RESULTS

Stability study

Analytical method

Chromatogram of blank NSS and cisplatin standard solution 200 µg/ml were shown in Figure 5. The retention time is 3.48 minutes. No interfering peak can be seen.

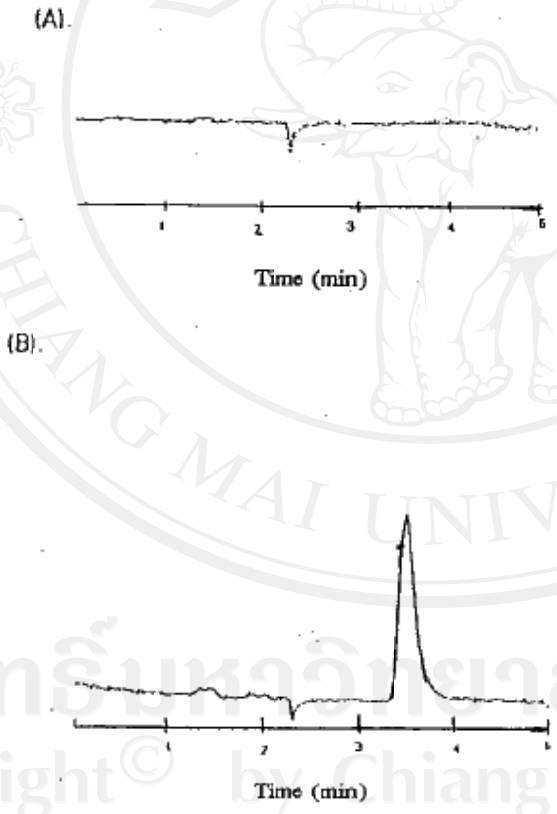

Figure 5. Typical chromatograms of blank NSS (A) and cisplatin standard solution 200 µg/ml (B).

Standard curve which is in a range 0-1,000 µg/ml is shown in Figure 6. A linear relationship is observed as Y = 2370 + 968.34 X, with correlation coefficient of 0.9999.

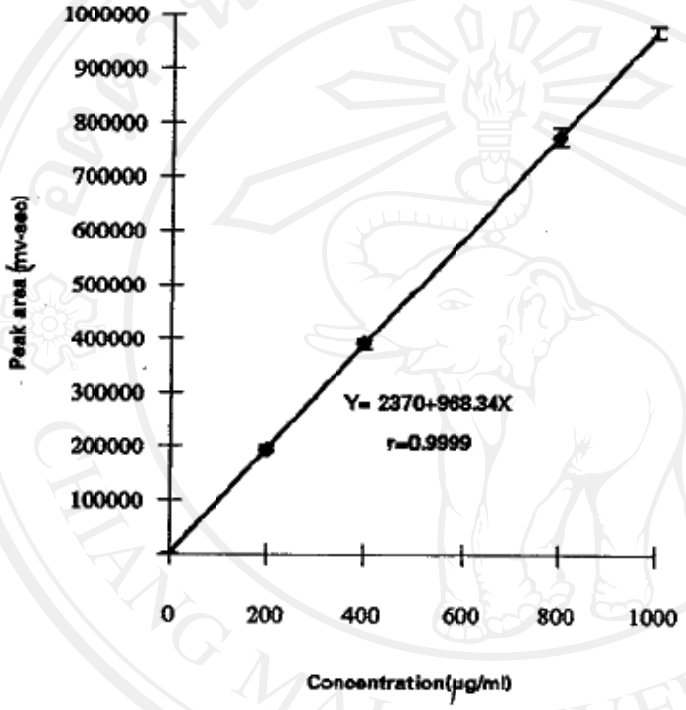

Figure 6. Standard curve of cisplatin in NSS

Inter-day and intra-day variation results are shown in Table 4. Both inter-day and intra-day variation are less than 3 %.

Table4. Inter-day and intra-day variation of cisplatin solution 200 µg/m (n=6)

Compound	Conc(µg/ml)	% C.V.	
		Intra-day	Inter-day
Standard cisplatin in NSS	200	2.61	2.17

Cisplatin labelled amount

The concentrations and the labelled amount percentages of three commercial cisplatin solutions are listed in Table 5.

