CHAPTER 3

RESULTS

3.1 Subjects

The demographic characteristics of 12 subjects enrolled in the study are shown in Table 2. Every subject was healthy on the basis of medical history, physical and biochemical investigations. All subjects completed the study.

3.2 Validation of HPLC method

3.2.1 Specificity

Chromatogram of isoflavone-free plasma is shown in Figure 5, whereas chromatogram of plasma containing 2,400 ng/mL of diadzein and genistein as well as 50,000 ng/mL of fluorescein (IS) is presented in Figure 6. The retention times (k) of diadzein, genistein and fluorescein were 9.980, 12.541 and 6.425 min, respectively. All peaks were clearly separated and no interference from endogenous substances was observed.

Table 2 The demographic characteristics of 12 subjects enrolled in this study

Subject No.	Age (y)	Weight (kg)	Height (m)	BMI (kg/m^2)	FSH (IU/L)
1	52	57.00	1.60	22.27	49.20
2	53	52.50	1.53	22.43	48.50
3	54	54.00	1.51	23.68	33.50
4	54	60.00	1.56	24.65	49.40
5	56	48.50	1.45	23.07	38.60
6	56	42.00	1.44	20.25	68.50
7	49	58.50	1.66	21.23	128.20
8	55	58.00	1.53	24.78	68.80
9	57	48.50	1.50	21.56	101.00
10	62	60.50	1.60	23.63	53.33
11	59	49.00	1.51	21.50	40.79
12	62	48.00	1.42	23.81	63.49
Mean	55.75	53.04	1.53	22.74	61.94
SD	3.70	5.63	0.07	1.37	26.43

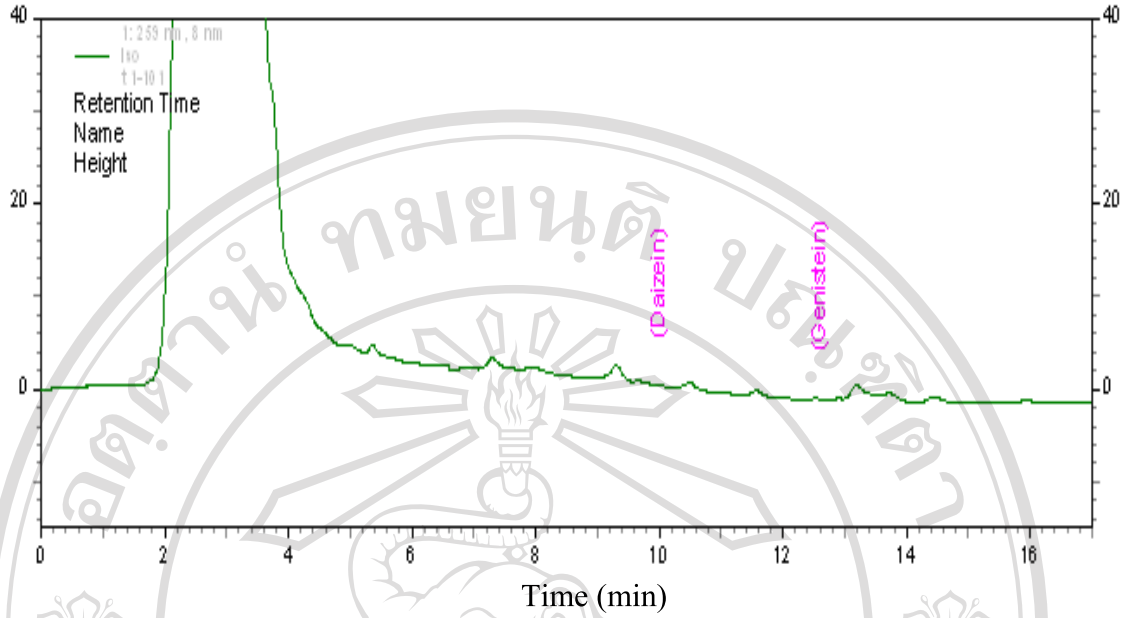

Figure 5 Chromatogram of isoflavone-free plasma

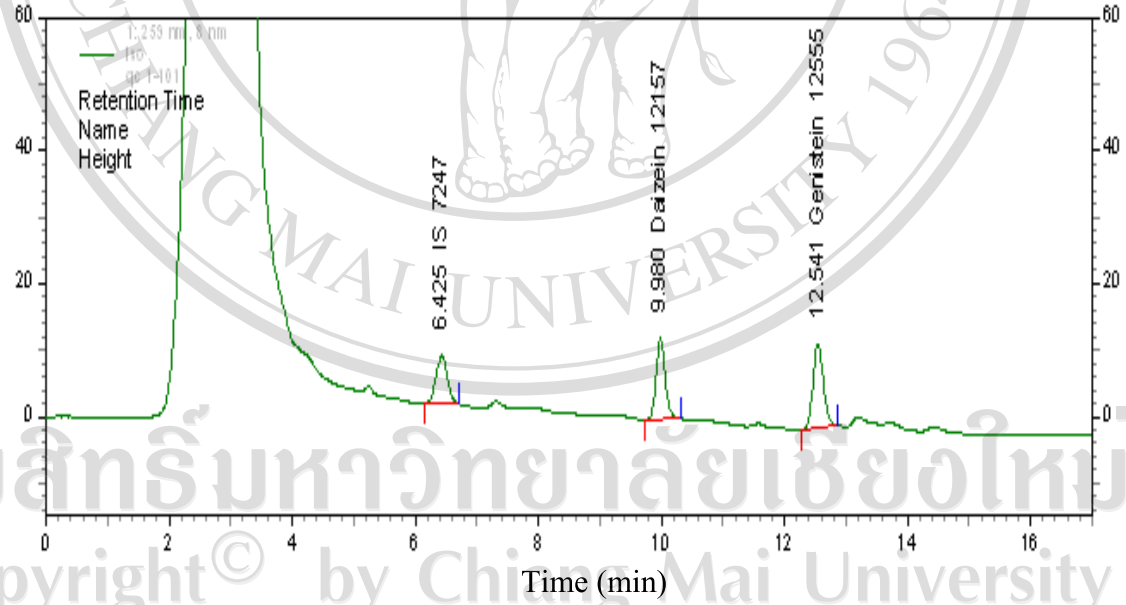

Figure 6 Chromatogram of plasma sample containing 2,400 ng/mL of diadzein (k = 9.980) and genistein (k = 12.541) as well as 50,000 ng/mL of fluorescein (IS, k = 6.425) (k = retention time)

3.2.2 Calibration curve and linearity

Calibration curve of standard plasma containing 37.5-2,400 ng/mL of isoflavones was constructed from the measured peak height ratios of isoflavones and fluorescein chromatograms. Linearity of the calibration curve was determined by regression and correlation coefficient (r^2) analyses. The data of plasma isoflavones used for constructing calibration curve are shown in Table 3. The regression equations for testing the linearity of standard calibration curves are shown below:

Daidzein $y = 0.0607x + 0.4064, \quad r^2 = 0.9999$

Genistein $y = 0.0597x + 2.0933, \quad r^2 = 0.9998$

The linearity with good correlation coefficient (r^2) of calibration curves is also demonstrated (Figures 7A, 7B).

Table 3 Calibration curve data of daidzein and genistein in plasma

No.	Conc. (ng/ml)	IS (peak ht)	Daid (peak ht)	Gen (peak ht)	Daid/IS (peak ht ratio)	Gen/IS (peak ht ratio)
1	37.5	10343	318	502	0.03	0.05
2	75	10703	553	724	0.05	0.07
3	150	9665	931	1079	0.10	0.11
4	300	9411	1792	1930	0.19	0.21
5	600	9262	3330	3355	0.36	0.36
6	1200	9579	6928	7059	0.72	0.74
7	2400	9484	13904	13814	1.47	1.46

Pooled calibration curves from 5 replicated calibration data of daidzein and genistein are shown in Figures 8A and 8B.

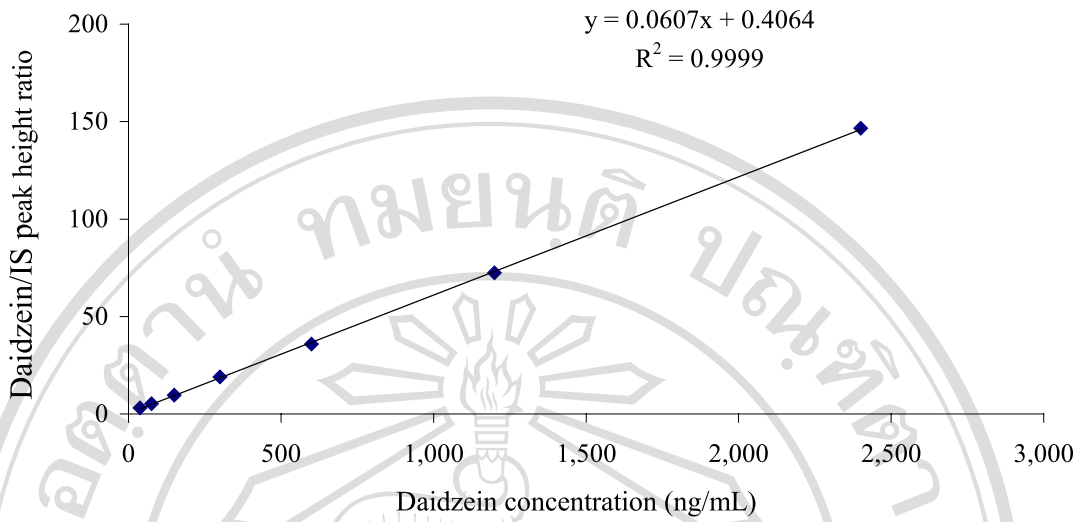

Figure 7A Standard calibration curve of peak height ratio of daidzein/IS versus plasma diadzein concentrations

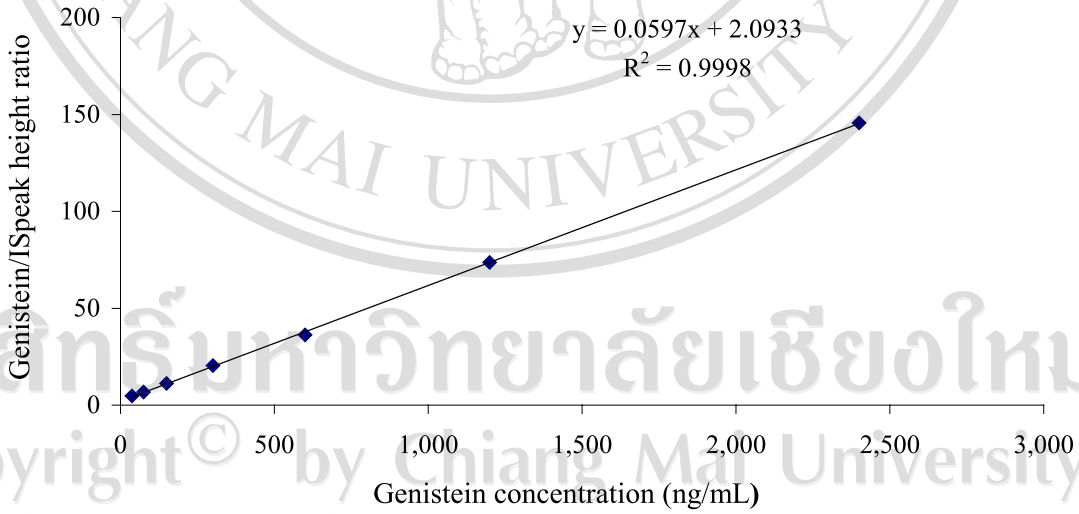

Figure 7B Standard calibration curve of peak height ratio of genistein/IS versus plasma genistein concentrations

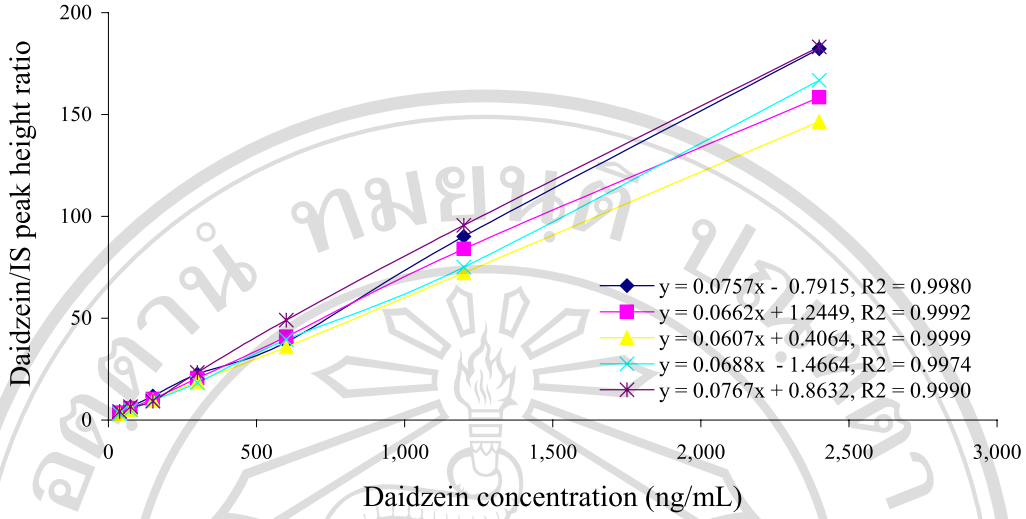

Figure 8A Pool of 5 replicates of calibration curve of peak height ratios of daidzein and IS versus plasma daidzein concentration

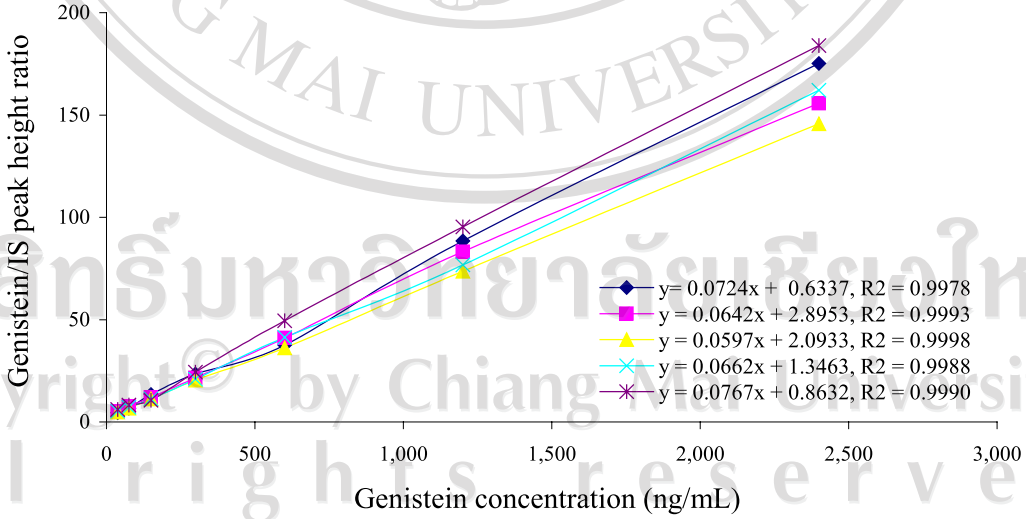

Figure 8B Pool of 5 replicates of calibration curve of peak height ratios of genistein and IS versus plasma genistein concentration

3.2.3 Precision and accuracy

Intra-day and inter-day precision were determined using 3 quality control (QC) samples of daidzein and genistein in plasma (112.5, 1100, 2200 ng/mL). Intra-day precision was determined in one day by assaying 5 replicates of each concentration whereas inter-day precision was determined daily with one sample of each concentration for 5 days.

