CHAPTER 3

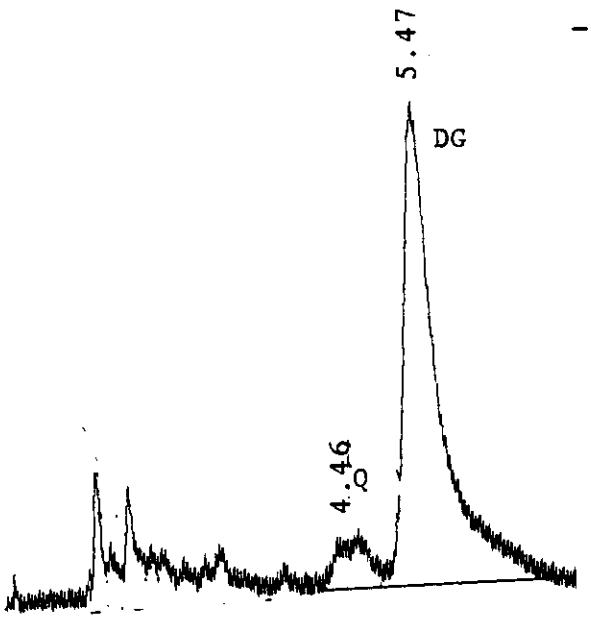
RESULTS AND DISCUSSION

ASSAY VALIDATION

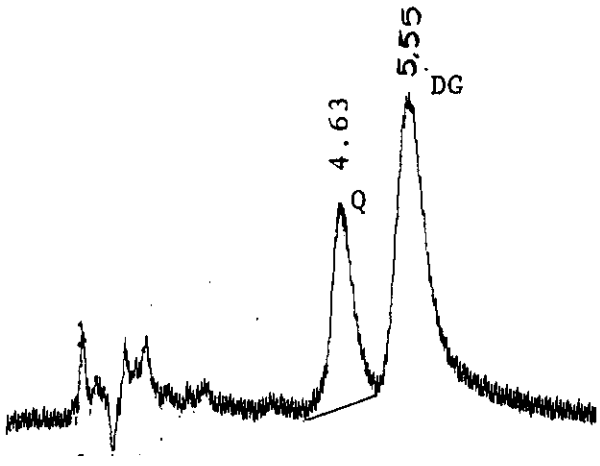
The stability indicating capacity of the chromatographic method employed in this study was determined using heat-decomposed solution of quinine. Quinine was nearly completely decomposed after direct flame heating for 15 min. (Figure 1A). The decomposition products were well resolved from the peak for quinine and diazepam, internal standard as shown in Figure 1. The retention times of diazepam, quinine and degradation product in water were 1.91, 4.58 and 5.55 minutes respectively. The presence of dextrose and sodium chloride in sample solution did not change the retention times of quinine and internal standard but the retention time of degradation product shifted from 5.55 to 6.4 minutes if quinine was mixed in infusion fluid containing sodium chloride solution (Figure 1B and 1C). It is likely that sodium chloride increases ionic strength of solution and decreases the solubility of degradation product in mobile phase. As a result, degradation product can retain on stationary phase for a longer time and its retention time shifted from 5.55 to 6.4 minutes with the presence of sodium chloride (11).

The typical chromatograms obtained from the analysis of quinine were shown in Figure 2. Electrolytes and dextrose in infusion fluid have no effect on retention time of quinine and diazepam. However peak response of quinine in IV fluid containing 0.9% saline solution (D/NSS and NSS) was lower than that of quinine in D5W and D/1/2NSS (Figure 2). This may due to 'salt effect'(12). Increasing in ionic strength can change the quinine-solvent interaction and cause slight differences in the absorption of quinine (12). Because of this reason, we constructed standard curves of quinine in both water and NSS for determining the sample concentration.