Table 5. Concentrations and the labelled amount percentages of three commercial cisplatin solutions

Vial no	Concentration of cisplatin (µg/ml)		
	Platinol	Cisplatin	Cisplatinum
1	527.31	495.33	496.53
2	513.01	490.33	496.20
3	518.35	494.78	493.30
4	513.85	504.80	499.32
5	513.41	504.91	493.69
6	528.89	498.80	501.01
Mean± S.E.M	519.14 ± 7.20	498.16 ± 5.83	496.68 ± 3.04
Labelled amount percentage (%)	103.83	99.63	99.33

Note. vial 1-3 are the same lot numbers and vial 4-6 are the same lot numbers.

A one-way analysis of variance (ANOVA) results are shown in Table 6. It shows that cisplatin concentrations of 3 commercial cispaltin solutions is significant difference ($p<0.05$).

Table 6. One-way ANOVA of the mean concentration of 3 commercial cisplatin solution

Source	Sum of square	Degree of freedom	Mean Square	F
Between Groups (treatment)	1882.62	2	941.31	29.812
Within Groups (error)	473.78	15	31.58	
Total	2356.4	17		

F 0.05 =3.68

Efffect of light and temperature on cisplatin stability

Graph of the percentage remaining cisplatin solution versus time of group 2, group 3 and group 4 is shown in Figure 7(A). Two phase of the percentage remaining of cisplatin solution can be seen. Log remaining cisplatin solution of group 2, group 3 and group 4 plotted versus time is shown in Figure 7 (B). Llinear relationship at all the times of study is obtained for group 3 and 4. For group 2, linear relationship is obtained only at the first 5 days following with a plateau region. From the results, the first order of degradation had been seen. Degradation equation, degradation rate constants (K), half-lives ($T_{1/2}$), and shelf-lives (T_{90}) of cisplatin solution of group 2 (only for the first 5 days), group 3 and group 4 were shown in Table 7. .

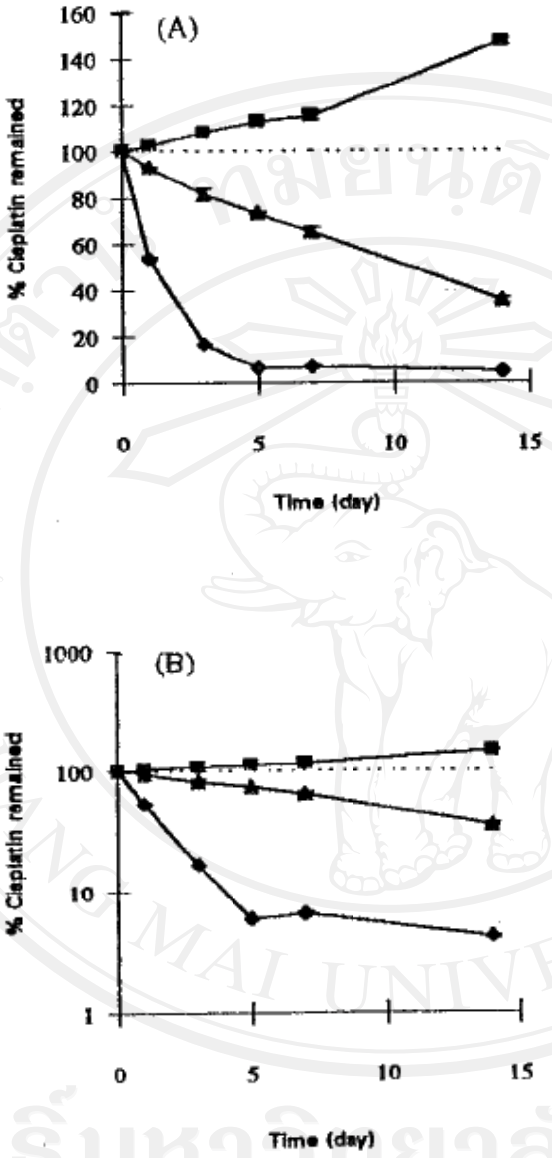

Figure 7. The percentage remaining of cisplatin solution versus time in 3 conditions ; expoded to light in a distance 30 cm (—◆—), exposed to light in a distance 100 cm (—▲—) and kept at 40°C (—■—) compared to the control (- - - -). (A) in arithmetic scale, (B) in semilogarithmic scale.

