Bioequivalence of Two Formulations of Amoxicillin in Human Healthy Volunteers on (HPLC) Technique

Alaa K. Jabbar Alhamd[*, 1]

[*] Department of Medicinal Sciences, College of Pharmacy, Al-Mustansirya University, Baghdad , Iraq

Abstract

Amoxicillin is commercially available in the form of capsules and tablets containing 250mg or 500mg for oral administration. It is also available in the form of suspension containing "25mg/ml" . Amoxicillin is presently used as the most common antibiotics .Ten healthy Human volunteers were characterized respected to their pharmacokinetic and bioavailability of two formulations of Amoxicillin from two sources of industrial companies after a single dose administration was given orally. A procedure is described for determination the concentration levels of Amoxicillin in human plasma of healthy volunteers using high performance liquid chromatography (HPLC) with reversed-phase isocratic column at low wave length of UV-visible detection "230nm". An efficient drug extraction procedure was used for the separation of Amoxicillin after simple extraction with cold methanol using ODS-C_{18}-DB column. The pharmacokinetic 500mg of Amoxicillin capsule orally administrated treatment through 10 hours has been examined. The Amoxicillin was eluted for "10.0 minutes" at flow Rate "1.5ml/min." and Temperature equal to 298 K .The retention time of Amoxicillin was observed at 7.0 minutes. The mean absolute recovery of Amoxicillin in blood plasma of all healthy volunteers were 94.1% at 1.0ppm, 102% at 5.0ppm, 103% at 10.0ppm 102% at 20ppm, 99.3% at 40ppm and 104% at 50ppm respectively. The assay showed excellent relationships between area under the curve ratios and drug concentration levels (P>0.002) .Oral Amoxicillin administration in ten healthy volunteers gave maximum concentration peak plasma at two hours and decline through ten hours. Treatment with Iraqi formulation Amoxicillin produced higher area under the curve "AUC" and maximum concentration "C (max)" of Amoxicillin than Indian formulation.

Key word: Amoxicillin, Bioequivalence, ODS -DB column.

الخلاصة

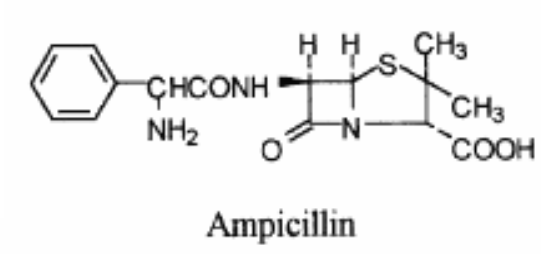
يتوفر الاموكسيلين تجاريا على شكل كبسولات و حبوب تحتوي الاولى على ٢٥٠ ملي غرام والثانية على ٥٠٠ ملي غرام كما ويتوفر على شكل محلول عالق بتركيز ٢٥ ملي غرام / ملي لتر حيث توخذ الجرعة عن طريق الفم ويعتبر الاموكسلين المضاد الحيوي الاكثر أستخداما . درست حركية الدواء والتوافر الحيوي على توليفتين من كبسولات الاموكسلين لشركتين مختلفتين طبقت على عشرة من الناس الاصحاء المتطوعين بجرعة ٥٠٠ ملي غرام اخذت عن طريق الفم . توضح الطريقة التي حسبت فيها مستويات تراكيز الاموكسيلين في بلازما الدم لعدد من الناس الاصحاء والمتطوعين باستخدام جهاز كروموتوغرفيا السائل ذات الاداء العالي (HPLC) على نماذج بلازما الدم استخلص منها الاموكسيلين بالميثانول المبرد على عمود من نوع (ODS-C_{18}-DB). عينت مستويات تراكيز الاموكسيلين على جهاز كروموتوغرافيا السائل ذات الاداء العالي بزمن قدره عشر دقائق وبسرعة طور متحرك ١.٥ ملي ليتر / دقيقة وبمحيط حراري ٢٩٨ درجة كلفن و وقت الاحتجاز للاموكسيلين ٧ دقائق , حسبت النسبة المئوية للاسترجاع في بلازما الدم للتراكيز(١ , ٥ , ١٠ , ٢٠ , ٤٠ , ٥٠ بي بي أم)على التوالي (٩٤.١ , ١٠٢ , ١٠٣ , ١٠٢ , ٩٩.٣ , ١٠٤%) بمستوى ثقة (P>0.002) اشارت النتائج بان اعلى تركيز في بلازما الدم تكون خلال ساعتين من وقت الجرعة المأخوذة عن طريق الفم ثم يبدأ التركيز بالنزول حتى الساعة العاشرة من وقت الجرعة كما وان النتائج اشارت بان سلوك التكافوء الحيوي للتوليفتين غير مشابه والاختلاف في المساحة والتركيز الاعلى للتوليفتيين لان التوليفة العراقية اعلى توافر حيوي من التوليفة الهندية .

