



## Pharmacokinetics of Amoxicillin/Clavulanic Acid Combination after Oral Administration of New Suspension Formulations in Human Volunteers

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### Abstract

The pharmacokinetic properties of amoxicillin and clavulanic acid when used alone or in combination may be different and show interaction between these two agents that might decrease the absolute bioavailability of clavulanic acid. In an open, randomized, replicated Latin square under fasting condition, pharmacokinetics of new formulations of amoxicillin/clavulanic acid were compared with a reference formulation after single dose administration in 15 healthy male volunteers. Subjects were given equal molar doses of new suspension formulations of amoxicillin/clavulanic acid (312 mg/5 ml or 156 mg/5 ml) or Augmentin<sup>®</sup> (312 mg/5 ml) as the reference product. The wash-out period was one week between the administrations of these antibacterial agents. Blood samples were collected exactly before and after drug administration of each of the formulations at different time points up to 6 h. The concentrations of the antibiotics in plasma were measured by validated HPLC methods. Three formulations exhibited a similar mean  $C_{max}$  of about  $7.5 \pm 1.6$  mg/l after  $T_{max}$  of about  $75 \pm 25$  min. for amoxicillin and  $C_{max}$  of about  $2.5 \pm 0.6$  mg/l after  $T_{max}$  of about  $61 \pm 15$  min. for clavulanic acid. The  $AUC_{0-inf}$  (total area under the curve) for amoxicillin was about  $1278 \pm 172$  g.min/ml and it was about  $354 \pm 66$  g.min/ml for clavulanic acid. There were no significant differences in pharmacokinetic parameters among these formulations. Pharmacokinetic parameters of amoxicillin and clavulanic acid found in this study were similar to previously published data. The two generic formulations investigated in this study proved to be bioequivalent with brand-name Augmentin<sup>®</sup> with regard to the pharmacokinetic parameters  $C_{max}$ ,  $AUC_{0-t}$ ,  $AUC_{0-inf}$  and  $T_{max}$ . Moreover, the parametric confidence intervals (90%) for the ratio of the  $C_{max}$ ,  $T_{max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-\infty}$  values lie between 0.8-1.2 based on log transformed values. We may conclude that the two new formulations are bioequivalent with the reference suspension and could be considered equally effective in medicinal practice. Moreover, there were no interaction in pharmacokinetic parameters between amoxicillin and clavulanic acid. No serious adverse event was observed with the studied drugs.

**Keywords:** Amoxicillin; Clavulanic acid; Pharmacokinetics; Suspension.

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## 1. Introduction

Clavulanic acid is added to amoxicillin to inhibit  $\beta$ -lactamase and increase the antibacterial effect of amoxicillin, and their combination is used as a broad spectrum antibiotic for treatment of a wide range of bacterial infections, including upper and lower respiratory tract infections and infections of the skin and soft tissue structures [1-4]. It was first introduced into clinical medicine in Europe in 1981 and in United States in 1984. Since its release, combination of amoxicillin and clavulanate has been extensively used in patients of all ages including infants, children and adults [5-7]. The highly desirable antibacterial spectrum of the drug combined with its favorable pharmacokinetic and safety profiles underscore its rapid acceptance as one of the most commonly prescribed antibiotics. In many countries, the standard regimen for pediatric patients aged over 3 months for the treatment of mild to moderate infections is now amoxicillin/clavulanic acid 25 mg/3.6 mg per kg/day, divided in either two or three doses. For more severe infections such as acute otitis media, the standard regimen is amoxicillin/clavulanic acid 45 mg/6.4 mg per kg/day divided in two doses [8]. Reported data support a nonlinear absorption process for amoxicillin. Saturable transport mechanisms, limited solubility and the existence of an absorption window are possibly involved in the gastrointestinal absorption of amoxicillin leading to a decrease in the pharmacokinetic parameters of this drug. Furthermore, a possible interaction between amoxicillin and clavulanic acid that might decrease the absolute bioavailability of clavulanic acid is reported [9]. New oral suspensions of this combination were

prepared; therefore, their pharmacokinetic characteristics need to be evaluated and compared to the same product produced by the innovator. In this regard, pharmacokinetic parameters of active ingredients of this product were measured and compared with the reference standard (Augmentin<sup>®</sup>) available in the market. Then the data were analyzed to determine whether the test and the reference products yield comparable values.

