CHAPTER 4

RESULTS

1. Assay Validation

1.1 Linearity of the standard calibration curve

The standard calibration curve for DEC at the concentrations of 25, 50, 100, 250, 500, 1000 and 2000 ng/ml was linear with the coefficient of determination (r^2) of 0.999 (Figure 11). The equation of linear regression line of DEC was Y = 0.006X – 0.0053. The slope and intercept were 0.006 and 0.0053, respectively.

1.2 Precision

The intra-day assay was repeated 5 times per day and the CV for DEC was 3.66 - 4.33 % (Table 1), whereas the inter-day assay was assessed on 5 different days; the CV was 7.64 – 9.68% (Table 2).

1.3 Recovery

The recovery of DEC in plasma was 102.35-104.96% (Table 3).

1.4 Accuracy

The accuracy of DEC in plasma was 94.15 – 97.67% (Table 4). The result reflects that the method is obviously accurate and this ensures reliable result.

1.5 Limit of detection

The limit of detection (LOD) and limit of quantification (LOQ) for DEC were approximately 10 and 25 ng/ml, respectively.

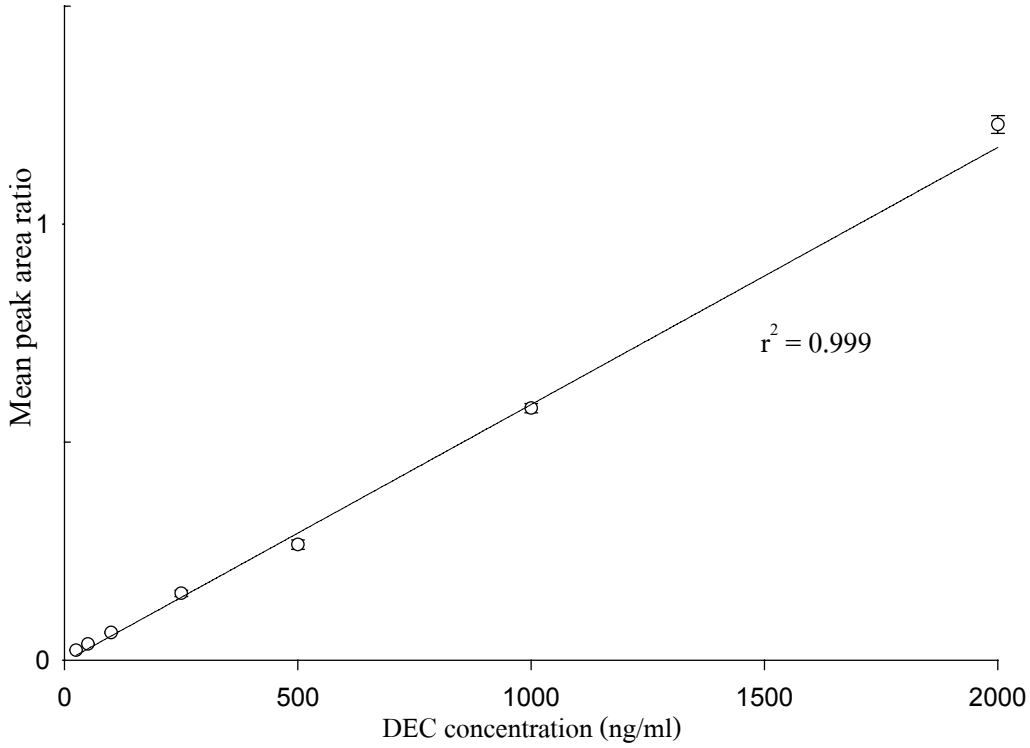

Figure 11 Correlation between peak area ratio of DEC to quinidine sulfate (internal standard) in plasma and DEC concentration. The coefficient of determination (r^2) of regression line for DEC is 0.999. Each point represents mean ± SD.

Table 1. The intra-day variation of three different DEC concentrations in plasma[a].

Concentration (ng/ml)	Mean peak area ratio ± SD (n = 5)	% CV[b]
100	0.051415 ± 0.001883	3.66
500	0.306358 ± 0.014271	4.66
2000	1.284467 ± 0.055578	4.33

[a]Various concentrations of standard DEC were added to drug-free human plasma sample prior to precipitation as described in text.

[b]Standard deviation divided by mean, expressed in percent.

Table 2. The inter-day variation of three different DEC concentrations in plasma[a].

