CHAPTER IV

RESULTS AND DISCUSSION

In vitro studies

All four brands of simvastatin tablets were tested for content of active ingredient and uniformity of dosage units. The result indicates that each brand was within the limits of 85.0-115.0 % labeled amount (% LA) as shown in Table 5.

Table 5 Percentge of labeled amount from four brands of simvastatin tablets (n=3).

Brand	% Labeled Amount			Mean(SD)
	1	2	3	
A	97.33	97.74	97.65	97.58(0.22)
B	100.81	104.80	97.86	101.16(3.48)
C	106.85	101.53	102.71	103.70(2.80)
D	97.82	95.99	97.54	97.11(0.99)

For content uniformity analysis, all ten tablets of each brand met the general requirements that the content of each tablet was within the limits of 85.0-115.0 % LA and the percent coefficient of variation (% C.V.) of these content was less than 6.0 % as shown in Table 6.

Table 6 Content uniformity of dosage units from four brands of simvastatin tablets (n=10).

Tablet No.	% Labeled amount			
	Brand A	Brand B	Brand C	Brand D
1	99.04	103.76	106.85	98.62
2	92.50	93.56	105.66	98.90
3	100.46	101.85	108.42	97.05
4	96.75	105.10	103.02	97.50
5	97.23	100.65	104.44	93.03
6	99.25	104.24	105.18	94.55
7	96.93	109.52	102.45	100.38
8	97.96	92.59	92.76	99.79
9	95.86	101.07	97.12	102.52
10	98.86	99.90	100.49	90.31
Mean	97.48	101.22	102.64	97.26
S.D.	2.23	5.11	4.75	3.68
% C.V.	2.29	5.05	4.62	3.79

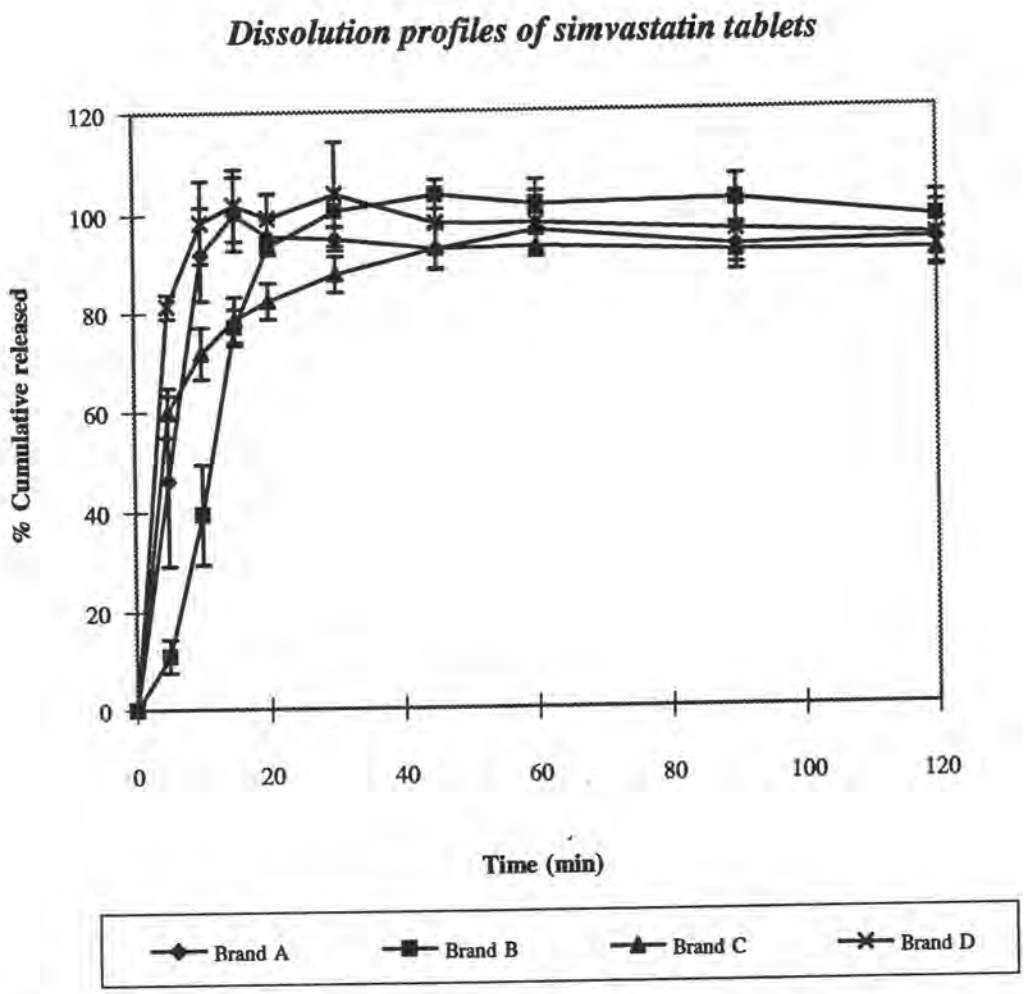


Figure 6 Dissolution profiles of four brands of simvastatin tablets in dissolution medium.

Table 7 Mean percent dissolved of simvastatin in dissolution medium at each sampling time.

Sampling time (min)	Mean percent dissolved (S.D.) (n = 6 tablets)			
	Brand A	Brand B	Brand C	Brand D
5	46.20(17.04)	10.96(3.44)	59.81(4.97)	81.31(2.41)
10	91.87(9.50)	39.44(10.03)	71.75(5.30)	98.35(8.22)
15	100.00(7.29)	77.05(3.68)	78.54(4.70)	101.76(7.08)
20	95.66(3.36)	93.55(1.83)	82.24(3.67)	99.10(4.76)
30	94.94(2.42)	100.42(2.93)	87.64(3.68)	103.74(10.27)
45	92.87(4.34)	103.53(2.85)	92.79(4.09)	98.03(6.09)
60	96.33(3.33)	101.50(4.74)	93.20(2.28)	97.82(6.22)
90	93.09(3.87)	102.19(4.73)	91.88(4.11)	96.12(4.69)
120	93.82(3.79)	98.30(4.69)	91.65(3.83)	94.70(6.31)

Table 8 Dissolution rate constants (K_d) of four brands of simvastatin tablets in dissolution medium.

Tablet No.	Dissolution rate constant (K_d) (min^{-1})			
	Brand A	Brand B	Brand C	Brand D
1	0.269	0.129	0.060	0.075
2	0.189	0.105	0.076	0.323
3	0.251	0.086	0.057	0.332
4	0.030	0.080	0.063	0.284
5	0.209	0.092	0.085	0.240
6	0.187	0.126	0.033	0.384
Mean	0.189	0.103	0.062	0.273
S.D.	0.085	0.020	0.018	0.108

Table 9 Statistical differences of dissolution rate constant of simvastatin tablets assessed by one-way analysis of variance (α=0.05).

Source of variation	Degree of freedom	Sum of squares	Mean squares	F ratio
Between group	3	0.1579	5.262×10^{-2}	10.70*
Within group	20	9.836×10^{-2}	4.918×10^{-3}	
Total	23	0.2562		

* = Significant ($p < 0.05$)

where tabulated $F_{0.05}(3,20) = 2.42$

Dunnett's test results comparing the mean dissolution rate constant of each local brand with brand A (α=0.05).

Brand	Mean dissolution rate constant (S.D.)	$\lvert$Difference of $K_d\rvert$ (Local VS brand A)	Significant level
A	0.189(0.085)	-	-
B	0.103(0.020)	0.086	NS
C	6.25×10^{-2}(0.018)	0.126	S
D	0.273(0.108)	0.084	NS

$$D' = t' \sqrt{S^2 (1/N_1 + 1/N_2)} = 0.104$$

where tabulated $t'_{0.05}(3,20) = 2.57$

D' = The critical difference for a 2-sided test for any of the comparisons VS control.

S^2 = Within mean square from ANOVA.

N_1, N_2 = number of each sample group (= 6 in each group).

The dissolution testing of simvastatin film-coated tablets was a crucial factor for systemic drug availability because simvastatin has a low water solubility. Since the method of dissolution testing for simvastatin was not described elsewhere, the new procedure was tried out. For characterizing the dissolution profiles and the dissolution rate constant (K_d), the method described below was found to be the most practical. The dissolution testing was conducted according the paddle method provided in USP 23 for coated-tablet using 0.5 % SLS in 0.01 M monobasic sodium phosphate buffer pH 5.5 ± 0.02 as the dissolution medium.

The dissolution profiles and the mean percent drug dissolved in dissolution medium at various times of four brands of simvastatin tablet were illustrated in Figure 6 and Table 7. The mean percent drug dissolved of brand A and D was 100 % within 15 min and that of brand B was 100 % within 30 min, but, the mean percent drug dissolved of brand C was rather low. The dissolution rate constants (K_d) were calculated from the slopes of the first order plots between the amount of undissolved simvastain (X_∞-X_t) versus time according to the method in Appendix F and were summarized in Table 8. Statistical comparison, as presented in Table 9, indicates that there was a significant difference in the dissolution rate constants of the four brands. Using Dunnett's test to compare the dissolution rate constant between each locally-manufactured product and the innovator's product, the result showed that only brand C had a significantly different dissolution rate constant from the innovator's product. As seen in Figure 6 and Table 8, different brands of simvastatin film-coated tablets resulted in different dissolution rates. This was very common and always seen when a practically insoluble drug was manufactured as a film-coated tablet. This variation might be due to different solubility of coating material, the film thickness, different manufacturing

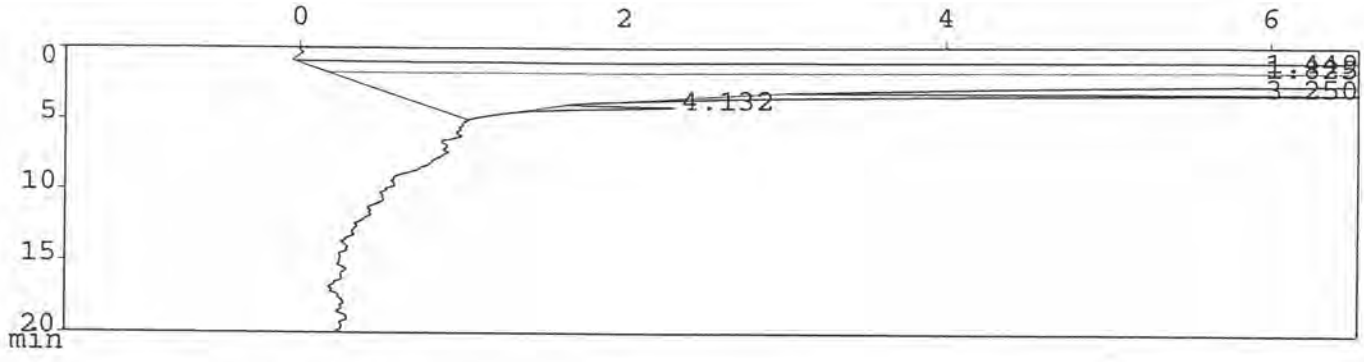
process, sources of active ingredients and tablet formulation. These problems could be overcome if the formulator(s) used appropriate manufacturing process and raw materials. Finally, the in vitro rate of drug release from the dosage forms is influenced greatly by the condition of the test include stirring rate and characteristics of dissolution medium such as pH, concentration, viscosity, surface tension, additives and volume that affect markedly the dissolution rate (Abdou, H. M., 1989). Therefore, if the dissolution condition is changed, the dissolution rate constants and their rank would be differ from the present data.

