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APPENDICES

APPENDIX I

Validation of the HPLC Method

Validation of the HPLC Method

Analytical parameters validated were precision, accuracy and linearity. The validation of an analytical method was the process by which shown the characteristics of the method were established to meet the USP 27,2004 requirements for the intended analytical applications.

1. Precision

The precision of an analytical method was the degree of agreement between individual tests results when the method was applied repeated to multiple samplings of a homogenous sample. The precision of an analytical method was usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. The determination of precision of the analysis of asiaticoside by the HPLC method was performed by analyzing the coefficient of variation of three sets of five standard solutions.

Table 17 and 18 illustrate the data of within run precision and between run precision, respectively. The value of coefficient of variation of the within run and between run precisions were 0.71-1.09 % and 0.48-1.81% respectively. The coefficient of variation of an analytical method could generally be less than 2% (USP27,2004). Since all the values were less than 2%, the HPLC method could be used for quantitative analysis of asiaticoside in the range studied.

Table 17 The Data of Within Run Precision

Concentration	Peak Area			Mean	SD	%CV
(mg/ml)	Set No.1	Set No.2	Set No. 3			
0.15	212503	214521	209987	212337	1854.72	0.87
0.25	326540	332102	331023	329888	2408.26	0.73
0.30	402314	398756	395412	398827	2818.18	0.71
0.45	542365	556245	545210	547940	5986.28	1.09

Table 18 The Data of Between Run Precision

Concentration (mg/ml)	Peak Area									Mean	SD	%CV
	Day 1			Day 2			Day 3					
	Set No.1	Set No.2	Set No. 3	Set No.1	Set No.2	Set No. 3	Set No.1	Set No.2	Set No. 3			
0.15	225013	221540	220479	225412	227520	226312	223456	226874	228217	224980	2514	1.12
0.25	324455	323565	329026	326540	329307	326412	326273	320839	327957	326042	2569	0.79
0.30	382959	384940	387412	384512	387551	385246	389432	385109	386848	386001	1851	0.48
0.45	556129	558789	556974	586367	572018	563312	572079	582737	568548	568550	10297	1.81
0.50	631324	617939	645967	648895	636087	647123	650164	654842	639227	641285	10812	1.69

2. Accuracy

The accuracy of an analytical method was the closeness of test results obtained to the true value. The accuracy could be established across its range. The determination of accuracy of analysis of asiaticoside by HPLC method was performed by analyzing the percentages of analytical recovery of three sets of five standard solutions. The percentages of analytical recovery of asiaticoside are shown in Table 19. The mean and % CV of the percentages of analytical recovery of all asiaticoside concentrations were 101.01 % and 0.41 % respectively, indicating that this method could be used for analysis of asiaticoside in all concentrations studied with high accuracy. The mean of the percentage of analytical recovery could generally be 98-102% (USP27,2004).

Table 19 The Percentages of Analytical Recovery of Asiaticoside Analysis by HPLC Method

Concentration	Estimated concentration (mg/ml)			Mean ±SD	% Recovery
mg/ml	1	2	3		
0.15	0.15	0.15	0.15	0.15	101.56
0.25	0.25	0.25	0.25	0.25	101.20
0.3	0.30	0.30	0.30	0.30	101.00
0.45	0.45	0.45	0.45	0.45	100.30
0.5	0.5	0.51	0.51	0.505	101.00
Mean					101.01
SD					0.41
%CV					0.46

3. Linearity

The linearity of an analytical method was the ability to elicit test results that were directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity could be established across the range of the analytical procedures. It should be established initially by visual examination of a plot of signals as a function of analyte concentration. If there appeared to be a linear relationship, test results could be established by calculation of regression line by the method of least squares. The calibration curve data is shown in Table 20. A plot of asiaticoside concentration versus the peak area ratios of asiaticoside (Figure 12) illustrated the linear correlation in the concentration range studied. The coefficient of determination (r^2) of this line was 0.9956, and the corresponding equation was Y= -0.01452 + 8.0687 e-07 x. These results indicated that the HPLC method was acceptable for quantitative analysis of asiaticoside in the concentration range studied.