Concentration (ng/ml)	Mean peak area ratio ± SD (n = 5)	% CV[b]
100	0.049941 ± 0.003813	7.64
500	0.295446 ± 0.021504	7.28
2000	1.236661 ± 0.120975	9.78

[a]Various concentrations of standard DEC were added to drug-free human plasma sample prior to precipitation as described in text.

[b]Standard deviation divided by mean, expressed in percent.

Table 3. Relative percent recovery of standard DEC in human plasma.

Concentration (ng/ml)	Mean peak area ratio ± SD (n = 5) in methanol[a]	Mean peak area ratio ± SD (n = 5) in plasma[b]	% Recovery[c]
100	0.064753 ± 0.000987	0.06659 ± 0.000987	102.84
500	0.322191 ± 0.00543	0.338175 ± 0.017744	104.96
2000	1.277383 ± 0.078013	1.307383 ± 0.078013	102.35

[a]Various concentrations of standard DEC in methanol were directly injected.

[b]Various concentrations of standard DEC were added to drug-free human plasma samples prior to extraction.

[c]Mean peak area ratio divided by mean peak area ratio in methanol, expressed in percent.

Table 4. The accuracy of three different DEC concentrations in plasma[a].

Concentration (ng/ml)	Mean peak area ratio ± SD (n = 5)	% CV[b]	% accuracy[c]
100	0.051415 ± 0.001883	3.66	95.59
500	0.306358 ± 0.014271	4.66	97.67
2000	1.284467 ± 0.055578	4.33	94.15

[a]Various concentrations of standard DEC were added to drug-free human plasma sample prior to precipitation as described in text.

[b]Standard deviation divided by mean, expressed in percent.

[c]Accuracy, detected value divided by theoretical value, expressed in percent.

2. Chromatograms

The chromatograms showed that the peak of DEC and quinidine sulfate (internal standard) were well separated from the other peaks in plasma (Figure 12-14). The retention times of DEC and quinidine sulfate were 5.15 min and 10.16 min, respectively.

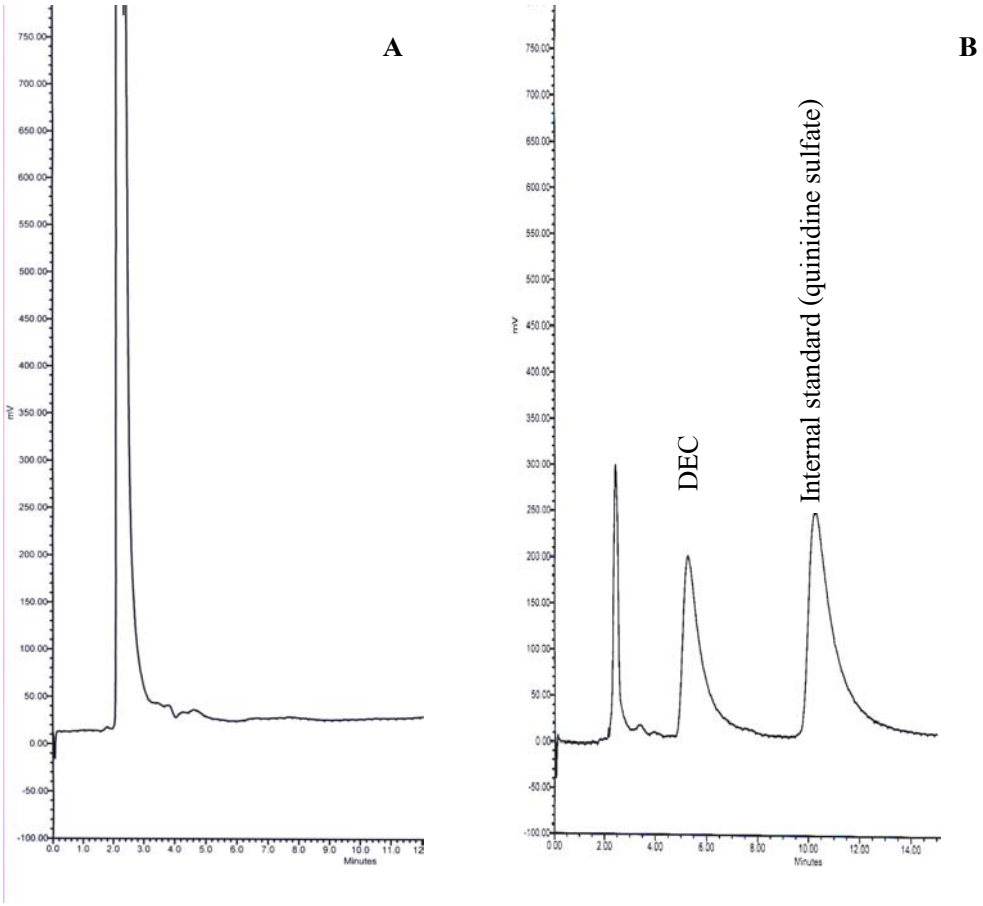

Figure 12 Representative chromatograms of 20 µl human plasma.