## 2. Materials and methods

### 2.1. Materials

Amoxicillin/clavulanate potassium (Co-amoxiclav<sup>®</sup>) for oral suspensions; 312 mg/5 ml (250 mg amoxicillin plus 62.5 mg clavulanic acid, T<sub>1</sub>) and 156 mg/5 ml (125 mg amoxicillin plus 62.5 mg clavulanic acid, T<sub>2</sub>) from Farabi Pharmaceutical Company, Isfah, Iran, were evaluated. These formulation were compared with the reference product (R) Augmentin<sup>®</sup> produced by Beecham Pharmaceutical Company, England, for oral suspension; 312 mg/5 ml (250 mg amoxicillin plus 62.5 mg clavulanic acid).

Solvents used for drug measurement, were of HPLC grade; while other chemicals and reagents were of analytical grade. Amoxicillin and clavulanic acid powder were purchased from Beecham Pharmaceuticals, Brantford, England. Other materials were purchased from local market.

### 2.2. Study subjects

Fifteen healthy adult male subjects were enrolled in the fasting study. Fourteen subjects completed all three phases of the study. Subjects ranged in ages from 21 to 38 years, in body weight from 57 to 85 kg and in height from 163 to 185 cm. All subjects were in good health as indicated by medical history (history

**Table 1.** Pharmacokinetic parameters of amoxicillin for three formulations (mean $\pm$  S.D.)

Formulations	C <sub>max</sub> (mg/l)	T <sub>max</sub> (h)	T <sub>1/2</sub> (h)	Cl <sub>PO</sub> (l/h)	AUC <sub>0-∞</sub> (mg.h/l)
T1	7.59 $\pm$ 1.45	1.2 $\pm$ 0.40	1.166 $\pm$ 0.16	26.34 $\pm$ 3.68	19.32 $\pm$ 2.66
T2	7.32 $\pm$ 1.68	1.26 $\pm$ 0.45	1.286 $\pm$ 0.14	25.65 $\pm$ 4.26	18.57 $\pm$ 3.11
R	7.30 $\pm$ 1.72	1.23 $\pm$ 0.35	1.140 $\pm$ 0.10	27.66 $\pm$ 4.77	20.00 $\pm$ 3.37

or evidence of hepatic, renal, gastrointestinal and hematological disorders, acute or chronic diseases or allergy to  $\beta$ -lactam antibiotics), physical examination and clinical laboratory tests (hematology and blood biochemistry). Also, the subjects were not permitted to smoke, to take any drug and to do hard physical activity from two weeks before to the end of study and not to have beverages and foods containing caffeine during the study.

The volunteers were informed about the risk and the aim of the study and signed the written informed consent statement before entering the study.

### 2.3. Drug administration and sample collection

The study was designed based on a single-dose, replicated Latin square under fasting condition. After an overnight fasting (10 h), subjects were given equal molar doses (10 ml of T<sub>1</sub>, 20 ml of T<sub>2</sub> or 10 ml of R) of the drug followed by 250 ml water. They were fasted over 2 h post-dose then they received the same breakfast and lunch according to the time scheduled. Therefore, all subjects received equivalent 500 mg amoxicillin and 125 mg clavulanic acid on three occasions separated by a 7-days wash out period.

To determine amoxicillin and clavulanic acid concentrations, samples of venous blood (8 ml) were collected at 0 h pre-dose and at 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, and 6 h post-dose, and transported to the laboratory on dry ice for analysis.