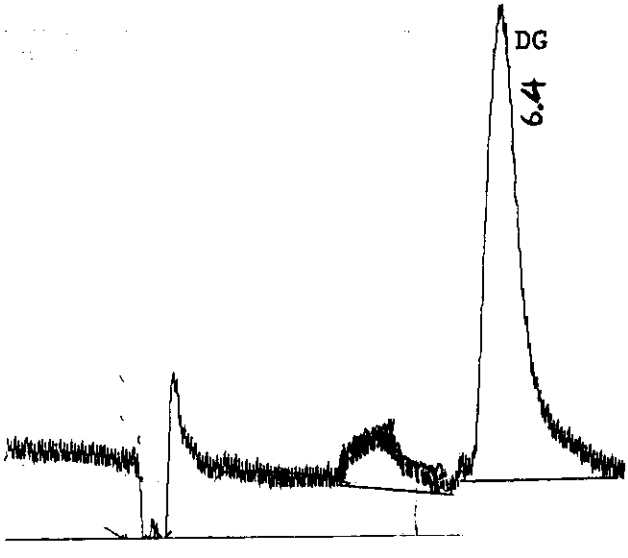
The within run precision and between run precision over the calibration range were summarized in Tables 1-8. The within run and between run precision of quinine in all concentrations were less than 2.5%. The standard curves were linear over the concentration range expected to be encountered in the study (Figure 3 and 4).