Introduction

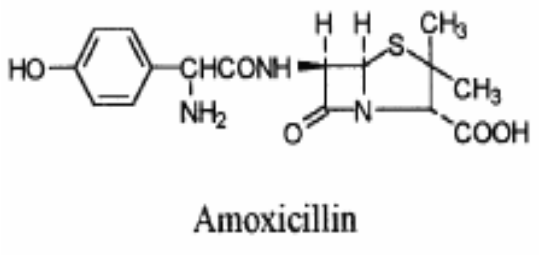
Amoxicillin capsules and Amoxicillin suspensions were analyzed for their drug content as described in the united state pharmacopeia [(1)]. Amoxicillin is the most commonly prescribed antibiotic for the treatment of phyaryngitis in the US.[(2)] Pharmacokinetic and bioavailability may be vital to ensure successful protocol in the clinic as well as in researches. Often the clinical evaluation of drugs is carried out on the basis of some secondary response because of the non existence of directly measurable parameter which is related to the treatment of disease by the drugs [(3)] .Many times no response is measured at all, and the clinician attempts to make objective and subjective assessment of the patients general welfare. A dosage regimen for a new drug may in fact be based on such an evaluation and may not include a comparison with a standard drug or analog, a dosage regimen based upon such studies can be only a rough approximation at best. this point is well illustrated if one compares drug dosage regimens (e.g. sulfonamides) calculated

1Corresponding author E- mail : prof_alaaalhamd@yahoo.com
Received : 11 /3 /2009
Accepted : 28/12/2009

from pharmacokinetic data to those commonly used in the clinic. The same importance from this point of view does not concern only medicines but all other compounds including pharmaceuticals which are introduced to the organism for the diagnostic purposes. There is a through pharmacokinetic examination of a diagnostic agent which is extremely important from the point of view of decreasing of the possible risk [4].Pharmacokinetics are the studies of the movement of drugs in the body through the time course; it must not be hidden in mathematics itself. It is extremely important for everybody who is interested in pharmacotherapeutics in drug researches and in drugs productions to understand what the pharmacokinetics really means [5]. Pharmacokinetic study of children that assessed the single-dose administration of an investigational oral Amoxicillin sprinkle designed to sequentially deliver an immediate-release and multiple delayed-release pulses of Amoxicillin to provide prolonged plasma concentrations of Amoxicillin [6]. A new pharmacokinetically enhanced formulation of Amoxicillin has recently become available [7]. The new formulation can maintain the main Amoxicillin serum concentration about 49% of the dosing interval in contrast with only 34% for three times daily regimen [7,8]. The pharmacodynamic and pharmacokinetic properties of the new formulation predict high rates of success against respiratory tract pathogens [8]. Amoxicillin (AMO) is oral semi-synthetic penicillin structurally related to Ampicillin as shown below:



