Pharmacokinetics of dimemorfan phosphate following single- and multiple-dose oral administration in healthy Chinese volunteers

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Abstract An LC-MS/MS method was developed to investigate the pharmacokinetic parameters in healthy Chinese volunteers following single and multiple oral administration of dimemorfan phosphate. A randomized, two-period and crossover study of 12 healthy volunteers was conducted with single-dose of 10 mg or 40 mg of dimemorfan phosphate. Another 12 volunteers were administered with 20 mg. The values of $AUC_{0-48\ h}$, $t_{1/2}$, and c_{max} were (11.81 ± 14.46), (52.60 ± 96.01) and (34.70 ± 29.59) ng·h/mL, (12.11 ± 2.54), (12.16 ± 2.01) and (12.77 ± 1.27) h, and (0.965 3 ± 0.817 8), (3.150 ± 3.451) and (2.167 ± 1.650) ng/mL for 10 mg, 40 mg and 20 mg oral administration. The same 12 healthy volunteers as the group of single-dose of 20 mg participated in multiple-dose study, who were administered with dimemorfan phosphate 20 mg, three-time a day until the day-8, $AUC_{0-48\ h}$, $t_{1/2}$, and c_{max} were (115.9 ± 135.2) ng·h/mL, (11.22 ± 1.61) h, and (7.418 ± 7.010) ng/mL. The accumulation parameters $R_{c_{max}}$ and R_{AUC} were (3.14 ± 1.34) and (3.38 ± 1.22), respectively. Dose proportional of c_{max} and AUC was not concluded ranging from 10 mg to 40 mg after confidence interval criteria method. Accumulation occurred after multiple-dose administration. And the results demonstrated significant individual difference.

Key words dimemorfan phosphate; pharmacokinetics; human plasma; mass spectrometry

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磷酸二甲啡烷在中国健康人体的口服药代动力学

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摘 要 采用 LC-MS/MS 研究单次及多次口服给药后磷酸二甲啡烷在中国健康受试者体内药代动力学特征。单剂量试验的 12 例受试者采用两周期、交叉设计,分别口服本品 10 mg、40 mg,以及其余 12 例受试者单次口服 20 mg 后的药代动力学参数:$AUC_{0-48\ h}$(11.81 ± 14.46)、(52.60 ± 96.01)、(34.70 ± 29.59) ng·h/mL;$t_{1/2}$(12.11 ± 2.54)、(12.16 ± 2.01)、(12.77 ± 1.27) h;c_{max}(0.965 3 ± 0.817 8)、(3.150 ± 3.451)、(2.167 ± 1.650) ng/mL。多剂量试验的 12 例受试者接受单剂量 20 mg 后,开始每天 3 次服用磷酸二甲啡烷 20 mg,连服至第 8 天晨采集血样的药代动力学参数:$AUC_{0-48\ h}$(115.9 ± 135.2) ng·h/mL;$t_{1/2}$(11.22 ± 1.61) h;c_{max}(7.418 ± 7.010) ng/mL;累积常数 $R_{c_{max}}$ 为(3.14 ± 1.34),R_{AUC} 为(3.38 ± 1.22)。置信区间法表明磷酸二甲啡烷在 10 ~ 40 mg 范围内,药代动力学参数与剂量无线性关系;多次给药后在体内存在蓄积;磷酸二甲啡烷在中国健康受试者中存在显著的个体差异。

关键词 磷酸二甲啡烷;药代动力学;人体血浆;质谱法

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Cough is a sudden and often repetitively occurring reflex which helps to clear the large breathing passages from secretions, irritants, foreign particles and microbes[1]. Frequent coughing usually indicates the presence of a disease. Dextromethorphan may be modestly effective in decreasing cough, nevertheless, psychotomimetic reactions and toxicity have been reported in children related to high-dose ingestion due to dextrorphan, a major metabolite of dextromethorphan, which has been reported to be abuse potential in adolescents. Dimemorfan (3-methyl-17-methylmorphinan)[2] is recognized as a nonopioid antitussive[3] and an analog of dextromethorphan. Whereas, dimemorfan phosphate maintains its anticonvulsant and neuroprotective activities[4-5] without being converted into dextrorphan *in vivo*.