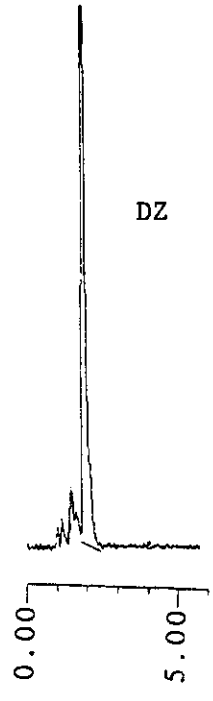
PANEL 1A



PANEL 1B

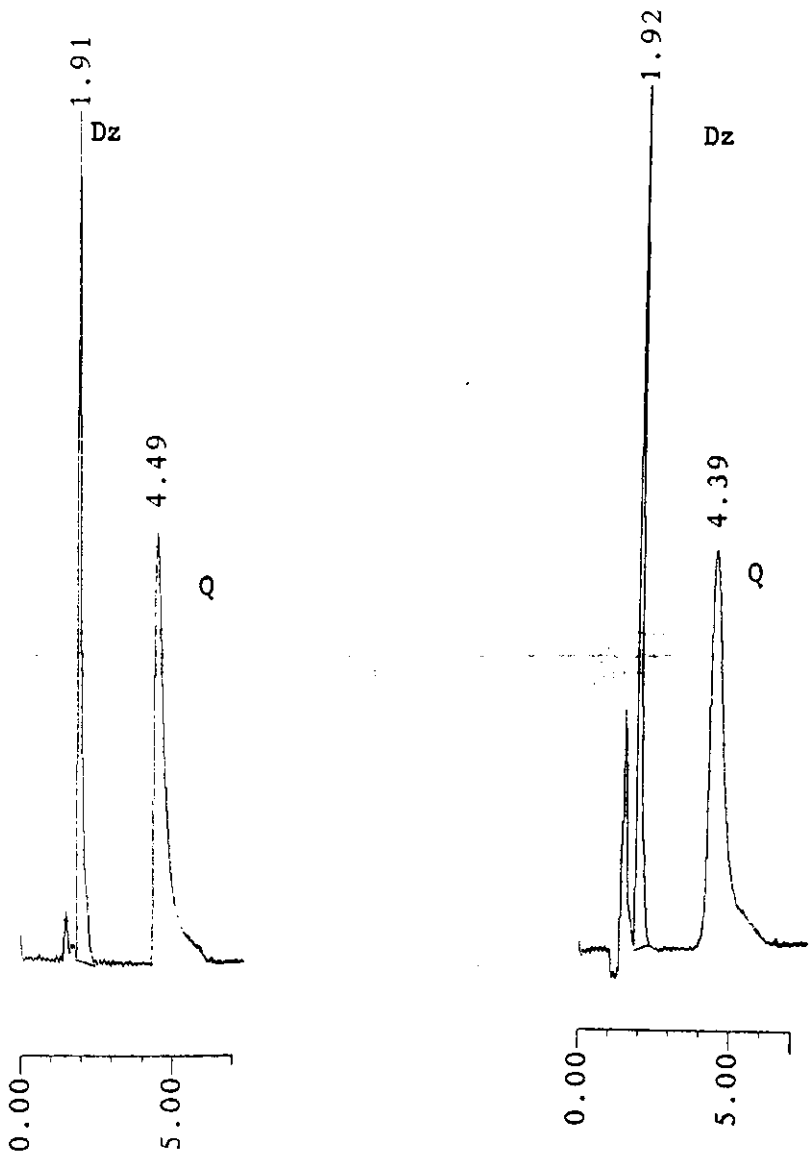


PANEL 1C



PANEL 1D

Figure 1 chromatograms of quinine and degradation product
Panel 1A Quinine in aqueous solution (1.2 mg/ml) after direct flame heating for 15 minutes.
Panel 1B Quinine was spiked to heated solution (1A).
Panel 1C Quinine and degradation product in normal saline solution.
Panel 1D Diazepam in methanol.
Q represents quinine, DZ represents diazepam and DG represents degradation product.



PANEL 2A PANEL 2B

Figure 2 Chromatograms of quinine and internal standard.
Panel 2A 1.2 mg/ml Quinine standard in aqueous solution.
Panel 2B 1.2 mg/ml Quinine standard in normal saline solution.
Q represents quinine, DZ represents diazepam.

Table 1 Within run precision for quinine from three replicated standard curves of PART 1 in water.

CONCENTRATION (microgram/ml)	PEAK HEIGHT RATIO (mean±SD)	%CV	%THEORY
0	0	-	-
48.88	0.21±0.004	2.16	102.01
73.32	0.31±0.001	0.31	102.04
97.76	0.41±0.002	0.66	103.37
122.20	0.50±0.006	1.49	101.63
195.52	0.78±0.014	2.28	99.58
244.40	0.96±0.013	1.69	99.10
	AVERAGE	1.43	

1. Mean percent theory was 101.29±1.48 with %CV 1.46
2. Determination coefficient (r^2) = 0.9989
3. Regression line: Peak height ratio = 0.0039(conc)+0.018
4. Percent theory = $\frac{(\text{Peak height ratio}-0.018)*100}{(0.0039)\ (\text{conc})}$

Table 2 Between run precision for quinine from three replicated standard curves of PART1 in water.

CONCENTRATION (microgram/ml)	PEAK HEIGHT RATIO (mean±SD)	%CV	% THEORY
0	0	-	-
48	0.21±0.004	2.25	104.5
72	0.30±0.005	1.86	101.1
96	0.39±0.006	1.56	99.97
120	0.49±0.002	0.57	99.93
192	0.77±0.007	1.00	99.88
240	0.97±0.014	1.51	99.86
	AVERAGE	1.46	

1. Mean percent theory was 100.89±1.70 with %CV 1.69
2. Determination coefficient (r^2) = 0.9997
3. Regression line: Peak height ratio = 0.0040(conc)+0.0086
4. Percent theory = $\frac{(\text{Peak height ratio}-0.008)*100}{(0.0040)\ (\text{conc})}$

Table 3 Within run precision for quinine from three replicated standard curves of PART1 in normal saline solution

CONCENTRATION (microgram/ml)	PEAK HEIGHT RATIO (mean±SD)	%CV	%THEORY
0	0		
48	0.16±0.002	1.76	99.18
72	0.23±0.001	0.25	99.02
96	0.33±0.001	0.54	106.46
120	0.39±0.004	1.21	101.03
192	0.61±0.011	1.86	99.10
240	0.77±0.010	1.38	99.44
	AVERAGE	1.17	

1. Mean percent theory was 100.70±2.66 with %CV 2.64
2. Determination coefficient (r^2) = 0.9986
3. Regression line: Peak height ratio = 0.0032(conc)+0.010
4. Percent theory = $\frac{(\text{Peak height ratio}-0.010)*100}{(0.0032)\ (\text{conc})}$

Table 4 Between run precision for quinine from three replicated standard curves PART1 in normal saline solution.