Ampicillin



Amoxicillin

The present of benzyl ring in the side chain extends the antibacterial activity to gram-negative bacteria [9]. Amoxicillin presents as a highly absorption after oral administration and is not altered by the concomitant ingestion with food [10]. Amoxicillin exhibits low binding with plasma proteins and is quickly distributed through the body. It has an elimination half life of one hour [11]. Amoxicillin pharmacokinetics was obtained across pregnancy states. Oral clearance and renal clearance were higher while the half life was shorter during pregnancy, these changes suggest that the Amoxicillin exposure will be less while pregnancy that maintenance of trough concentrations will be difficult [12]. Anew per oral Amoxicillin \ Clavulanate therapeutic system was developed and evaluated by in vivo bioavailability study [13,14,15]. Amoxicillin was effective in reducing oral micro organism level up to 12 hour post-dose [16]. The treatment with Amoxicillin for 3 to 7 days had similar clinical efficiency and also similar selection of oral streptococci with reduced susceptibility to Amoxicillin [17]. Amoxicillin is commercially available in the form of capsules and tablets (250 or 500 mg) for oral administration and also available in the form of suspensions containing "25 or 50 mg / ml". Amoxicillin is one as the most commonly used as antibiotic. To understand the bioavailability and pharmacokinetic behavior of this drug in human which is needed reliable qualitative and quantitative methods. There are several high performance liquid chromatography (HPLC) methods were used for separation and determination of Amoxicillin in body fluids .Some of these methods were developed to use direct UV-visible detection at low wave length (225-229nm) [18—22]. The bioavailability of two brands of melixican (7.5mg and 15mg) tablets and to obtained pharmacokinetic parameters of this molecules on Mexican population using modified and validated high performance liquid chromatography "HPLC" technique pharmacokinetic parameters AUC , C(max) , and T(max) were determined from plasma concentration levels of both formulations that the results indicated in a C(max) 120% larger and T(max) 65% faster than those reported[23,24]. Others were used fluorimetric detection [25—28]. Some others special techniques have been used to enhance the sensitivity and selectivity such as ion-pairing reagents and post column derivatization [21, 28, 29]. There are several different methods used to preparation the sample that have been applied prior to chromatographic analysis mostly based on

liquid- liquid extraction [28] , deproteinization by precipitation [19,25] and solid phase extraction [18,30,31]. Plasmatic Amoxicillin concentration levels were determined by combined reversed-phase liquid chromatography and mass spectrometry with positive ion electro spray ionization using the selective ion monitoring technique [32, 33]. The paper presents the favored approach of clinical studies involved in qualitative and quantitative assay of pharmacokinetics of two Amoxicillin formulations firstly, Iraqi formulation of capsule containing 500mg Amoxicillin compared with Indian formulation capsule containing 500mg Amoxicillin. After evaluation of the various condition of the (HPLC) assay, a suitable and simple assay for the determination of Amoxicillin in human plasma of healthy volunteers was developed using reversed-phase isocratic (HPLC) at direct low wave length of UV-visible detection (230nm) and temperature equal to 298 K for subsequent study of pharmacokinetics and bioavailability.

Experimental
Materials and methods

Chemicals and drugs

All chemicals used in this study were the highest analytical grade purchased from commercial sources and used without any further purification. The deionized distilled-water was used for all preparation. Methanol (Absolute MeOH) and acetonitrile (Absolute ACN) "HPLC grade" were purchased from (FLUKA). Amoxicillin capsules from two sources one from Iraq (S D I, Iraq) and the other from India(MICRO LABS LIMITED ,Bangalore ,India) Potassium dihydrogen phosphate (KH_2PO_4), Dipotassium hydrogen phosphate (K_2HPO4), Phosphoric acid and 1-octane sulphonic acid sodium salt were purchased from (BDH, England). Sepelco-ODS-C_{18}-DB column (250 X 4.6mm I.D.) was purchased from (sepelco, United Kingdom).