Table 20 Data of Calibration Curve of Asiaticoside Analysis by HPLC Method

Concentration	Peak area ratio			Mean	SD	%CV
mg/ml	1	2	3			
0.15	201532	204210	203254	202999	1357.14	0.668544
0.25	335400	334267	331023	333563	2271.76	0.681058
0.3	398412	397542	391204	395719	3934.51	0.994269
0.45	542365	556245	545210	547940	7331.66	1.338041
0.5	652102	659072	652130	654435	4016.07	0.613671

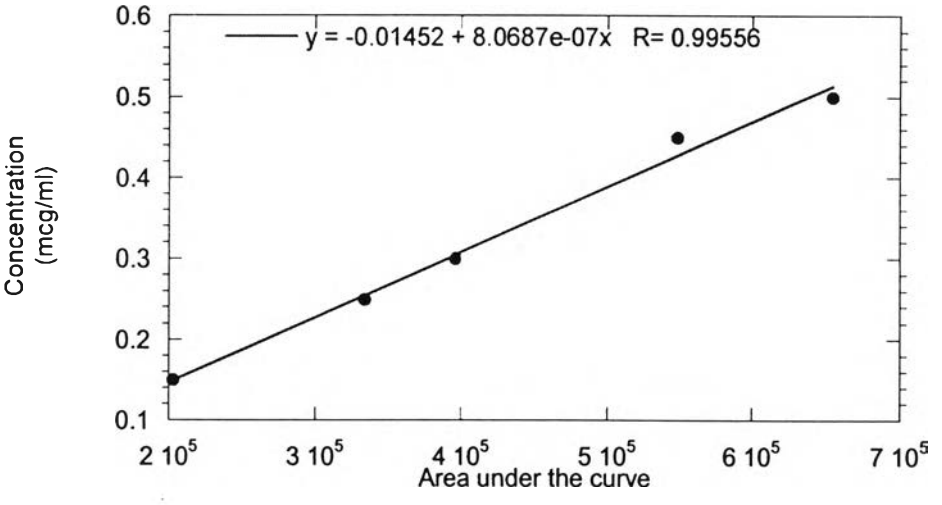

Figure 12 Calibration Curve of Asiaticoside Analysis Using HPLC Method

APPENDIX II

Statistical Analysis Data

Table 20 The Statistical Data of Moisturizing Effect Using SAS Program

OBS	SUBJECT	MWK0	MWK1	MWK2	MWK3	MWK4	DMWK1	DMWK2	DMWK3	DMWK4
1	1	14.98	15.2	14.3	10.7	9.5	0.22	-0.68	-4.28	-5.48
2	1	16.20	14.8	10.5	11.5	9.5	-1.40	-5.70	-4.70	-6.70
3	1	15.30	16.3	9.0	13.4	7.9	1.00	-6.30	-1.90	-7.40
4	2	11.20	9.5	14.6	10.6	8.5	-1.70	3.40	-0.60	-2.70
5	2	12.50	10.4	11.0	11.5	7.9	-2.10	-1.50	-1.00	-4.60
6	2	12.70	10.9	8.8	8.9	7.9	-1.80	-3.90	-3.80	-4.80
7	3	13.90	19.1	14.6	13.9	15.3	5.20	0.70	0.00	1.40
8	3	11.60	14.5	11.0	11.0	16.4	2.90	-0.60	-0.60	4.80
9	3	12.10	11.3	8.8	10.5	14.9	-0.80	-3.30	-1.60	2.80
10	4	7.30	7.4	7.5	9.1	9.3	0.10	0.20	1.80	2.00
11	4	5.00	6.5	5.8	6.3	7.3	1.50	0.80	1.30	2.30
12	4	4.30	4.2	4.6	5.0	7.1	-0.10	0.30	0.70	2.80
13	5	11.60	8.5	7.4	13.4	10.1	-3.10	-4.20	1.80	-1.50
14	5	9.70	13.3	6.1	7.4	10.4	3.60	-3.60	-2.30	0.70
15	5	12.60	12.1	6.7	13.8	12.1	-0.50	-5.90	1.20	-0.50
16	6	16.70	12.6	12.9	9.5	6.2	-4.10	-3.80	-7.20	-10.50
17	6	13.40	10.3	9.3	8.5	5.9	-3.10	-4.10	-4.90	-7.50
18	6	9.80	10.5	13.7	9.0	6.1	0.70	3.90	-0.80	-3.70
19	7	29.10	19.4	10.4	13.9	13.2	-9.70	-18.70	-15.20	-15.90
20	7	29.80	25.7	9.1	14.0	11.4	-4.10	-20.70	-15.80	-18.40
21	7	21.40	23.3	9.1	13.6	11.4	1.90	-12.30	-7.80	-10.00
22	8	9.00	19.1	7.2	12.0	5.9	10.10	-1.80	3.00	-3.10
23	8	9.10	12.6	7.7	7.2	5.5	3.50	-1.40	-1.90	-3.60
24	8	6.90	17.1	7.1	9.5	5.3	10.20	0.20	2.60	-1.60
25	9	8.40	9.4	6.6	8.5	6.5	1.00	-1.80	0.10	-1.90
