.09	0.52	1.09	0.88	0.23
24	0.70	0.87	0.92	1.03	0.45	1.00	0.83	0.22

Table 11B Permeation of Glucosamine hydrochloride in Tween 80 microemulsion across pig ear skin (GS HCl concentration in donor = 0.5 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.17	0.51	0.95	4.76	9.44

$$Y = 0.0376X + 0.0451$$

$$R^2 = 1.0000$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	13.54	19.74	12.11	19.62	17.69	15.08	16.29	3.21
6	23.37	34.23	20.49	35.96	27.08	31.02	28.69	6.12
12	43.03	26.74	49.44	40.50	47.61	55.36	43.78	9.82
18	46.08	45.70	40.45	41.53	43.48	43.57	43.47	2.22
24	44.84	44.81	41.49	46.03	45.08	47.28	44.92	1.93

Table 12B Permeation of Glucosamine hydrochloride in Tween 80 microemulsion across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.17	0.51	0.95	4.76	9.44

$$Y = 0.0376X + 0.0451$$

$$R^2 = 1.0000$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	21.37	24.09	21.07	17.88	13.92	16.78	19.18	3.68
6	38.46	41.84	42.33	42.03	39.96	37.29	40.32	2.10
12	45.17	45.84	44.47	53.43	46.61	47.21	47.12	3.24
18	44.34	47.46	50.36	48.84	46.97	44.47	47.08	2.38
24	44.66	47.83	58.33	45.82	46.46	43.58	47.78	5.37

Table 13B Permeation of Glucosamine hydrochloride in Tween 80 microemulsion across pig ear skin (GS HCl concentration in donor = 10 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.16	0.53	1.05	4.57	9.01

$$Y = 0.0356X + 0.1089$$

$$R^2 = 0.9999$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	7.99	12.23	14.14	11.55	12.40	16.75	12.51	2.90
6	2.84	20.47	24.43	22.58	23.08	29.29	20.45	9.12
12	17.84	31.02	36.66	34.42	32.40	41.93	32.38	8.09
18	19.80	29.89	42.76	30.06	30.28	41.43	32.37	8.53
24	24.41	33.19	34.57	30.85	32.19	37.47	32.11	4.40

Table 14B Permeation of Glucosamine hydrochloride in Tween 80 microemulsion gel across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.32	0.60	0.99	4.63	8.94

$$Y = 0.0352X + 0.1795$$

$$R^2 = 0.9998$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	25.14	20.43	23.39	13.31	14.90	21.41	19.76	4.70
6	33.67	28.28	33.50	23.78	24.98	32.17	29.40	4.36
12	45.32	44.24	49.43	44.24	39.31	46.29	44.81	3.31
18	43.00	40.95	49.88	44.39	35.70	46.90	43.47	4.91
24	58.08	42.52	44.89	46.76	40.22	42.58	45.84	6.40

Table 15B Permeation of Glucosamine hydrochloride in Tween 80 microemulsion (w/o) across pig ear skin (GS HCl concentration in donor = 2 %)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.13	0.51	0.96	4.78	9.36

$$Y = 0.0374X + 0.0442$$

$$R^2 = 0.9999$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	6.48	6.61	7.46	6.71	6.72	8.70	7.11	0.85
6	10.11	14.49	15.24	13.43	13.03	15.60	13.65	2.00
12	20.87	25.65	23.77	24.07	24.32	25.21	23.98	1.68
18	21.91	24.80	26.03	23.66	25.38	25.21	24.50	1.49
24	23.42	24.07	24.06	25.70	26.44	27.06	25.12	1.48

Table 16B Permeation of Glucosamine hydrochloride in micelle system of Tween 80 and water across pig ear skin (GS HCl concentration in donor = 2%)

Calibration curve data

Concentration(μg/ml)	0	2.5	12.5	25	125	250
Peak area ratio	0	0.23	0.55	0.10	4.60	8.84

$$Y = 0.0351X + 0.115$$

$$R^2 = 0.9997$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	9.40	11.26	11.75	10.04	12.24	9.73	10.74	1.17
6	13.81	18.70	19.58	17.22	20.30	19.86	18.25	2.43
12	28.90	33.03	31.78	31.11	35.85	32.56	32.20	2.30
18	31.27	30.71	32.96	33.28	33.29	33.76	32.54	1.24
24	30.03	29.92	32.00	31.63	32.41	32.74	31.46	1.20