Standard solutions

Amoxicillin (1.0 mg) was dissolved in 100ml of freshly prepared mixture of water: methanol (95:5) "10000 ppm". The standard solution was filtered, degassed and stored at 253 K for further use. The standards were prepared freshly every month. Stock solution of Amoxicillin (1mg/ml) was prepared in a mixture of (water: methanol) (95: 5). The applied standard solutions were prepared from stock solution by sequential dilution with the same mixture to produce final concentrations (1, 5, 10, 20, 40, 50ppm). The stock and applied solutions were protected from light and stored at 253 K. Calibration standard curve were performed to achieve the concentration of (0.1, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0ppm) Figure-1.

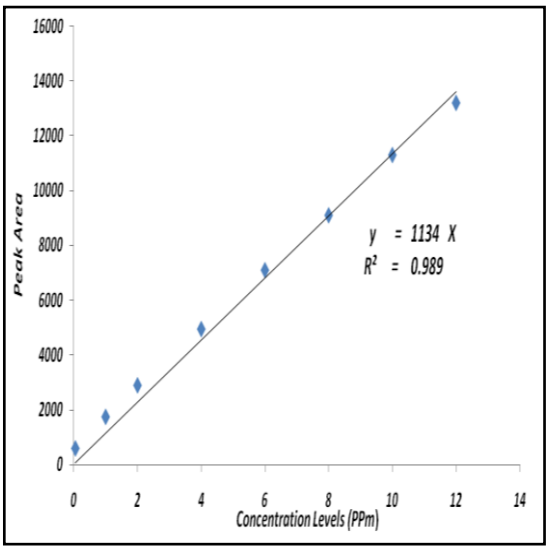


Figure 1: Linearity of different concentration levels of Amoxicillin using HPLC technique on ODS-DB column

Extraction of Amoxicillin

Blood sample (3--5ml) were drawn from vein by syrings in to hyparinized blood tubes, then transferred immediately into polyproplene tubes and centrifuged within 5 min. at 500G for 15 min. One milliliter of sodium metabisulphate, (pH equal to 8.0) was added for each one milliliter of plasma. Amoxicillin was extracted from human plasma samples by deproteinization using precipitation process. A 500µl aliquot form each plasma sample was transferred to a 5.0ml polypropylene tube. One milliliter of cold methanol was added. After slightly vortex mixing, the tubes were centrifuged for 15min. at 500G. A 100µl aliquot of the supernatant was transferred to the injection vials and 10µl were injected into chromatographic system. All samples from volunteers were analyzed on the same day in order to avoid inter-assay variation. Plasma solutions were protected from the light and stored in a deep freezer at (203 K).

HPLC Instrumentation

This study was performed on Shimadzu instruments model LC-6A HPLC system. The unit was operated in the isocratic model using solvent reservoirs fitted with 0.22 µm stainless steel filter at the end of polytriflouroethylene (PTFE) tubes, transferring the mobile phase from reservoirs to the pump, the system also involved an injector with 50µL sample loop

model (Reseadyre 7125) , Column in type of ODS-C_{18}-DB "250 X 4.6mm I.D.", Thermostatic oven model CTO-6A Shimadzu, UV-visible detector model "SPD" and chromatopac unit model R_4-6A Shimadzu.

HPLC Operation Condition

For routine HPLC analysis of Amoxicillin use the following estimation condition. The mobile-phase was phosphate buffer conc. 10mM that containing 0.1mM 1-octane Sulphonic acid sodium salt: methanol (95:5) (v/v), pH buffer equal to six, column temperature 298 K, flow rate equal to (1.5ml/min.) and UV-visible detection at 230nm. The typical chromatograms of standard solution and blood plasma samples of Amoxicillin are shown in fig-2 and fig-3 respectively.