CONCENTRATION (microgram/ml)	PEAK HEIGHT RATIO (mean±SD)	%CV	% THEORY
0	0	-	-
48	0.16±0.003	1.88	103.62
72	0.22±0.004	1.82	97.47
96	0.32±0.005	1.56	103.34
120	0.38±0.002	0.53	98.12
192	0.61±0.006	0.98	99.56
240	0.77±0.011	1.43	100.31
	AVERAGE	1.36	

1. Mean percent theory was 100.41±2.36 with %CV 2.35
2. Determination coefficient (r^2) = 0.9994
3. Regression line: Peak height ratio = 0.0032(conc)+0.0023
4. Percent theory = $\frac{(\text{Peak height ratio}-0.002)*100}{(0.0032)\ (\text{conc})}$

Table 5 Within run precision for quinine from three replicated standard curves of PART 2 in water.

CONCENTRATION (microgram/ml)	PEAK HEIGHT RATIO (mean±SD)	%CV	%THEORY
0	0	-	-
132	0.31±0.005	1.87	108.34
264	0.56±0.006	1.15	101.06
330	0.69±0.003	0.57	100.72
396	0.83±0.004	0.51	100.92
495	1.00±0.005	0.51	98.22
	AVERAGE	0.92	

1. Mean percent theory was 101.85±3.40 with %CV 3.34
2. Determination coefficient (r^2) = 0.9978
3. Regression line: Peak height ratio = 0.0020(conc)+0.022
4. Percent theory = $\frac{(\text{Peak height ratio}-0.022)*100}{(0.0020)\ (\text{conc})}$

Table 6 Between run precision for quinine from three replicated standard curves of PART2 in water.

CONCENTRATION (microgram/ml)	PEAK HEIGHT RATIO (mean±SD)	%CV	% THEORY
0	0	-	-
132	0.30±0.006	2.18	106.38
264	0.55±0.002	0.52	101.64
330	0.68±0.006	0.92	100.61
396	0.83±0.001	0.13	101.93
495	0.98±0.012	1.24	97.60
	AVERAGE	1.00	

1. Mean percent theory was 101.63±2.82 with %CV 2.77
2. Determination coefficient (r^2) = 0.9975
3. Regression line: Peak height ratio = 0.0020(conc)+0.0207
4. Percent theory = $\frac{(\text{Peak height ratio}-0.0207)*100}{(0.0020)\ (\text{conc})}$

Table 7 Within run precision for quinine from three replicated standard curves of PART2 in normal saline solution

CONCENTRATION (microgram/ml)	PEAK HEIGHT RATIO (mean±SD)	%CV	%THEORY
0	0		
132	0.23± 0.003	1.48	105.51
264	0.43± 0.001	0.37	98.04
330	0.56± 0.002	0.49	103.85
396	0.65± 0.001	0.27	100.54
495	0.79± 0.002	0.26	98.20
	AVERAGE	0.58	

1. Mean percent theory was 101.23±3.00 with %CV 2.96
2. Determination coefficient (r^2) = 0.9976
3. Regression line: Peak height ratio = 0.0016(conc)+0.012
4. Percent theory = $\frac{(\text{Peak height ratio}-0.012)*100}{(0.0016)\ (\text{conc})}$

Table 8 Between run precision for quinine from three replicated standard curves of PART2 in normal saline solution.

CONCENTRATION (microgram/ml)	PEAK HEIGHT RATIO (mean±SD)	%CV	% THEORY
0	0	-	-
132	0.23±0.002	1.15	105.34
264	0.42±0.005	1.18	97.89
330	0.56±0.004	0.80	104.34
396	0.64±0.007	1.14	100.48
495	0.78±0.010	1.40	98.03
	AVERAGE	1.14	

1. Mean percent theory was 101.22±3.11 with %CV 3.07
2. Determination coefficient (r^2) = 0.9972
3. Regression line: Peak height ratio = 0.0016(conc)+0.0121
4. Percent theory = $\frac{(\text{Peak height ratio}-0.012)*100}{(0.0016)\ (\text{conc})}$