26	9	7.20	6.4	5.1	9.5	7.1	-0.80	-2.10	2.30	-0.10
27	9	6.10	9.0	5.6	8.7	6.2	2.90	-0.50	2.60	0.10
28	10	16.70	15.5	13.2	11.2	7.4	-1.20	-3.50	-5.50	-9.30
29	10	13.20	14.5	10.5	9.0	4.4	1.30	-2.70	-4.20	-8.80
30	10	10.70	13.8	11.0	9.4	7.2	3.10	0.30	-1.30	-3.50
31	11	22.70	23.0	18.9	20.4	14.9	0.30	-3.80	-2.30	-7.80
32	11	26.50	17.8	22.0	18.5	11.0	-8.70	-4.50	-8.00	-15.50
33	11	27.60	12.4	20.3	17.3	10.3	-15.20	-7.30	-10.30	-17.30
34	12	12.50	14.0	14.7	11.9	11.3	1.50	2.20	-0.60	-1.20
35	12	14.20	12.7	14.7	10.8	8.2	-1.50	0.50	-3.40	-6.00
36	12	11.20	12.9	14.3	8.1	8.4	1.70	3.10	-3.10	-2.80
37	13	11.60	14.5	9.5	11.9	10.2	2.90	-2.10	0.30	-1.40
38	13	9.00	12.2	8.2	9.2	9.5	3.20	-0.80	0.20	0.50
39	13	7.10	12.8	6.6	7.7	8.9	5.70	-0.50	0.60	1.80
40	14	7.50	9.4	7.1	8.2	7.4	1.90	-0.40	0.70	-0.10
41	14	7.00	7.2	8.0	7.1	5.7	0.20	1.00	0.10	-1.30
42	14	7.50	7.4	8.7	7.5	7.4	-0.10	1.20	0.00	-0.10
43	15	6.20	4.1	5.3	5.8	4.2	-2.10	-0.90	-0.40	-2.00
44	15	6.60	3.5	2.8	2.3	3.5	-3.10	-3.80	-4.30	-3.10
45	15	6.20	5.4	3.4	5.4	2.4	-0.80	-2.80	-0.80	-3.80
46	16	6.10	6.0	4.5	4.3	2.3	-0.10	-1.60	-1.80	-3.80
47	16	6.90	5.6	4.7	5.5	3.9	-1.30	-2.20	-1.40	-3.00
48	16	5.80	5.9	4.9	6.3	4.1	0.10	-0.90	0.50	-1.70
49	17	9.80	7.4	4.6	5.4	4.2	-2.40	-5.20	-4.40	-5.60
50	17	7.40	6.1	4.2	4.4	3.8	-1.30	-3.20	-3.00	-3.60
51	17	7.10	4.3	3.0	3.2	2.9	-2.80	-4.10	-3.90	-4.20
52	18	14.40	11.4	7.1	5.3	4.5	-3.00	-7.30	-9.10	-9.90
53	18	17.60	11.3	7.7	4.3	3.8	-6.30	-9.90	-13.30	-13.80
54	18	15.9	10.2	6.8	3.6	3.2	-5.7	-9.1	-12.3	-12.7
55	19	15.5	10.4	8.1	6.6	5.2	-5.1	-7.4	-8.9	-10.3
56	19	14.3	12.3	8.8	5.5	4.8	-2.0	-5.5	-8.8	-9.5
57	19	16.1	13.5	8.5	3.8	4.5	-2.6	-7.6	-12.3	-11.6
58	20	10.3	7.0	4.2	5.4	4.0	-3.3	-6.1	-4.9	-6.3
59	20	7.8	5.4	4.2	4.1	4.2	-2.4	-3.6	-3.7	-3.6
60	20	6.9	4.1	4.8	4.8	3.8	-2.8	-2.1	-2.1	-3.1

Table 21 The t-Test for Moisturizing Effect of PG Eye-Patch

N	Mean	Std Dev	Std Error	T	Prob>|T|
60	-4.6246667	5.2713455	0.6805278	-6.7957060	0.0001