Although dimemorfan has an established safety record in humans at antitussive doses, adverse effects in psycho-neurologic and digestive systems are observed at high doses. Therefore, methods for quantifying dimemorfan phosphate concentration in human blood samples are required for optimum dosage adjustments and for clinical and forensic toxicological analysis.

Previous reports mainly focus on the quantification of dimemorfan phosphate in plasma, instead of its pharmacokinetics (PK). GC/MS[6] was time-consuming and labor-intensive as solid-phase extraction was used to prepare the samples. The LC-MS/MS[7] showed lower limit of quantification (LLOQ) of 0.04 ng/mL, which was not sensitive enough for determination of dimemorfan phosphate in healthy volunteers. In this paper, a simple and sensitive HPLC-MS/MS method was developed and applied to study the pharmacokinetic of dimemorfan phosphate in healthy Chinese volunteers following oral administration of single-dose (10, 40 mg) and multiple-dose (20 mg). Pharmacokinetic characteristics of dimemorfan phosphate such as elimination half time and pharmacokinetic linearity were revealed, which could facilitate further research and development of dimemorfan phosphate.

1 Experimental

1.1 *Chemicals and reagents*

The reference standard of dimemorfan phosphate (purity > 99%) was obtained from Sichuan Baili Pharmaceutical Co., Ltd. (Chengdu, China). Indapamide (internal standard, IS) was purchased from Tianjin Pacific Chemical & Pharmaceutical Co., Ltd. (Tianjin, China). Methanol was HPLC grade and purchased from Merck KGaA (Darmstadt, Germany). Formic acid and ether (analytical grade) were purchased from Nanjing Chemical Reagents Co., Ltd. (Nanjing, China). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA) and used throughout the study.

1.2 *LC-MS/MS analysis*

Chromatographic condition Chromatographic separation was performed at 30 °C using a Zorbax Eclipse XDB-Phenyl column (2.1 mm × 150 mm, 5 μm, Agilent, USA). The mobile phase was comprised of methanol and deionized water containing 0.2% formic acid (75 ∶ 25) in isocratic mode at a flow rate of 0.2 mL/min.

Mass spectrometric conditions After chromatographic separation, column effluent was directed to a triple-quadrupole tandem mass spectrometer of Shimadzu MS-8040 system (Kyoto, Japan) and operated in positive electrospray ionization (ESI) mode. Quantification was performed using multiple reaction monitoring (MRM) of transitions of protonated precursor ion at *m/z* 256.25→155.10 at −38 V collision energies for dimemorfan and at *m/z* 366.15→132.25 at −14 V for IS.

1.3 *Preparation of standard and quality control samples*

Stock solutions (1 mg/mL) of dimemorfan phosphate and IS were prepared separately in the mobile phase and stored at 4 °C until analysis. Working solutions of dimemorfan phosphate and IS were prepared by serial dilution of the stock solution in the mobile phase, and freshly prepared. Calibration standards were prepared by spiking the appropriate standard solutions into blank human plasma to obtain final concentration levels of 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 4, 6 ng/mL. Quality control (QC) samples were prepared in the same way as the calibration standards, to achieve low, medium and high concentrations of 0.05, 0.5 and 4 ng/mL.

1.4 *Sample preparation*

All samples were stored in a freezer at −20 °C and allowed to thaw at room temperature before analysis. 10 μL of IS (500 ng/mL) was added into a glass tube, then 500 μL of plasma sample and 100 μL of 0.1 mol/L sodium hydroxide were added and vortexe d. The mixture was then extracted with 4 mL diethyl ether by vortex-mixing for 5 min. After centrifugation

at 4 000 r/min for 10 min, the supernatant was evaporated to dryness at 30 °C under a slight stream of nitrogen. Then the residue was reconstituted with 100 μL of mobile phase and vortex-mixed for 30 s. After centrifugation at 14 000 r/min for 10 min, a 5 μL of the supernatant was injected into the HPLC-MS/MS system for analysis.

1.5 *Method validation*

Linearity The calibration curves of dimemorfan phosphate were performed with nine levels in the range of 0.02-6 ng/mL. Peak area ratios of dimemorfan phosphate to IS were calculated and the calibration curves were established by fitting these ratios to the nominal concentrations using weighted ($1/c^2$) least squares linear regression. Standard curves were considered acceptable when the correlation coefficient (R) was greater than 0.99 and the deviations of the calculated concentrations were within ± 15% of the nominal concentrations.