### 2.4. Chromatographic conditions

Serum samples were analyzed to measure amoxicillin and clavulanic acid concentrations. The validated HPLC chromatographic methods for each active

ingredient [10, 11] were used. The HPLC system was consisted of 515 isocratic pump Waters® equipped with 717 plus auto sampler with heater/cooler system and dual-absorbance UV-visible detector connected to a Millennium® 32 software data integrator. Chromatographic separation was performed on a reversed 2.1x150 mm i.d. stainless steel C<sub>18</sub>-bondapack (3.5  $\mu$ m particle size) column connected to a C18 guard column. The mobile phase composed of potassium dihydrogen phosphate 0.05 M (pH=2.75), methanol and acetonitrile (94:3.5:2.5 %V/V).

### 2.5. Calibration curve

Standard curve was produced by preparing nine plasma standards over the range of 0.2-20 g/ml or 0.05-10 g/ml for either amoxicillin or clavulanic acid, respectively. Standards were analyzed in triplicates (n=9).

### 2.6. Pharmacokinetic data analysis

Pharmacokinetic analysis was performed by model independent method using SPSS® and MS Excel® softwares. The maximum amoxicillin concentrations ( $C_{max}$ ) and the corresponding Tmax were determined by the inspection of the individual drug plasma concentration-time profiles. The elimination rate constant (Kel) was obtained as the slope of the linear regression of the log-transformed plasma concentration values versus time data in the terminal phase.  $T_{1/2}$  was calculated as  $0.693/Kel$ . AUC to the last measurable concentration ( $AUC_{0-t}$ ) was calculated by the linear trapezoidal rule. AUC extrapolated to infinity ( $AUC_{0-\infty}$ ) was calculated by equation  $AUC_{0-t} + C_t/Kel$  where  $C_t$  is the last measurable concentration. Oral clearance ( $Cl_{p.o.}$ ) was calculated as  $D/AUC_{0-\infty}$ , and volume of distribution (Vd/F) was calculated by dividing corresponding  $Cl_{p.o.}$ . The relative bioavailability was calculated by dividing

**Table 2.** Pharmacokinetic parameters of clavulanic acid for three formulations (mean $\pm$  S.D.)

Formulations	$C_{max}$ (mg/l)	$T_{max}$ (h)	$T_{1/2}$ (h)	$Cl_{p.o}$ (l/h)	$AUC_{0-\infty}$ (mg.h/l)
T1	2.60 $\pm$ 0.68	1.00 $\pm$ 0.18	1.03 $\pm$ 0.12	22.20 $\pm$ 5.90	6.11 $\pm$ 1.38
T2	2.43 $\pm$ 0.50	1.00 $\pm$ 0.23	1.02 $\pm$ 0.15	22.98 $\pm$ 3.68	5.91 $\pm$ 1.20
R	2.44 $\pm$ 0.53	1.05 $\pm$ 0.33	1.06 $\pm$ 0.07	22.73 $\pm$ 5.59	5.68 $\pm$ 0.72

**Table 3.** Statistical parameters for pharmacokinetic parameters of amoxicillin after two treatments.

Formulations	Parameter	ANOVA		90% Confidence interval
		F <sub>calculated</sub>	F <sub>critical</sub>	
T1	C <sub>max</sub>	0.22	4.23	95-116%
	T <sub>max</sub>	0.07	4.23	80-116%
	AUC <sub>0-6</sub>	0.35	4.23	90-104%
	AUC <sub>0-∞</sub>	1.48	4.23	86-100%
T2	C <sub>max</sub>	0.01	4.23	92-109%
	T <sub>max</sub>	0.07	4.23	86-119%
	AUC <sub>0-6</sub>	1.37	4.23	88-98%
	AUC <sub>0-∞</sub>	3.35	4.23	84-94%

AUC<sub>0-∞</sub> values of the active ingredient of new formulations over the same values after administration of the standard formulation (Augmentin®).