Key : (A) blank human plasma; (B) spiked with standard DEC 1,000 ng/ml.

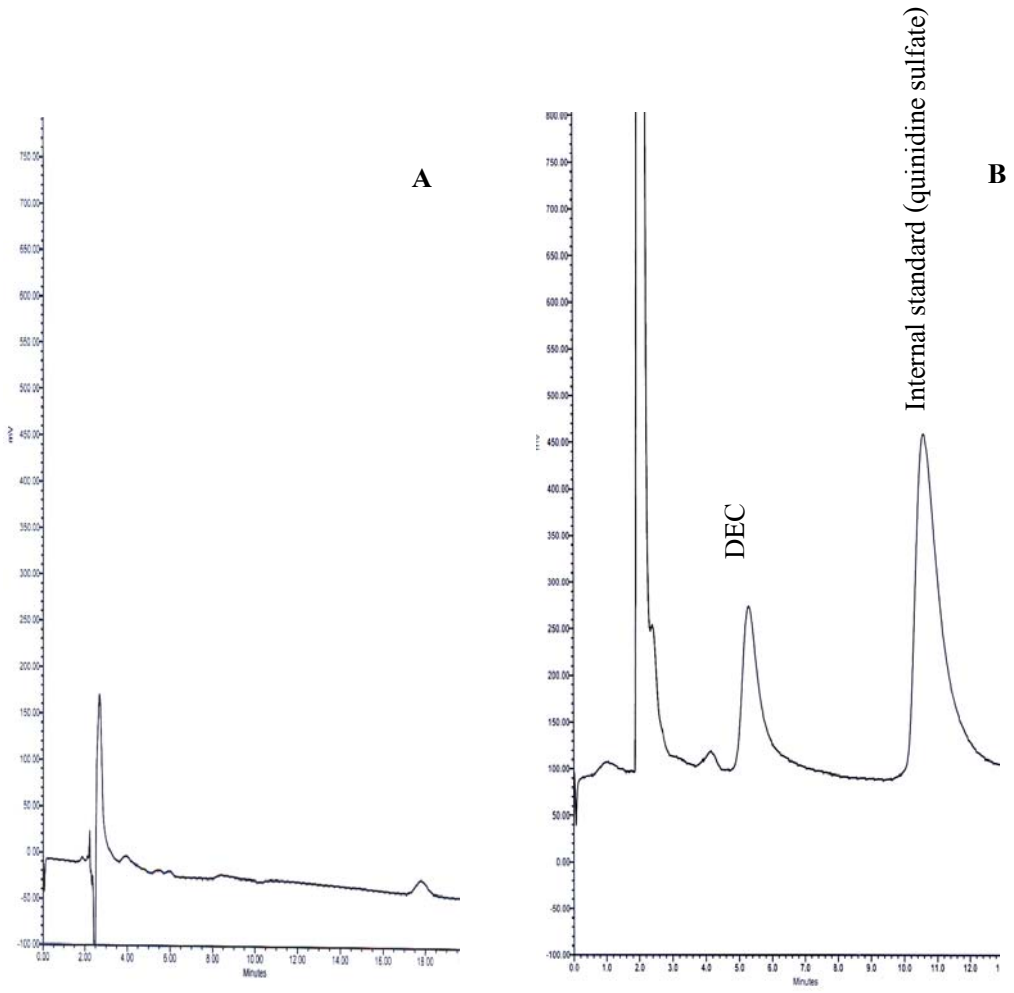

Figure 13 Representative chromatograms of 20 µl human plasma.

Key : (A) blank human plasma; (B) plasma obtained from a subject receiving 6 mg/kg DEC alone at 0.25 h.