Dilution of plasma sample was required if the concentration of dimemorfan phosphate in studied samples were higher than the upper limit of quantification (ULOQ). Dilution integrity experiments were carried out by a 5-fold dilution of the plasma samples. Dilution of high concentration samples was considered acceptable if precision and accuracy of replicate (n = 5) values varied by less than 15%.

Recovery, matrix effect, precision and accuracy Extraction recovery at three QC levels was assessed by comparing the peak area ratio of the dimemorfan phosphate and IS obtained from the extracted plasma samples with the ratio from the blank plasma extracts spiked with standard solution and internal standard. Matrix effect was defined as the alteration or interference in response to the presence of unintended analytes or other interfering substances in the sample. The matrix effect was investigated by comparing the peak areas of the analytes dissolved in blank sample (the final solution of blank plasma after being extracted and re-dissolved) with those obtained by injection of standard solutions at the same concentration. The blank plasmas used in this study were from six different batches of human blank plasma. If the ratio <85% or >115%, a matrix effect was implied.

The intra-day accuracy (relative error, RE) and precision (relative standard deviation, RSD) of the method were assessed by determining the QC samples five times on a single-day, and the inter-day accuracy and precision were estimated over three consecutive days. The intra-and inter-day accuracy should within 15% and precision should not exceed 15% for the QC samples.

1.6 *Pharmacokinetic study*

The clinical investigation was conducted in accordance with the principles of the *Declaration of Helsinki* and approved by the local Ethics Committee. Twenty-four healthy Chinese volunteers were fully informed about the purpose of the study and gave written consent prior to being enrolled. And the single-dose and multiple-dose group volunteers were no significant difference in body weight, age and BMI of (61.5 ± 11.6) kg *vs* (61.2 ± 10.8) kg, (22 ± 2) *vs* (23 ± 3), and (21.08 ± 1.60) *vs* (21.30 ± 1.65).

Single-dose study 12 healthy volunteers (half male and half female) participated in an open-label, randomised, single-dose, two-way crossover single-dose study. A computer-generated randomization scheme was used to assign eligible volunteers to receive single-oral doses of dimemorfan phosphate granules 10 or 40 mg. Then 10 or 40 mg were administered alternately in the second period. The wash-out period between the two periods was one week.

After a 10-hour overnight fast, the volunteers received a single-oral dose dimemorfan phosphate granules with 250 mL of water. For the determination of plasma concentrations, venous blood samples (4 mL) were collected into heparinized tubes before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 36 and 48 h after administration of study drug. Plasma was separated by centrifugation at 3 500 r/min for 5 min and stored at −20 °C until analysis.

Multiple-dose study Another 12 volunteers (half male and half female) received 20 mg dimemorfan phosphate granules on the first day and day-8, and continued to receive the dose drug three times a day from day-3 to day-7. This dose was chosen for the multiple-dose study because it is likely to be the most commonly used dose in clinical practice. Volunteers were confined to the clinic for the duration of the study. Blood samples were collected before dosing on days 6 and 7. On the last day (day-8), blood samples were drawn at the same times as in the single-dose study. All other experimental conditions were the same as those in the single-dose study.

1.7 *Pharmacokinetic analysis*

Maximum concentration (c_{max}) and time point of maximum plasma concentration (t_{max}) were observed directly from the data. Other PK parameters were esti-

mated using the non-parametric model analysis. All individual PK parameters were summarized using descriptive statistics in the program DAS 2.0 (Mathematical Pharmacology Professional Committee of China, Shanghai, China) as described.

In the single-dose study, t_{max} and $t_{1/2}$ were tested via the Kruskal-Wallis test; and AUC and c_{max} were tested via Homogeneity of variance test and t test after natural logarithmic transformation. Dose linearity was also tested for individual log-transformed and standardized c_{max} and AUC by a comparison of the 90% confidence interval (CI) of the slopes with a criterion CI, based on a power model. Inferences were made based on the theoretical slope of 1 with the confidence limits of 0.8 and 1.25 for AUC and 0.75 and 1.33 for c_{max}, respecively. The criterion intervals for testing the slope of the power model $\beta = 1$ were as follows: $[1 + \ln(\theta_L)/\ln(r)]$, $[1 + \ln(\theta_H)/\ln(r)]$, where θ_L = 0.8 for AUC and 0.75 for c_{max}, θ_H = 1.25 for AUC and 1.33 for c_{max}, r = high/low: high = 40 mg (highest dose) and low = 10 mg (lowest dose)[8].