Precision determines as percentage of coefficient of variation (CV) and % deviation of intra-day for low (112.5 ng/mL), medium (1100 ng/mL), and high (2200 ng/mL) plasma daidzein and genistein concentrations are shown in Table 4, whereas the % CV and % deviation of inter-day precision for plasma daidzein and genistein concentrations are shown in Table 5. All these values were within range of ±15% recommended by U.S. Food and Drug Administration guidance for bio-analytical method validation.

3.2.4 Recovery

The mean values of % recovery of the analytical procedure for daidzein, genistein, and IS are presented in Table 6. At the concentrations of 112.5, 1100, and 2200 ng/mL, the mean vales of % recoveries of daidzein were 102.46%, 81.44% and 85.00%, respectively, whereas, those of genistein were 97.24%, 84.73%, and 83.77%, respectively. The overall mean values of % recoveries of daidzein, genistein, and IS were 89.63%, 88.58%, and 91.29%, respectively.

Table 4 Intra-day precision and deviation of the analysis for daidzein and genistein in plasma

Spiked concentration (ng/mL)	Calculated concentration of daidzein (ng/mL)							Precision (% CV)	Deviation (%)
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No. 5	Mean	SD		
112.5	120.47	110.91	102.12	112.71	114.81	112.21	6.68	5.96	-0.26
1100	1180.32	1127.35	1172.22	1187.13	1200.87	1173.58	27.90	2.38	6.69
2200	2302.24	2246.13	2346.04	2493.02	2313.09	2340.11	92.75	3.96	6.37
						Average		4.10	4.27

Spiked concentration (ng/mL)	Calculated concentration of genistein (ng/mL)							Precision (% CV)	Deviation (%)
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No 5	Mean	SD		
112.5	113.60	116.35	120.05	115.12	126.17	118.26	5.02	4.25	5.12
1100	1267.37	1173.73	1253.14	1270.83	1205.17	1234.05	42.72	3.46	12.19
2200	2429.09	2350.44	2539.14	2689.18	2410.52	2483.68	133.60	5.38	12.89
						Average		4.36	10.37

Table 5 Inter-day precision and deviation of the analysis for daidzein and genistein in plasma

Spiked concentration (ng/mL)	Calculated concentration of daidzein (ng/mL)							Precision	Deviation
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	(% CV)	(%)
112.5 (n=5)	112.21±6.68	114.73±6.16	109.53±1.52	126.87±5.73	116.94±5.01	115.76	6.65	5.74	3.16
1100 (n=5)	1173.58±27.90	1069.19±17.58	1068.53±17.25	1218.06±86.18	1092.10±75.37	1121.10	67.83	6.05	-4.77
2200 (n=5)	2340.11±92.75	2277.48±80.62	2125.46±61.52	2689.04±165.34	2378.48±144.59	2348.28	206.64	8.80	0.37
						Average		6.86	-0.41

Spiked concentration (ng/mL)	Calculated concentration of genistein (ng/mL)							Precision	Deviation
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	(% CV)	(%)
112.5 (n=5)	118.26±5.02	114.20±7.45	118.45±9.49	100.28±5.10	120.16±4.43	114.03	8.12	7.12	1.36
1100 (n=5)	1234.05±42.72	1174.83±18.02	1163.53±16.65	1309.62±96.17	1192.53±85.28	1213.78	59.34	4.89	10.34
2200 (n=5)	2483.68±133.60	2482.30±99.97	2342.99±62.58	2551.53±117.18	2293.78±103.91	2428.93	107.83	4.44	10.41
						Average		5.48	7.37

Table 6 Recovery of daidzein, genistein and IS in plasma

Concentration (ng/mL)	Peak ht of daidzein		Peak ht of genistein		Peak ht of IS	
	in mobile phase	in plasma	in mobile phase	in plasma	in mobile phase	in plasma
112.5	672	655	672	662	7392	7083
112.5	685	677	732	679	7184	7250
112.5	811	724	795	710	7425	7371
112.5	626	742	636	641	7050	7285
112.5	581	660	605	653	6500	6857
Mean	675	692	688	669	7110	7169
SD	86.37	39.18	76.21	26.79	374.14	203.45
% Recovery		102.46		97.24		100.83
1100	6746	5273	6881	5636	8016	6905
1100	7142	5676	7232	6045	7925	6349
1100	6459	5286	6512	5609	7526	6135
1100	6995	5346	7161	5625	8004	6931
1100	6350	5858	6610	6230	8130	7291
Mean	6738	5488	6879	5829	7920	6722
SD	338.27	264.31	320.62	289.28	232.18	470.27
% Recovery		81.44		84.73		84.87
2200	14098	11984	13050	10786	6505	6121
2200	14336	11889	13607	11133	7940	6740
2200	13577	11722	12896	11059	7555	6819
2200	14073	12185	13331	11411	7867	6623
2200	14214	11970	13492	11217	8035	7115
Mean	14060	11950	13275	11121	7580	6684
SD	289.31	167.69	297.79	228.87	627.54	363.25
% Recovery		85.00		83.77		88.17
Average recovery (%)		89.63		88.58		91.29

3.2.5 Freeze/thaw (F/T) stability

The stability of diadzein and genistein in plasma at the concentrations of 112.5 and 2,200 ng/mL kept frozen at -80 ºC before and after freeze and thaw stability test is presented in Table 7. The remaining of daidzein at the concentrations of 112.5 and 2,200 ng/mL after 3 freeze-thaw cycles was 91.16% and 101.73%, respectively, whereas that of genistein was 89.65% and 98.51%, respectively. The overall freeze/thaw stability of daidzein and genistein was 96.45%, and 94.08%, respectively.

3.2.6 Short-term stability

Short-term stability test of diadzein and genistein in plasma kept at room temperature for 8 h against those of freshly prepared samples is presented in Table 8. Daidzein remaining at the concentrations of 112.5 and 2,200 ng/mL kept at room temperature for 8 h was 100.54% and 94.67%, respectively, whereas that of genistein was 90.27% and 90.46%, respectively when compared with the corresponding freshly prepared samples. The overall remaining of daidzein was 97.60% and was 90.36% for genistein.

Table 7 Concentrations of daidzein and genistein in plasma before and after 3 cycles of freeze and thaw (F/T) stability

Concentration (ng/mL)	Before F/T		After F/T	
	Peak ht of daidzein	Peak ht of genistein	Peak ht of daidzein	Peak ht of genistein
112.5	866	1072	712	859
112.5	793	970	689	841
112.5	763	905	807	942
Mean	807	982	736	881
SD	52.97	84.18	62.55	53.87
%Remaining[1]			91.16	89.65
2200	13663	14190	13784	13782
2200	12907	13420	13531	13666
2200	13063	13434	13005	12984
Mean	13211	13681	13440	13477
SD	399.14	440.57	397.39	431.16
% Remaining[1]			101.73	98.51
Average F/T stability (%)[2]			96.45	94.08

[1] (mean value of concentration after F/T) x100/(mean value of concentration before F/T)

[2] (% remaining of low concentration + % remaining of high concentration)/2

Table 8 Short-term stability of daidzein and genistein in plasma

Concentration (ng/mL)	Freshly prepared sample					Sample kept at room temperature for 8 h				
	Peak ht			Daid/IS	Gen/IS	Peak ht			Daid/IS	Gen/IS
	IS	Daid	Gen			IS	Daid	Gen		
112.5	7724	811	1029	0.10	0.13	7417	899	1008	0.12	0.14
112.5	7739	811	1041	0.10	0.13	8519	896	983	0.11	0.12
112.5	6929	1078	1059	0.16	0.15	7542	1063	968	0.14	0.13
Mean				0.12	0.14				0.12	0.13
SD				0.03	0.01				0.02	0.01
% Remaining[1]									100.54	90.27
2200	7466	13486	12862	1.81	1.72	6745	11981	10875	1.78	1.61
2200	6856	11940	11347	1.74	1.66	7822	12150	11113	1.55	1.42
2200	7364	11403	10902	1.55	1.48	8101	12113	11030	1.50	1.36
Mean				1.70	1.62				1.61	1.46
SD				0.13	0.13				0.15	0.13
% Remaining[1]									94.67	90.46
Average short term stability (%)[2]									97.60	90.36

[1] (mean value of concentration after 8 h) x 100/(mean value of concentration of freshly prepared sample)

[2] (% remaining of low concentration + % remaining of high concentration)/2

3.2.7 Post-preparative stability

Post-preparative stability test of diadzein and genistein in plasma samples standing in the auto-sampler for injection onto the analytical column against freshly prepared samples is presented in Table 9. The remaining of daidzein at the concentrations of 112.5 and 2200 ng/mL after waiting in the auto-sampler for injection onto the analytical column was 101.66% and 102.20%, respectively, whereas that of genistein was 97.87% and 100.60%, respectively. The overall post-operative stability of daidzein and genistein was 101.93%, 99.24%, respectively.

Table 9 Post-preparative stability test of diadzein and genistein in plasma

Concentration (ng/mL)	Freshly prepared sample					Sample standing in auto-sampler				
	IS	Daid	Gen	Daid/IS	Gen/IS	IS	Daid	Gen	Daid/IS	Gen/IS
112.5	8724	795	1062	0.09	0.12	8564	792	1039	0.09	0.12
112.5	8592	799	1099	0.09	0.13	9241	844	1089	0.09	0.12
112.5	7039	671	910	0.10	0.13	7417	733	949	0.10	0.13
112.5	8780	775	1043	0.09	0.12	9241	844	1089	0.09	0.12
112.5	8724	795	1062	0.09	0.12	8564	792	1039	0.09	0.12
Mean				0.09	0.12				0.09	0.12
SD				0.003	0.005				0.003	0.004
% Remaining[1]									101.66	97.87
2200	7887	14160	15835	1.80	2.01	7887	14160	15835	1.80	2.01
2200	8319	13819	15636	1.66	1.88	8126	13912	15438	1.71	1.90
2200	8874	14368	16273	1.62	1.83	8710	14437	16051	1.66	1.84
2200	7659	13517	15325	1.76	2.00	8014	14693	16155	1.83	2.02
2200	7823	15207	17114	1.94	2.19	7608	15055	16757	1.98	2.20
Mean				1.76	1.98				1.80	1.99
SD				0.127	0.138				0.124	0.138
% Remaining[1]									102.20	100.60
Average short term stability (%)[2]									101.93	99.24

[1] (mean value of concentration after waiting in the auto-sampler for injection onto the analytical column) x100/(mean value of concentration before auto-sampler injection onto the analytical column)

[2] (% remaining of low concentration + % remaining of high concentration)/2

3.3 Pharmacokinetics of daidzein and genistein

The individual as well as mean plasma daidzein and genistein concentration-time profiles from 12 subjects after "single ISO", "single ISO + D_3-calcium", or "continuous D_3-calcium/single ISO" are presented in Tables 10A, 11A, 12A and 10B, 11B, 12B, respectively. The plasma concentration-time curves of daidzein and genistein in three phases from each subject are shown in Figure 9.

Composites of individual plasma daidzein and genistein concentration-time curves from 12 subjects after "single ISO", "single ISO + D_3-calcium", or "continuous D_3-calcium/single ISO" are respectively depicted in Figures 10A, 11A, and 12A and 10B, 11B, and 12B, respectively.

The plasma concentration-time profiles of daidzein and genistein after taking various dosage regimens of soy extract showed wide intra-individual and inter-individual variation. However, the mean plasma concentration-time profiles of daidzein and genistein exhibited much less difference among different soy extract dosage regimens. The plasma concentration-time profiles of daidzein and genistein were typically biphasic in every individual regardless of soy extract regimens taken. The first and second peak concentrations of daidzein and genistein were generally attained at 2-4 h and 6-8 h. The second peak concentrations of both isoflavones were usually higher than the first peak concentrations.

The pharmacokinetic parameters of daidzein and genistein (C_{max}, AUC_{0-32}, $AUC_{0-\infty}$, T_{max}, $t_{1/2}$) after treatment with "single ISO", "single ISO + D_3-calcium", or "continuous D_3-calcium/single ISO" were determined and are shown in Tables 13A and 13B.

The phase A study was to obtain the pharmacokinetic parameters of the aglycones (daidzein and genistein) following "single ISO". The results demonstrated that mean elimination $t_{1/2}$ of daidzein and genistein were 6.80 ± 2.98 h and 8.31 ± 4.90 h, respectively. The mean AUC_{0-32} was 3474.05 ± 931.53 ng.h/mL for daidzein and 5804.19 ± 2643.87 ng.h/mL for genistein, whereas, $AUC_{0-\infty}$ was 4905.23 ± 1150.99 ng.h/mL for daidzein and 6787.62 ± 2793.66 ng.h/mL for genistein, respectively. Pharmacokinetic analysis of the plasma concentration time curves showed the mean T_{max} was 5.50 ± 2.11 h for daidzein and 4.58 ± 2.11 h for genistein. In addition, C_{max} was 506.09 ± 299.62 ng.h/mL for daidzein and 593.33 ± 303.31 ng.h/mL for genistein, respectively.