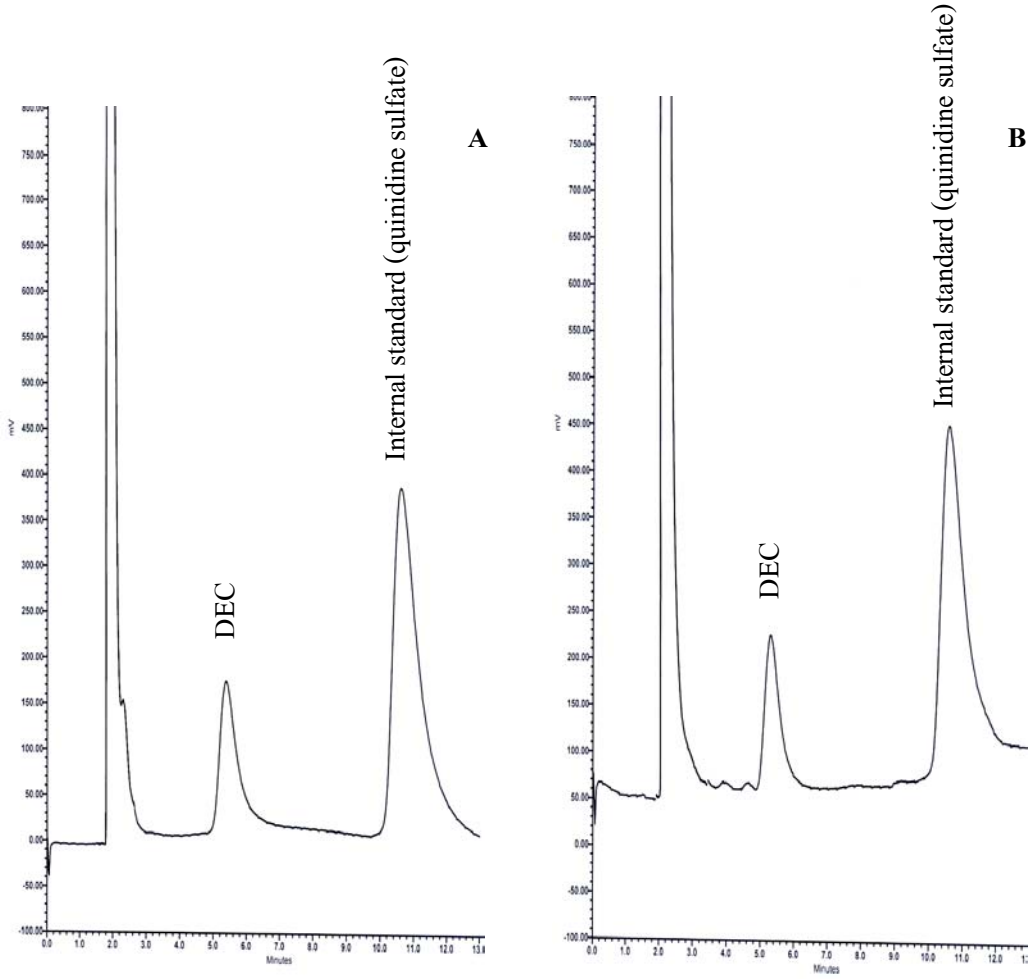

Figure 14 Representative chromatograms of 20 µl human plasma.

Key : (A) plasma obtained from a subject receiving 6 mg/kg DEC at 0.25 h after pretreatment with rifampicin; (B) plasma obtained from a subject receiving 6 mg/kg DEC at 0.25 h after pretreatment with ketoconazole.

3. The plasma concentration-time data of DEC

According to the wide inter-individual variations of the subjects, the non-compartment model was used to determine the pharmacokinetics data of DEC in this study. The mean plasma concentration-time profiles of DEC after receiving DEC alone and pretreatment with rifampicin or ketoconazole were shown in Figure 15.

4. The effect of period and sequence on the pharmacokinetic parameters

The period and sequence had no significant on the pharmacokinetic parameters (see Appendix B).

5. Adverse effect

Twelve male healthy volunteers were enrolled and completed in this study. No serious side effects were observed after drug administration.

6. Pharmacokinetics

6.1 Pharmacokinetics of a single oral dose of 6 mg/kg of DEC in subjects

Mean plasma concentration-time profiles of DEC in subjects receiving a single dose of 6 mg/kg DEC alone was shown in Figures 15. DEC concentrations were detectable in plasma at least 15 min after administration and decreased to undetectable levels after 36 and 48 h post-administration in two subjects and three subjects, respectively.