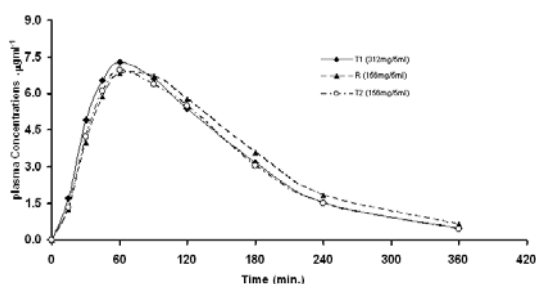
## 2.7. Sample preparation for HPLC injection

### 2.7.1. Amoxicillin

Each serum sample was transferred to a filter tube for centrifugation at 5 °C and 5000×g for 40 min. A 20 l aliquot from the filtrated liquid was injected to the chromatograph by an autosampler. Wavelength of UV detection was set at 229 nm. The mobile phase was pumped at a flow rate of 0.2 ml/min. and the run time was regulated at 12 min.

### 2.7.2. Clavulanic acid

Each serum sample was transferred to a filter tube for centrifugation at 5 °C and 5000×g for 40 min. A 100 l aliquot of imidazole buffer was added to 400 l of the filtrated sample. The obtained solutions were vortexed and kept at 30 °C for 13 min. Then,



**Figure 1.** Mean concentration-time profiles of the amoxicillin in three formulations.

10 l was injected into the column by auto sampler. The wavelength of UV detection was set at 320 nm and flow rate of mobile phase was 0.1 ml/min. Run time was regulated at 10 min.

## 2.8. Statistical analysis

For the purpose of pharmacokinetic analysis, for each active ingredient, AUC<sub>0-t</sub>, AUC<sub>0-inf</sub>, C<sub>max</sub>, T<sub>max</sub>, t<sub>1/2</sub>, Cl<sub>p.o.</sub> and V<sub>d</sub> were compared as the pharmacokinetic variables. The difference between two related parameters was considered statistically significant for a p ≤ 0.05. After logarithmic transformation C<sub>max</sub>, AUC<sub>0-t</sub>, and AUC<sub>0-∞</sub> (or AUC<sub>0-Inf</sub>) were analyzed as per current FDA guidelines [12, 13].

## 3. Results and discussion

New formulations were tolerated well by the volunteers. Unexpected incidents that could have influenced the outcomes of the study did not occur. All volunteers who had started the study and continued to the end were discharged in good health. All formulations were readily absorbed from the gastrointestinal tract, and active ingredients were measurable at the first sampling time (15 min.) in all volunteers. The mean concentration-time profiles for the three formulations of amoxicillin and clavulanic acid are shown in Figures 1 and 2. A sharp peak in serum amoxicillin concentrations at ~1 to 1.5 h after administration, with a sharp decline thereafter was observed indicating a

**Table 4.** Statistical parameters for pharmacokinetic parameters of clavulanic acid after two treatments.

Formulations	Parameter	ANOVA		90% Confidence interval
		F <sub>calculated</sub>	F <sub>critical</sub>	
T1	C <sub>max</sub>	0.45	4.23	93-118%
	T <sub>max</sub>	0.21	4.23	82-111%
	AUC <sub>0-6</sub>	0.98	4.23	93-119%
	AUC <sub>0-∞</sub>	1.14	4.23	94-119%
T2	C <sub>max</sub>	0.01	4.23	87-113%
	T <sub>max</sub>	0.11	4.23	84-114%
	AUC <sub>0-6</sub>	0.35	4.23	92-115%
	AUC <sub>0-∞</sub>	0.38	4.23	92-114%

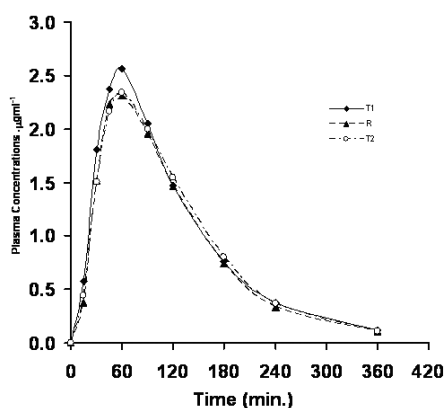
prompt distribution to the peripheral compartment. The terminal elimination half life of amoxicillin of about 1.2 h was similar in all formulations. The reduced terminal elimination half life (1-2 h in all formulations) indicates that amoxicillin was rapidly eliminated from the body and no accumulation occurred after repeated doses in subjects with normal renal function.