Table 7. Degradation parameters in stability study

Condition	Degradation equqtion	K (day^{-1})	$T_{1/2}$(day)	T_{90}
Expose to light at a distance 30 cm	Log c = - 0.247t+1.9824	0.5679	1.22	4.40 hr
Expose to light at a distance 100 cm	Log c = - 0.297t+2.0051	0.0684	10.13	1.54 day
Temp 40°C	Log c = 0.01t+1.9824	-0.023	n	n

c = concentration

t = time

n= not calculated

Pharmacokinetic study

Analytical method

Chromatogram of UF plasma and cisplatin in UF plasma concentration 2.5 µg /ml are shown in Figure 8. In blank UF plasma, unknown peak can be seen inconsistency. A linear relationship was observed for peak area and concentration. It was linearity in range 0-10 µg/ml. Standard curve of cisplatin in UF plasma were developed by analyzing peak areas against various concentrations of cisplatin (0.5, 2.5, 5.0 µg/ml) as shown in Figure 9. A linear relationship is observed as y = 27159.3 + 165546 X, with correlation coefficient of 0.9957. Intra-day and inter-day variation results are shown in Table 8.

Table 8. Intra-day and inter-day variation of cisplatin in UF plasma

Compound	Conc. (µg/ml)	% C.V.	
		Intra-day	Inter-day
Standard cisplatin in UF plasma	1	18.56	21.24
	5	1.70	4.60

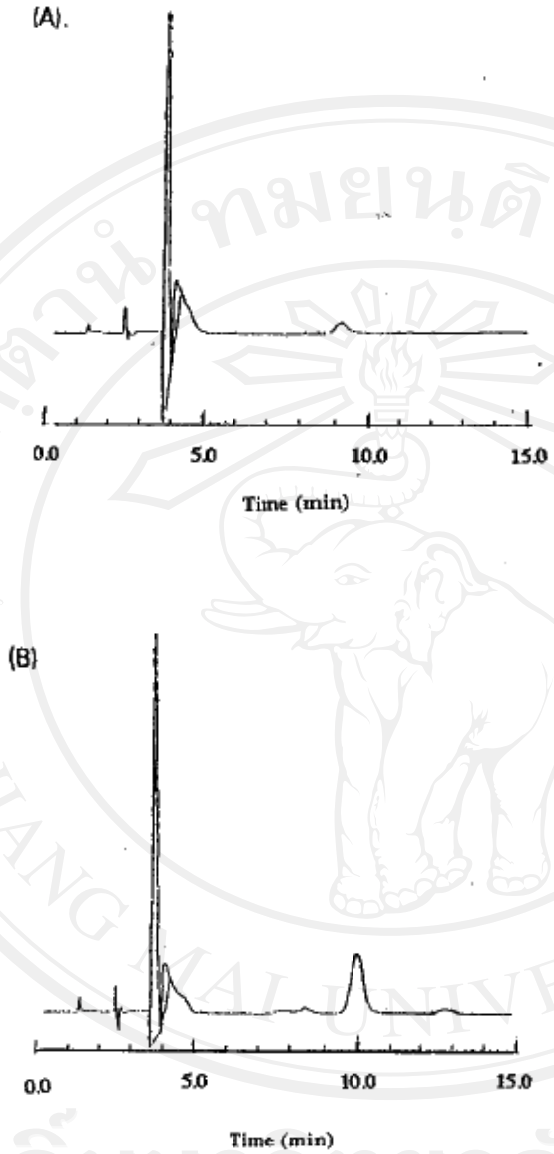

Figure 8. Chromatogram of blank UF plasma (A), and cisplatin 2.5 µg/ml in UF plasma (B).

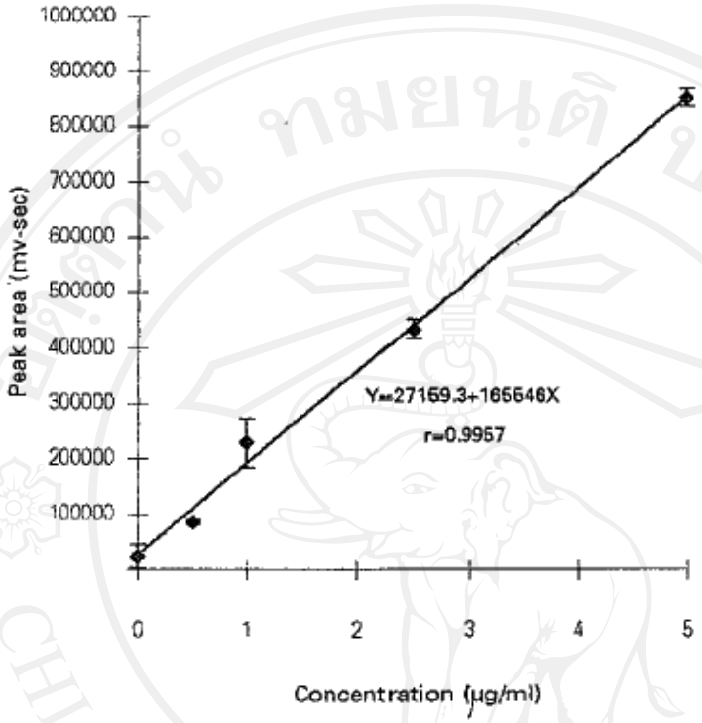

Figure 9. Standard curve of cisplatin in UF plasma

Accuracy and percentage recovery of cisplatin in UF plasma are shown in Table 9.