After a single oral dose of 6 mg/kg of DEC alone in twelve volunteers, the pharmacokinectic parameters of DEC (C_{max}, t_{max}, $AUC_{0\text{-}48}$, $AUC_{0\text{-}\infty}$, $t_{1/2}$, k_a, k_e, V_d/F, Cl/F) analyzed using one-compartment model was shown in Table 5.

6.2 Pharmacokinetics of a single oral dose of 6 mg/kg of DEC in subjects after pretreatment with 600 mg rifampicin once daily for 5 days

Mean plasma concentration-time profiles of a single oral dose of 6 mg/kg of DEC in subjects after pretreatment with 600 mg rifampicin once daily for 5 days was shown in Figures 9. DEC concentrations were detectable in plasma within 15 min after administration and decreased to undetectable levels after 36 and 48 h post-administration in one subjects and eight subjects, respectively. In the remainder, DEC was still found in plasma until to 48 h.

Results of pharmacokinetic parameters of DEC (C_{max}, t_{max}, $AUC_{0\text{-}48}$, $AUC_{0\text{-}\infty}$, $t_{1/2}$, k_a, k_e, V_d/F, Cl/F) analyzed using one-compartment model was shown in Table 6.

6.3 Pharmacokinetics of a single oral dose of 6 mg/kg of DEC in subjects after pretreatment with 400 mg ketoconazole once daily for 5 days

Mean plasma concentration-time profiles of a single oral dose of 6 mg/kg of DEC in subjects after pretreatment with 400 mg ketoconazole once daily for 5 days was shown in Figures 9. DEC concentrations were detectable in plasma within 15 min after administration and decreased to undetectable levels after 48 h post-administration in seven subjects. In the rest five subjects, DEC was still found in plasma until to 48 h.

Results of pharmacokinetic parameters of DEC (C_{max}, t_{max}, AUC_{0-48}, $AUC_{0-\infty}$, $t_{1/2}$, k_a, k_e, V_d/F, Cl/F) analyzed using one-compartment model was shown in Table 7.

Statistical analysis using two-way ANOVA indicated that neither rifampicin nor ketoconazole significantly altered the mean C_{max}, t_{max}, AUC_{0-48}, $AUC_{0-\infty}$, $t_{1/2}$, k_a, k_e, V_d/F, Cl/F (see Table 5).

The plasma concentration of DEC during 0-48 h interval in each subjects receiving a single oral dose of 6 mg/kg DEC alone, DEC after pretreatment with 600 mg rifampicin, and DEC after pretreatment with 400 mg ketoconazole were shown in Table 9, 10, and 11, respectively.

6.4 Urine pH

The urine pH of each subject was recorded at the time 0, 1, 4, 12, 24, 36, and 48 h (Figure 15).

The plasma concentration of DEC and urine pH during 0-48 h interval in each subjects receiving a single oral dose of 6 mg/kg DEC alone, DEC after pretreatment with 600 mg rifampicin, and DEC after pretreatment with 400 mg ketoconazole were shown in Table 12, 13, and 14, respectively.

6.4.1 Urine pH of subjects receiving a single oral dose of 6 mg/kg DEC alone

The mean of urine pH value of each subject receiving a single oral dose of 6 mg/kg DEC alone was shown in Table 15.

6.4.2 Urine pH of subjects receiving a single oral dose of 6 mg/kg DEC after pretreatment with 600 mg rifampicin.

The mean of urine pH value of each subject receiving a single oral dose of 6 mg/kg DEC after pretreatment with 600 mg rifampicin was shown in Table 16.

6.4.3 Urine pH of subjects receiving a single oral dose of 6 mg/kg DEC after pretreatment with 400 mg ketoconazole.

The mean of urine pH value of each subject receiving a single oral dose of 6 mg/kg DEC after pretreatment with 400 mg ketoconazole was shown in Table 17.

Statistical analysis using two-way ANOVA, the results indicated that there were no significant differences among the mean urine pH values in 3 phases of study (see Table 5).