In vivo studies

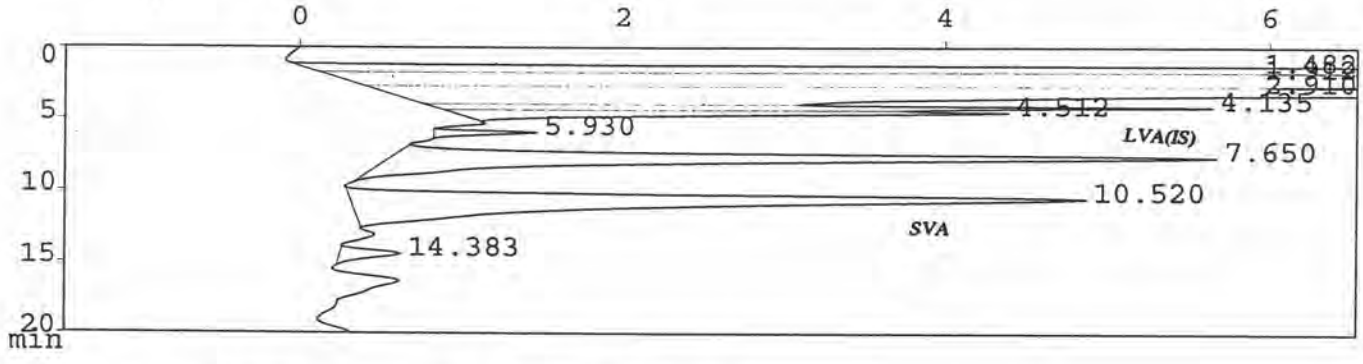
1. Assay validation

The typical chromatograms in mobile phase 44% acetonitrile in 0.025 M monobasic sodium phosphate pH 4.5, after alkaline hydrolysis, of blank dog plasma, dog plasma spiked with simvastatin (SV) and internal standard, and plasma sample taken at 1.5 hr postdose from a dog orally administered with a single dose of 200 mg simvastatin are shown in Figure 7a. Both simvastatin hydroxy acid (SVA) and lovastatin hydroxy acid (LVA) peaks were well separated from the endogenous substance peaks with the retention times of 10.5 and 7.6 min, respectively. Figure 7b shows previous chromatogram of SV before (SV) and after (SVA) alkaline hydrolysis, in dog plasma in mobile phase 55% acetonitrile in 0.025 M monobasic sodium phosphate pH 4.5 which were eluted at 8.86 and 5.29 min. It can be seen from this chromatogram that SV peak did not interfere with SVA peak and that hydrolysis of SV to SVA was completed in all samples since no peak of SV was observed. The standard curves showed good linearity in the concentration range employed (25-500 ng/ml) (r^2 = 0.99) as shown in Appendix B. The method was validated by determining the within-run and between-run precisions, the efficiency of

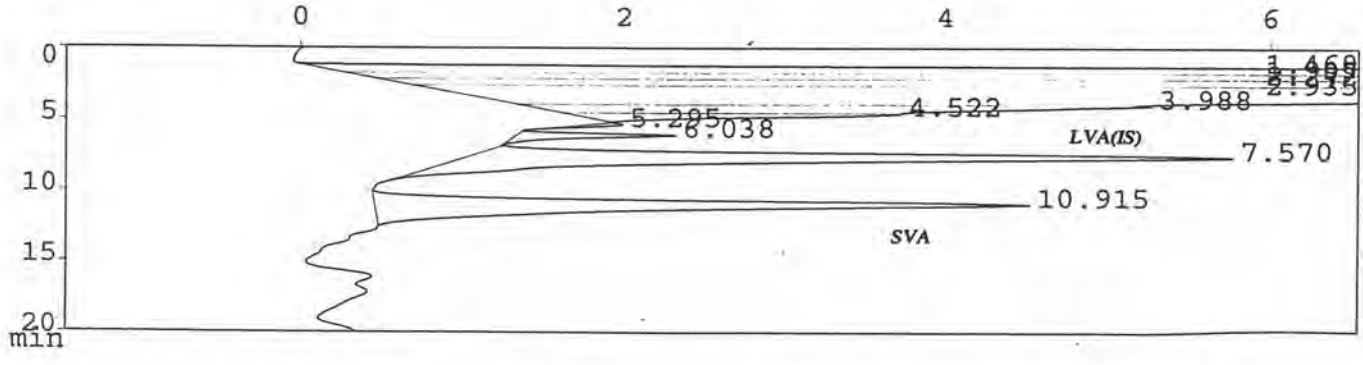
separation technique, and the lowest limit of quantitation. The results for both within the same day and between the different days demonstrated good reproducibility and precisions in the concentration range, with % CV at all concentrations tested less than 15% except at the lowest limit of quantitation (25 ng/ml). However, at this concentration %CV was not greater than 20% (Shah et. al., 1992). The % CV for the within-run and between-run precisions were 5.27-18.46 and 9.71-19.13 % as shown in Table 10 and 11, respectively. The efficiency of plasma protein separation technique was evaluated by calculating the percentage of physical recoveries. Physical recoveries were determined from the peak area ratios (SVA/LVA) of the spiked plasma with those of the standard aqueous solution of the same concentration, similary treated. The overall average percent recovery of simvastatin hydroxy acid was found to be 93.23 ± 5.22 % (n = 30) over a range of five concentrations studied as shown in Table 12. Table 13 shows the PAR values of SVA to LVA after 10 separate HPLC analysis of plasma spiked with 25 ng/ml SV. The % CV of the PAR values was calculated to be 19.68. Since the maximum % CV allowable for the limit of quantitation (LOQ) was proposed to be 20 %, the standard SV concentration of 25 ng/ml was considered to be the appropriate LOQ for this assay procedure (Shah et. al., 1992).



Blank dog plasma.

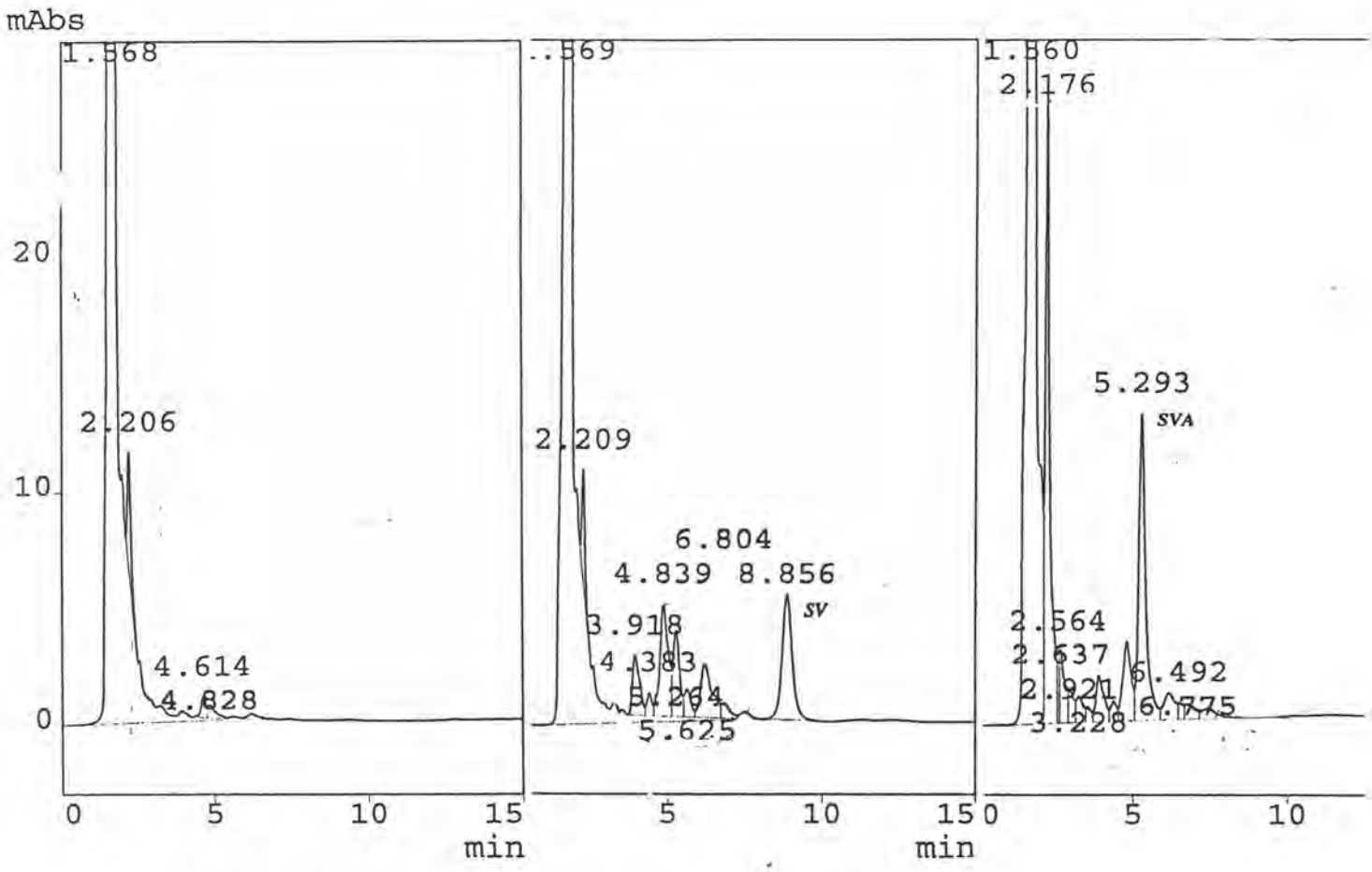


Spiked dog plasma (SVA conc. 250 ng/ml).



Dog plasma sample taken at 1.5 hr postdose (SVA observed conc. 150 ng/ml).

Figure 7a High performance liquid chromatographic chromatograms of simvastatin hydroxy acid (SVA) and lovastatin hydroxy acid (LVA internal standard) in mobile phase 44% acetonitrile in 0.025 M monobasic sodium phosphate pH 4.5 (attenuation 3).



blank dog plasma spiked SV only SVA after alkaline hydrolysis

Figure 7b High performance liquid chromatographic chromatograms of simvastatin (SV) and simvastatin hydroxy acid (SVA) in mobile phase 55% acetonitrile in 0.025 M monobasic sodium phosphate pH 4.5 (attenuation 5).

Table 10 Within-run results of the SVA HPLC assay.

Conc.	Drug / Internal standard peak area ratio			Mean (S.D.)	%C.V.
(ng/ml)	1	2	3		
25	0.232	0.307	0.337	0.292(0.054)	18.46
50	0.339	0.434	0.378	0.384(0.048)	12.48
100	0.502	0.424	0.489	0.472(0.042)	8.89
250	0.825	0.889	0.915	0.876(0.046)	5.27
500	1.7825	1.919	1.681	1.794(0.119)	6.66

Table 11 Between-run results of the SVA HPLC assay.

Conc.	Drug / Internal standard peak area ratio			Mean (S.D.)	%C.V.
(ng/ml)	1	2	3		
25	0.292	0.202	0.230	0.242(0.046)	19.13
50	0.384	0.405	0.462	0.417(0.040)	9.71
100	0.472	0.630	0.605	0.569(0.085)	14.97
250	0.915	1.068	1.198	1.060(0.141)	13.34
500	1.794	2.098	2.307	2.066(0.258)	12.48

Table 12 Relative physical recoveries results of the SVA HPLC assay (n = 6).

Conc.	Relative physical recoveries						Mean(SD)	%CV
(ng/ml)	1	2	3	4	5	6		
25	89.93	104.0	97.69	90.16	88.0	104.75	95.74(7.47)	7.80
50	82.92	93.45	91.04	86.35	82.32	82.81	86.48(4.75)	5.49
100	94.72	91.94	95.25	102.16	105.04	103.09	98.75(5.83)	5.46
250	86.11	98.33	88.22	83.98	90.37	92.26	89.88(5.08)	5.65
500	87.16	86.94	94.47	93.34	99.89	91.75	92.26(4.88)	5.28
					Overall	mean	93.23(5.22)	5.60

Table 13 % Coefficient of variation (% CV) results of the total SVA HPLC assay at concentration of 25 ng/ml (n = 10).