The phase B study was "single ISO + D_3-calcium". Pharmacokinetic analysis of the plasma concentration time curves showed the mean elimination $t_{1/2}$ of daidzein and genistein were 6.34 ± 5.29 h and 7.44 ± 2.27 h, respectively. The mean AUC_{0-32} was 2968.71 ± 627.60 ng.h/mL for daidzein and 6574.74 ± 2625.30 ng.h/mL for genistein, whereas, $AUC_{0-\infty}$ was 4938.40 ± 1611.00 ng.h/mL for daidzein and 7434.65 ± 2748.42 ng.h/mL for genistein, respectively. The mean T_{max} was 8.00 ± 1.79 h for daidzein and 7.64 ± 2.19 h for genistein. In addition, the mean C_{max} was 396.32 ± 115.37 ng/mL for daidzein and 578.99 ± 240.98 ng/mL for genistein, respectively.

The phase C study was "continuous D_3-calcium/single ISO". Pharmacokinetic analysis of the plasma concentration time curves showed the mean elimination $t_{1/2}$ of daidzein and genistein were 6.26 ± 3.73 h and 8.86 ± 4.09 h, respectively. The mean AUC_{0-32} was 4620.55 ± 2148.09 ng.h/mL for daidzein and 7758.56 ± 5068.69 ng.h/mL for genistein, whereas, $AUC_{0-\infty}$ was 5961.22 ± 2546.45 ng.h/mL for daidzein

and 9091.58 ± 5394.67 ng.h/mL for genistein, respectively. The mean T_{max} was 6.08 ± 2.50 h for daidzein and 6.08 ± 2.78 h for genistein. In addition, the mean C_{max} was 488.05 ± 165.90 ng/mL for daidzein and 688.56 ± 285.42 ng/mL for genistein, respectively.

Of all pharmacokinetic parameters, only T_{max} of daidzein and genistein after "single ISO + D_3-calcium" and "continuous D_3-calcium/single ISO" were significantly longer than "single ISO", of which the T_{max} of both daidzein and genistein derived from "single ISO + D_3-calcium" was the longest. Other pharmacokinetic parameters (C_{max}, AUC_{0-32}, $AUC_{0-\infty}$, and $t_{1/2}$ of daidzein and genistein after 3 different dosage regimens were not different significantly.

Table 10A Plasma concentrations of daidzein after "single ISO" at various sampling times in 11 subjects completed the study without protocol deviation[#]

Subject No.	Concentration of daidzein at specified sampling time (h)										
	0.00	0.50	1.00	2.00	4.00	6.00	8.00	10.00	12.00	24.00	32.00
1	<LLQ	185.76	216.43	410.89	446.63	238.43	206.23	160.41	149.17	<LLQ	<LLQ
2	<LLQ	167.38	230.91	249.25	264.2	237.1	195.1	179.92	93.11	<LLQ	<LLQ
3	<LLQ	170.64	234.79	254.15	265.18	240.04	198.14	178.15	<LLQ	<LLQ	<LLQ
4	<LLQ	166.06	370.93	486.51	509.37	300.27	180.62	124.82	113.34	<LLQ	<LLQ
5	<LLQ	191.29	224.08	168.21	139.61	278.97	309.17	235.92	204.05	<LLQ	<LLQ
6	<LLQ	50.47	155.81	1321.14	270.24	284.45	452.67	326.05	254.87	<LLQ	<LLQ
7	<LLQ	209.65	242.25	162.47	760.04	239.4	182.29	136.78	81.41	<LLQ	<LLQ
8	<LLQ	214.95	346.47	243.93	182.55	286.7	296.15	227.79	186	105.94	<LLQ
10	<LLQ	<LLQ	256.5	154.9	148.03	304.53	669.27	219.01	158.98	<LLQ	<LLQ
11	<LLQ	270.72	278.97	224.72	258.64	144.13	442.09	345.81	237.69	<LLQ	<LLQ
12	<LLQ	117.59	190.88	187.82	287.8	407	276.77	244.16	184.15	56.87	43.53
Mean	<LLQ	174.45	249.82	351.27	321.12	269.18	309.86	216.26	166.28	81.41	43.53
SD	<LLQ	58.97	62.95	338.28	184.37	64.18	154.18	71.21	58.49	34.70	<LLQ
% CV	<LLQ	33.80	25.20	96.30	57.42	23.84	49.76	32.93	35.18	42.62	<LLQ

LLQ = lower limit of quantification

Data from subject No.9 was not taken into account because isoflavones concentration was detected in plasma sample at baseline

Table 10B Plasma concentrations of genistein after "single ISO" at various sampling times in 11 subjects completed the study without protocol deviation[#]

Subject No.	Concentration of genistein at specified sampling time (h)										
	0.00	0.50	1.00	2.00	4.00	6.00	8.00	10.00	12.00	24.00	32.00
1	<LLQ	149.28	140.86	527.75	916.69	555.04	518.49	394.36	404.43	70.45	<LLQ
2	<LLQ	191.35	245.16	280.42	327.08	289.33	219.29	160.73	70.56	<LLQ	<LLQ
3	<LLQ	193.87	249.44	286.47	332.14	295.12	223.12	163.7	<LLQ	<LLQ	<LLQ
4	<LLQ	116.96	331.65	440.83	510.18	274.05	226.32	149.01	118.3	<LLQ	<LLQ
5	<LLQ	128.28	208.43	197.88	172.78	264.62	234.59	181.32	172.12	74.2	<LLQ
6	<LLQ	<LLQ	64.24	1248.77	353.11	441.51	526.18	381.46	299.19	96.7	69.88
7	<LLQ	156.89	285.55	255.15	547.25	186.24	162.41	145.67	108.23	35.07	29.65
8	<LLQ	155.42	360.57	337.37	274.77	260.64	274.1	227.08	204.3	128.76	116.23
10	<LLQ	49.73	269.94	215.88	258.34	557.81	794.66	396.56	289.89	84.49	40.35
11	<LLQ	193.02	226.93	232.98	525.13	224.54	894.65	713.68	507.18	137.88	74.6
12	<LLQ	78.11	195.04	211.87	488.55	453.44	303.45	267.45	208.94	78.87	<LLQ
Mean	<LLQ	141.29	234.35	385.03	427.82	345.67	397.93	289.18	238.31	88.30	66.14
SD	<LLQ	48.98	83.42	304.31	203.78	132.06	251.68	173.14	138.29	32.99	33.88
% CV	<LLQ	34.66	35.59	79.04	47.63	38.20	63.25	59.87	58.03	37.36	51.22

LLQ = lower limit of quantification

Data from subject No.9 was not taken into account because isoflavones concentration was detected in plasma sample at baseline

Table 11A Plasma concentrations of daidzein after "single ISO + D_3-calcium" at various sampling times in 11 subjects completed the study without protocol deviation[#]

Subject No.	Concentration of daidzein at specified sampling time (h)										
	0.00	0.50	1.00	2.00	4.00	6.00	8.00	10.00	12.00	24.00	32.00
1	<LLQ	91.15	126.79	204.43	220.77	315.68	329.21	405	296.32	LLQ	LLQ
2	<LLQ	<LLQ	76.58	121.04	181.78	242.55	220.59	375.48	162.38	LLQ	LLQ
3	<LLQ	<LLQ	<LLQ	156.82	178.9	240.49	584.76	381.26	289.67	LLQ	LLQ
4	<LLQ	<LLQ	171.29	173.65	247.88	311.84	199.74	154.54	LLQ	LLQ	LLQ
5	<LLQ	115.11	282.69	192.48	251.11	387.64	359.88	265.26	193.31	LLQ	LLQ
6	<LLQ	186.03	270.11	179.42	559.69	646.26	358.02	261.74	202.05	LLQ	LLQ
7	<LLQ	188.23	208.26	217.3	318.34	255.8	183.13	373.75	285.99	LLQ	LLQ
8	<LLQ	<LLQ	136.62	172.57	240.77	340.83	250.58	189	152.56	LLQ	LLQ
10	<LLQ	179.82	227.4	142.66	97.48	188.25	273.35	349.11	267.6	LLQ	LLQ
11	<LLQ	149.61	284.95	207.29	185.24	266.79	290.25	209.87	196.99	LLQ	LLQ
12	<LLQ	223.4	225.06	227.29	249.13	244.85	294.63	262.91	208.79	LLQ	LLQ
Mean	<LLQ	161.9	201.0	181.4	248.3	312.8	304.0	293.4	225.6	LLQ	LLQ
SD	<LLQ	46.05	71.37	32.57	117.75	123.79	110.46	87.38	54.31	LLQ	LLQ
% CV	<LLQ	28.44	35.51	17.96	47.43	39.57	36.33	29.78	24.08	LLQ	LLQ

LLQ = lower limit of quantification

Data from subject No.9 was not taken into account because isoflavones concentration was detected in plasma sample at baseline

Table 11B Plasma concentrations of genistein after "single ISO + D_3-calcium" at various sampling times in 11 subjects completed the study without protocol deviation[#]

Subject No.	Concentration of genistein at specified sampling time (h)										
	0.00	0.50	1.00	2.00	4.00	6.00	8.00	10.00	12.00	24.00	32.00
1	<LLQ	<LLQ	<LLQ	86.18	230.21	498.73	618.04	886.94	712.23	215.57	115.99
2	<LLQ	<LLQ	<LLQ	59.33	230.99	247	354.25	631.24	424.54	111.08	<LLQ
3	<LLQ	<LLQ	<LLQ	<LLQ	245.17	273.6	1061.07	618.91	418.86	108.46	<LLQ
4	<LLQ	57.95	176.66	223.05	242.3	259.7	162.56	139.42	88.19	<LLQ	<LLQ
5	<LLQ	109.34	287.09	257.35	353.68	565.48	397.27	261.89	190.02	43.72	42.24
6	<LLQ	114.73	259.04	227.3	606.78	511.8	302.93	214.93	195.85	<LLQ	<LLQ
7	<LLQ	331.43	341.57	375.16	579.34	299.42	294.97	650.7	286.57	113.75	67.65
8	<LLQ	30.28	97.58	204.59	240.17	277.23	210.76	197.3	153.04	76.21	48.99
10	<LLQ	148.07	232.37	172.15	133.88	286.75	441.84	588	412.14	124.56	61.47
11	<LLQ	139.85	277.3	246.52	301.79	449.06	417.16	334.88	283.89	109.75	48.77
12	<LLQ	167.96	157.14	251.95	271.8	287.58	392.72	318.65	276.12	108.44	75.99
Mean	<LLQ	137.45	228.59	210.36	312.37	359.67	423.05	440.26	312.86	112.39	65.87
SD	<LLQ	90.86	79.70	89.80	148.76	119.94	244.04	243.32	173.42	45.91	25.08
% CV	<LLQ	66.10	34.87	42.69	47.62	33.35	57.69	55.27	55.43	40.85	38.07

LLQ = lower limit of quantification

Data from subject No.9 was not taken into account because isoflavones concentration was detected in plasma sample at baseline

Table 12A Plasma concentrations of daidzein after "continuous D_3-calcium/single ISO" at various sampling times in 11 subjects completed the study without protocol deviation[#]

Subject No.	Concentration of daidzein at specified sampling time (h)										
	0.00	0.50	1.00	2.00	4.00	6.00	8.00	10.00	12.00	24.00	32.00
1	<LLQ	<LLQ	171.28	316.18	176.46	295.34	408.97	308.35	240.16	<LLQ	<LLQ
2	<LLQ	<LLQ	170.15	253.6	120.72	165.92	415.83	351.36	214.58	<LLQ	<LLQ
3	<LLQ	<LLQ	118.36	174.6	262.35	172.1	446.61	349.19	212.62	<LLQ	<LLQ
4	<LLQ	<LLQ	145.84	173.79	252.39	475.25	261.46	153.78	<LLQ	<LLQ	<LLQ
5	<LLQ	278.89	323.69	231.89	170.61	308.58	365.8	249.1	196.6	123.84	<LLQ
6	<LLQ	175.1	179.94	906.44	456.53	348.15	234.53	148.18	105.9	<LLQ	<LLQ
7	<LLQ	76.87	166.84	328.05	226.56	586.58	522.41	407.02	355.37	146.04	<LLQ
8	<LLQ	113.42	131.61	382.25	357.33	190.65	184.39	121.43	101.14	<LLQ	<LLQ
10	<LLQ	167.92	379.09	242.94	136.44	269	310	265.94	207.06	<LLQ	<LLQ
11	<LLQ	129.53	374.27	255.91	698.56	175.75	342.15	351.29	305.44	82.3	<LLQ
12	<LLQ	112.8	146.74	236.88	168.78	341.16	585.5	444.75	367.36	174.32	<LLQ
Mean	<LLQ	150.65	209.80	318.41	275.16	302.59	370.70	286.40	230.62	131.63	<LLQ
SD	<LLQ	131.88	192.40	292.04	252.56	277.87	340.47	263.37	210.75	110.10	<LLQ
% CV	<LLQ	87.54	91.71	91.72	91.79	91.83	91.85	91.96	91.38	83.65	<LLQ

LLQ = lower limit of quantification

Data from subject No.9 was not taken into account because isoflavones concentration was detected in plasma sample at baseline

Table 12B Plasma concentrations of genistein after "continuous D_3-calcium/single ISO" at various sampling times in 11 subjects completed the study without protocol deviation[#]