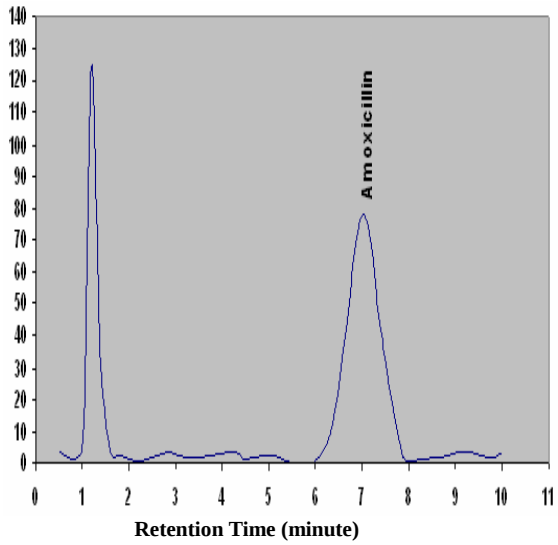


Figure 2: Typical chromatogram of HPLC analysis of Amoxicillin on ODS-DB column

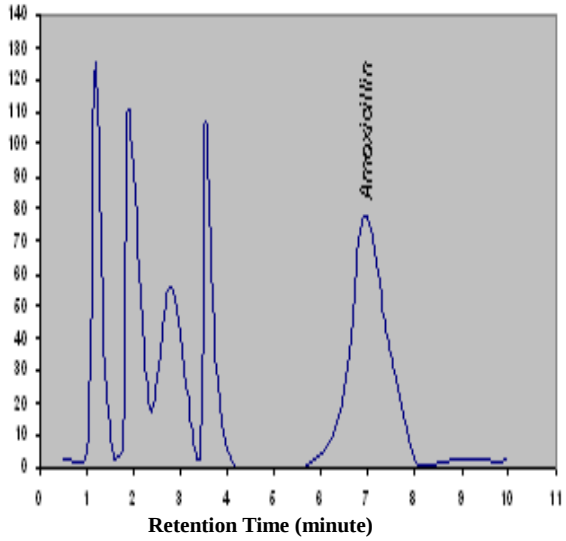


Figure 3: Typical chromatogram of HPLC analysis of plasma Amoxicillin on ODS-DB column

Pharmacokinetics and statistical analysis

The observation of maximum plasma concentration levels (C(max)) and time consuming to reach it (T(max)) were obtained from drug concentration versus time curves. The area under the curve "AUC" of the Amoxicillin concentration levels versus time from 5.0 minute to ten hours were estimated from "figure-4".

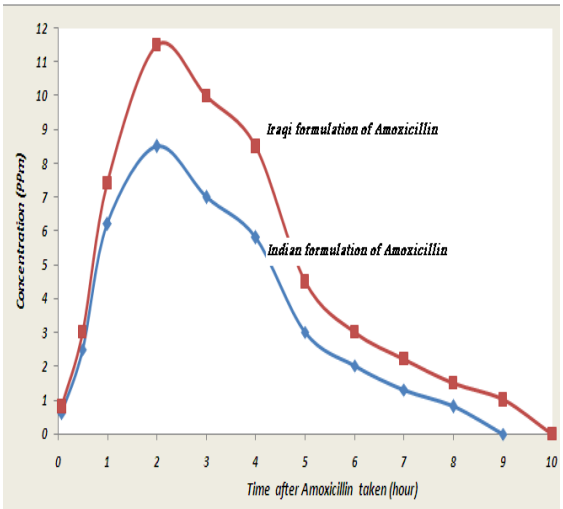


Figure 4: Pharmacokinetics of Iraqi formulation and Indian formulation Amoxicillin in blood plasma of healthy volunteers