Run number	Drug / Internal standard peak area ratio
1	0.443
2	0.449
3	0.341
4	0.331
5	0.347
6	0.269
7	0.307
8	0.232
9	0.353
10	0.337
Mean (S.D.)	0.341(0.067)
%C.V.	19.68

2. Pharmacokinetic studies

The concentration of simvastatin (SV) in ng/ml, analyzed as total simvastatin hydroxy acid (total SVA), in the plasma of the individual dog from 0-8 hr, after taking twenty tablets of brand A, B, C and D are recpectively shown in Tables 14-17. Comparison of the plasma SV concentration-time profiles in each of the twelve dogs are also illustrated in Figures 8-19. As can be seen from these Figures, the individual plasma drug concentration-time profiles are not smooth and vary from one dog to another as a result of high intersubject variation. Factors that contribute to the intersubject variability may include the dogs' activities after dosing as well as difference in their health conditions, bodyweight and age. These differences may lead to variation in the metabolism and clearance of SV in the individual dogs. However, the intersubject variability can be extracted from the error mean square during the analysis of variance since the crossover design was used in this study.

Some profiles also exhibited secondary peak plasma concentrations. These can be clearly seen in dogs number 2, 6 and 9 whereas a minor peak or "shoulder" was observed for most of the other dogs in the later period of drug administration. The occurrence of the secondary peak was not limited to a particular brand but could be observed in every product. As reported by Duggan and Vickers (1990), this could be due to the enterohepatic circulation of SVA, which allows the active metablite to be excreted via the bile duct into the duodenum and then reabsorbed through the hepatic portal vein. The presence of the enterohepatic circulation enables the drug to have a prolonged effect in lowering the plasma cholesterol and LDL level (Vicker et al., 1990a) despite the short plasma elimination half-life of its active metabolite SVA.

The mean plasma SV concentration-time profiles of each brand are illustrated in Figure 20 and the data are summarized in Table 18. The relative high standard deviation error values substantiate previous discussion that the plasma drug was highly fluctuated among the individual dogs. Since SV has an extensive first pass metabolism and a very high hepatic extraction ratio (> 94 %), a variation in the absorption among different dogs may lead to a wide variation in its biotransformation (Duggan and Vicker, 1990).

The bioavailability and other relevant pharmacokinetic parameters were taken or calculated from the individual plasma data. They were AUC, C_{max} and t_{max}. The values of plasma elimination rate constant and plasma elimination half-life were also calculated for some dogs, particularly when their plasma profiles of total SVA did not exhibit a distinct secondary peak in the elimination phase.

Table 14 Individual plasma concentration of simvastatin (SV) in ng/ml, analyzed as total simvastatin hydroxy acid (SVA), in twelve dogs after taking a 200 mg oral dose of simvastatin tablets (brand A). Data = Mean ± SEM.

Dog No.	Time (hr)								
	0.5	1	1.5	2	3	4	5	6	8
1	0	28.2	225.56	444.85	419.77	264.31	121.02	48.68	0
2	0	38.72	79.0	94.15	26.22	26.0	41.52	38.95	0
3	0	30.15	41.72	37.72	102.74	57.36	28.31	25.99	0
4	53.29	96.18	113.79	236.42	96.18	60.28	49.19	46.62	40.87
5	0	25.99	142.39	121.8	49.19	38.72	40.87	30.15	0
6	0	49.93	91.17	80.17	77.39	52.47	49.19	33.79	0
7	0	40.87	95.02	104.16	49.19	40.87	38.72	30.15	0
8	30.15	127.93	120.28	49.19	38.72	30.15	41.27	41.52	25.99
9	131.06	25.99	38.95	35.33	52.81	49.19	40.88	30.15	28.15
10	0	25.99	32.71	66.25	49.96	38.95	41.52	30.15	0
11	0	30.15	49.19	72.96	126.77	82.4	38.1	27.82	0
12	30.9	260.84	367.91	377.58	166.75	38.95	30.65	25.99	0
Mean	20.45	65.08	116.47	143.38	104.64	64.97	46.77	34.16	7.92
SEM	11.30	20.06	27.72	39.45	30.99	18.64	6.97	2.27	4.25

Note; Drug concentrations below the quantitation limit were considered negligble and assigned a zero value.

Table 15 Individual plasma concentration of simvastatin (SV) in ng/ml, analyzed as total simvastatin hydroxy acid (SVA), in twelve dogs after taking a 200 mg oral dose of simvastatin tablets (brand B). Data = Mean ± SEM.

Dog No.	Time (hr)								
	0.5	1	1.5	2	3	4	5	6	8
1	0	79.08	200.3	303.5	177.26	73.05	39.77	40.1	0
2	0	29.37	137.24	242.43	26.39	57.02	60.55	34.76	0
3	0	60.55	83.1	39.77	34.76	33.72	29.37	28.98	0
4	0	59.88	130.46	342.27	60.55	34.76	39.77	29.37	25.51
5	66.98	49.52	74.69	60.55	46.74	65.51	34.76	28.15	0
6	49.52	57.2	54.84	101.89	41.39	29.37	60.55	31.73	29.84
7	0	41.39	52.75	60.55	47.74	40.13	34.76	29.37	0
8	36.83	39.96	35.62	52.8	226.34	192.55	85.02	75.98	33.57
9	0	153.34	98.19	76.38	77.94	26.35	47.88	40.04	28.8
10	0	49.76	91.03	70.31	103.92	63.46	62.25	60.55	0
11	42.11	34.76	41.39	30.71	34.76	59.32	60.98	51.19	38.45
12	0	80.22	124.94	188.8	118.91	57.65	49.98	28.37	0
Mean	16.29	61.25	93.71	130.83	83.06	61.07	50.47	39.97	13.01
SEM	7.22	9.54	13.87	31.63	18.22	12.78	4.62	4.38	4.72

Note; Drug concentrations below the quantitation limit were considered negligble and assigned a zero value .

Table 16 Individual plasma concentration of simvastatin (SV) in ng/ml, analyzed as total simvastatin hydroxy acid (SVA), in twelve dogs after taking a 200 mg oral dose of simvastatin tablets (brand C). Data = Mean ± SEM.

Dog No.	Time (hr)								
	0.5	1	1.5	2	3	4	5	6	8
1	82.65	148.03	228.43	137.96	114.76	106.6	73.17	0	0
2	97.24	114.58	128.55	107.52	101.51	91.97	63.02	*	0
3	46.01	101.46	74.98	72.57	67.26	61.0	47.54	25.05	0
4	0	61.34	266.51	155.57	126.83	94.02	41.06	0	0
5	0	109.3	131.09	77.32	56.67	47.72	46.9	37.44	0
6	77.23	173.16	66.65	61.61	56.23	56.20	50.08	43.5	0
7	61.24	73.19	72.72	62.0	47.11	40.4	32.94	31.94	0
8	0	64.95	109.58	56.79	54.52	47.05	46.72	36.77	0
9	0	86.91	157.10	83.61	68.21	62.70	48.99	41.63	0
10	32.13	52.17	90.97	66.13	65.12	48.45	48.39	27.06	0
11	60.75	75.53	187.89	82.24	75.27	52.18	50.0	33.83	0
12	0	52.65	137.26	101.62	83.20	65.04	52.15	33.48	0
Mean	38.10	85.56	131.80	103.50	77.67	64.90	51.22	28.91	0
SEM	10.81	11.02	18.26	9.75	7.33	6.19	2.90	4.52	0

Note; Drug concentrations below the quantitation limit were considered negligble and assigned a zero value.

* = Missing data

Table 17 Individual plasma concentration of simvastatin (SV) in ng/ml, analyzed as total simvastatin hydroxy acid (SVA), in twelve dogs after taking a 200 mg oral dose of simvastatin tablets (brand D). Data = Mean ± SEM.

Dog No.	Time (hr)								
	0.5	1	1.5	2	3	4	5	6	8
1	32.93	96.62	246.27	202.63	50.14	47.5	52.05	65.73	39.39
2	0	37.81	64.45	60.48	294.05	41.61	46.90	31.35	0
3	42.28	46.90	83.21	34.88	30.46	36.91	37.81	29.39	0
4	172.97	503.22	460.0	407.44	130.16	65.79	47.50	37.81	37.70
5	0	64.0	109.10	81.35	25.57	28.63	0	0	0
6	90.24	103.14	83.08	82.42	93.0	66.17	40.39	28.86	0
7	37.81	61.23	75.2	62.13	51.99	48.39	38.86	28.65	27.93
8	29.44	29.28	149.64	47.50	38.45	38.84	33.59	25.32	28.36
9	38.05	44.28	60.71	48.45	47.15	41.16	36.39	37.81	25.47
10	0	51.49	46.90	65.15	67.95	45.41	44.96	47.50	41.50
11	0	0	158.93	204.93	117.15	120.26	40.42	40.79	0
12	0	63.84	64.93	105.36	106.27	86.63	59.97	37.81	28.68
Mean	39.98	91.30	132.17	117.92	87.80	56.11	40.30	34.13	20.11
SEM	14.63	38.24	33.88	31.04	21.30	7.46	4.20	4.43	5.06

Note; Drug concentrations below the quantitation limit were considered negligble and assigned a zero value.

Table 18 Mean plasma concentration of simvastatin (SV) in ng/ml, analyzed as total simvastatin hydroxy acid (SVA), in twelve dogs after taking twenty tablets equivalent to 200 mg oral dose of different brands of simvastatin tablets (Mean ± SEM).

Time (hr)	Brand A	Brand B	Brand C	Brand D
0.5	20.45(11.30)	16.29(7.22)	38.10(10.81)	36.98(14.63)
1	65.08(20.06)	62.71(9.54)	85.56(11.02)	91.30(38.24)
1.5	116.47(27.72)	93.71(13.87)	131.80(18.26)	132.16(33.88)
2	143.38(39.45)	130.83(31.63)	103.49(9.75)	117.92(31.04)
3	104.64(31.0)	83.06(18.22)	77.67(7.34)	87.80(21.30)
4	64.97(18.64)	61.07(12.78)	64.90(6.19)	56.11(7.46)
5	46.77(6.97)	50.47(4.62)	51.22(2.90)	40.30(4.20)
6	34.16(2.27)	39.96(4.38)	28.91(4.52)	34.13(4.43)
8	7.92(4.25)	13.01(4.72)	0	20.11(5.06)

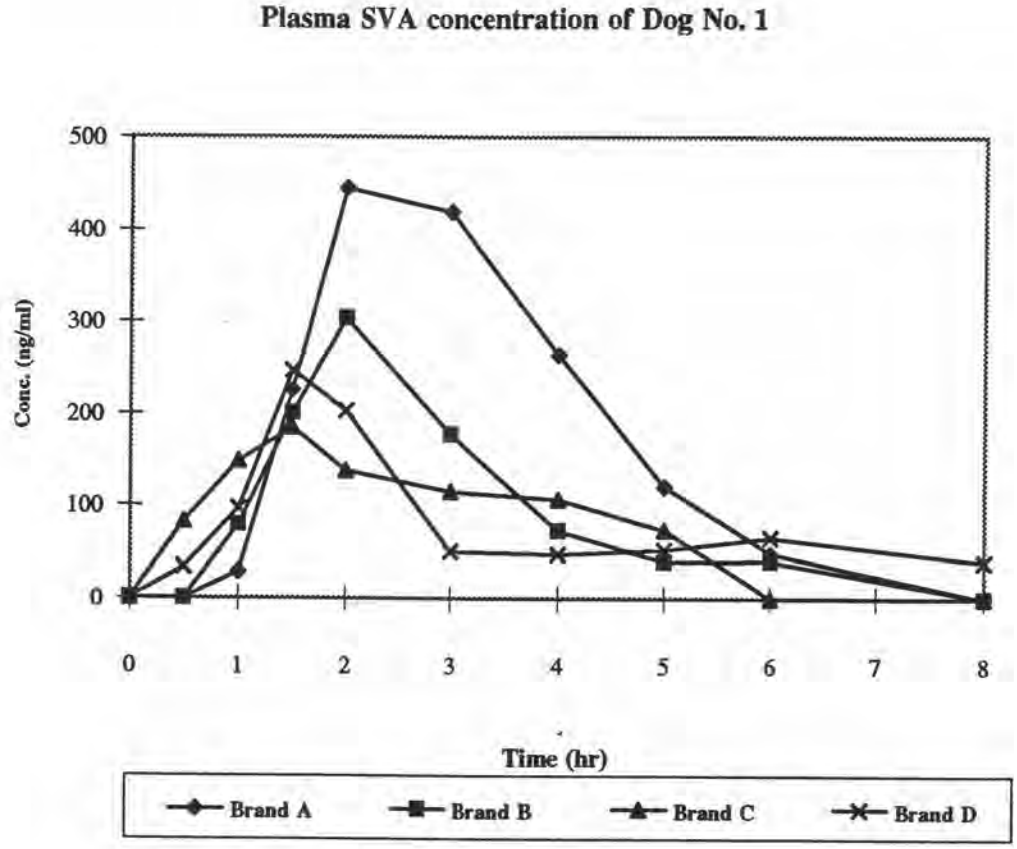


Figure 8 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 1 after taking 200 mg of different brands of simvastatin tablets.