Subject No.	Concentration of genistein at specified sampling time (h)										
	0.00	0.50	1.00	2.00	4.00	6.00	8.00	10.00	12.00	24.00	32.00
1	<LLQ	<LLQ	85.1	399	285.14	542.97	859.2	672.89	163.27	211.14	101.07
2	<LLQ	<LLQ	152.63	180.56	112.06	193.15	541.14	300.23	176.46	<LLQ	<LLQ
3	<LLQ	<LLQ	106.5	136.63	176.37	188.74	535.06	298.19	178.25	<LLQ	<LLQ
4	<LLQ	<LLQ	160.2	116.79	264.12	584.59	341.62	217.18	158.46	130.74	<LLQ
5	<LLQ	170.18	274.73	214.81	171.1	244.02	206.34	155.71	139.29	87.29	<LLQ
6	<LLQ	123.85	165.93	917.21	236.04	155.57	101.16	77.33	78.8	<LLQ	<LLQ
7	<LLQ	100.92	130.86	264.12	211.49	1105.07	1060.13	876.83	654.56	334.39	161.87
8	<LLQ	70.18	107.44	329.9	318.48	179.43	169.37	126.95	113.75	106.75	39.17
10	<LLQ	147.2	437.32	346.84	236.52	479.94	537.46	457.81	341.62	127.85	56.52
11	<LLQ	110.49	362.05	374.04	756.11	315.88	604.28	553.49	480.13	189.23	106.71
12	<LLQ	68.55	85.81	157.81	169.19	443.69	790.76	565.05	482.45	244.21	96.67
Mean	<LLQ	113.05	188.05	312.52	266.97	403.00	522.41	391.06	269.73	178.95	93.67
SD	<LLQ	37.68	118.29	223.80	172.68	280.55	302.77	254.40	190.10	82.90	42.98
% CV	<LLQ	33.33	62.90	71.61	64.68	69.61	57.96	65.05	70.48	46.33	45.88

LLQ = lower limit of quantification

Data from subject No.9 was not taken into account because isoflavones concentration was detected in plasma sample at baseline

Subject 1

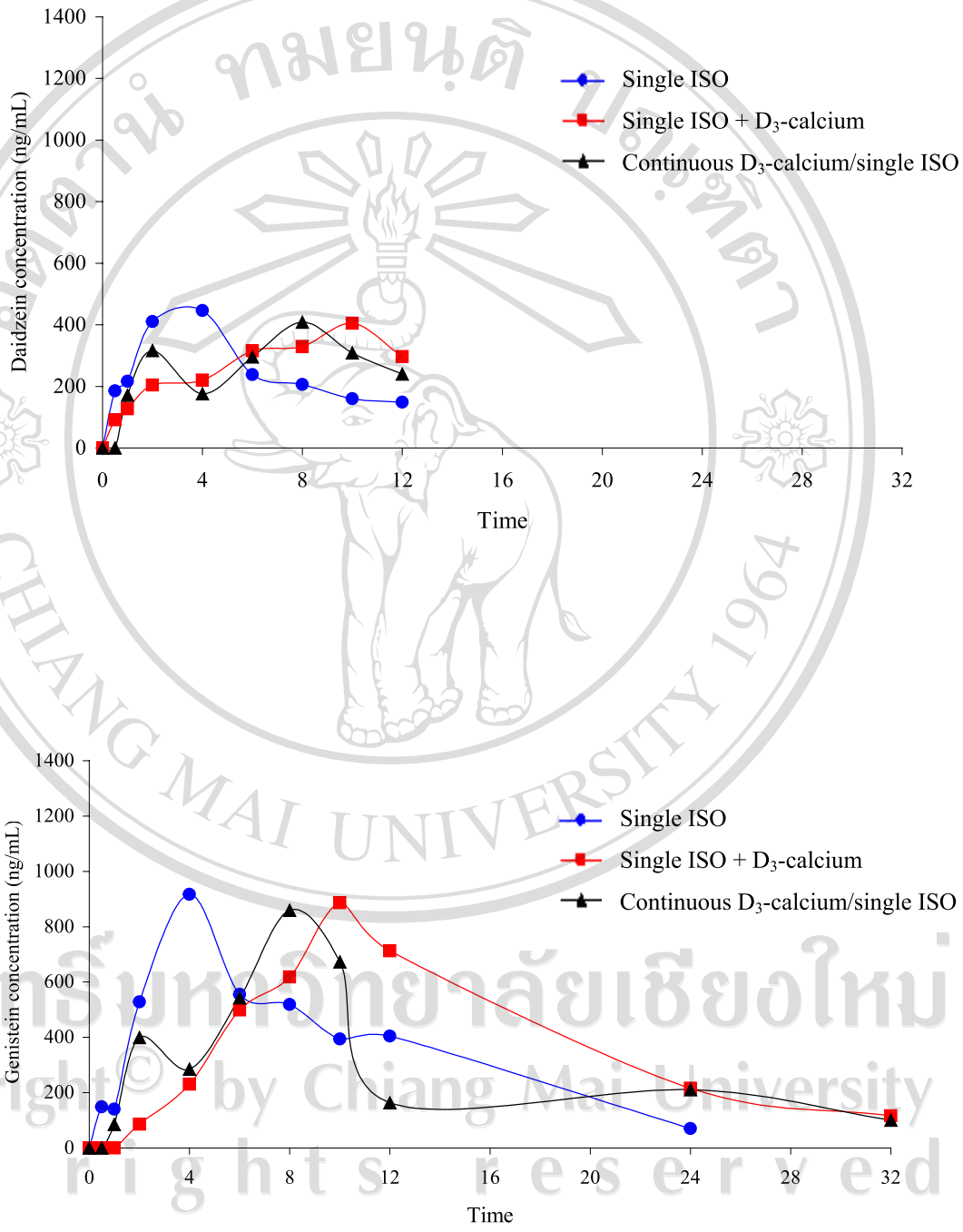

Figure 9 Plasma concentration-time curves of isoflavones after "single ISO", "single ISO + D_3-calcium", or "continuous D_3-calcium/single ISO" in each subject

Subject 2

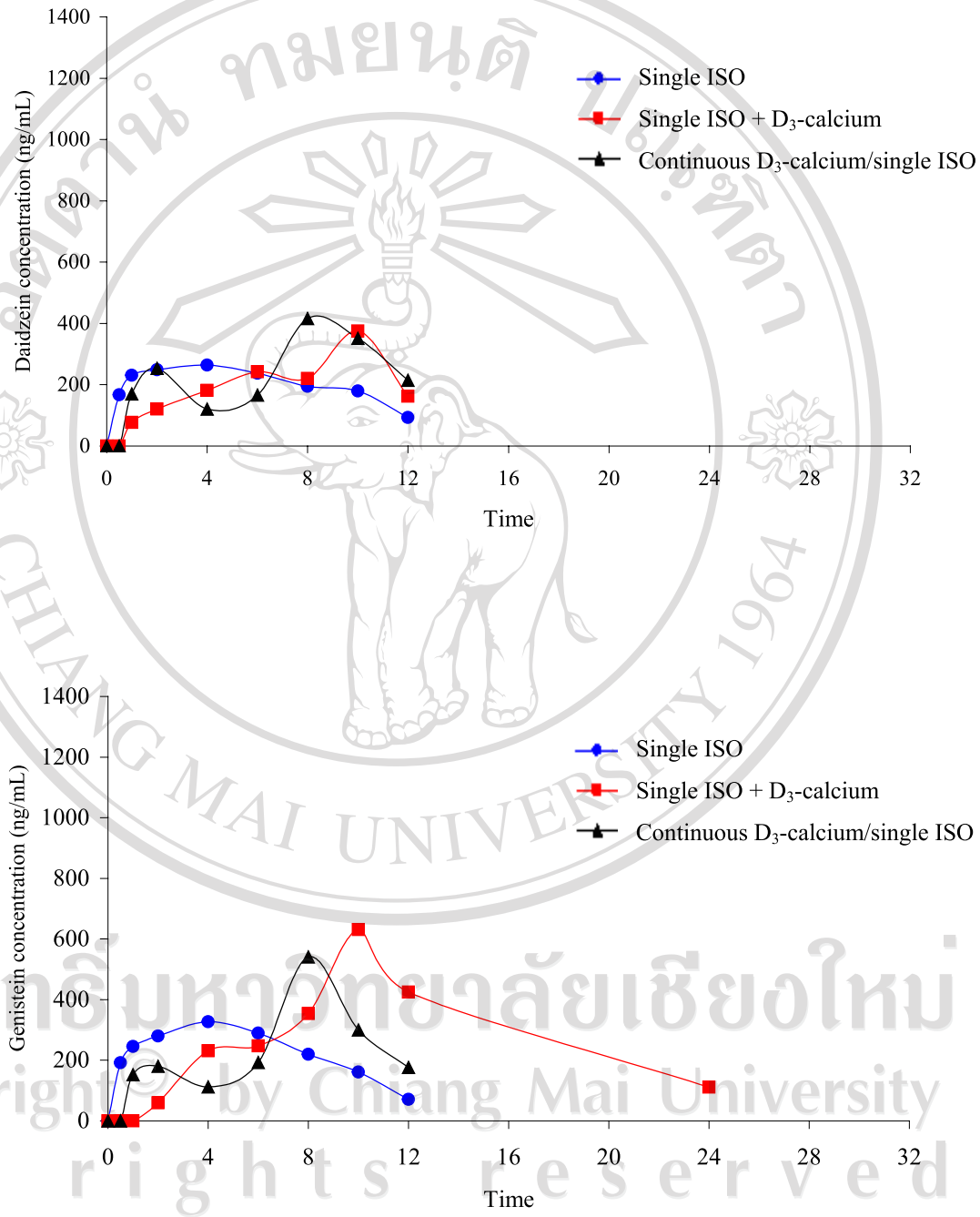

Figure 9 (Continued)

Subject 3

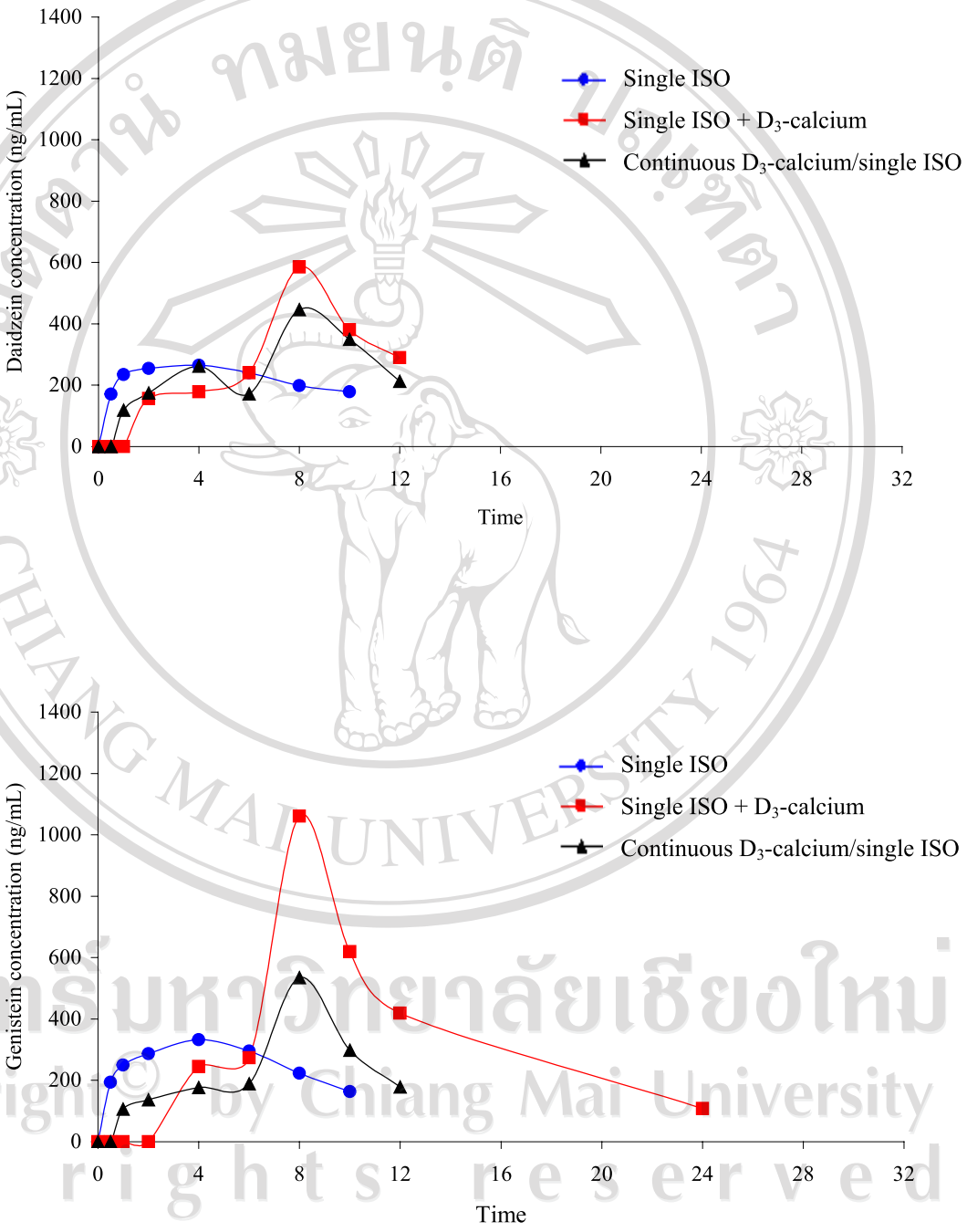

Figure 9 (Continued)