In the multiple-dose study, individual PK parameters were compared to determine any difference between day 1 and day 8. The tests for t_{max}, $t_{1/2}$, c_{max} and AUC were similar to single-dose study. When the P value was higher than 0.05, it would be considered "no statistically significant".

2 Results

2.1 *Method validation*

Linearity Calibration curve demonstrated good linearity at 0.02-6 ng/mL for dimemorfan phosphate. A mean equation of the calibration curves was $c = R \times 0.338\ 9 - 0.004\ 539$, $r = 0.999$, $w = 1/c^2$, where R was the peak area ratio of dimemorfan phosphate to IS and c was the concentration of dimemorfan phosphate. And the limit of detection (LOD) was 0.01 ng/mL. Representative MRM chromatograms of dimemorfan phosphate and IS were shown in Figure 1.

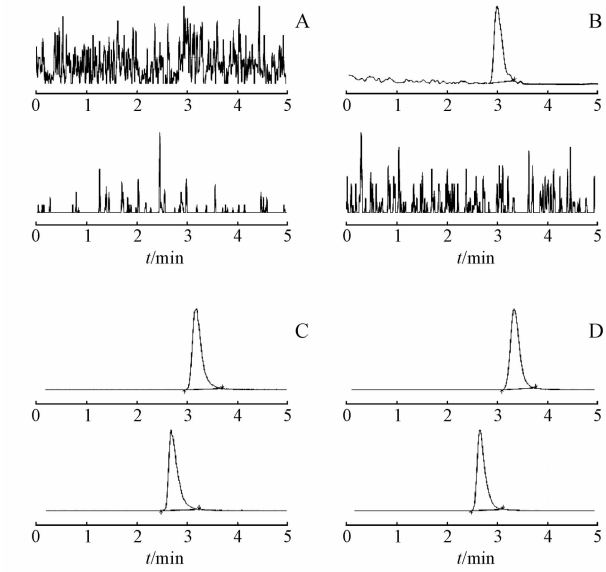


Figure 1 MRM chromatograms of dimemorfan phosphate
A: Blank plasma sample; B: LOD for dimemorfan phosphate; C: Blank plasma sample spiked with dimemorfan phosphate and IS; D: Plasma sample from a healthy volunteer with IS

For diluted samples, the precision was less than 3.86%, and the accuracy was between -6.63% and 3.33%. The results suggested that plasma samples whose concentrations exceed the ULOQ can be determined by an appropriate dilution.

Recovery, matrix effect, precision and accuracy The average measured extraction recoveries of dimemorfan phosphate (Table 1) indicated that the extraction procedure was consistent and reproducible. The results indicated no obvious matrix effect.

Table 1 Precision, accuracy, extraction and matrix effect for analysis of dimemorfan phosphate in human plasma ($\bar{x} \pm s$)

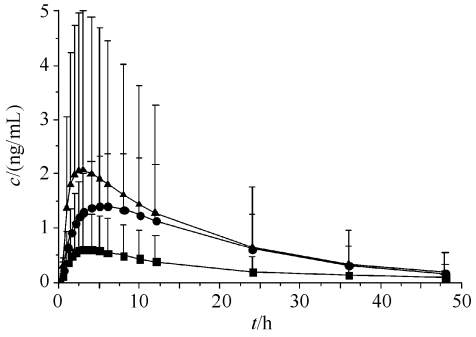
Nominal concentration /(ng/mL)	Intra-batch (n = 5)		Inter-batch(n = 3)		Extraction recovery/%	Matrix effect	
	Accuracy/%	RSD/%	Accuracy/%	RSD/%		Dimemorfan phosphate/%	IS/%
0.05	106.40 ± 4.94	4.64	102.08 ± 5.74	5.62	89.91 ± 6.63	97.72 ± 4.44	97.72 ± 1.30
0.5	99.89 ± 7.97	7.98	99.37 ± 6.20	6.24	90.00 ± 2.47	100.58 ± 3.14	
4	97.97 ± 11.91	12.16	97.44 ± 8.36	8.57	95.17 ± 3.88	109.29 ± 3.48	

The intra- and inter-day precision values (RSD) were less than 12.20%. Likewise, both intra- and inter-day accuracy were found to be 97.97%-106.40% and 97.44%-102.08%, with all samples located within general assay acceptability criteria according to guidelines.