APPENDIX C

Pig ear skin preparation

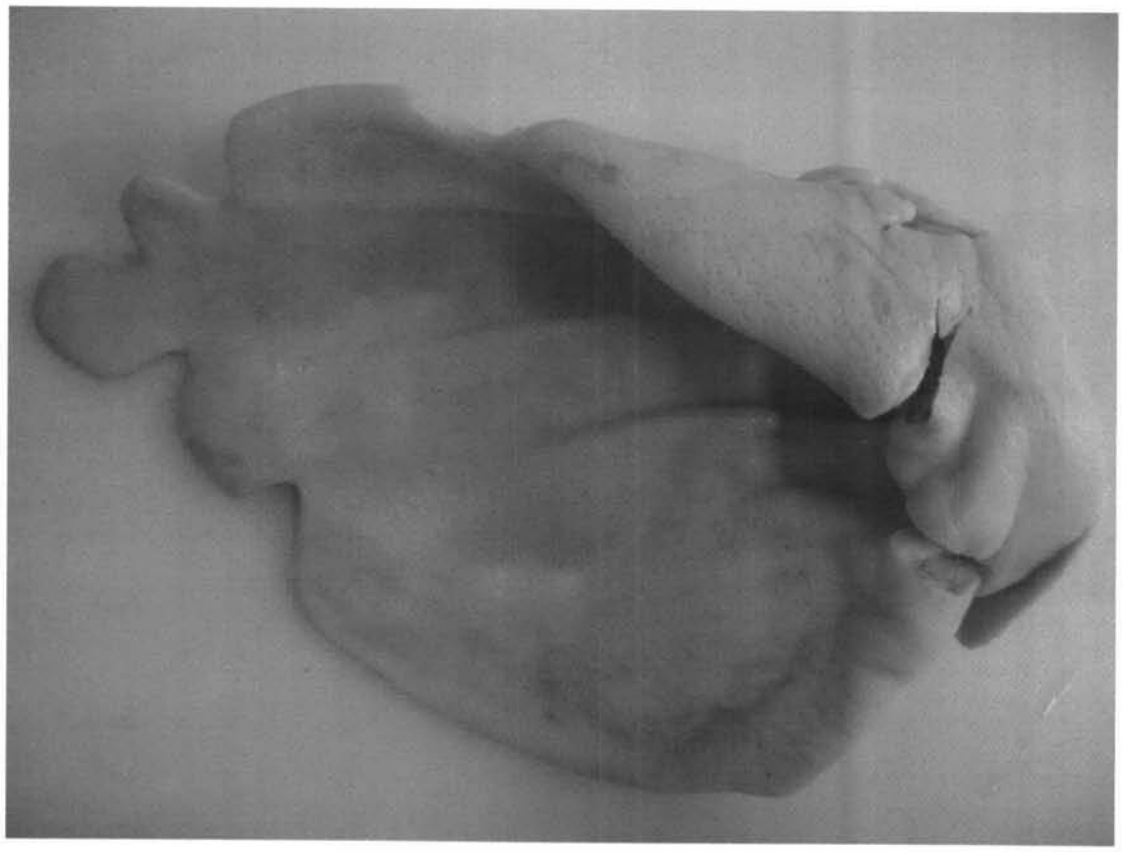
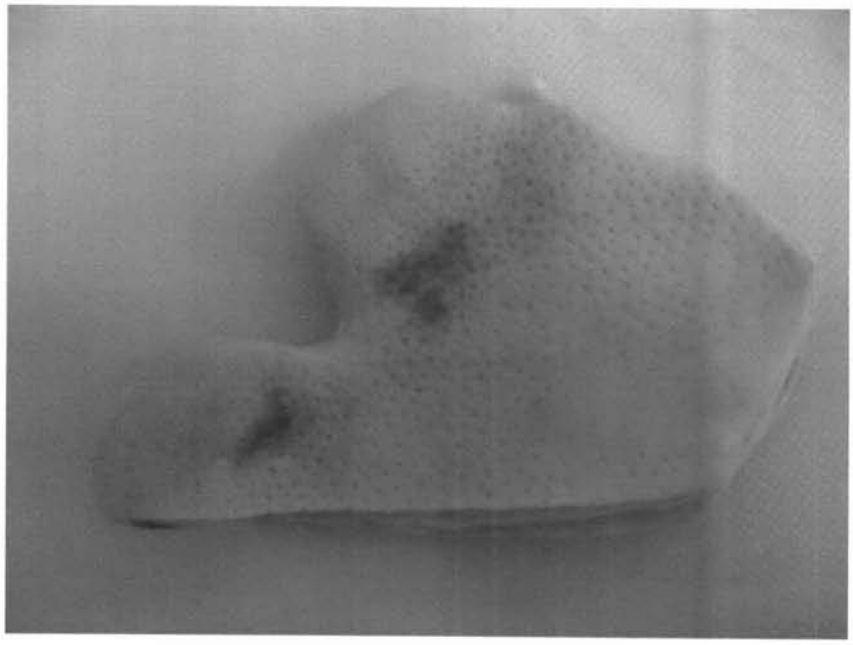


Figure 1C Pig ear obtained from local market in Chainat province of Thailand.