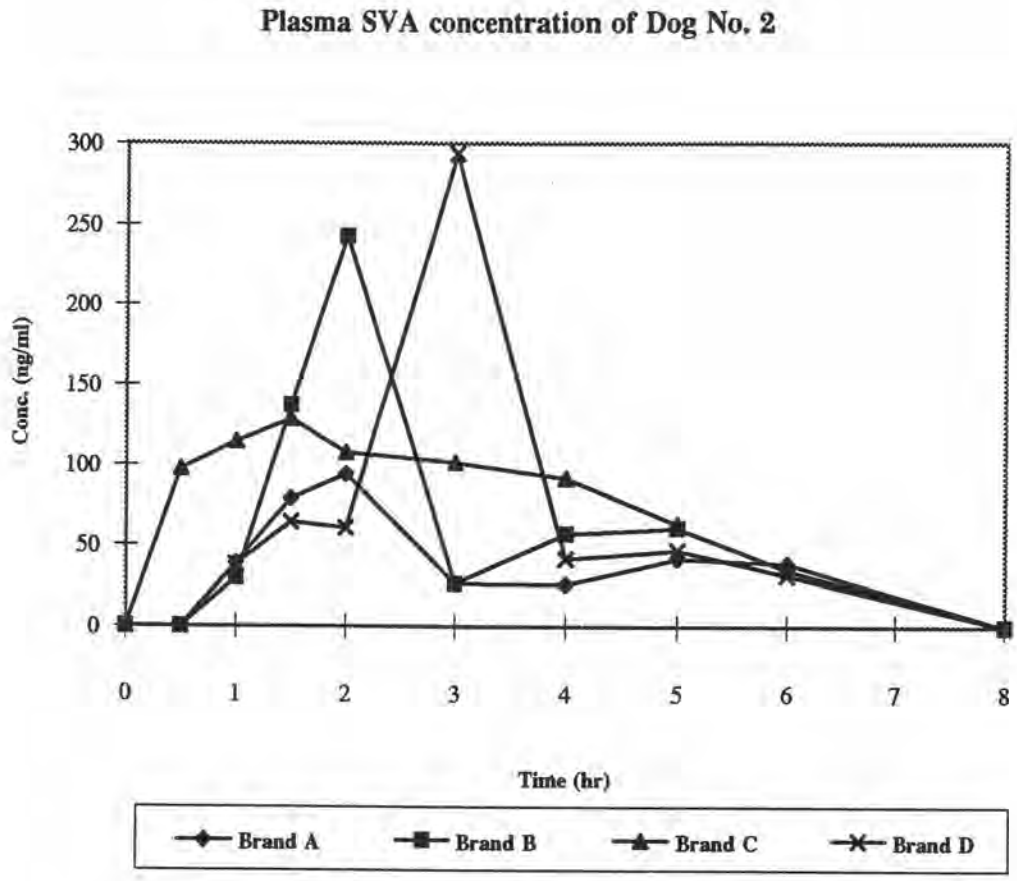


Figure 9 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 2 after taking 200 mg of different brands of simvastatin tablets.

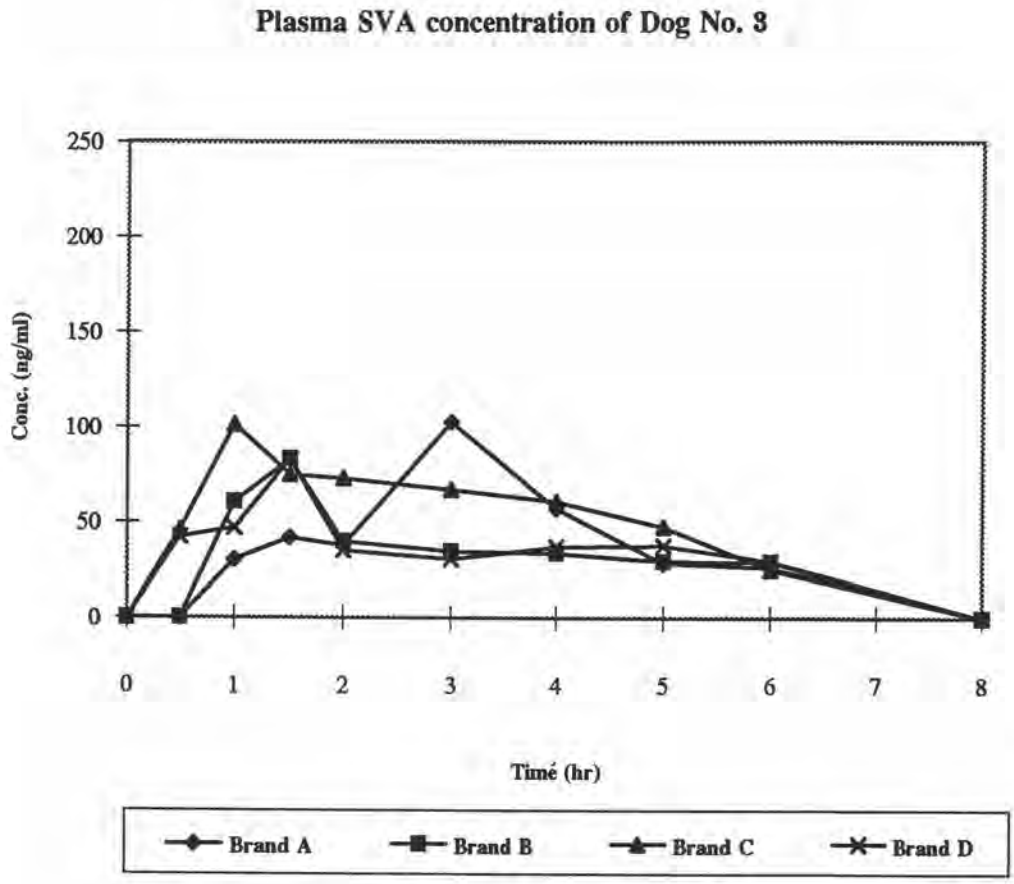


Figure 10 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 3 after taking 200 mg of different brands of simvastatin tablets.

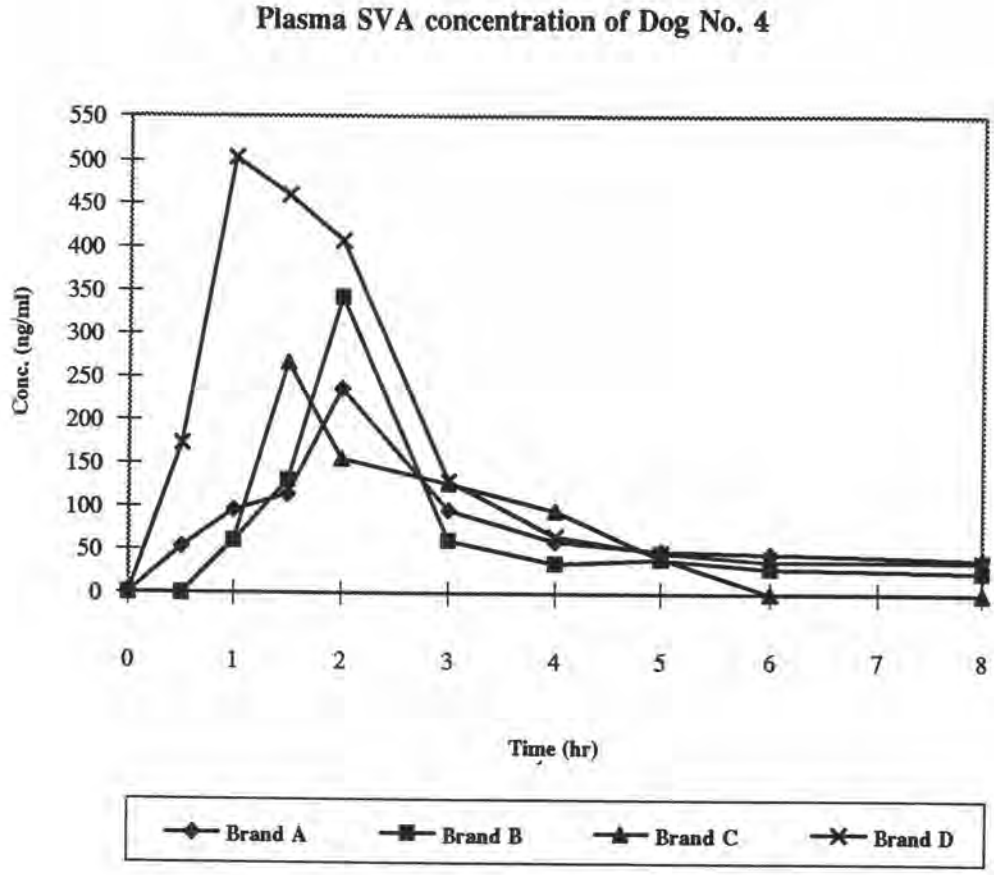


Figure 11 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 4 after taking 200 mg of different brands of simvastatin tablets.

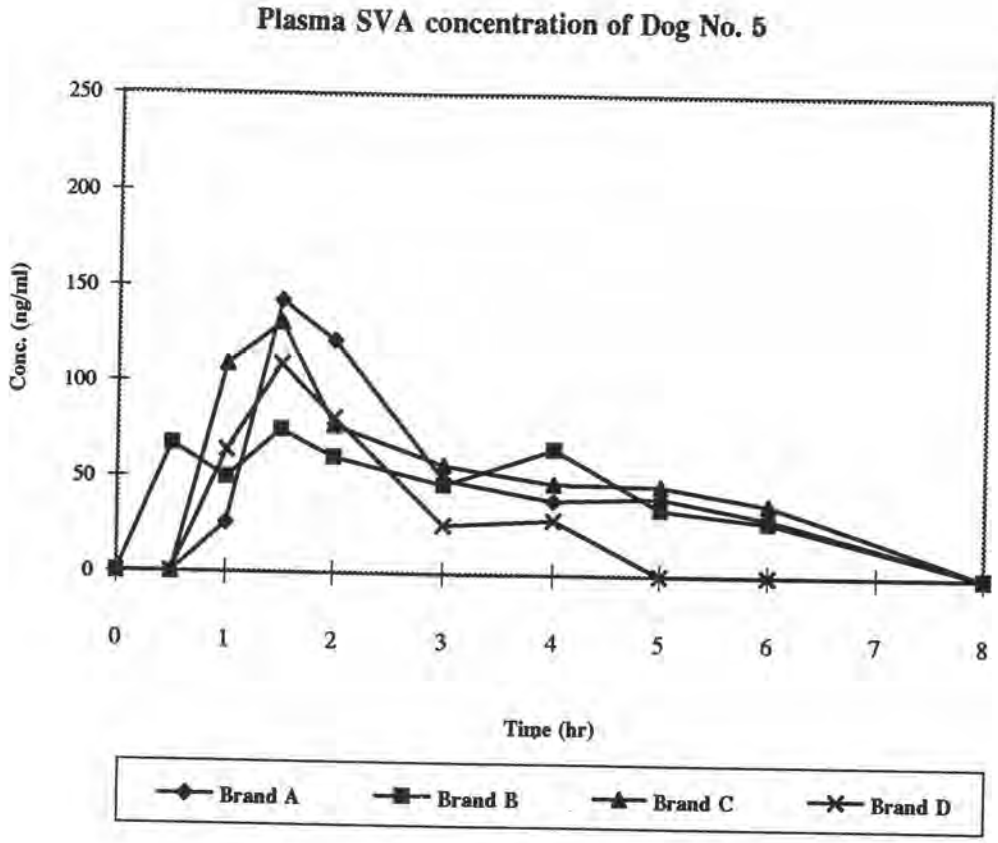


Figure 12 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 5 after taking 200 mg of different brands of simvastatin tablets.