Table 9. Accuracy and recovery of cisplatin in UF plasma (n =6)

	% Recovery	
Concentration of cisplatin (μg/ml)	Standard in NSS	UF plasma
5	100	84.18
2.5	100	100.32

Mean UF plasma concentration of cisplatin at each sampling time after IV infusion are presented in Table 10.

Table 10. Mean cisplatin UF serum concentrations at each sampling times following 100 mg/ m^2 infusion (n=6).

Time (hr)	0.25	0.50	1.00	2.00	3.00	4.00	5.00
Conc(μg/ml)	0.61	0.66	0.80	0.89	0.59	0.25	0.18
±	±	±	±	±	±	±	±
S.E.M	0.16	0.17	0.26	0.16	0.15	0.07	0.07

The pharmacokinetic parameters of cisplatin; C_{max}, $T_{1/2}$ and K_e were summarized in Table 11.

Table11. Pharmacokinetic parameters of cisplatin 100 mg/m^2 IV infusion in 6 Thai patients

Patient no.	Sex	Infusion time (hr)	C_{max} (µg/ml)	$T_{1/2}$ (hr)	K_e (hr^{-1})
1	F	2.25	0.47	0.78	0.89
2	M	2.50	1.16	1.50	0.46
3	F	3.25	1.31	1.70	0.41
4	F	2.17	1.85	1.42	0.49
5	F	2.00	0.78	1.60	0.43
6	M	2.67	0.52	2.24	0.31
mean ± S.E.M		2.47 ± 0.18	1.02 ± 0.22	1.54 ± 0.19	0.50 ± 0.08

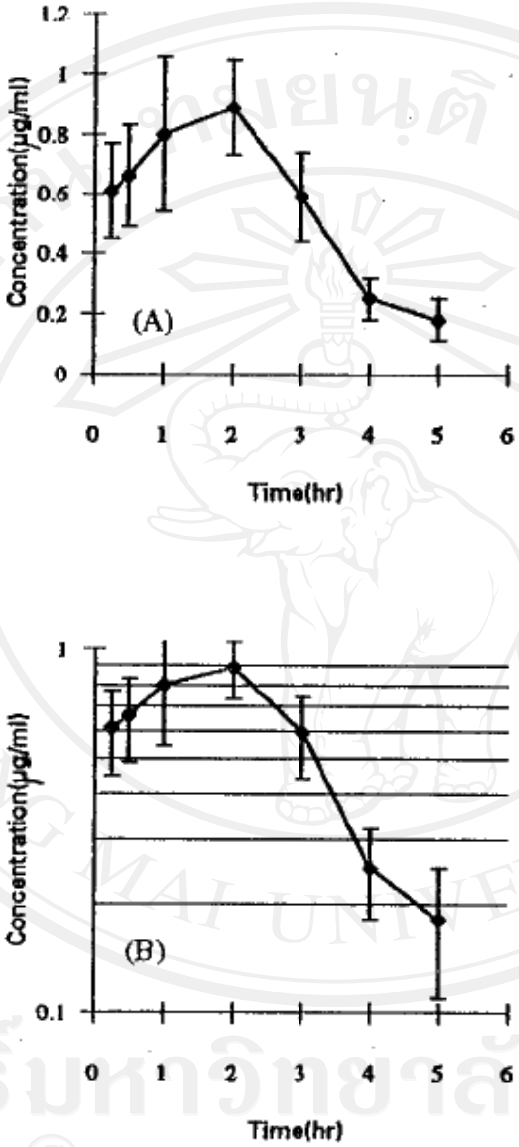

Figure 10. Mean DDTC-cisplatin concentration in UF serum versus time in 6 patients receiving cisplatin 100mg/m^2 as a 2-hours infusion. The verticle bars represent standard error of mean. (A) in arithmetic scale, (B) in semilogarithmic scale.