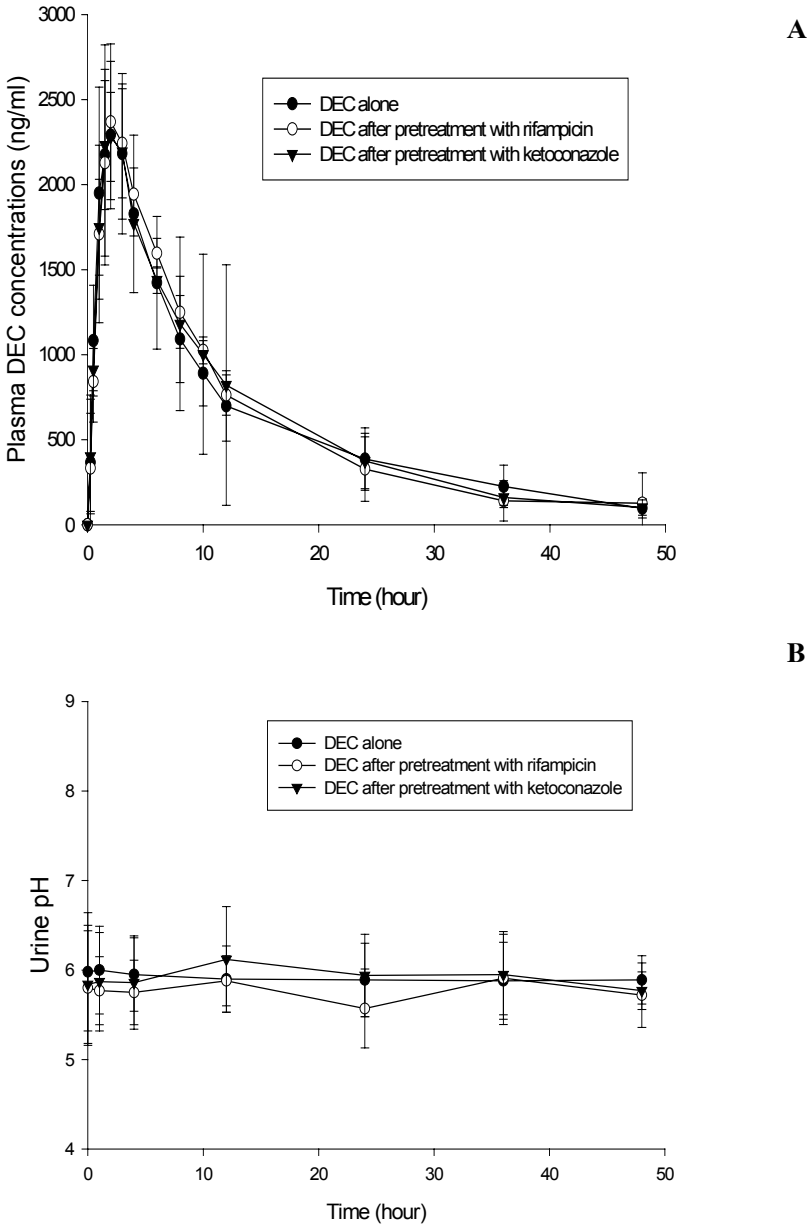

Figure 15 Mean plasma DEC concentration (A) and urine pH (B) after a single oral dose of 6 mg/kg DEC administration alone or after pretreatment with 600 mg rifampicin and 400 mg ketoconazole once daily for 5 days.

Table 8 Pharmacokinetic parameters and urine pH (mean ± SD.) of diethylcarbamazine in twelve subjects after receiving a single oral dose of 6 mg/kg or pretreatment with 600 mg rifampicin and ketoconazole orally for 5 days.

Parameters	**DEC (n=12)**	**DEC+RIF (n=12)**	**DEC+KET (n=12)**	***P*-value (two-way ANOVA)**
C_{max} (ng/ml)	2243.54 ± 393.83	2274.38 ± 526.55	2211.99 ± 335.63	0.475
t_{max}	2.31 ± 0.66	2.64 ± 0.78	2.42 ± 0.65	0.843
AUC_{0-48} (ng.h /ml)	20484.81 ± 8931.84	23610.91 ± 5790.97	24027.14 ± 8239.23	0.095
$AUC_{0-\infty}$(ng.h /ml)	21287.97 ± 9176.35	24249.57 ± 6097.74	24718.18 ± 8490.30	0.101
k_a (h^{-1})	1.126 ± 0.604	0.953 ± 0.697	1.075 ± 0.623	0.931
k_e (h^{-1})	0.154 ± 0.063	0.164 ± 0.072	0.152 ± 0.067	0.666
$t_{1/2}$	5.14 ± 1.84	4.83 ± 1.62	5.48 ± 2.54	0.414
V_d/F (L)	117.79 ± 24.87	108.02 ± 34.80	118.08 ± 30.97	0.757
Cl/F (L/h)	17.16 ± 5.02	16.01 ± 3.52	16.36 ± 4.06	0.351
Urine pH	5.93 ± 0.44	5.77 ± 0.44	5.91 ± 0.50	0.372