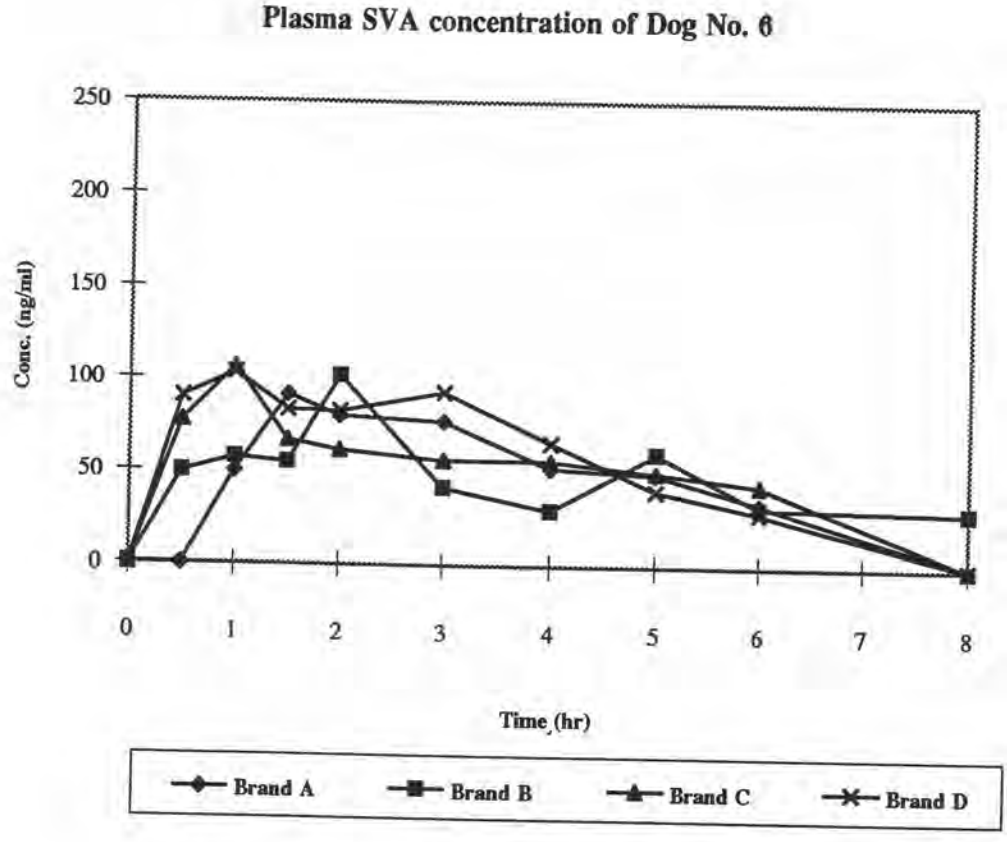


Figure 13 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 6 after taking 200 mg of different brands of simvastatin tablets.

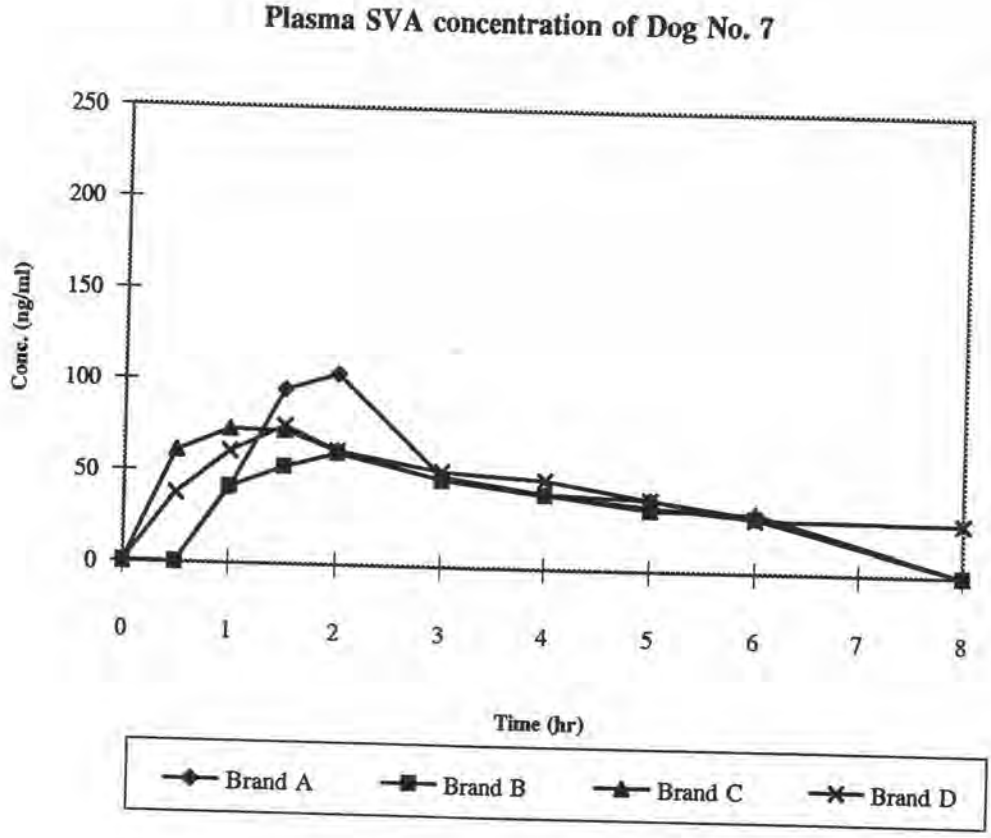


Figure 14 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 7 after taking 200 mg of different brands of simvastatin tablets.

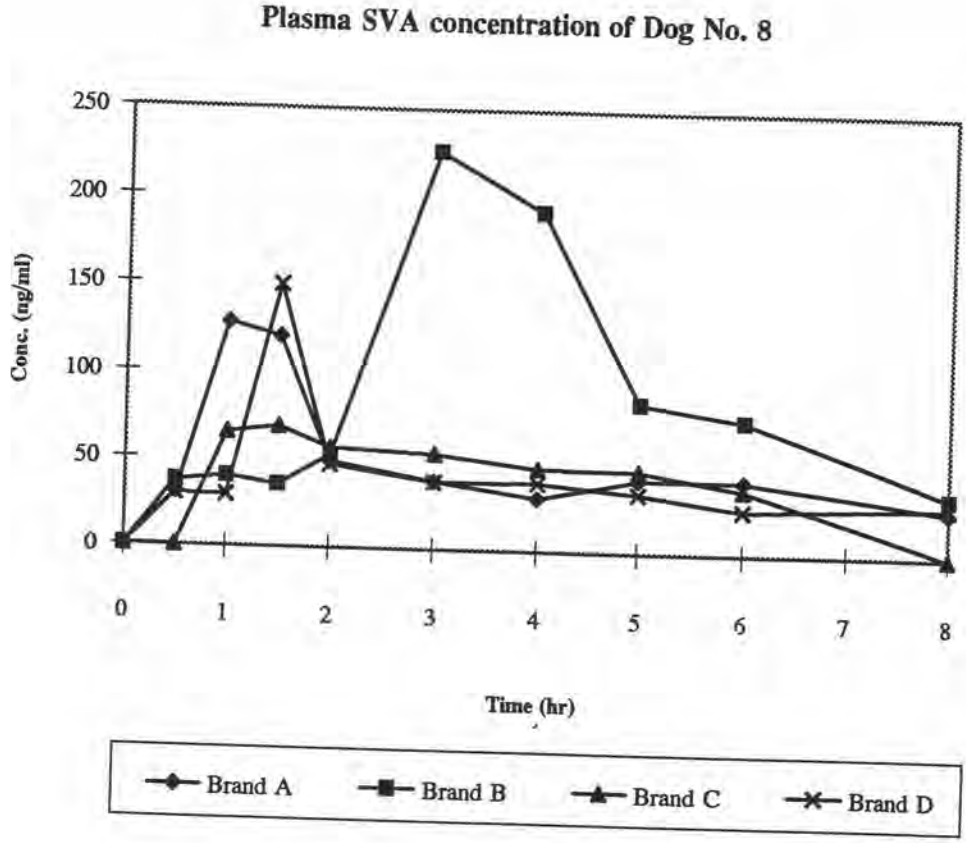


Figure 15 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 8 after taking 200 mg of different brands of simvastatin tablets.

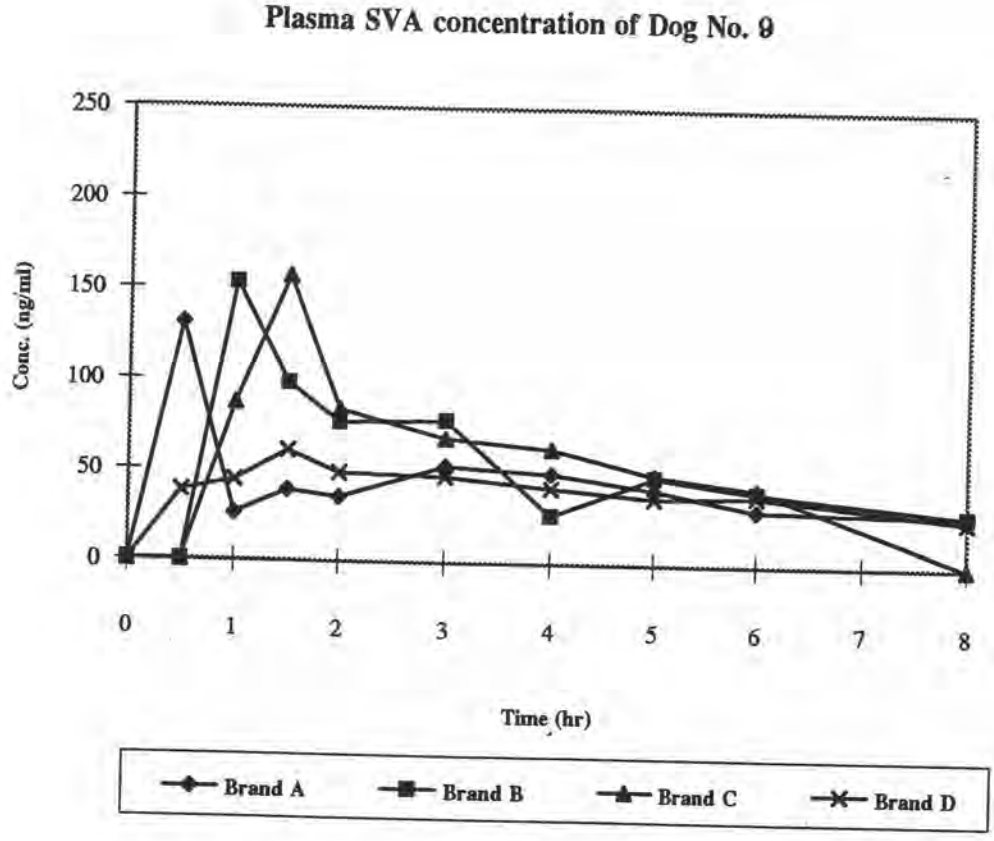


Figure 16 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 9 after taking 200 mg of different brands of simvastatin tablets.