2.2 *Pharmacokinetic analysis*

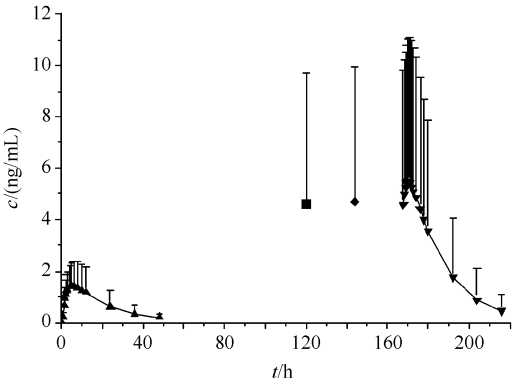
The valid and developed method was applied to dimemorfan phosphate concentration in the plasma of Chinese volunteers obtained following single-dose (10, 40 mg) and multiple-dose (20 mg) shown as Figure 2 and Figure 3, and also the partial main PK

parameters were shown in Table 2. The results revealed that non-parametric model analysis was better in data fitting.



—■—10 mg; —●—20 mg; —▲—40 mg

Figure 2 Mean plasma concentration-time profiles of dimemorfan phosphate in human plasma after single-dose oral administration of 10, 20, 40 mg dimemorfan phosphate granules ($\bar{x} \pm s$, $n = 12$)



—▲—day-1 to day-2; —■—day-6; —◆—day-7; —▼—day-8 to day-9

Figure 3 Mean plasma concentration-time profiles of dimemorfan phosphate in human plasma after multiple-dose oral administration of 20 mg dimemorfan phosphate granules ($\bar{x} \pm s$, $n = 12$)

Table 2 Main pharmacokinetic parameters[mean (SD)] after single-dose of 10, 20, 40 mg and multiple-dose of 20 mg dimemorfan phosphate granules ($\bar{x} \pm s$, $n = 12$)

Parameter	Single-dose			Multiple-dose
	10	40	20 mg	20 mg
c_{max}/(ng/mL)	0. 965 3(0. 817 8)	3. 150(3. 451)	2. 167(1. 650)	7. 418(7. 010)
$AUC_{0-48\ h}$/ (ng·h/mL)	11. 81(14. 46)	52. 60(96. 01)	34. 70(29. 59)	115. 9(135. 2)
$AUC_{0-\infty}$/ (ng·h/mL)	13. 19(16. 19)	55. 43(101. 17)	38. 09(32. 95)	122. 3(145. 0)
MRT/h	11. 89(2. 67)	13. 88(2. 19)	14. 86(1. 41)	13. 56(1. 35)
$t_{1/2}$/h	12. 11(2. 54)	12. 16(2. 01)	12. 77(1. 27)	11. 22(1. 61)
t_{max}/h	2. 67(0. 84)	2. 17(0. 54)	2. 79(1. 05)	2. 33(0. 75)
CL/F / (L/h)	1 771(1 278)	2 226(1 467)	868. 3(572. 6)	377. 1(292. 0)
$K_e/10^{-2}$	5. 967(1. 291)	5. 839(0. 942)	5. 481(0. 589)	6. 287(0. 857)
V	30 306(23 486)	41 583(33 226)	15 498(9 471)	6 449(5 408)
$c_{min,\ ss}$/ (ng/mL)	–	–	–	4. 565(5. 267)
$c_{av,\ ss}$/ (ng/mL)	–	–	–	5. 323(5. 597)
AUC_{ss}/ (ng·h/mL)	–	–	–	42. 58(44. 78)
DF	–	–	–	0. 61(0. 19)
$R_{c\ max}$	–	–	–	3. 14(1. 34)
R_{AUC}				3. 38(1. 22)