Subject 4

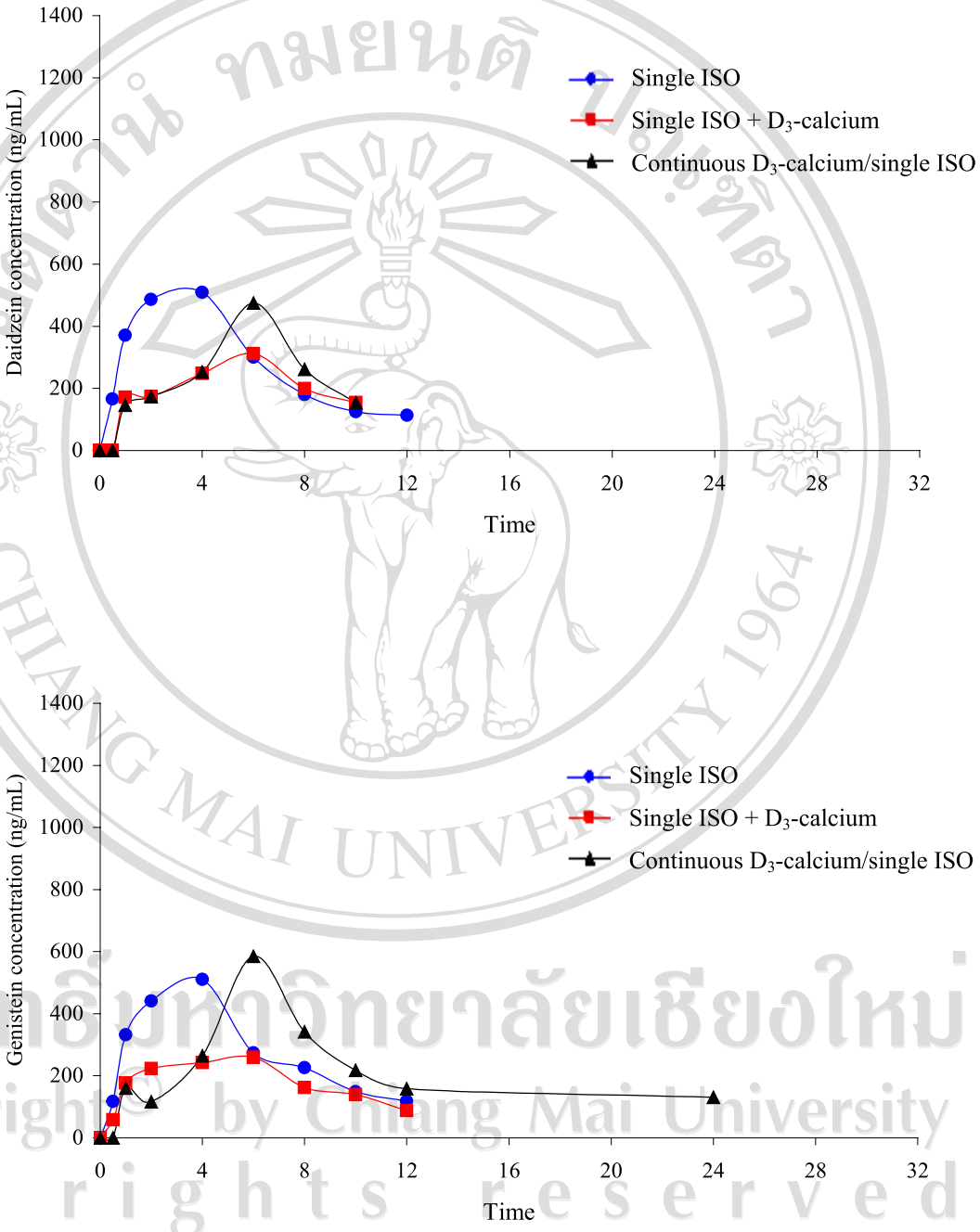

Figure 9 (Continued)

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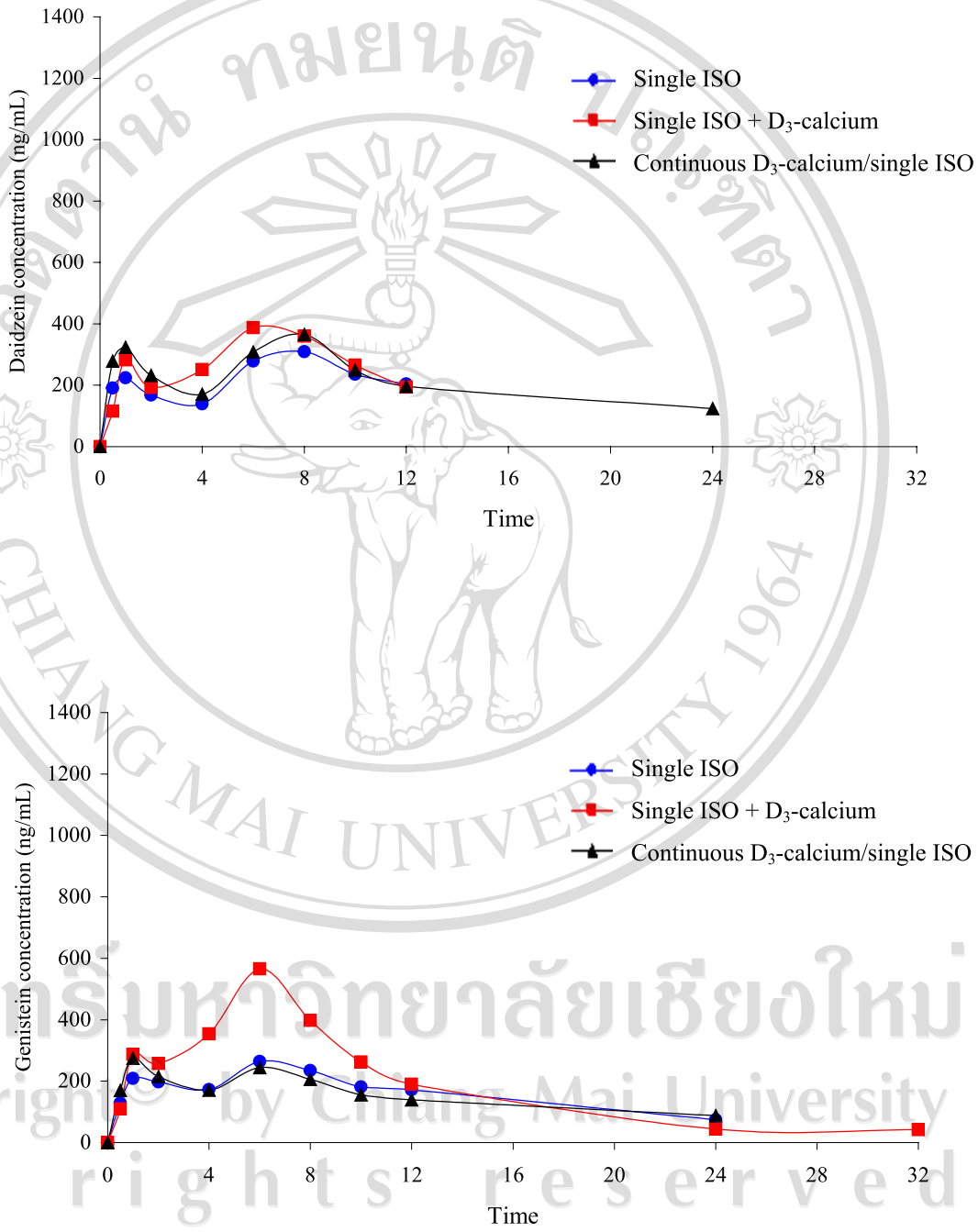

Figure 9 (Continued)

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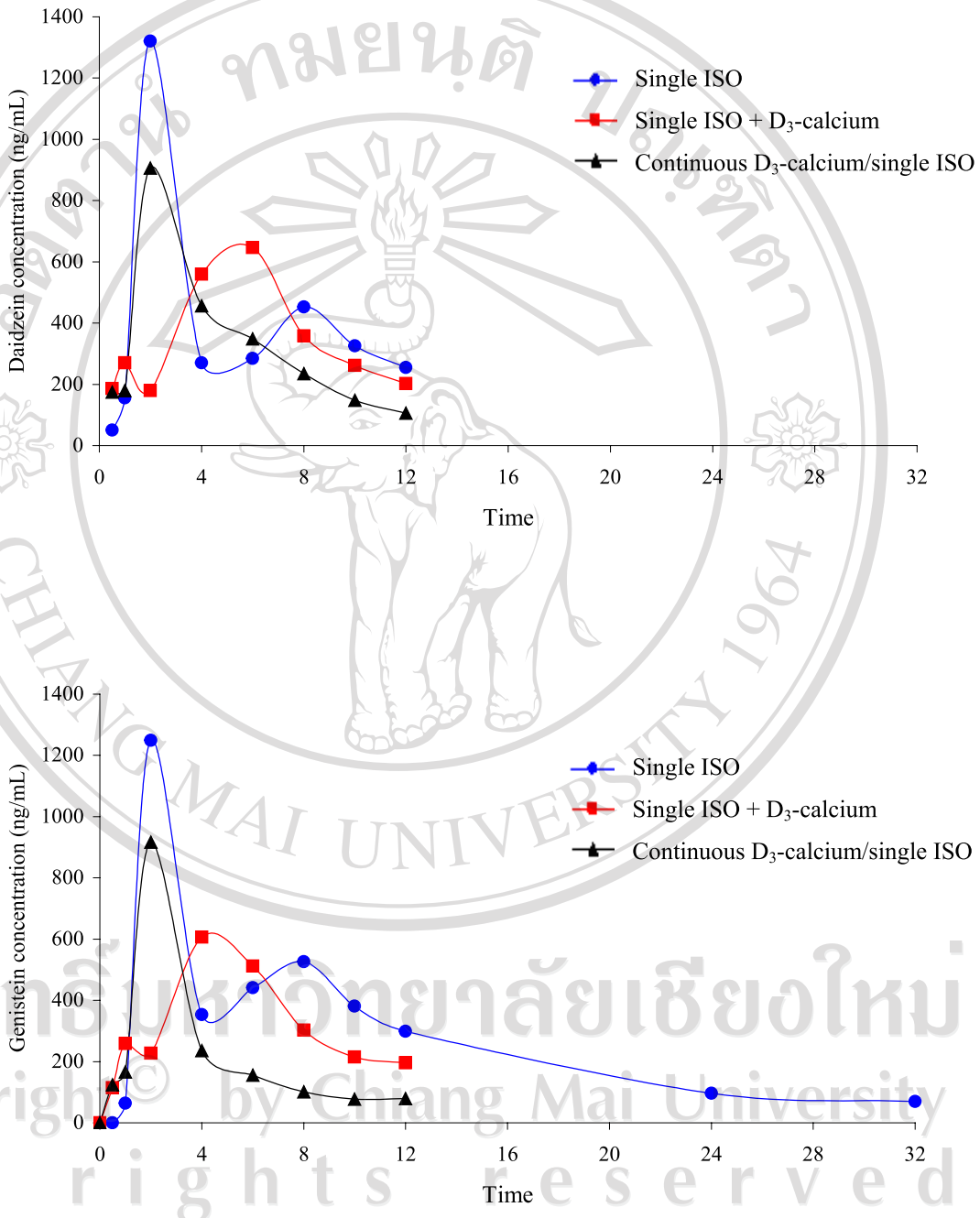

Figure 9 (Continued)

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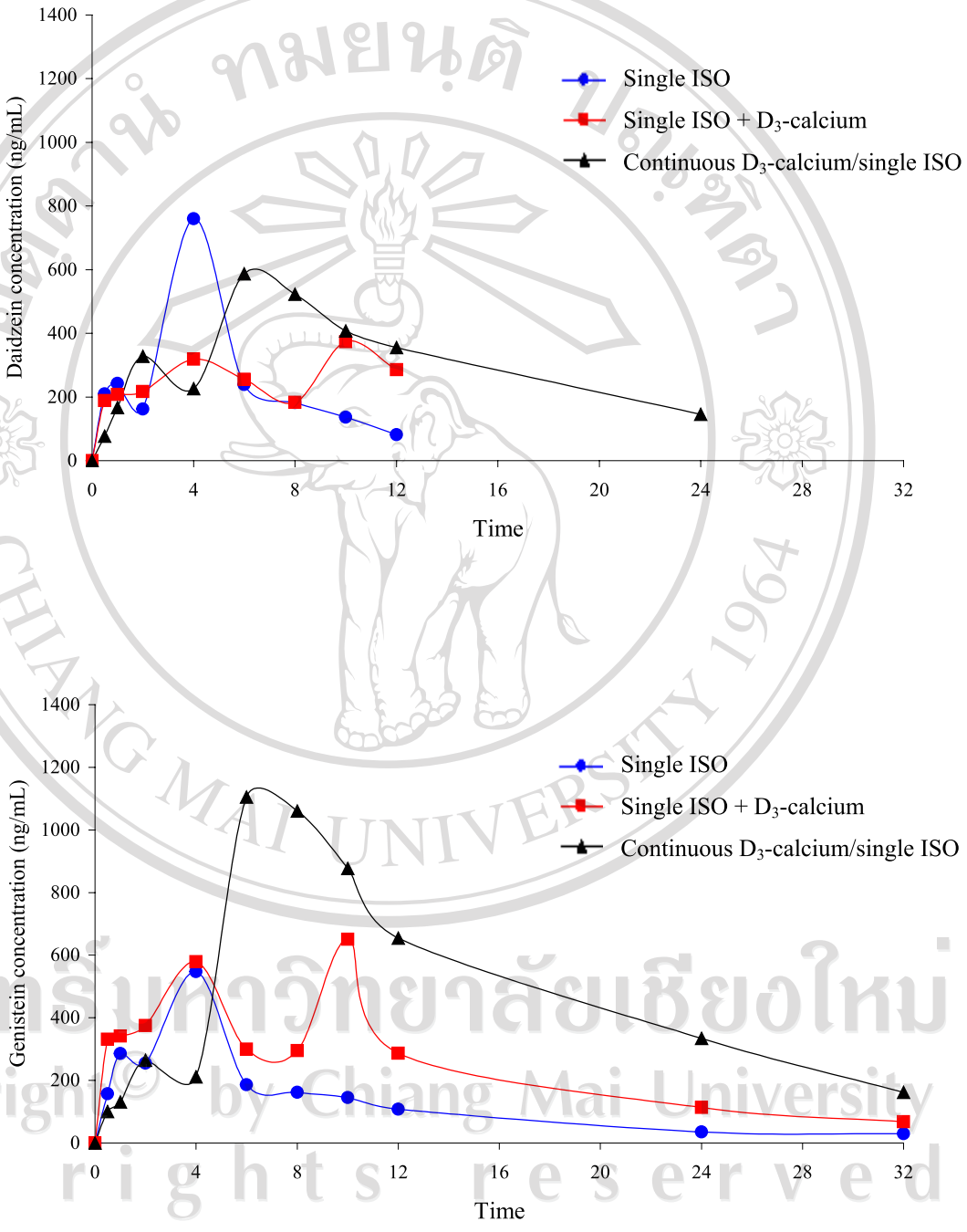

Figure 9 (Continued)