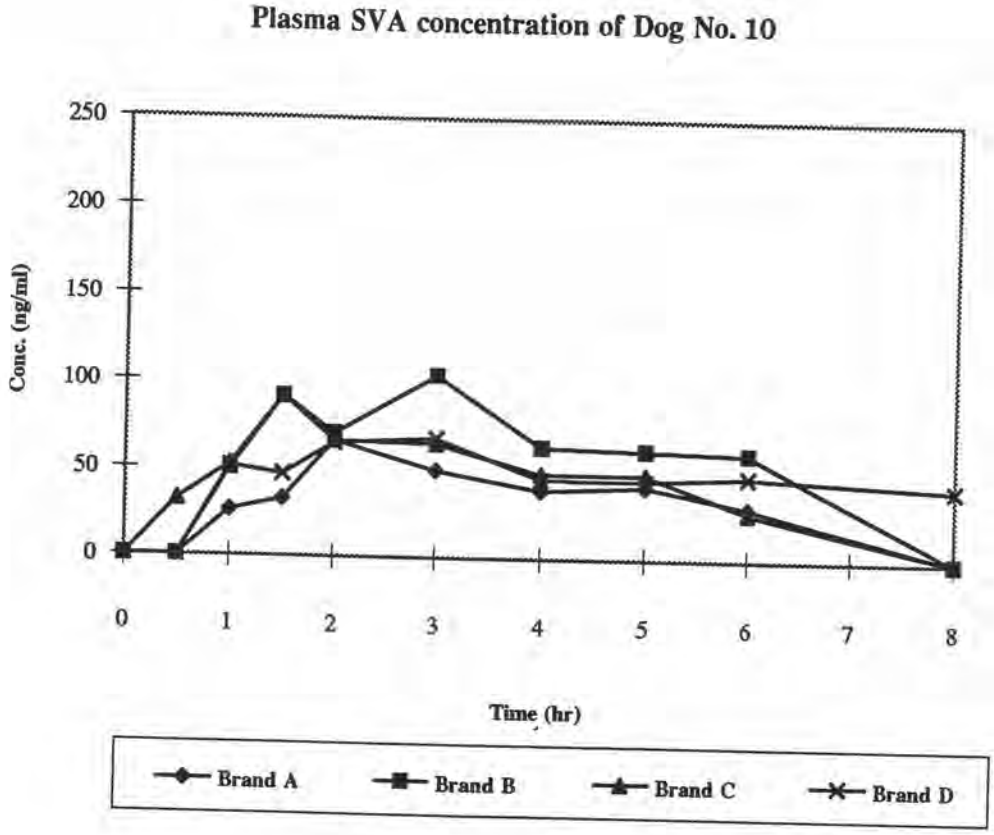


Figure 17 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 10 after taking 200 mg of different brands of simvastatin tablets.

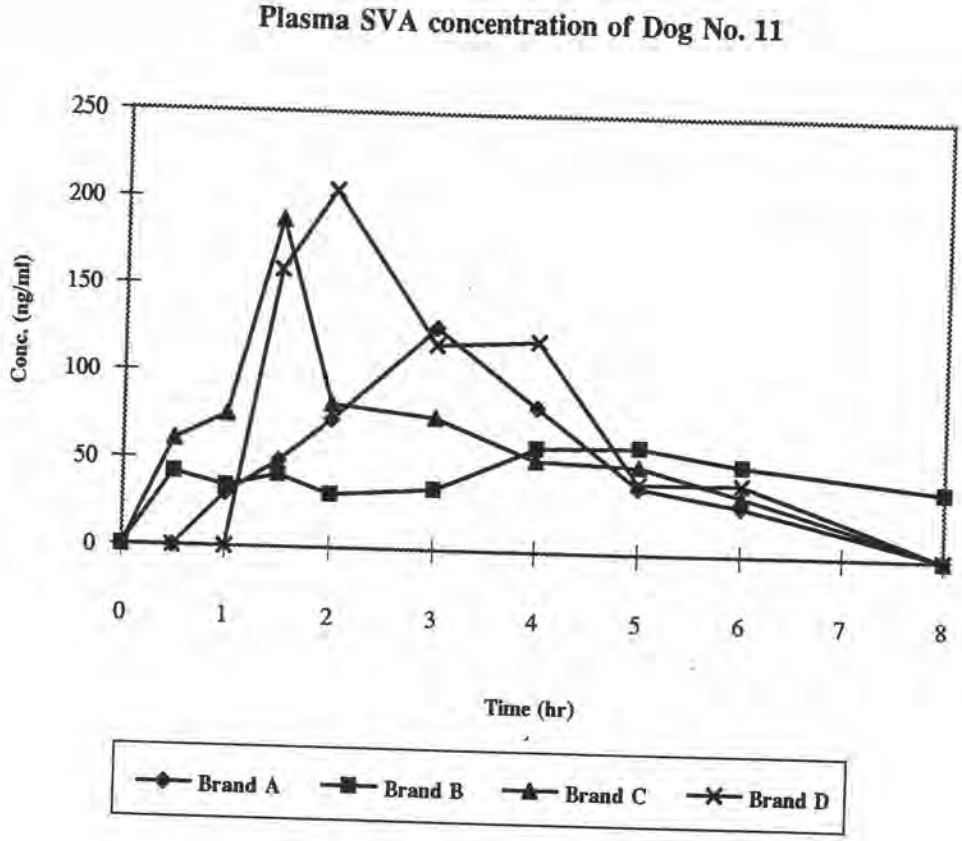


Figure 18 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 11 after taking 200 mg of different brands of simvastatin tablets.

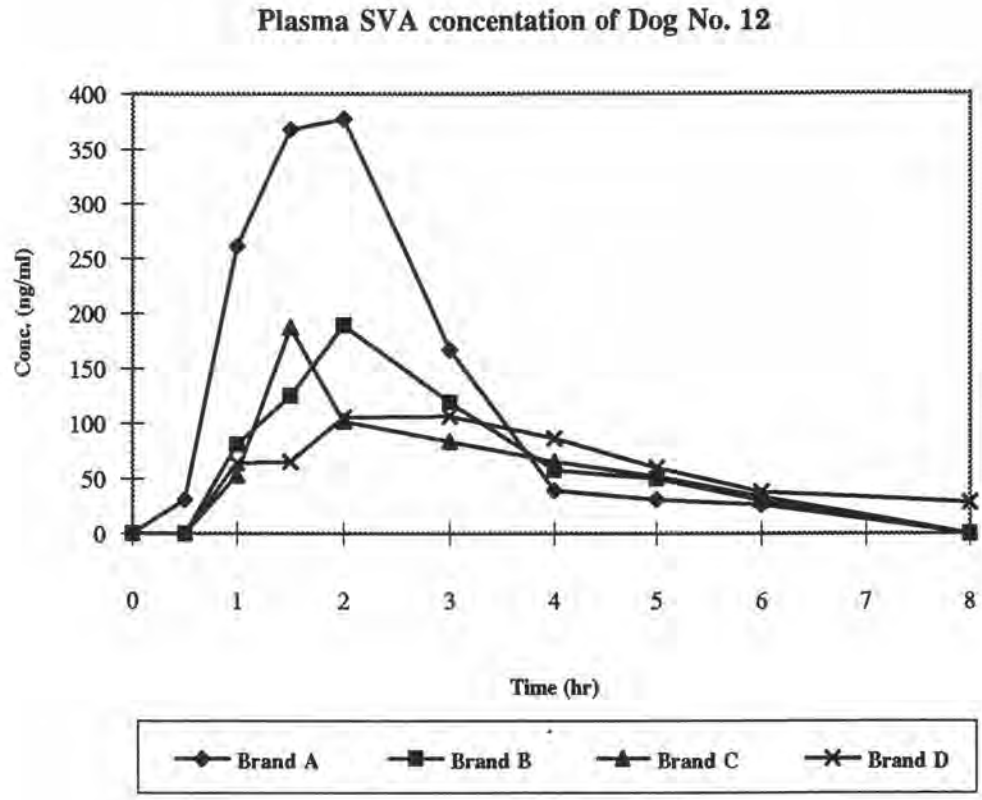


Figure 19 Plasma concentration time-profiles of simvastatin (SV) (ng/ml), analyzed as total simvastatin hydroxy acid (total SVA), in dog No. 12 after taking 200 mg of different brands of simvastatin tablets.

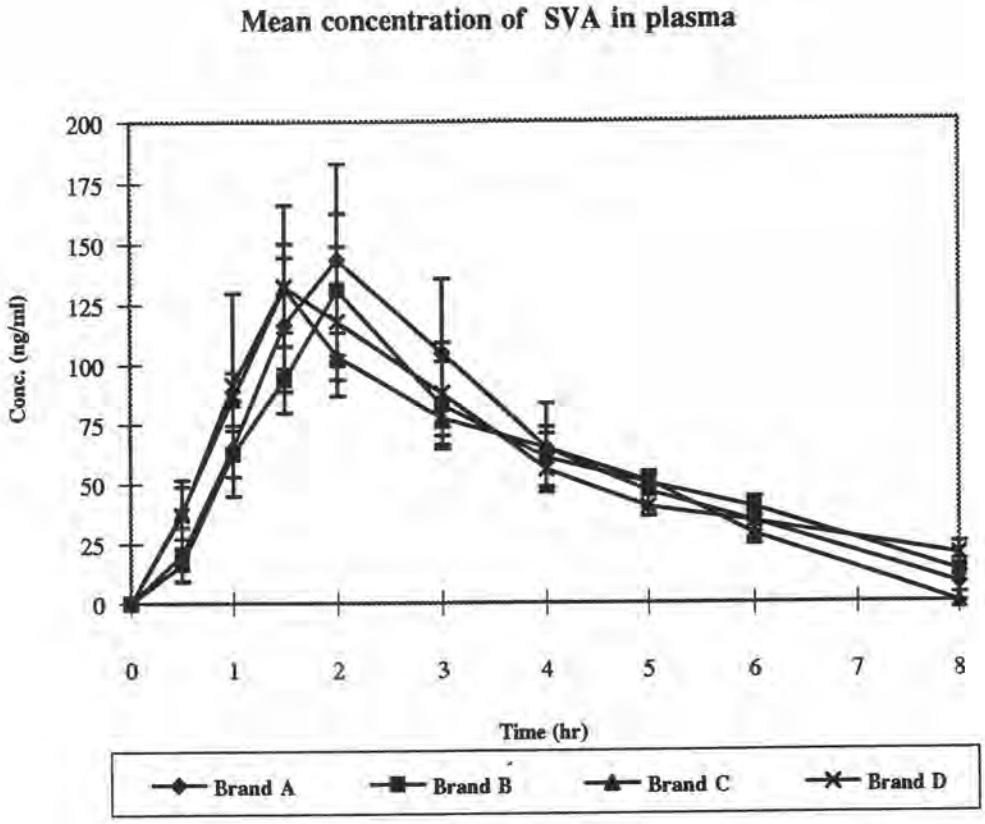


Figure 20 Comparison of mean plasma simvastatin time-profiles ,analyzed as total simvastatin hydroxy acid (total SVA), from 12 dogs after taking 200 mg of different brands of simvastatin tablets. (Data = Mean ± SEM)

3. Bioavailability evaluation

The principal parameters that were used to evaluate the bioavailability are peak plasma concentration (C_{max}), time to peak plasma concentration (t_{max}), and area under plasma concentration-time curve (AUC). The parameters C_{max} and t_{max} represent the rate of drug reaching the systemic circulation while the AUC value reflects the total amount of active drug that reaches the systemic circulation after first pass metabolism. According to the current criterion, two formulations are bioequivalent if end points of the 90% confidence interval for the ratio of the treatment means (test to reference) are within the 80-120% range or 80-125% for the logarithmically transformed data (USP 23, 1995). The AUC values are first focused because the sampling time points are usually not optimal. In generally, t_{max} data are more variable than C_{max} and both parameters are more variable than AUC. The relevant pharmacokinetic parameters obtained for the bioavailability comparison are as follows:

Area under plasma concentration-time curve (AUC)

The mean AUC (mean ± SEM) of total simvastatin hydroxy acid (total SVA), equivalent to simvastatin, after taking 200 mg drug for brands A, B, C and D were 484.08 ± 93.23, 451.28 ± 46.59, 438.03 ± 28.34, and 472.50 ± 76.78 ng hr/ml, respectively.