FIGURE 3
STANDARD CURVE OF QUININE IN PART1

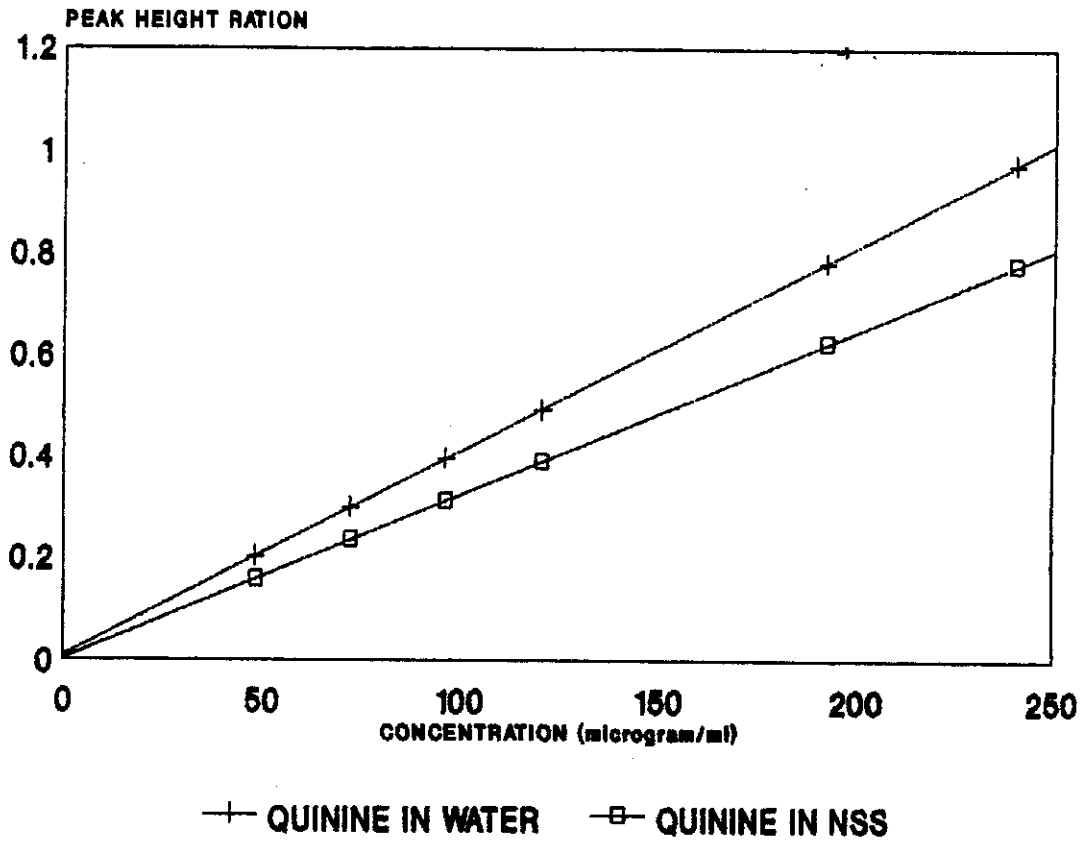
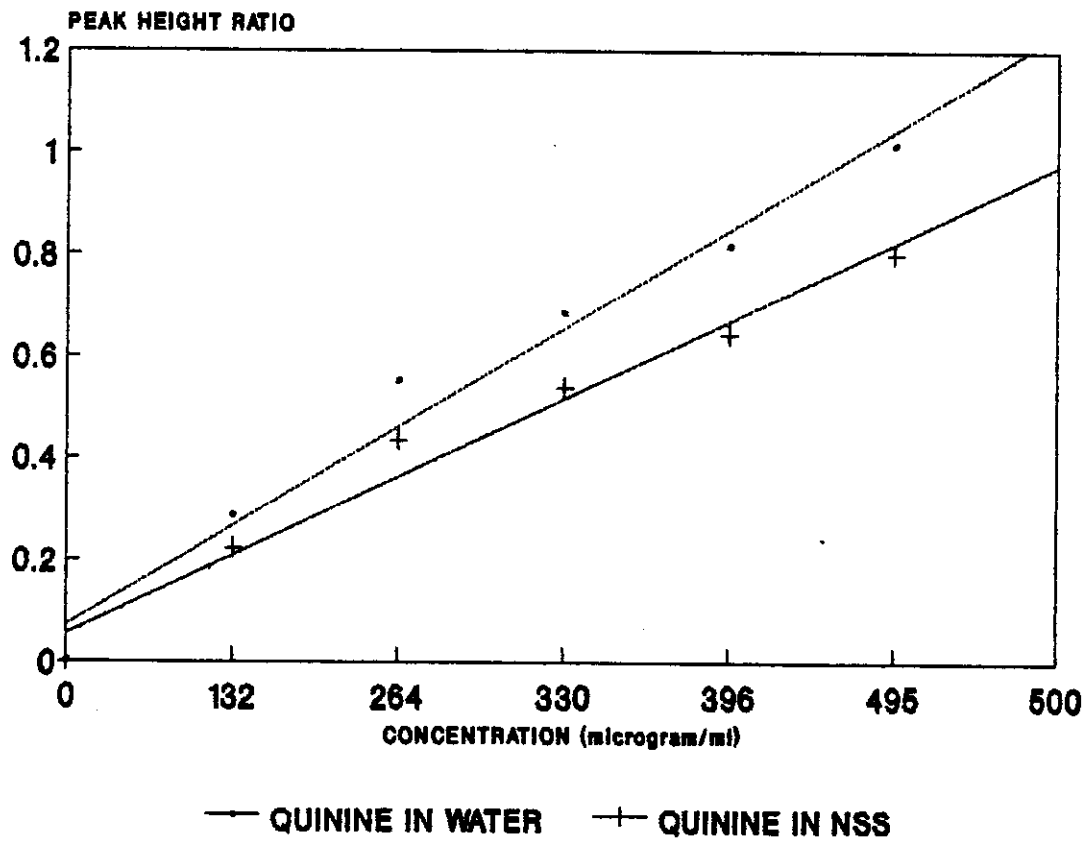