Results

The isocratic reverse-phase HPLC technique described and used here for estimation of drug provides the appropriated sensitivity, specificity and high sample accuracy for bioavailability and pharmacokinetic studies. Fig.1 shows the retention time of Amoxicillin standard solution that under described chromatographic condition. The retention time of Amoxicillin was 7.0 minutes. The optimal chromatogram of analysis was given an ideal shape, symmetrical, and good resolution of peak. Fig.-2 shows the typical chromatogram of Amoxicillin in blood plasma samples of healthy volunteers which was appeared no endogenous Interfering peaks at the retention time of interest compound. The mean absolute recovery of Amoxicillin in blood plasma was 94.1% at 1.0ppm, 102% at 5.0ppm, 103% at 10.0ppm 102% at 20ppm, 99.3% at 40ppm and 104% at 50ppm respectively. The calibration curve was linear with regression coefficient R^2 =0.989 (Table-1). The analytical precision and accuracy values was obtained from assays of six quality control (1, 5.0, 10.0, 20.0, 40.0, and 50.0 ppm) are shown in table-1 .The accuracy were 94.1% , 102% , 103% ,

102%, 99.3% and 100% respectively and there is not significant degradation of Amoxicillin was observed during the period of storage.

Table 1: the linearity, precision and accuracy of blood plasma Amoxicillin samples.

Spiked concentration (ppm)	1.0	5.0	10.0	20.0	40.0	50.0
Recovered average con. (ppm)	0.94	5.1	10.3	20.4	39.7	50.2

Slope	R^2	P.V
1134	0.989	0.001

Discussion

The HPLC technique presented in this study decreases the lower limit of quantitation of Amoxicillin to about 0.1ppm. It was appeared that is more sensitive than many other assays. The low limit of the estimation of the plasma concentration of Amoxicillin was sufficient to perform the pharmacokinetics study of drug. Amoxicillin plasma concentration levels were measured by several methods in combination with UV-visible detection. The lowest plasma concentration levels of Amoxicillin was obtained by UV-visible detection which was 0.05ppm but the process was consumed long time that was 30min. [10,14] .Charles et al were described a procedure for determination of Amoxicillin in urine[8]. Other complicated procedures for extraction and estimation of plasma concentration levels of Amoxicillin by using Solid-phase extraction has been also reported [7, 9, 18, &19]. Nevertheless, Solid-phase extraction (SPE) procedures are laborious and require SPE cartridges, increasing the cost of analysis. In order to improve the sensitivity, a column ion-pair HPLC with post column derivatization has been used[16]. Where the low limit of quatitation was 0.01ppm, this procedure was more complicated due to the more step of post column derivatization and their retention time that will be longer than 10min.which is compared with our procedure that the retention time of Amoxicillin peak was 7min. and the full time of process not greater than 10min. . Also these procedures cannot be used in pharmacokinetics studies in human where a large number of samples were analyzed.The pharmacokinetics study was done in "10 hours" and the results indicate that the Iraqi formulation has higher bioavailability compared to the Indian formulation depending on the area under the curve AUC and C(max) .Our technique was evaluated and produced the best results in terms of selectivity and sensitivity consideration the fact that the present technique involves a shorter running time and a simple sample preparation process.

Conclusion

Our HPLC technique was employed here proved to be fast, simple, precise, selective and sensitive enough to be used in clinical pharmacokinetic and bioavailability study for Amoxicillin in plasma human. The AUC and C(max) of Iraqi formulation are higher than Indian formulation of Amoxicillin and the T(max) of both two formulations are similar which is shown in table-2 and the relative bioavailability of Indian to Iraqi formulation was estimated equal to 64.11% .

Table 2 : The pharmacokinetic parameters " Cmax , Tmax and AUC" for the Iraqi and Indian formulations of Amoxicillin .

Pharmacokinetic parameter	**Indian formulation "A"**	**Iraqi formulation "B"**
C(max) "ppm"	8.9	11.5
T(max) "hour"	2	2
AUC	30.25	47.18

Acknowledgment

I wish to thank Miss. Kafa Q. Al-Obydee for carrying out the experiments that associated with preparation of samples and Kolood I. Mohamad for their technical assistance.