Peak plasma concentration (C_{max})

The mean C_{max} (mean ± SEM) of total SVA after taking 200 mg drug for brands A, B, C and D were 170.46 ± 34.84, 161.82 ± 28.24, 148.74 ± 16.53, and 167.0 ± 37.43 ng/ml, respectively.

Time to peak plasma concentration (t_{max})

The mean t_{max} (mean ± SEM) of total SVA after taking 200 mg drug for brands A, B, C and D were 1.88 ± 0.20, 2.25 ± 0.30, 1.38 ± 0.07, and 1.83 ± 0.22 hr, respectively.

Prior to the randomized block analysis of variance (ANOVA) on the three pharmacokinetic parameters, Bartlett's test was first carried out in order to ensure that the homogenity of variance and normal distrbution of the data sets have been met. If the Bartlet's test failed on original data sets, the parameters AUC and C_{max} would be logarithmically transformed and the test was then reapplied. After passing the Bartlett's test, parametric ANOVA was then performed on these two parameters. On the other hand, if t_{max} failed the fisrt Bartlett's test, the data would be analyzed by suitable statistical approach, the Friedman nonparametric ANOVA, as recommended by USP 23. The statistical results from the Bartlett's test on the relevant pharmacokinetic parameters are summarized in Table 19. The AUC, C_{max}, t_{max} of total SVA in each individual dog after taking four different brands of simvastatin tablet are respectively shown in Tables 20 to 22.

Table 19 Statistical results from Bartlett's test of the pharmacokinetic parameters.

Pharmacokinetic parameters	$\chi^2_{0.05,2}$
AUC	47.7*
C_{max}	35.02*
t_{max}	16.59*
log AUC	-2.63
log C_{max}	-5.14
log t_{max}	-2.02

where tabulated $\chi^2_{0.05,2} = 5.99$

* Significant difference at $p < 0.05$ (Failed the Bartlett's test)

Table 20 The area under plasma concentration-time curves (AUC) of total simvastatin hydroxy acid after taking four different brands of simvastatin tablets.

Dog No.	AUC					
	Brand A	Brand B	Brand C	Brand D	Mean	SEM
1	1338.65	717.56	627.58	627.57	827.83	171.59
2	281.65	461.24	570.36	526.09	459.83	63.48
3	291.63	242.98	379.35	261.63	293.90	30.19
4	625.40	556.52	543.52	1211.54	734.24	160.11
5	349.56	330.25	385.65	201.80	316.81	40.02
6	360.29	365.94	432.50	442.91	400.41	21.67
7	320.08	259.18	318.44	343.86	310.39	18.03
8	374.48	747.25	333.31	317.02	443.02	102.13
9	340.75	423.56	427.07	314.74	376.53	28.67
10	254.70	443.59	339.99	368.98	351.82	39.04
11	383.40	352.46	451.82	572.19	439.97	48.72
12	888.36	514.79	446.79	481.67	582.90	102.76
Mean	484.08	451.28	438.03	472.50	-	-
SEM	93.23	46.59	28.34	76.78	-	-

Table 21 The peak plasma concentrations (C_{max}) of total simvastatin hydroxy acid after taking four different brands of simvastatin tablets.

Dog No.	C_{max} (ng/ml)					
	Brand A	Brand B	Brand C	Brand D	Mean	SEM
1	444.85	303.5	228.43	246.63	305.85	49.01
2	94.15	242.43	128.55	294.05	189.80	47.03
3	102.74	83.1	101.46	83.21	92.63	5.48
4	236.42	342.27	266.51	503.22	337.10	59.68
5	142.39	74.69	131.09	109.10	114.32	14.91
6	91.17	101.89	173.16	103.14	117.34	18.8
7	104.16	60.55	73.19	75.20	78.28	9.22
8	127.93	226.34	109.58	149.64	153.37	25.66
9	131.06	153.34	157.10	60.71	125.55	22.36
10	66.25	103.92	90.97	67.95	82.27	9.16
11	126.77	60.98	187.59	204.93	145.07	32.66
12	377.58	188.80	137.26	106.27	202.48	60.80
Mean	170.46	161.82	148.74	167.00	-	-
SEM	34.87	28.24	16.53	37.43	-	-

Table 22 The time to peak plasma concentrations (t_{max}) of total simvastatin hydroxy acid after taking four different brands of simvastatin tablets.

Dog No.	t_{max} (hr)					
	Brand A	Brand B	Brand C	Brand D	Mean	SEM
1	2	2	1.5	1.5	1.75	0.14
2	2	2	1.5	3	2.12	0.32
3	3	1.5	1	1.5	1.75	0.43
4	2	2	1.5	1	1.62	0.24
5	1.5	1.5	1.5	1.5	1.50	0
6	1.5	2	1	1	1.38	0.24
7	2	2	1	1.5	1.62	0.24
8	1	3	1.5	1.5	1.75	0.43
9	0.5	1	1.5	1.5	1.12	0.24
10	2	3	1.5	3	2.38	0.38
11	3	5	1.5	2	2.88	0.77
12	2	2	1.5	3	2.12	0.32
Mean	1.88	2.25	1.38	1.83	-	-
SEM	0.20	0.30	0.07	0.22	-	-

The Bartlett's test for homogenity of variances showed that AUC, C_{max}, t_{max} failed to meet the criteria of constant variances. In order to obtain correct ANOVA determination, logarithmic transformation was applied to the AUC and C_{max} values. The logarithmically transformed values of AUC and C_{max} data are provided in Table 23 to 24. Bartlett's test was again applied to the log AUC and log C_{max}. The results in Table 19 indicate that the two parameters conformed well to the ANOVA requirement. This was in agreement with the US FDA guideline on bioavailability studies and the USP 23 which recommend that parameters like AUC and C_{max} should be logarithmically transformed prior to an ANOVA (but not t_{max}). On the other hand, the untransformed t_{max} values were analyzed using the non-parametric approach (Friedman ANOVA) although log t_{max} was previously shown to pass the second Bartlett's test. The reason for this is that t_{max} has been generally regarded as highly variable and its data tended to be far from normal distribution. Therefore, the non-parametric statistical approach has been proposed as the method of choice for t_{max} (Weiner and Yuh, 1994). Since many biological data correspond more closely to a log-normal distribution than to a normal distrubution. The plasma concentration data, including the derived parameters AUC and C_{max}, tend to be skewed, and their variances tend to increase with the mean. Log transformation is likely to remedy this situation and make the variance independent of the mean. In addition, frequency distributions skewed to the left are often made more symmetrical by log transformation. Following the recommendation in USP 23, the log AUC and log C_{max} values were statistically tested with randomized block ANOVA, Dunnett's test, and 90% confidence intervals and the results are summarized in Tables 25 and 26. The t_{max} values, which failed to meet the criteria of constant variances were statistically tested with nonparametric Friedman test and the results are summarized in Table 27.

Table 23 Log AUC values of total simvastatin hydroxy acid after taking four different brands of simvastatin tablets.

Dog No.	log AUC			
	Brand A	Brand B	Brand C	Brand D
1	3.127	2.856	2.798	2.798
2	2.450	2.664	2.756	2.721
3	2.465	2.386	2.579	2.418
4	2.796	2.745	2.735	3.083
5	2.544	2.519	2.586	2.305
6	2.557	2.563	2.636	2.646
7	2.505	2.414	2.503	2.536
8	2.573	2.873	2.523	2.501
9	2.532	2.627	2.630	2.498
10	2.406	2.647	2.531	2.567
11	2.584	2.547	2.655	2.758
12	2.949	2.712	2.650	2.683
Mean	2.624	2.629	2.632	2.626
SEM	0.06	0.04	0.03	0.06

Table 24 Log C_{max} values of total simvastatin hydroxy acid after taking four different brands of simvastatin tablets.

Dog No.	log C_{max}			
	Brand A	Brand B	Brand C	Brand D
1	2.648	2.482	2.359	2.392
2	1.974	2.385	2.109	2.468
3	2.012	1.920	2.006	1.920
4	2.374	2.534	2.426	2.702
5	2.153	1.873	2.118	2.038
6	1.960	2.008	2.238	2.013
7	2.018	1.782	1.864	1.876
8	2.107	2.355	2.040	2.175
9	2.117	2.186	2.196	1.783
10	1.821	2.017	1.959	1.832
11	2.103	1.785	2.273	2.312
12	2.577	2.276	2.138	2.026
Mean	2.155	2.134	2.144	2.128
SEM	0.07	0.08	0.05	0.08

Table 25 Statistical comparison of log AUC for four brands of simvastatin tablets assessed by randomized block ANOVA, Dunnett's test, and 90 % confidence intervals ($\alpha = 0.05$).

Randomized block ANOVA results

Source of variation	Degree of freedom	Sum of squares	Mean square	F ratio
Dog(block)	11	0.837	7.61×10^{-2}	4.94*
Product	3	3.662×10^{-2}	1.22×10^{-4}	7.92×10^{-3}
Error	33	0.508	1.54×10^{-2}	
Total	47	1.346		

* = significant ($p < 0.05$)

where tabulated $F_{0.05}(11,33) = 2.16$

$F_{0.05}(3,33) = 2.92$

Dunnett's test results

Product	Mean log AUC (SD)	|Difference of log AUC| [a] (local VS brand A)	Significant level
A	2.624(0.219)	-	-
B	2.629(0.154)	0.005	NS
C	2.632(0.094)	0.008	NS
D	2.626(0.204)	0.002	NS

[a] $D' = t'\sqrt{S^2(1/N_1+1/N_2)} = 0.127$ (calculated according to the method in Appendix E).

where tabulated $t'_{0.05}(3,33) = 2.50$

90% Exact confidence interval results

Product	Mean log AUC (SD)	90% Exact confidence interval [b]
A	2.624(0.219)	-
B	2.629(0.154)	83.00-123.11
C	2.632(0.094)	83.58-123.97
D	2.626(0.204)	82.41-122.24

[b] Results were calculated according to the method in Appendix E.

Table 26 Statistical comparison of log C_{max} for four brands of simvastatin tablets assessed by randomized block ANOVA, Dunnett's test , and 90 % confidence intervals ($\alpha = 0.05$).

Randomized block ANOVA results

Source of variation	Degree of freedom	Sum of squares	Mean square	F ratio
Dog(block)	11	1.758	0.160	5.619*
Product	3	5.188×10^{-3}	1.729×10^{-3}	6.08×10^{-3}
Error	33	0.939	0.028	
Total	47	2.702		

* = significant ($p < 0.05$)

where tabulated $F_{0.05}(11,33) = 2.16$

$F_{0.05}(3,33) = 2.92$

Dunnett's test results

Product	Mean log C_{max} (SD)	|Difference of log C_{max}| [a] (local VS brand A)	Significant level
A	2.155(0.252)	-	-
B	2.134(0.271)	0.022	NS
C	2.144(0.165)	0.012	NS
D	2.128(0.285)	0.027	NS

[a] $D' = t'\sqrt{S^2(1/N_1+1/N_2)} = 0.172$ (calculated according to the method in Appendix E).

where tabulated $t'_{0.05}(3,33) = 2.50$

90% Exact confidence interval results

Product	Mean log C_{max} (SD)	90% Exact confidence interval [b]
A	2.155(0.252)	-
B	2.134(0.271)	72.98-121.73
C	2.144(0.165)	74.51-127.29
D	2.128(0.285)	71.86-122.77

[b] Results were calculated according to the method in Appendix E.