FIGURE 4
STANDARD CURVE OF QUININE IN PART2



QUININE STABILITY

Quinine stability in 4 commonly used infusion fluids at two concentrations, 1.2 mg/ml and 3.6 mg/ml, was studied. A study period of twenty four hours was chosen, since most hospitals would be unlikely to store quinine admixtures for any longer period of time. Over the twenty four-hour study period, there was no significant change (more than 10 percent) in quinine concentration in any sample solution. Both concentrations remained greater than 90 percent of the time zero at twenty four hours (Table 9 and 10). All solution remained clear and colourless and none had any visible particles.

At the concentration of 1.2 mg/ml, percent of concentration remaining at 24 hr ranged from 91-98% (Table 9). Table 11 indicated statistical difference of percent of quinine (1.2 mg/ml) at 24 hr among four IV fluids studied. Quinine in D5W was more stable than that in NSS and D/1/2NSS but its stability at 24 hr was not statistically different from that of quinine in D/NSS.

At the concentration of 3.6 mg/ml,percent of the concentration remaining at 24 hr. ranged from 96-101 % (Table 10). Table 12 showed the analysis of variance of percent of quinine (3.6 mg/ml) remaining at 24 hr. Quinine in D5/NSS was more stable than quinine in the other IV solutions.

Although the statistical analysis indicated that there was statistically significant difference in stability of quinine in four IV fluids. Quinine concentration decreased less than 10% at 24 hr in all sample solution. Hence we can conclude that quinine is stable in four IV fluids studied for at least 24 hr.

The solutions of quinine were kept under usual laboratory fluorescent lighting at room temperature during the experiment and quinine concentration decreased less than 10% over 24 hr. on this condition. From this result, we can conclude that quinine infusion solutions do not require protection from light and can be stored at room temperature at least 24 hr.

Table 9: Quinine concentration remaining at various times.