Table 22 The Statistical Data of Young's Modulus in Elasticity Using SAS Program

OBS	SUBJECT	EWK0	EWK1	EWK2	EWK3	EWK4	DEWK1	DEWK2	DEWK3	DEWK4
1	1	12.97	9.43	8.85	10.81	9.41	-3.54	-4.12	-2.16	-3.56
2	2	14.99	15.58	15.31	15.22	13.43	0.59	0.32	0.23	-1.56
3	3	13.90	14.37	13.16	11.89	10.20	0.47	-0.74	-2.01	-3.70
4	4	9.37	12.08	12.60	12.01	10.78	2.71	3.23	2.64	1.41
5	5	4.20	3.29	4.49	6.60	3.99	-0.91	0.29	2.40	-0.21
6	6	12.56	11.95	11.83	11.10	9.37	-0.61	-0.73	-1.46	-3.19
7	7	11.62	11.08	11.16	10.72	11.28	-0.54	-0.46	-0.90	-0.34
8	8	12.49	10.08	13.14	9.20	10.45	-2.41	0.65	-3.29	-2.04
9	9	12.51	11.28	12.99	9.81	12.47	-1.23	0.48	-2.70	-0.04
10	10	13.64	13.49	14.12	12.87	13.22	-0.15	0.48	-0.77	-0.42
11	11	10.39	9.93	10.97	12.12	12.06	-0.46	0.58	1.73	1.67
12	12	11.03	7.85	7.83	10.49	8.12	-3.18	-3.20	-0.54	-2.91
13	13	14.28	14.11	14.35	12.53	14.51	-0.17	0.07	-1.75	0.23
14	14	13.56	11.78	13.26	13.12	11.78	-1.78	-0.30	-0.44	-1.78
15	15	13.53	13.35	12.95	13.97	11.25	-0.18	-0.58	0.44	-2.28
16	16	14.10	11.81	13.35	13.18	12.17	-2.29	-0.75	-0.92	-1.93
17	17	11.18	12.08	10.33	11.49	10.20	0.90	-0.85	0.31	-0.98
18	18	15.31	9.43	8.12	8.81	7.87	-5.88	-7.19	-6.50	-7.44
19	19	12.99	13.53	13.28	13.74	12.80	0.54	0.29	0.75	-0.19
20	20	9.56	8.81	9.20	7.08	7.20	-0.75	-0.36	-2.48	-2.36

Table 23 The t-Test of Young's Modulus in Elasticity of PG Facial-Patch

N	Mean	Std Dev	Std Error	T	Prob>|T|
20	-1.5810000	2.0620733	0.4610936	-3.4288048	0.0028

Data Analysis of Skin Irritation

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
SUBJECT	200	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200
TREATMENT	2	c p

Number of observations in data set = 400

Analysis of Variance Procedure

Dependent Variable: irritation

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	200	1.00000000	0.00500000	1.01	0.4859
Error	199	0.99000000	0.00497487		
Corrected Total	399	1.99000000			

R-Square	C.V.	Root MSE	Irritation Mean
0.502513	1410.656	0.07053279	0.00500000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Subject	199	0.99000000	0.00497487	1.00	0.5000
Treatment	1	0.01000000	0.01000000	2.01	0.1578

Analysis of Variance Procedure

T Confidence Intervals for variable: IRRITATION

Alpha= 0.05 Confidence= 0.95 df= 199 MSE= 0.004975

Critical Value of T= 1.97

Half Width of Confidence Interval= 0.009835

TIME	N	Lower Confidence Limit	Mean	Upper Confidence Limit
Patch	200	0.000165	0.010000	0.019835
Control	200	-0.009835	0.000000	0.009835

Analysis of Variance Procedure

T tests (LSD) for variable: Irritation

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 199 MSE= 0.004975

Critical Value of T= 1.97

Least Significant Difference= 0.0139

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	0.010000	200	Patch
A			
A	0.000000	200	Control

VITA

Flying Officer Thitiporn Rungnava was born on December 31, 1977 in Bangkok, Thailand. She received her Bachelor Degree in Pharmaceutical Science from Department of Pharmacy, Faculty of Pharmacy, Chulalongkorn University in 2001. After graduation, she worked at Wing 21 Hospital, 2nd Air Division, Air Combat Command in Ubonratchathani for 2 years before enrolled in her Master's Degree Program in Pharmaceutical Technology at Chulalongkorn University.