Table 27 Statistical comparison of t_{max} for four brands of simvastatin tablets assessed by Friedman test ($\alpha = 0.05$).

Dog No.	Brand A		Brand B		Brand C		Brand D	
	t_{max} (hr)	assigned rank	t_{max} (hr)	assigned rank	t_{max} (hr)	assigned rank	t_{max} (hr)	assigned rank
1	2	3.5	2	3.5	1.5	1.5	1.5	1.5
2	2	2.5	2	2.5	1.5	1	3	4
3	3	4	1.5	2.5	1	1	1.5	2.5
4	2	3.5	2	3.5	1.5	2	1	1
5	1.5	2.5	1.5	2.5	1.5	2.5	1.5	2.5
6	1.5	2	2	3	1	1.5	1	1.5
7	2	3.5	2	3.5	1	1	1.5	2
8	1	1	3	4	1.5	2.5	1.5	2.5
9	0.5	1	1	2	1.5	3.5	1.5	3.5
10	2	2	3	3.5	1.5	1	3	3.5
11	3	3	5	4	1.5	1	2	2
12	2	2.5	2	2.5	1.5	1	3	4
Mean	1.88	-	2.25	-	1.38	-	1.83	-
Rank sums	-	31	-	37	-	19.5	-	30.5

$\chi^2 = 2.02$ (were calculated according to the method in Appendix E) = NS

where $\chi^2_{0.95,3} = 7.81$

Comparison of the log AUC values of total SVA in Table 25 showed that there were no statistically significant differences ($p > 0.05$) between log AUC values of brands A, B, C and D. Using Dunnett's test to compare log AUC values between each locally-manufactured and the innovator's product, the result also supported that no brand had a significant difference log AUC from that of brand A. The 90% exact confidence interval for the ratios of AUC values of brands B, C and D relative to that of brand A were 83.0-123.1, 83.6-124.0, and 82.4-122.0 %, respectively, that passed the USP equivalency test. This clearly demonstrated that for a poorly soluble drug, sound formulations play an important role which could provide adequate drug absorption into systemic circulation despite its undesirable intrinsic solubilitiy.

Comparison of the log C_{max} values of total SVA in Table 26 showed that there were no statistically significant differences ($p > 0.05$) between log C_{max} values of brands A, B, C and D. Using Dunnett's test to compare log C_{max} values between each locally-manufactured and the innovator's product, the result also further supported that no brand had a significant difference log C_{max} from that of brand A. This might be said that brand A, B, C and D could seemingly produce the same intensity of action. However, the 90% exact confidence interval for the ratios of C_{max} value of brand B to that of brand A was 73.0-121.73 % which was below the conventional lower limit of 80%. The 90% exact confidence interval for the ratios of log C_{max} value of brand C to that of brand A was 74.5-127.3 % which exceed both the lower and upper limits. Similarly, the 90% exact confidence interval for the ratios of log C_{max} value of brand D to that of brand A was 71.9-122.8 % which was below the 80% lower limit.

Comparison of the t_{max} values of total SVA in Table 27 showed that there were no statistically significant differences ($p > 0.05$) between t_{max} values of brands A, B, C and D.

Referring to the previous results, the 90% exact confidence interval for ratios of AUC of brand B, C, and D relative to those of brand A which passed the USP equivalency test indicated that they have equal extent of absorbed drug entering the systemic circulation. The absence of statistically significant difference ($p > 0.05$) between t_{max} values of brands A, B, C and D indicates that they also have similar rates of drug absorption. Although the 90% exact confidence intervals for the ratios of C_{max} of brand B, C and D relative to those of brand A fell the USP equivalency test of 80-125 %, this could be due to biological variation of this drug, especially during the absorption phase, which led to high standard deviation and rather large confidence intervals. However, such difference in the absorption phase may have little impact on the therapeutic outcome since the cholesterol lowering effect requires chronic administration of simvastatin. These associated details could be seen from the latter cholesterol reduction results. Hence, fluctuation in the absorption phase and C_{max} might not necessarily be considered a critical factor in bioequivalence determination of special pharmacologic drugs like simvastatin.

In general, factors influencing the bioavailability of dosage form are numerous. The first factor is the physical characteristics of dosage form itself which include tablet compression force, crystal structure, formulation, particle size and dissolution rate. The second factor contains certain design of experiment which can affect the drug level profile. For example, the choice and spacing of the sampling time chosen can affect the accuracy of the determined drug concentration because the peak concentration might be higher and occur at some time point other than one of the chosen sampling time.

Moreover, in this study, some dogs have initial borderline high SGPT value and/or Microfilaria infection as shown in Appendix D. Despite no statistically significant difference in the pre- and post- treatment SGPT values as examined in Appendix E, this health conditions may have clinical significance and contribute to fluctuation in plasma drug concentrations.

Other pharmacokinetic parameters of simvastatin hydroxy acid observed in this study are as follows:

Elimination rate constant (K_{el})

The elimination rate constant was determined by linear regression analysis of log-linear terminal phase of the plasma concentration-time profile. The average elimination rate constants (mean ± SEM) obtained from individual plasma data of brands A, B, C and D were 1.31 ± 0.26, 1.12 ± 0.24, 1.92 ± 0.26 and 1.05 ± 0.28 hr^{-1} , respectively, as shown in Table 28.

Plasma elimination half-life ($t_{1/2}$) .

Plasma elimination half-life was calculated as natural log of 2 divided by the terminal elimination rate constant. The average elimination half-lives (mean ± SEM) obtained from individual plasma data of brands A, B, C and D were 0.99 ± 0.42, 1.34 ± 0.42, 0.47 ± 0.08 and 1.65 ± 0.43 respectively, as shown in Table 29.

These two data sets could be calculated for only some dogs in which estimation of K_{el} could be made without strong interferences from the secondary peak. Thus, variation in enterohepatic circulation between the individual dogs is another factor which may have contributed to the variability

in the total SVA plasma levels and C_{max} values. This phenomenon is also accountable for the large differences in the plasma drug elimination kinetics among different dogs. Therefore, the values of K_{el} and $t_{1/2}$ could be obtained only from dogs which did not show distinct enterohepatic circulation and sharp secondary peak.

Table 28 Elimination rate constants (K_{el}) of total simvastatin hydroxy acid after taking four different brands of simvastatin tablets.

Dog No.	K_{el} (hr^{-1})[a]			
	brand A	brand B	brand C	brand D
1	2.07	1.54	1.71	-
2	2.97	2.17	2.43	2.99
3	1.76	0.79	2.98	1.54
4	0.26	0.35	2.92	0.42
5	1.34	2.23	1.29	1.78
6	1.70	-	0.76	1.75
7	1.33	1.26	0.73	0.16
8	-	0.41	0.83	-
9	0.14	0.22	1.69	0.11
10	1.27	1.73	2.99	-
11	-	0.15	3.01	1.48
12	1.57	1.46	1.73	0.28
Mean	1.44	1.60	1.92	1.17
SEM	0.26	0.24	0.26	0.28

[a] obtained only from dogs which did not show distinct enterohepatic circulation and sharp secondary peak.

Table 29 Plasma elimination half-lives ($t_{1/2}$) of total simvastatin hydroxy acid after taking four different brands of simvastatin tablets.

Dog No.	$t_{1/2}$ (hr) [a]			
	brand A	brand B	brand C	brand D
1	0.33	0.45	0.40	-
2	0.23	0.32	0.28	0.23
3	0.39	0.87	0.23	0.45
4	2.64	1.96	0.24	1.64
5	0.52	0.31	0.54	0.39
6	0.41	-	0.92	0.40
7	0.52	0.55	0.95	4.41
8	-	1.71	0.83	-
9	4.88	3.19	0.41	6.08
10	0.54	0.40	0.23	-
11	-	4.56	0.23	0.47
12	0.44	0.47	0.40	2.48
Mean	0.99	1.34.	0.47	1.65
SEM	0.42	0.42	0.08	0.43

[a] obtained only from dogs which did not show distinct enterohepatic circulation and sharp secondary peak.

4. Acute plasma cholesterol-lowering effect

The acute pharmacological effect in lowering plasma cholesterol after 200 mg single oral dose of simvastatin tablets, as determinated from the difference in total cholesterol (TC) level at time 0 before drug administration and at 2 hr post dose, are summarized in Table 30. The mean percent reduction in TC (mean ± SEM) of brands A, B, C and D were 1.8 ± 1.3, 3.3 ± 2.2, 2.6 ± 5.0 and 1.7 ± 1.4, recpectively. Statistical comparison by paired student's t-test in Table 31 indicates that there was no statistically significant reduction of TC at 2 hr post dose from the initial value in each brand. The results apparently indicate that the plasma cholesterol lowering effect of simvastatin was not an acute effect. Therefore, the comparative plasma cholesterol lowering effect of simvastatin tablets must be performed in a multiple dose administration study.

These results are consistent with the previous data in beagle dogs (McCleland, et al., 1991) that the steady state effect in lowering plasma cholesterol was observed after multiple dose administration (100 mg/day) of SVA ammonium salt for 28 days.

Table 30 The difference in total cholesterol (TC) level at 2 hr after 200 mg sigle oral dose of four different brands of simvastatin tablets (expressed as a percentage change from the initial value).

Dog No.	% Change in TC			
	brand A	brand B	brand C	brand D
1	-1.4	+6.6	-1.3	+3.6
2	-0.7	-1.1	-0.3	-7.5
3	-0.8	+7.4	-29.9	+2.0
4	+0.2	-11.1	+9.4	-6.0
5	-8.3	-5.6	-7.7	-3.0
6	-7.2	+2.3	+19.5	+1.4
7	-2.2	-8.0	-4.0	+4.2
8	+0.6	-14.5	+0.3	-4.0
9	+1.8	-4.2	-0.6	0
10	-8.0	+3.0	+28.2	+2.9
11	-5.4	-14.3	-13.7	-10.1
12	+5.5	0	-31.4	-4.1
Mean	-1.8	-3.3	-2.6	-1.7
SD	4.4	7.6	17.4	4.7
SEM	1.3	2.2	5.0	1.4

+ = increase from the initial value

- = decrease from the initial value

Table 31 Statistical comparison of percent change in TC at 2 hours relative to time zero for four brand of simvastatin tablets as assessed by paired t-test ($\alpha = 0.05$).

Product	t value [a]	Significant level
A	1.42	NS
B	1.50	NS
C	0.52	NS
D	1.25	NS

where tabulated $t_{0.05}(11) = 1.80$

[a] Results were calculated according to the method in Appendix E.

5. *In vitro - in vivo* correlation

The correlation study between the *in vitro* and *in vivo* data for brands A, B, C and D are presented in Table 32. Test for zero correlation was performed in Appendix E, Sect. 7. The results demonstate that there was no statistically significant correlation between the two data sets ($p > 0.05$). Therefore, under this dissolution condition, the dissolution rate constant (K_d), percent drug dissolved at 15 and 30 min ($\%Q_{15}$ and $\%Q_{30}$) could not be used as the preliminary tool to predict the bioavailability of simvastatin film-coated tablets.

Table 32 *In vitro - in vivo* correlations.

Correlation (n = 4)	Correlation coefficient [a]	t value [b]	Statistical significant
Dissolution rate versus AUC	0.986	2.430	NS
Dissolution rate versus C_{max}	0.790	1.820	NS
Dissolution rate versus t_{max}	0.625	1.130	NS
$\%Q_{15}$ versus AUC	0.883	2.665	NS
$\%Q_{15}$ versus C_{max}	0.726	1.492	NS
$\%Q_{15}$ versus t_{max}	0.401	0.619	NS
$\%Q_{30}$ versus AUC	0.116	0.165	NS
$\%Q_{30}$ versus C_{max}	0.397	0.612	NS
$\%Q_{30}$ versus t_{max}	0.706	0.290	NS

where tabulated $t_{0.05}(2) = 4.303$

[a, b] Results were calculated according to the method in Appendix E.

$\%Q_t$ = the amount of drug dissolved at time t expressed as percentage of the labeled amount.