Tables 1 and 2 show the pharmacokinetic parameters for the three tested products for amoxicillin and clavulanic acid, respectively. All calculated pharmacokinetic values were in good agreement with previously reported studies that contain the same unit dose of the drug [14, 15]. Drug clearance is more than the average glomerular filtration due to tubular secretion, thus explaining why constant administration of probenecid decreases the urinary excretion of amoxicillin leading to a slower elimination rate. Amoxicillin is rapidly

and completely absorbed, and a high fraction of the dose reaches the systemic circulation within a short time ( $t_{max} < 76$  min. to give a C<sub>max</sub> of about 7.5 g/ml) under both fasting and non-fasting conditions [16].

The disposition of clavulanic acid is also characterized by the initial rapid phase, indicating easy distribution to the peripheral compartment. The short half life (~ 1 h) is the consequence of the rapid elimination from the body due to metabolism and renal excretion. Distribution studies reported for amoxicillin/clavulanic acid have shown that the access of clavulanic acid to ascetic fluid, synovial fluid, bone tissues, gynecological tissues, and sputum is similar to that reported for amoxicillin. However, the distribution of clavulanic acid is slightly lower than that established for amoxicillin which may be contributed to lower lipid solubility of clavulanic acid than amoxicillin.

To measure the relative bioavailability of new formulations, the 90% confidence intervals for the natural log-transformed data were also calculated according to the FDA guidelines [13] and the results are shown in Tables 3 and 4. The means and standard deviations of AUC<sub>0-t</sub>, AUC<sub>0-inf</sub>, C<sub>max</sub>,  $t_{1/2}$ , Cl<sub>p.o.</sub> and T<sub>max</sub> of the two test products in comparison to the reference product did not show any significant differences for either amoxicillin or clavulanic acid, suggesting that the plasma profiles generated by Co-Amoxiclav<sup>®</sup> suspensions are comparable to those produced by Augmentin<sup>®</sup>. Statistical

**Figure 2.** Mean concentration-time profiles of the clavulanic acid in three formulations.

analysis for these parameters did not differ significantly between each test with reference formulations. The pharmacokinetic behavior of clavulanate in these formulations also showed no differences from those of the existing formulation of Augmentin®.

The 90% confidence intervals also demonstrated that the ratios of  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ ,  $C_{max}$  and  $T_{max}$  of formulations and for the three periods lie within the FDA acceptable range of 80-125% for both ingredients.

On the bases of the plasma levels of the 14 volunteers completed the study, the mean relative bioavailability of amoxicillin for  $T_1$  and  $T_2$  were 96.61% and 92.83% for  $AUC_{0-t}$ , 92.93% and 89.32% for  $AUC_{0-\infty}$ , 103.89% and 100.21% for  $C_{max}$ , 97.10% and 102.90% for  $T_{max}$  clearly indicated no significant difference between tests and reference products in any of the calculated pharmacokinetic parameters. The confidence intervals (CIs) for the ratios of mean  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ ,  $C_{max}$ , and  $T_{max}$  indicated that these values are entirely within the bioequivalence acceptable range of 80%-125% (using log transformed values). Similar results were observed for clavulanic acid as well (Table 4).

#### 4. Conclusion

Pharmacokinetic parameters of amoxicillin and clavulanic acid in formulations used in this study were similar to previously published data [17, 18]. Furthermore, the new formulations of Co-Amoxiclav® (312 and 156 mg/5 ml) suspensions is bioequivalent to the reference formulation (Augmentin® 312 mg/5 ml) manufactured by Beecham, England. Therefore, the three products evaluated in this study may be considered equally effective in medicinal practice by using the same molar doses.

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