References

1. US pharmacopeial ,(2005), USP 28\NF 23 .The united states pharmacopeial convention, Inc, ROCKVILLE, MD , USA.
2. IMS American Ltd, (2003) , National disease and therapeutic Index data Junuary -2003 , IMS America Ltd. Ambler, PA.
3. Notari R. E. , Pharmaceutics and pharmacokinetics , 2nd edition , New York (1975).
4. David l. Nelson , Michal M. Cox , Lehninger principles of Biochemistry , 4thedition,New York (2005).
5. Gibaldi M. , Pharmacokinetic aspect of drug metabolism Ann. Y. Acd. Sci. , (1979) 197 : 19--31 .
6. M. E. Pichichero ,J. R. Casey, S. L. Block et al , Pharmacodynamic analysis and clinical trials of Amoxicillin sprinkle

administrated one daily for 7 days compared to pencillin V potassium administrated four times daily for 10 days in the treatment of tonsillopharyngitis to streptococous pyrogenes in children . J. Antimicrobial Agents Chemother. (2008) 52(7):2512---2520.

7. Kaye CM ,Allen A, Perry S. et al , The clinical pharmacokinetics of a new pharmacokinetically enhance formulation of Amoxicillin \ Clavulanate , Clin. Ther., (2001) 23: 578---584.

8. White AR , Kaye CM, Poupard J. et al , Augmentin (Amoxicillin| Clavulanate) in the treatment of community – acquired respiratory tract infection : a review of the continuing development of an antimicrobial agent , J. Antimicrob. Chemother. (2004) 53: 13---20 .

9. Waxman D. J. , Strominger J. L. , Penicillin – binding proteins and the Metabolisms of action of beta-lactum antibiotics , Annu. Rev. biochem. ,(1983)52: 825 .

10. Kosmids J., Willians D., Andrews J. , Amoxicillin –Pharmacology,bacteriology and clinical studies , Br. J. Clin. Pract . (1972) 26: 341-346 .

11. Neu H. C. , Antimicrobial activity and human pharmacology of Amoxiciliin , J. Infect. Dis. , (1974) 129: 123-131 .

12. Andrew MA, Easterling TR , Carr DB, et al , Amoxicillin pharmacokinetic in pregnant woman and simulation of dosage strategies , Clin. Pharmacol. Ther., (2007) 81 : 547---556.

13. Kerc J., Opara J. , A new Amoxicillin \ Clavulanate therapeutic system : preparation , in vitro and pharmacokinetic evaluation . Int. J. Pharm. , (2007) 335(2): 106---113.

14. Wirongnong Chierakul, Jinda Wangboonskul, Thida Singtoroj et al , Pharmacokinetic and pharmacodynamic assessment of co-amoxiclav in treatment of meliodosis, J. Antimicrobial Chemother. , (2006) 58(6): 1215---1220.

15. Guillterme Suarez-kurtz , Frederico Mota Ribero, Flaviol Vicente and Claudio J. Struchiner , Development and validation of limited – sampling strategies for predicating Amoxicillin pharmacokinetic and pharmacodynamic preparation ,Antimicrobial Agent and Chemother. , (2001) 45(11): 3029---3036.

16. Baglie S. , Del Ruenis AP. , Motta RH, et al , Plasma and salivary Amoxicillin concentration and effect against oral micro organism , Int. J. Clin. Pharmacol.Ther., (2007): 45(10) 556---562.

17. Chardin H., Yasukawu K. , Nonucer N. , et al , Reduced susceptibility to Amoxicillin of oral streptococci following Amoxicillin exposure . J. Med. Microbio. , (2009) 58(8): 1092-1097.

18. Krauwinkel W. J. ,Volkers-kamermans N. J. , Van Zijtveld J. , Determination of Amoxicillin on human plasma by hjgh performance liquid chromatography and solid phase extraction , J. Chromatogr., (1993) 617: 334-338.