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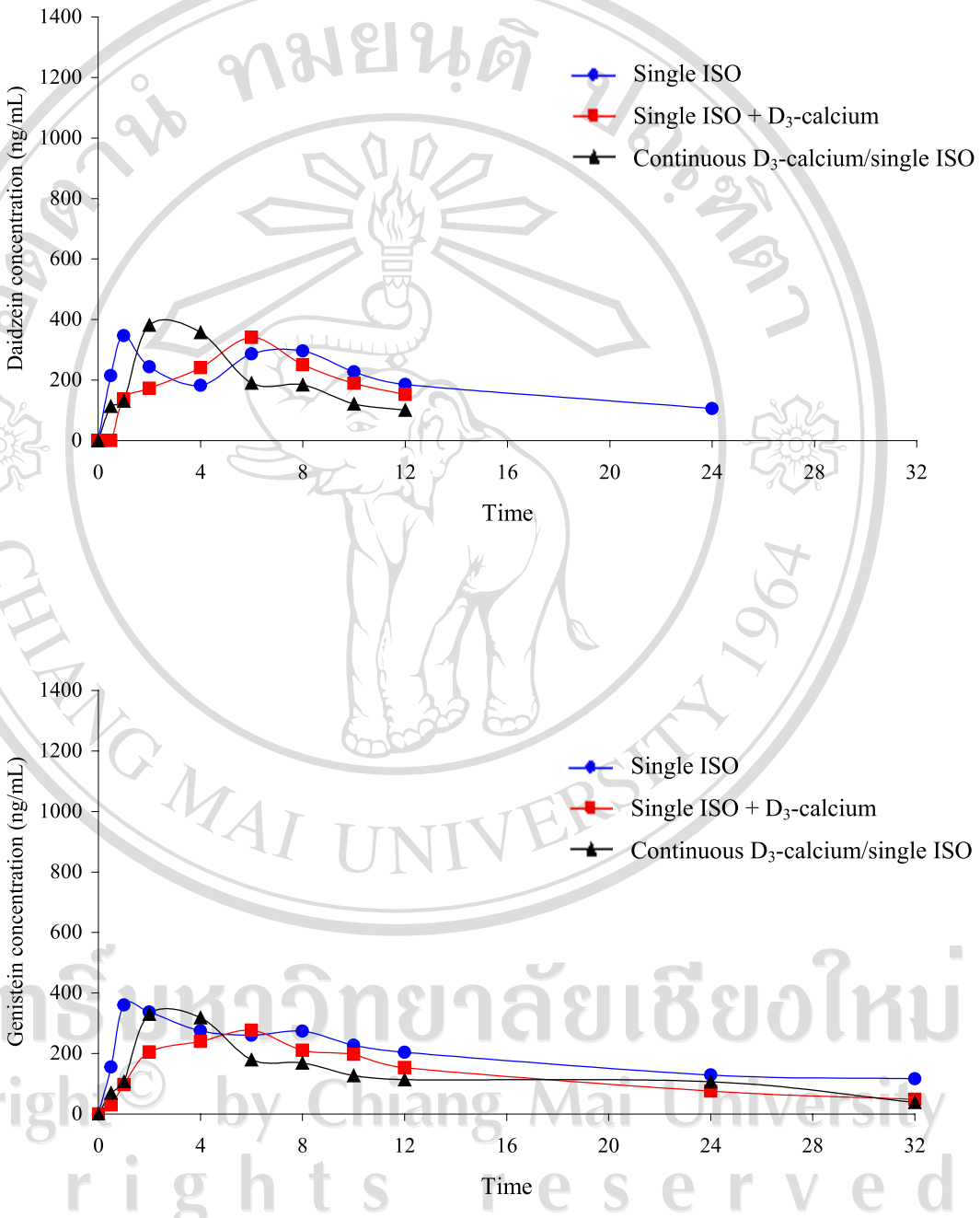

Figure 9 (Continued)

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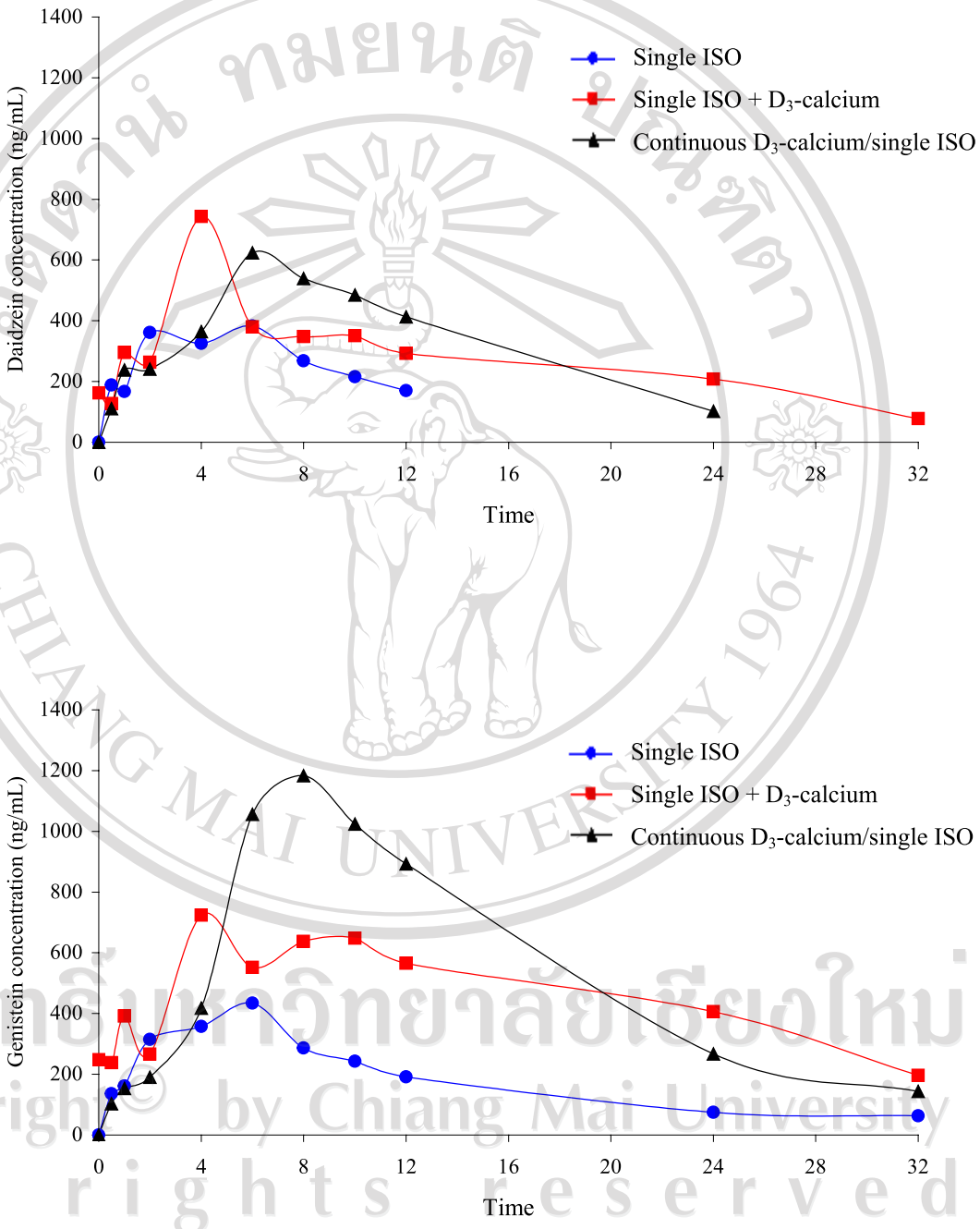

Figure 9 (Continued)

Subject 10

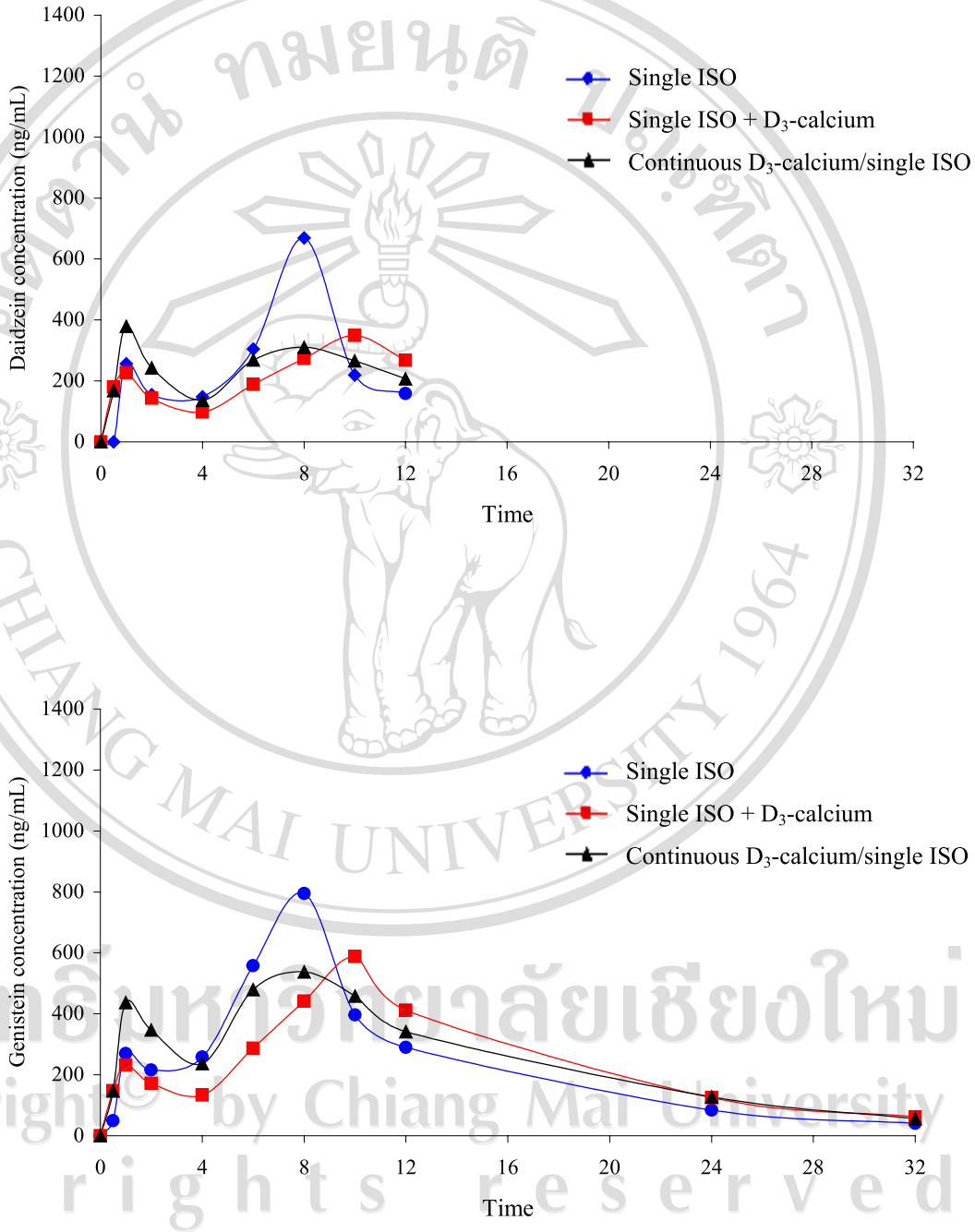

Figure 9 (Continued)

Subject 11

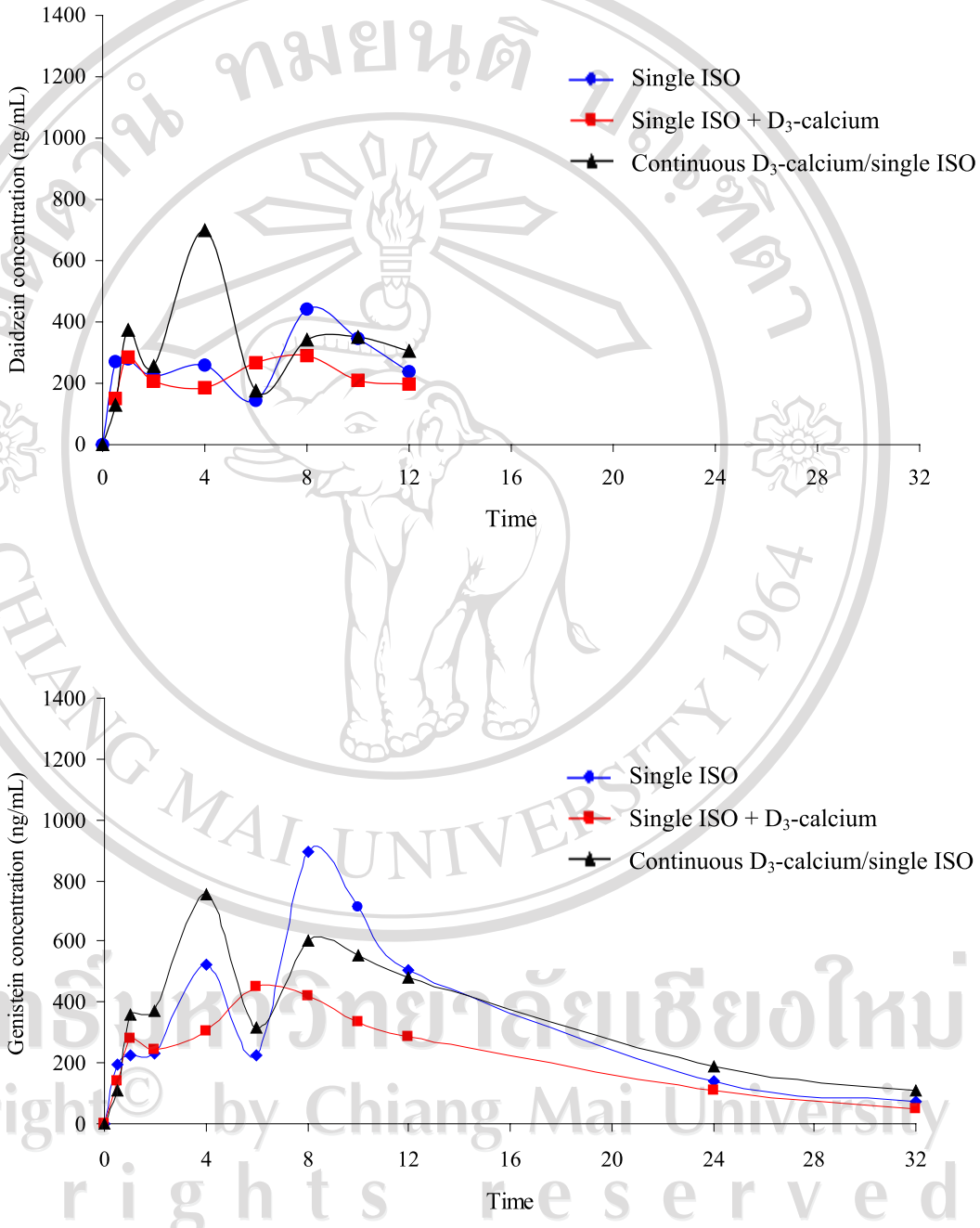

Figure 9 (Continued)