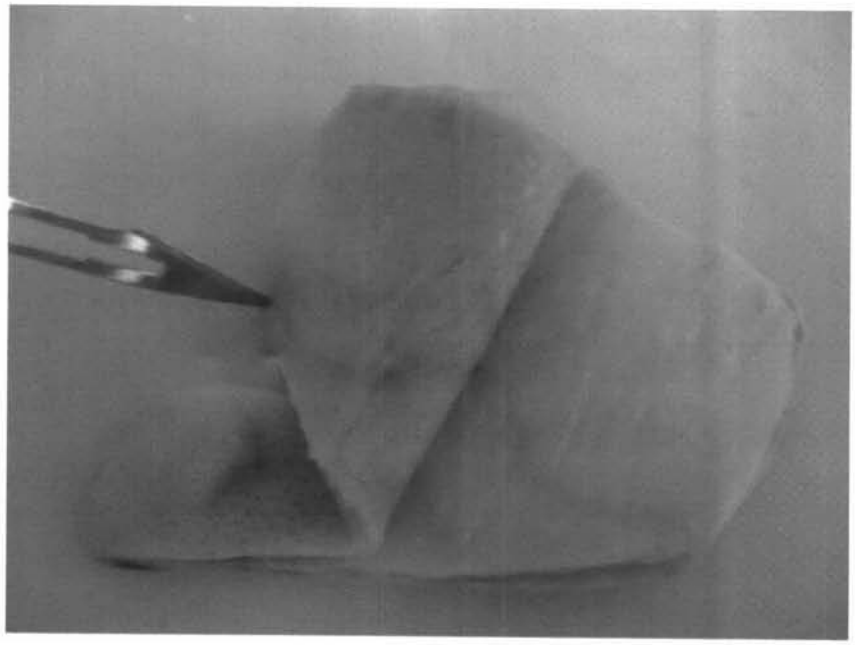


Figure 2C Subcutaneous fat and other extraneous tissues adhering to the dermis were completely removed and trimmed if necessary using forceps and scissors.