19. Charles B. , Chulavatnatol S. , Simple analysis of Amoxicillin in plasma byhigh performance liquid chromatography with internal standardization and ultra- violet detection . , Biomed. Chromatogr. (1993) 7: 204-207 .

20. Yaun Z. , Russlie H. Q. , Canafax D. M. , Sensitive assay for measuring Amoxicillin in human plasma and middle ear fluid using solid phase extraction and reversed phase high performance liquid chromatography ., J. Chromatogr. B, (1995) 674: 93-99 .

21. Muth P. , Metz R. , Beck H. ,Bolten W. W. , Verdin H. , Improved high performance liquid chromatographic determination of Amoxicillin in human plasma by means of column switching . J (1996) 259-266 .

22. Sourgens H. , Stinbrede H. , Verschoor J. S. , Bertola M. A. , Rayer B. , Bioequivalence study of a novel solutab tablet formulation of Amoxicillin / Clavulanic acid versus the originator film-coated tablet. Int. J. Clin.pharmacol. ther. (2001) 39: 75-82 .

23. G. Marcelin – Jimenez , Jose A. Hernandexz , Alionka P. Angeles , et al , Bioquivalance evaluation of two brands of meloxican tablet (promotion and mobicox): Pharmacokinetics in healthy female Mexican population , Biopharm. Drug Dispos., (2005) 26 : 167---171.

24. Foroutan S. M. , Zarghi A. , Shafaai A. , Khaddam A. , et al , Simultaneous determination of Amoxicillin and Clavulaic acid in human plasma by isocratic reversed-phase HPLC using UV detection , J. Pharm. Biomed. Analysis(UK) , (2007) 45:370---374.

25. Hoizy G. , lamiable D. , Frances C. , Trenque T. , Kaltenbach M., Denis J. , Millart H. , Simultaneous determination of Amoxicillin and Clavulanic acid in human plasma by HPLC with UV

detection . J. Pharm. Biomed. Anal.. (2002) 30: 661-666.

26. Miyazaki K. , Ohtani K. , Sunada K. , Arita T., . Dtermination of Ampicillin,Amoxicillin , Cephalexin and Cephradine in plasma by high performanceliquid chromatography using fluorimetric detection . J. Chromatogr. , (1986) 276: 478-482 .

27. Marscher H. , Kikuta C. , Determination of Amoxicillin in plasma by high performance liquid chromatography with fluorescence after online oxidation, J.Chromatogr. A , (1990) 506: 417-421 .

28. Carlqvist J. , Westerlund D. , Automated determination of Amoxicillin in biological fluids by column switching in ion-pair reversed phase liquid chromatographic system with post column derivation , J. Chromatogr. (1985) 344:285-296 .

29. Henion J. , Brewer E. , Rule G. , Simple preparation for LC/MS/MS analyzing biological and environmental samples , Anal. Chem. (1998) 70: 650-656 .

30. Maurer H. H., Liquid chromatography & mass spectrometry in forensic and clinical toxicology , J. Chromatogr. , B , (1998) 713: 3-25 .

31. Oliveira C. H. , Abib E. Vannuchi Y. B. , Sucupira M. , Llha J. , De Nucci G. , Comparative bioavailability of 4 Amoxicillin Formulations in healthy human volunteers after asingl dose administration .Int. L. Clin. Pharm. And Ther. (39), 4 : (2001) 167-172 .

32. Baglie S. , Rosalen PL. , Franco LM , et al , Comparative bioavailability of 875 mg Amoxicillin tablets in healthy human volunteers , Int. J. Clin. Pharmacol. Ther., (2005) 43(7) : 350---354.

33. Wibawa J. , powkes D., Shaw P. N. , Barrett D. A. , Measurement of Amoxicillin in plasma and gastric samples using High performance liquid chromatography with fluorimetric detection . J. Chromatogr. B , Anlyt.Technol. Biomed. Life Sci. (2002) 774: 141-148.