Subject 12

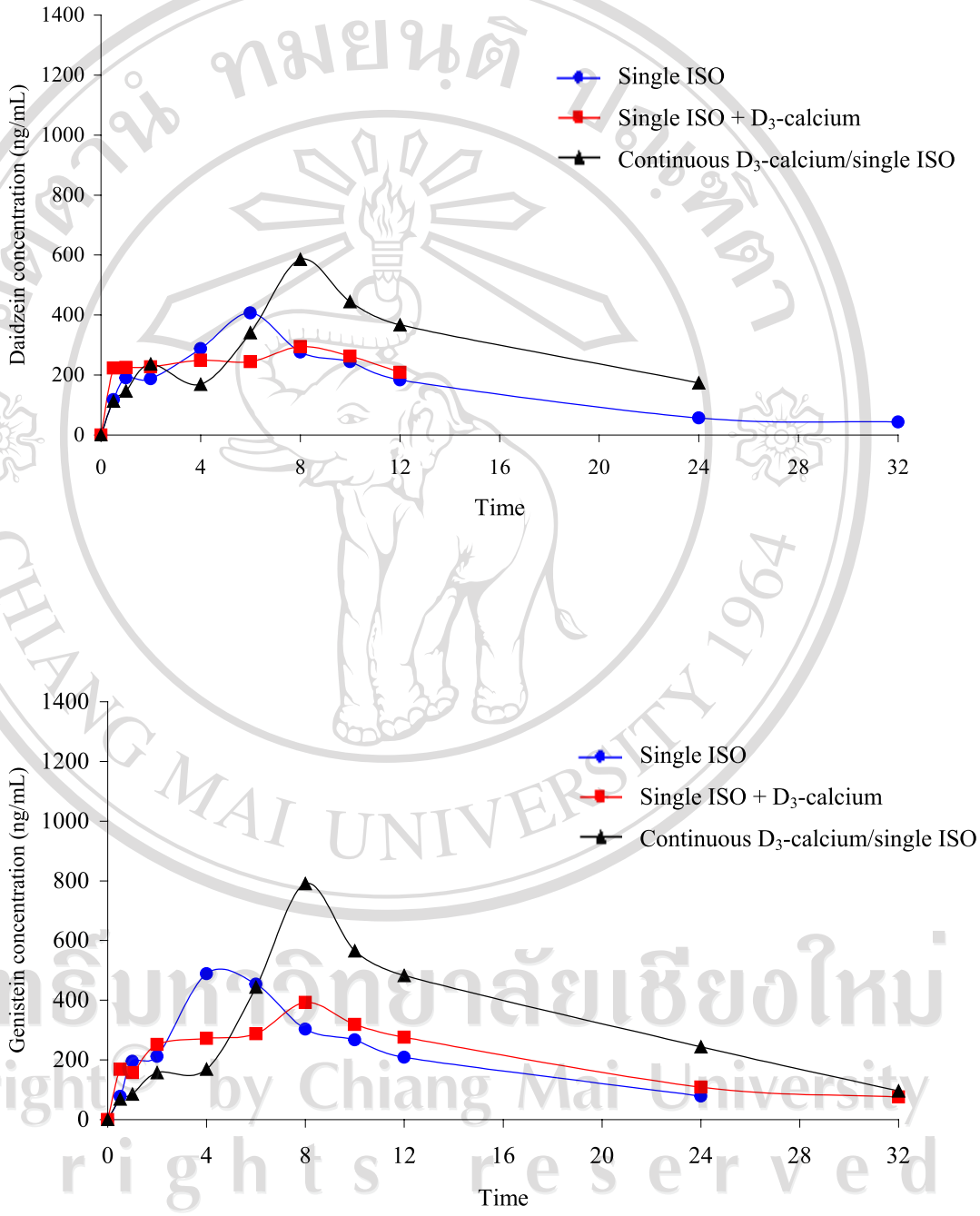

Figure 9 (Continued)

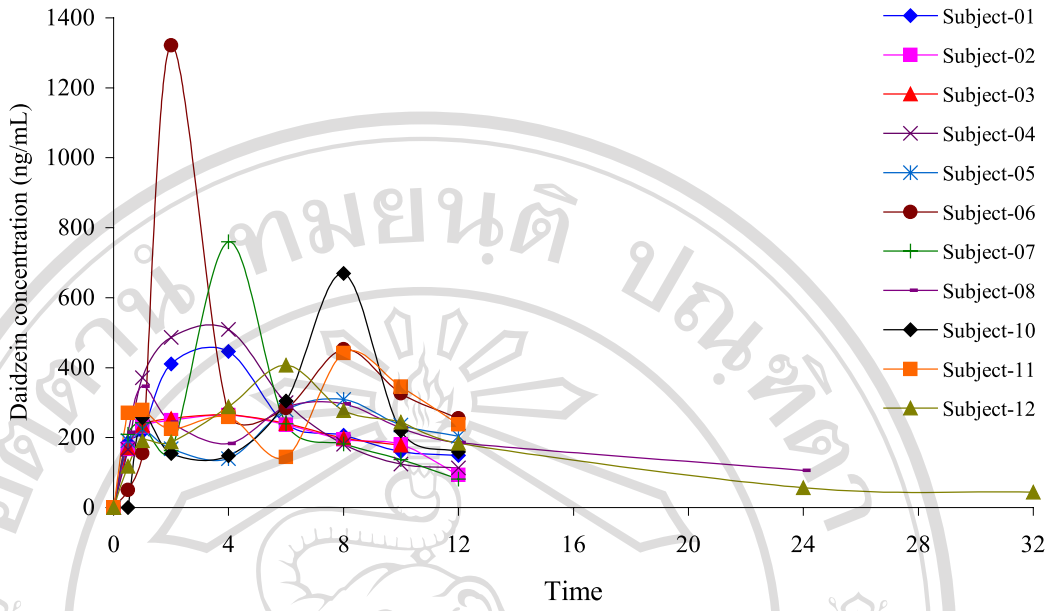

Figure 10A Composite of plasma daidzein concentration-time curves after "single ISO" in individual subject complted the study without protocol diviation (n=11)

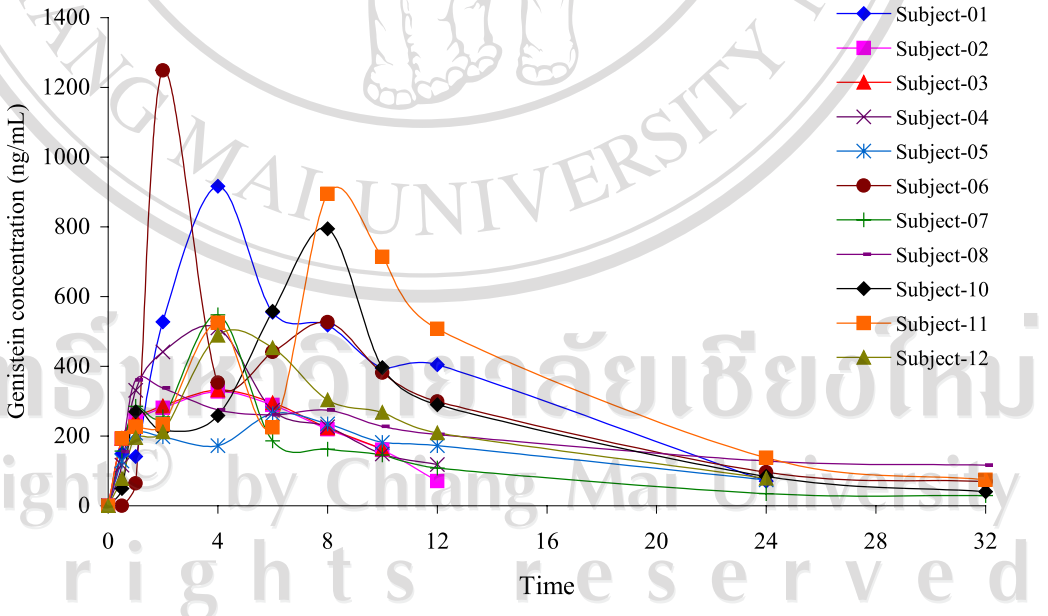

Figure 10B Composite of plasma genistein concentration-time curves after "single ISO" in individual subject complted the study without protocol diviation (n=11)

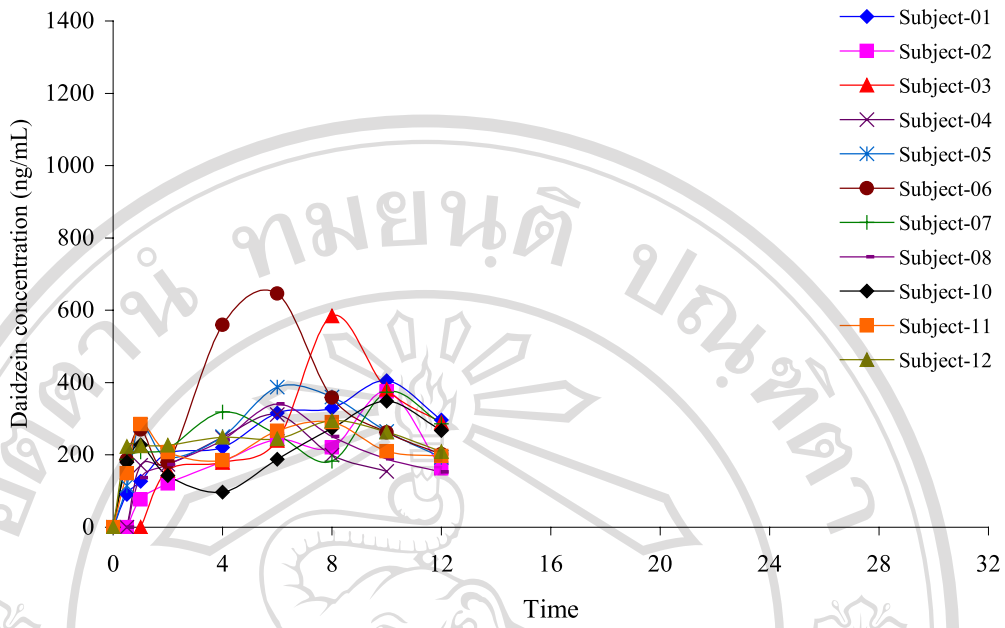

Figure 11A Composite of plasma daidzein concentration-time curves after "single ISO + D_3-calcium" in individual subject complted the study without protocol diviation (n=11)

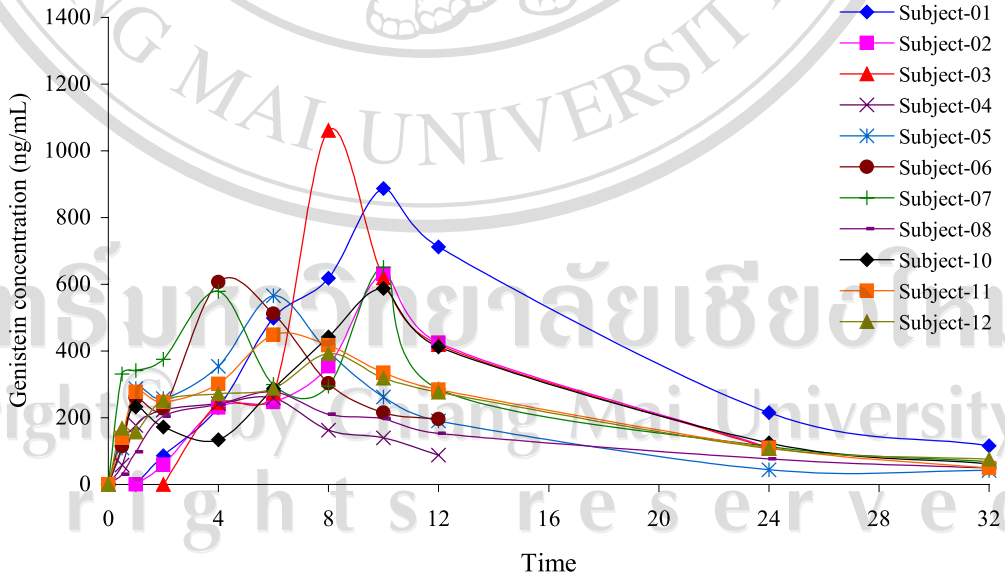

Figure 11B Composite of plasma genistein concentration-time curves after "single ISO + D_3-calcium" in individual subject complted the study without protocol diviation (n=11)

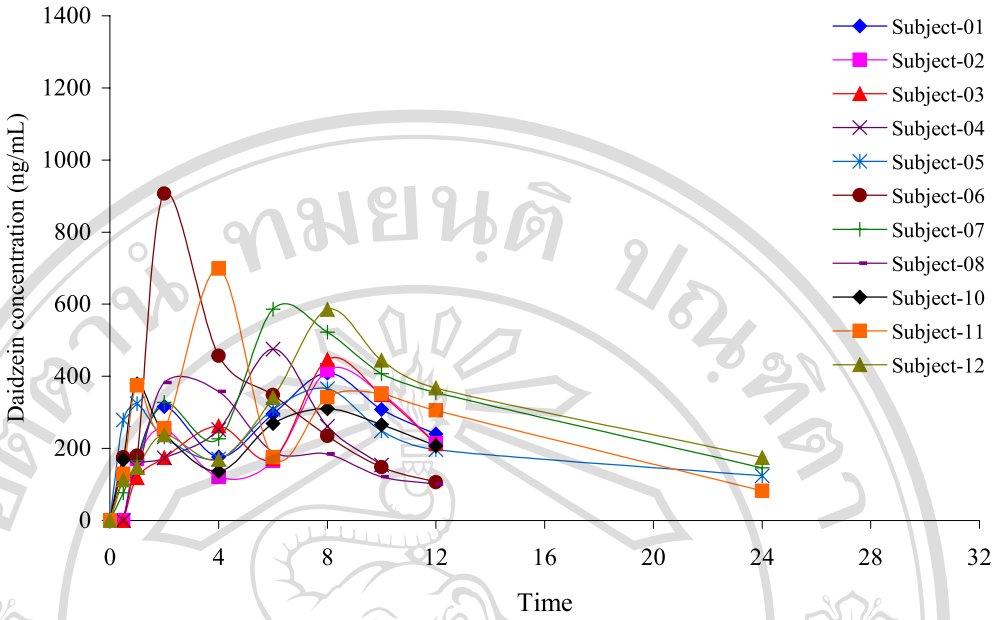

Figure 12A Composite of plasma daidzein concentration-time curves after "continuous D_3-calcium/single ISO" in individual subject complted the study without protocol diviation (n=11)