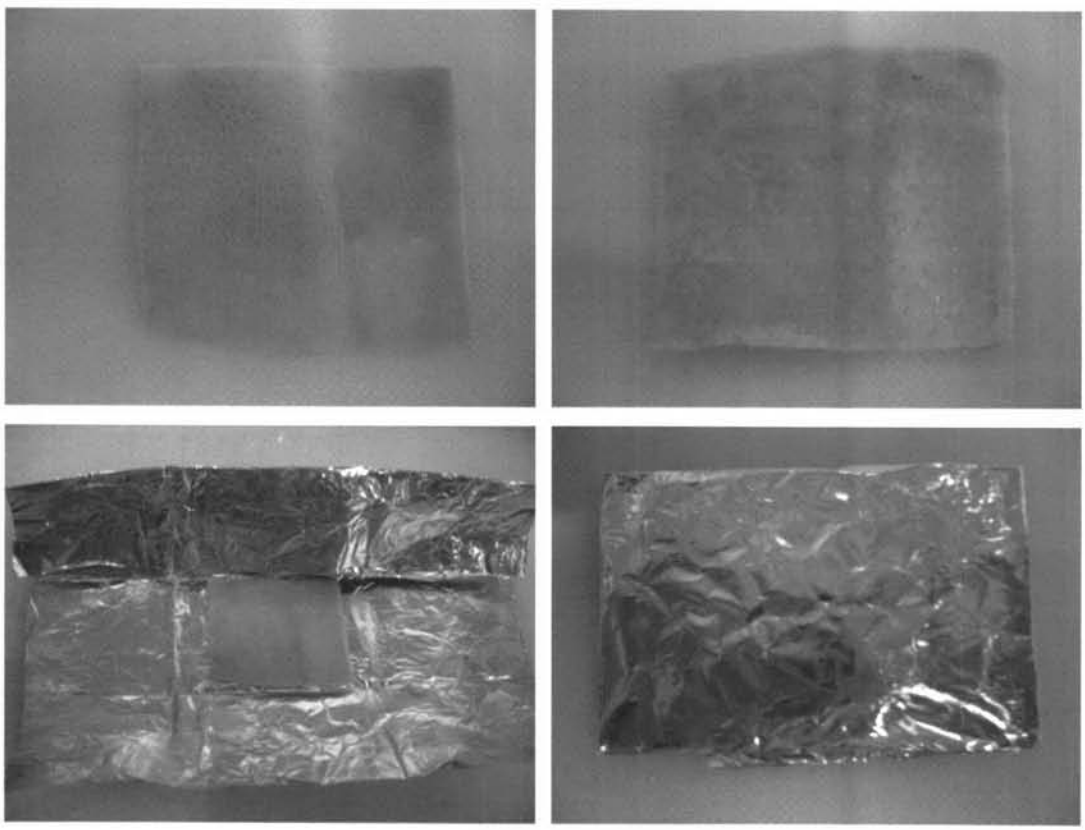


Figure 3C The skin specimen was cut to the size of 4x4 cm^2 wrapped in aluminium foil and stored at -20°c.

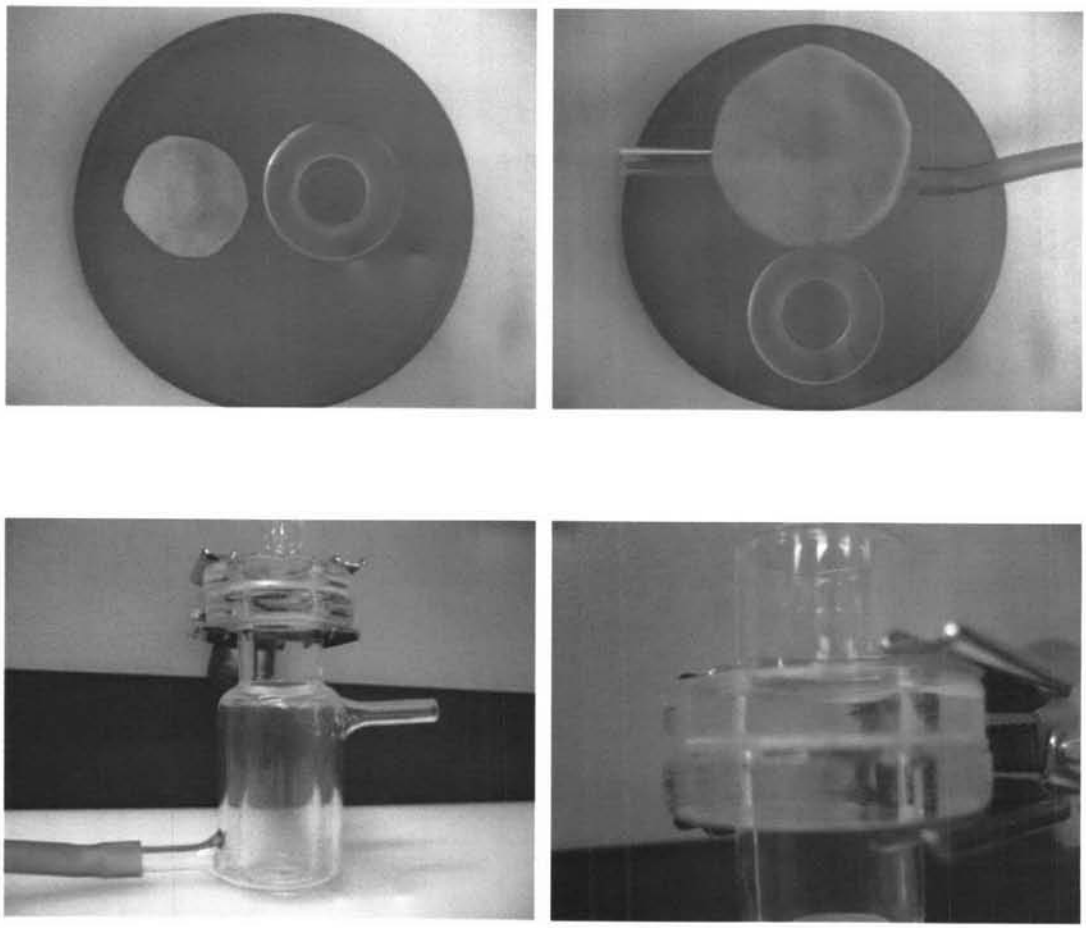


Figure 4C The excised pig ear skin was mould between the donor and the receptor compartment of franz-diffusion cell.

VITA

Miss Punlapa Anusitthakul was born on September 8, 1977 in Chainat, Thailand. She obtained her Bachelor degree in Pharmacy (B.Pharm.) from the Faculty of Pharmacy, Silpakorn University, Nakornpathom, Thailand in 2001. She has been working in Chainat hospital from 2002 until now.