$t_{1/2} = 0.693/K_e$; $CL/F = K_e/V$; " – " indicate no parameter in this administration

Single-dose PK analysis The terminal phase half-life ($t_{1/2}$) estimated to be 11-13 h and was consistent over the three-dose levels. And $t_{1/2}$ tested via Kruskal-Wallis test indicated no obvious difference. $\bar{x} \pm s$ of t_{max} indicated that there was no significant difference of three-dose levels ($P > 0.05$). Additionally, no differences were detected between groups with respect to the dose-normalized c_{max} and AUC using the Homogeneity of variance test and T test. Pearson correlation analysis for $\ln c_{max}$, $\ln AUC_{0-48\ h}$ and $\ln AUC_{0-\infty}$ to lnDose, showed moderate relation with Pearson correlation coefficient 0. 511 5, 0. 455 6 and 0. 439 7. Furthermore, power model analysis displayed dose non-proportional increases in the c_{max} and AUC within a dose range of dimemorfan phosphate 10 to 40 mg. So the criterion interval was (0. 792 5, 1. 205 7) for c_{max} and (0. 839 0, 1. 160 9) for AUC. Using the power model, the 90% CIs for the slope were 0. 511 5 (0. 261 0, 0. 759 7) for c_{max}, 0. 455 6 (0. 196 8, 0. 713 2) for $AUC_{0-48\ h}$, 0. 439 7 (0. 177 1, 0. 698 5) for $AUC_{0-\infty}$.

Multiple-dose PK analysis 12 healthy volunteers after multiple oral administration of 20 mg dimemorfan phosphate, the PK parameters of t_{max} showed no significant difference ($P > 0.05$). The c_{max} was obviously different and AUC presented a trend of accumulation

with accumulation constant of (3.14 ± 1.34) for $R_{c\max}$ and (3.38 ± 1.22) for R_{AUC}.

Gender difference Mann-Whitney U tests were used for PK parameters such as t_{max} and $t_{1/2}$ and independent-samples t test for AUC, c_{max}, CL/F between male and female. The consequence of data analysis indicated no significant between sexes.

3 Discussion and Conclusion

PK parameters were calculated from the plasma concentration-time data. The values of $AUC_{0-48\,h}$ and c_{max} of single-dose of 10, 20 and 40 mg showed great variance for different volunteers after oral administration of dimemorfan phosphate. From the analysis, it was concluded that dose proportional of c_{max} and AUC of dimemorfan phosphate was not concluded for comparing the 90% CIs to the criterion interval.

Compard with previous reports[7], the PK parameters $t_{1/2}$ and t_{max} were similar. But the standard deviations of AUC and c_{max} were higher than those reported due to the individual difference. The reasons attributed to the individual difference were probably gene polymorphism of enzymes in the translation of dimemorfan phosphate. Studies have found that oxidation and *N*-demethylation of dimemorfan were involved in CYP450 liver enzymes. Research showed the multiple P450 forms including CYP2D6 and CYP3A4 played predominant roles in this metabolic pathway. In healthy male volunteers, more than 98% of dimemorfan was metabolized and no metabolites had antitussive effect[9-10]. These findings were similar to a previous report about CYP2D6 genotype affecting the pharmacokinetics of dimemorfan phosphate[11]. Even the adverse reactions of dimemorfan phosphate are mild[12], so it is possible to adjust the dosage for special people like children, elder and so on.

As for multiple-dose study, the result demonstrated that there may be drug accumulation, thus the dose interval should be increased, for the $t_{1/2}$ about 12 h was longer than the dose interval, to reduce the phenomenon of accumulation, which may cause undesirable side-effects in various degrees. The activity of CYP2D6 and 3A4 in the metabolism of dimemorfan were enzyme saturated conditions[9-11].

The elimination rate constant (K_e) and apparent volume of distribution (V) of single-dose study showed no significant difference. There were different volunteers for single-dose and multiple-dose study, so V of two groups was significantly different to some degree. Also observed after multiple dosing were significantly lower apparent volume of distribution of dimemorfan phosphate, high plasma protein binding of drugs and less organization distribution which caused the high blood drug concentration. Thus it also explained drug accumulation after multiple-dose administration.

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