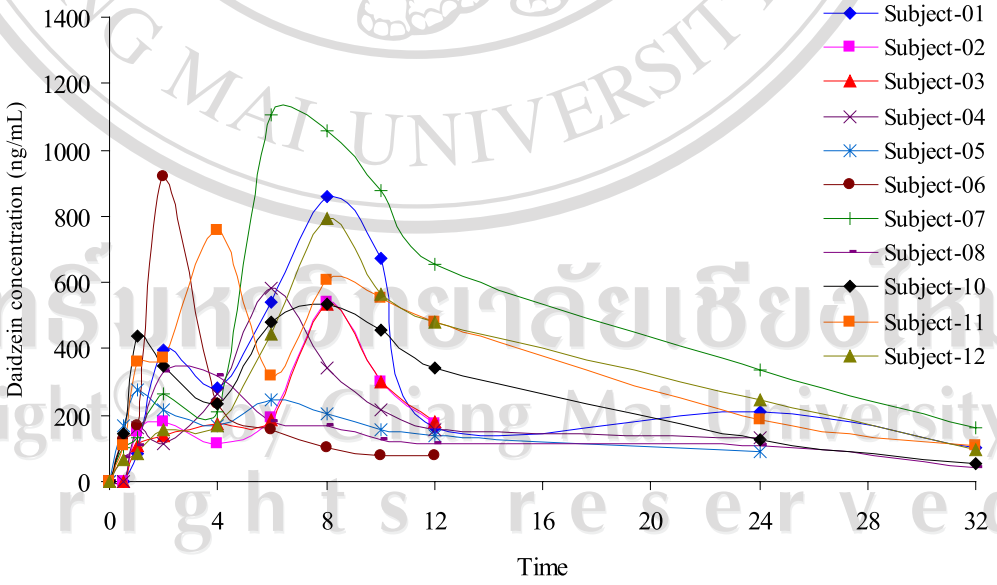

Figure 12B Composite of plasma genistein concentration-time curves after "continuous D_3-calcium/single ISO" in individual subject complted the study without protocol diviation (n=11)

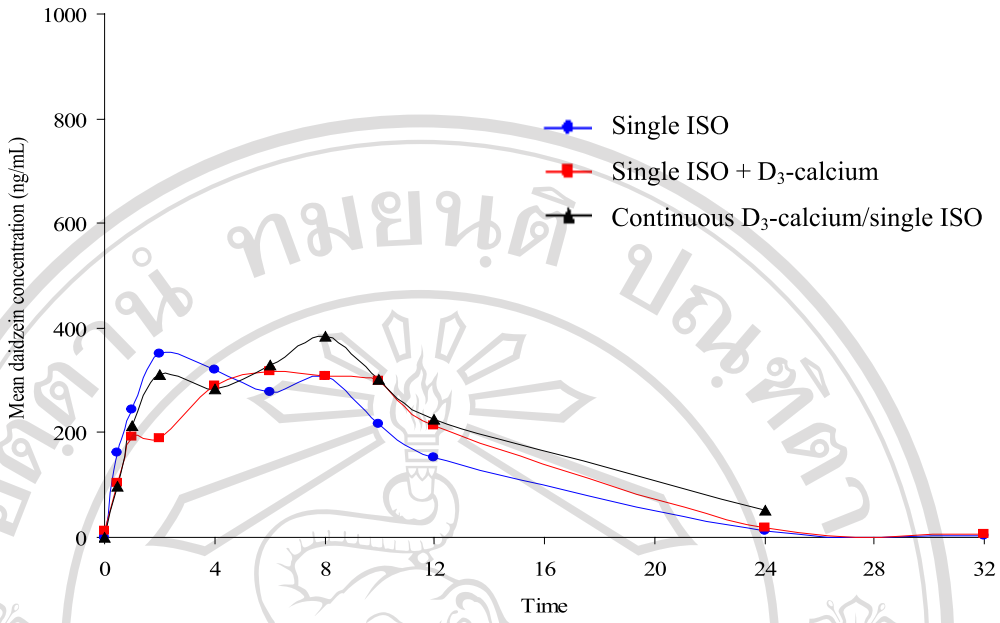

Figure 13A Mean plasma daidzein concentration-time curves from 11 subjects receiving "single ISO", "single ISO + D_3-calcium", and "continuous D_3-calcium/single ISO"

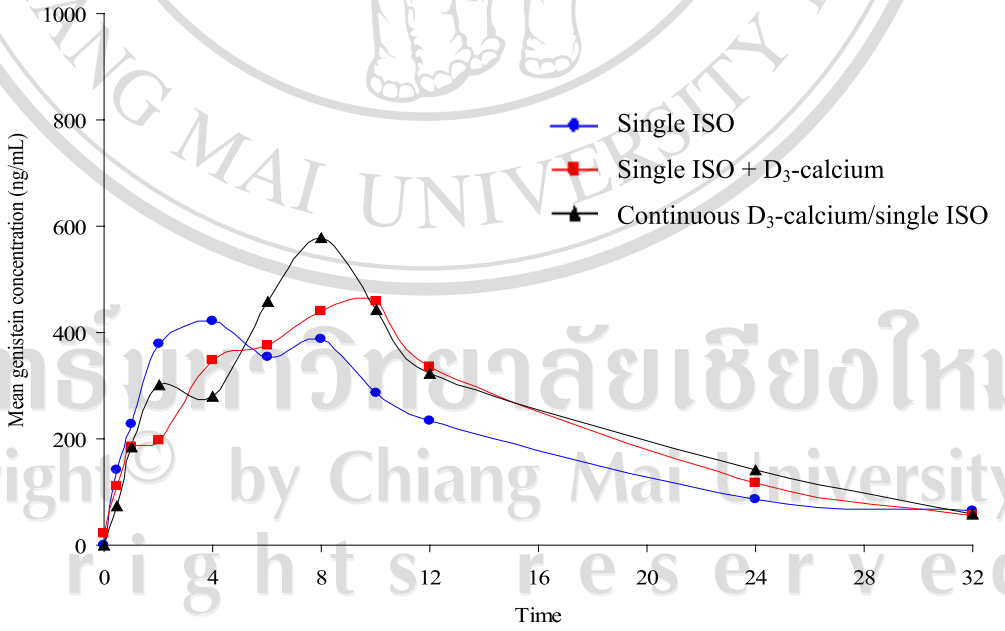

Figure 13B Mean plasma genistein concentration-time curves from 11 subjects receiving "single ISO", "single ISO + D_3-calcium", and "continuous D_3-calcium/single ISO"

Table 13A Pharmacokinetic parameters of daidzein after "single ISO", "single ISO + D_3-calcium", and "continuous D_3-calcium/single ISO" obtained from 11 subjects completed the study without protocol deviation[#]

Subject No.	C_{max} (ng/mL)			AUC_{0-32} (ng.h/mL)			$AUC_{0-\infty}$ (ng.h/mL)			T_{max} (h)			$t_{1/2}$ (h)		
	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO
1	446.63	405.00	408.97	3124.11	3284.95	3221.13	4923.26	5181.72	5142.95	4.00	10.0	8.0	8.4	4.4	5.6
2	264.20	375.48	415.83	2476.50	2442.18	2830.25	3121.95	2829.59	3700.53	4.00	10.0	8.0	4.8	1.7	2.8
3	265.18	584.76	446.61	2227.51	3295.72	3023.82	4617.34	5404.41	3880.97	4.00	8.0	8.0	9.3	5.1	2.8
4	509.37	311.84	475.25	3434.49	2062.40	2502.05	4122.99	3267.09	3081.52	4.00	6.0	6.0	4.2	5.4	2.6
5	309.17	387.64	365.80	2647.41	3279.38	5037.47	5459.42	4523.58	7733.11	8.00	6.0	8.0	9.6	4.5	15.1
6	1321.14	646.26	906.44	5045.49	4418.20	4062.85	7115.11	5830.96	4623.68	2.00	6.0	2.0	5.6	4.9	3.7
7	760.04	373.75	586.58	3248.65	3124.29	7504.61	3710.79	5261.49	9401.79	4.00	10.0	6.0	3.9	5.2	9.0
8	296.15	340.83	357.33	4657.24	2556.24	2537.53	6695.74	3786.02	3217.37	8.00	6.0	4.0	13.3	5.6	4.7
10	669.27	349.11	310.00	3265.39	2558.44	2902.51	4257.96	4571.27	4557.25	8.00	10.0	8.0	4.3	5.2	5.5
11	442.09	290.25	374.27	3300.70	2700.74	6496.71	4568.64	8921.28	7249.81	8.00	8.0	1.0	3.7	21.9	6.3
12	407.00	294.63	585.50	4947.01	2933.26	7205.59	5478.57	4745.02	9884.35	6.00	8.0	8.0	8.5	6.0	10.7
Mean	506.09	396.32	488.05	3474.05	2968.71	4620.55	4905.23	4938.40	5961.22	5.50	8.00	6.08	6.80	6.34	6.26
SD	299.62	115.37	165.90	931.53	627.60	2148.09	1150.99	1611.00	2546.45	2.11	1.79	2.50	2.98	5.29	3.73
%CV	59.20	29.11	33.99	26.81	21.14	46.49	23.46	32.62	42.72	38.37	22.36	41.15	43.85	83.36	59.54
Max	1321.14	646.26	906.44	5045.49	4418.20	8122.04	7115.11	8921.28	9884.35	8.00	10.00	8.00	13.30	21.90	15.10
Min	264.20	290.25	310.00	2227.51	2062.40	2502.05	3121.95	2829.59	3081.52	2.00	6.00	1.00	3.70	1.65	2.61
Median	424.55	373.75	431.22	3283.05	2933.26	3641.99	4704.14	4745.02	4883.32	5.00	8.00	7.00	5.83	5.18	5.55

Data from subject No.9 was not taken into account because isoflavones concentration was detected in plasma sample at baseline

Table 13B Pharmacokinetic parameters of genistein after "single ISO", "single ISO + D_3-calcium", and "continuous D_3-calcium/single ISO" obtained from 11 subjects completed the study without protocol deviation[#]

Subject No.	C_{max} (ng/mL)			AUC_{0-32} (ng.h/mL)			$AUC_{0-\infty}$ (ng.h/mL)			T_{max} (h)			$t_{1/2}$ (h)		
	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO	Single ISO	Single ISO + D_3-calcium	Continuous D_3-calcium / single ISO
1	916.69	886.94	859.20	8994.78	12202.38	9041.30	9586.46	13470.82	10826.03	4.0	10.0	8.0	5.8	7.6	12.2
2	327.08	631.24	541.14	2763.60	6654.22	2854.93	3074.16	7648.41	3519.00	4.0	10.0	8.0	3.1	6.0	2.6
3	332.14	1061.07	535.06	2578.18	7980.28	2859.79	3689.24	8882.00	3552.64	4.0	8.0	8.0	4.7	5.8	2.7
4	510.18	259.70	584.59	3405.88	2192.20	5004.01	4211.14	2769.09	7762.08	4.0	6.0	6.0	4.7	4.5	14.6
5	264.62	565.48	274.73	3873.94	5748.95	3666.46	4946.77	6187.07	5480.56	6.0	6.0	1.0	10.0	7.2	14.4
6	1248.77	606.79	917.22	8666.71	4061.33	2781.21	9549.46	5275.93	3325.95	2.0	4.0	2.0	8.8	4.3	4.8
7	547.25	650.70	1105.07	3985.38	8047.58	15625.13	4371.33	8979.89	17808.76	4.0	10.0	6.0	9.0	9.6	9.4
8	360.57	277.23	329.90	6110.29	4275.51	4219.41	9816.04	5072.06	4912.80	1. 0	6.0	2.0	22.1	11.3	12.3
10	794.66	588.00	537.46	7601.41	7783.94	8241.23	7994.29	8427.59	8856.06	8.0	10.0	8.0	6.8	7.3	7.5
11	894.65	449.06	604.28	10559.64	6933.27	11027.41	11288.38	7494.49	12443.49	8.0	6.0	8.0	6.8	8.0	9.2
12	488.55	392.72	790.76	5464.72	6442.46	10479.66	6325.45	7573.83	11744.86	4.0	8.0	8.0	7.6	10.3	9.1
Mean	593.33	578.99	688.56	5804.19	6574.74	7758.56	6787.62	7434.65	9091.58	4.58	7.64	6.08	8.31	7.44	8.86
SD	303.31	240.98	285.42	2643.87	2625.30	5068.69	2793.66	2748.42	5394.67	2.11	2.16	2.78	4.90	2.27	4.09
Median	51.12	41.62	41.45	45.55	39.93	65.33	41.16	36.97	59.34	46.01	28.25	45.67	58.90	30.47	46.17
Mean	1248.77	1061.07	1183.36	10559.64	12202.38	17302.19	11288.38	13470.82	18866.74	8.00	10.00	8.00	22.10	11.33	14.60
SD	264.62	259.70	274.73	2578.18	2192.20	2781.21	3074.16	2769.09	3325.95	1.00	4.00	1.00	3.05	4.30	2.61
Median	499.37	588.00	594.44	5555.26	6654.22	6622.62	6462.11	7573.83	8309.07	4.00	8.00	8.00	7.17	7.26	9.14

Data from subject No.9 was not taken into account because isoflavones concentration was detected in plasma sample at baseline