INFUSION FLUID	THEORETICAL CONCENTRATION (mcg/ml)	ACTUAL INITIAL CONCENTRATION (mcg/ml)	CONCENTRATION AT INDICATED TIME (hr) 2	4	6	10	24
D5W	1200	1253.6±42.3	1291.5±27.2	1254.9±49.7	1236.4±37.8	1224.4±19.6	1240.2±30.0
D/NSS	1200	1157.6±36.9	1117.5±16.0	1031.2±44.7	1123.1±19.3	1027.2±25.6	1098.8±29.0
D/1/2NSS	1200	1290.0±40.9	1264.1±07.5	1187.3±16.8	1225.1±10.1	1204.1±16.3	1183.3±10.6
NSS	1200	1039.9±15.2	1007.0±21.4	987.6±10.2	1005.2±08.8	996.4±15.7	940.1±11.7
D5W	3600	3761.8±52.1	3706.1±50.0	3611.9±45.1	3676.9±41.2	3611.3±41.0	3641.2±37.3
D/NSS	3600	3631.9±18.3	3569.8±07.0	3642.7±04.1	3551.3±51.6	3693.4±40.1	3703.3±65.6
D/1/2NSS	3600	3609.9±23.6	3423.9±57.7	3451.4±53.0	3454.1±60.5	3394.3±33.5	3441.0±22.9
NSS	3600	3791.1±24.0	3759.1±36.9	3737.7±93.3	3716.6±84.8	3644.2±75.5	3700.8±99.5

Table 10: Percent of initial quinine concentration remaining at various times.

INFUSION FLUID	THEORETICAL CONCENTRATION (µg/ml)	ACTUAL INITIAL CONCENTRATION (µg/ml)	PERCENT OF CONCENTRATION AT INDICATED TIME (hr)				
			2	4	6	10	24
D5W	1200	1253.6±4.23	103.10±2.86	100.12±2.87	98.64±0.46	97.73±1.87	98.96±1.01
D/NSS	1200	1157.6±3.69	96.60±2.22	99.05±1.57	97.15±4.35	98.79±2.60	94.99±3.32
D/1/2NSS	1200	1290.0±4.09	98.07±2.59	92.11±2.59	95.06±3.15	93.42±2.70	91.85±3.59
NSS	1200	1039.9±1.52	96.82±0.82	94.98±1.02	96.68±1.48	95.82±0.72	90.40±0.29
D5W	3600	3761.8±5.21	98.52±0.15	96.02±1.14	97.75±0.26	96.00±0.38	96.79±0.36
D/NSS	3600	3631.9±1.83	98.29±0.56	100.29±2.78	97.78±1.31	101.70±1.59	101.96±1.32
D/1/2NSS	3600	3609.9±2.36	94.84±1.01	95.60±0.86	95.68±1.07	94.03±0.35	95.32±0.39
NSS	3600	3791.1±2.40	99.28±3.81	98.65±2.72	98.11±3.13	96.19±2.54	97.65±1.42

Table 11 Statistical differences of percent of quinine that remaining at 24 hr assessed by oneway analysis of variance and LSD. (at 1.2 mg/ml of quinine)

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	129.3355	43.1118	4.5950	.0376
Within Groups	8	75.0580	9.3822		
Total		11	204.3935		

Rank order of IV fluids in terms of percent remaining at 24 hr

SUBSET 1

IV fluid	NSS	D5/1/2NSS	D5/NSS
Mean	90.4033	91.8467	94.9900

SUBSET 2

IV fluid	D5/NSS	D5/W
Mean	94.9900	98.9567

IV fluids grouped in the same subset are not statistically different. (P>0.05).

Table 12 Statistical differences of percent of quinine that remaining at 24 hr assessed by oneway analysis of variance and LSD. (at 3.6 mg/ml of quinine)

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	73.2986	24.4329	16.0634	.0010
Within Groups	8	12.1682	1.5210		
Total		11	85.4668		

Rank order of IV fluids in terms of percent remaining at 24 hr

SUBSET 1

IV fluid	D5W/1/2NSS	D5W
Mean	95.3233	96.7933

SUBSET 2

IV fluid	D5W	NSS
Mean	96.7933	97.6500

SUBSET 3

IV fluid	D5/NSS
Mean	101.9633

IV fluids grouped in the same subset are not statistically different. (P>0.05).