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Title: Development and Evaluation of a Sustained Release Amoxicillin Dosage Form.

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A sustained release (SR) dosage form of amoxicillin trihydrate was developed by mixing the drug with stearic acid which provide an oily barrier to slow water penetration and dissolution of the drug. Sustained drug release was shown by an *in vitro* dissolution test. The percentage of stearic acid in the mixture is a key factor in controlling the release rate of amoxicillin. A SR dosage form for amoxicillin was developed containing 14.5% stearic acid that released nearly 100% of the amoxicillin during an 8 hour period. An HPLC analytical procedure for determination of amoxicillin concentrations in human urine using a reverse phase column (C18, 5 μm) with UV detection at 229 nm is described. Immediate release (IR) capsules, suspension, and SR tablets of amoxicillin were evaluated in one human subject. The desired sustained release patten was not shown *in vivo*. A relative bioavailability from these formulations of about 30-40% was found, when compared to the IR

capsules. Results of oral administration of amoxicillin suspension in repeated small doses at frequent times to mimic sustained release input support the presence of an absorption window.

Development and Evaluation of a Sustained Release Amoxicillin Dosage Form

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CHAPTER 1
INTRODUCTION

Amoxicillin [D-(–)-α-amino-ρ-hydroxybenzyl-penicillin trihydrate], a semisynthetic penicillin, was approved for use by the U.S. Food and Drug Administration (FDA) in 1974. There is evidence from *in vitro* research and *in vivo* animal experiments that the efficacy of β-lactam antibiotics depends mainly on the length of time that bacteria are exposed to antibiotic concentrations above the minimum inhibitory concentration (MIC). Consequently, a sustained release dosage form of β-lactam antibiotics might be therapeutically more efficacious than the existing conventional products, which are rapidly absorbed to produce transient peaks in serum drug concentrations. Currently, no commercial sustained release dosage forms of amoxicillin are available.

Several attempts have been made by others to develop a sustained release amoxicillin product with little success. Hilton and Deasy (1) reviewed the literature relative to efforts to formulate sustained release amoxicillin. They developed a product with an excellent *in vitro* sustained release patten, but the product *in vivo* was only 64.4% bioavailable in comparison with a commercial immediate release product. Llabres et al. (2, 3, 4) reported that fat matrix and fat-silica matrix sustained release formulations resulted in only 13-50% of the dose excreted in the urine. This would be a bioavailability of about 19-74%, by comparison to the commercial

immediate release product. Arancibia et al. (5) reported development of a controlled released amoxicillin formulation, but details of the composition of the formulation were not provided. The controlled release formulation had rather low bioavailability in comparison with the conventional product and both gave no detectable drug concentrations in plasma after 8 hours of administration. Delgado Charro and Vila Jato (6) tested a formulation containing amoxicillin and Gelucire 64/02. The formulation showed adequate sustained release properties *in vitro*. *In vivo*, the amount of unchanged amoxicillin excreted in the urine decreased progressively as the Gelucire 64/02 increased in the formulation. They conclude that there may be an absorption window for this drug.

The purpose of this research was to develop and evaluate a new oral sustained release dosage form of amoxicillin for use in human subjects. Chapter 1 is an introduction. Chapter 2 deals with development of sustained release tablets containing amoxicillin, and *in vitro* dissolution studies for the tablets. Tablets with the desired controlled released rate *in vitro* were developed. Chapter 3 deals with development of an HPLC analysis for determination of amoxicillin in urine. The HPLC procedure was modified to achieve optimal peak resolution and retention time. The assay method was used to evaluate both the sustained release and immediate release dosage forms of amoxicillin in human subjects. Results of oral administration of amoxicillin suspension in repeated low doses over short time intervals to simulated continuous oral input supports the suggestion of an absorption window for amoxicillin.

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CHAPTER 2
DEVELOPMENT AND CHARACTERIZATION OF A SUSTAINED RELEASE AMOXICILLIN DOSAGE FORM

ABSTRACT

Amoxicillin trihydrate was mixed with stearic acid to control the released rate of amoxicillin. The percentage of stearic acid in the mixture is a key factor in controlling release rate of the drug. Dissolution was evaluated using the USP paddle method. A sustained release dosage form for amoxicillin was developed containing 14.5% stearic acid that released nearly 100% of the amoxicillin during an 8 hour period.

A sustained released dosage form of amoxicillin with stearic acid and ethylcellulose was also tested. The release rate was too low to release a practical amount of drug during a 12 hour period.

INTRODUCTION

Amoxicillin [D-(–)-α-amino-ρ-hydroxybenzyl-penicillin trihydrate], synthesized from 6-aminopenicillanic acid, is an orally absorbed, acid stable (pH 4.8), broad spectrum antimicrobial agent. The drug is a member of the aminopenicillin class of penicillin derivatives. Aminopenicillins have an amino group (NH_2) on the carbon atom of the side chain of the penicillin molecule, which enhances activity against gram negative bacteria while retaining activity against gram positive bacteria (1).

Typical of penicillins, the bactericidal activity of amoxicillin is characterized by an initial rise in the killing rate with increasing concentrations, but only until concentrations reach four to five times the minimum inhibitory concentration (MIC). Very high concentrations of β-lactam antibiotics in serum and tissue do not result in more rapid killing of bacteria. Furthermore, as soon as amoxicillin concentrations fall below the MIC, most pathogens rapidly recover and start to grow again. Only with *staphylococci* have prolonged *in vivo* postantibiotic effects been consistently observed for β-lactam antibiotics. Thus, the duration of time that drug concentrations in serum and tissue exceed the MIC has been shown to be a pharmacokinetic parameter of major importance. The goal of dosing β-lactams would be to maximize the time of microbial exposure to active drug concentrations. According to Schumacher's method, the hours above the MIC during a 72 hour period is 71 hours for 750 mg amoxicillin, twice a day, zero order input and 62 hours for 500 mg amoxicillin, three time a day, first order input (2). Therefore, an effective sustained release formulation for amoxicillin would be a most suitable form for this purpose.

Also, reduced frequency of administration is more convenient for patients and may thereby improve compliance (3, 4, 5, 6, 7).

First, the release rate of amoxicillin was modified with a stearic acid and ethylcellulose matrix. By varying the percentage of stearic acid and ethylcellulose, the desirable release rate could be approached (8). However, results show that ethylcellulose has a strong retarding effect on the drug release rate *in vitro*. A formulation using a mixture of drug and stearic acid alone was found to give a desired *in vitro* dissolution curve.

MATERIALS AND METHODS

Materials

Amoxicillin trihydrate USP, compacted, Mfg. lot No. 6453-XS, was kindly supplied by Biocraft Laboratories, Inc. 95% stearic acid was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI 53233, USA). Stearic acid USP was purchased from J. T. Baker Inc. (Phillipsberg, NJ 08863, USA). Magnesium stearate, purified, was purchased from Fisher Scientific Company (Pittsburgh, 15219, USA). 95% myristic acid, and 99% stearic acid were purchased from Sigma Chemical Company (ST. Louis, MO 63178, USA). Ethylcellulose, viscosity 45, was purchased from The Dow Chemical Company (Midland, MI 48674, USA).

Formulations

Formulations consisted of a mixture of amoxicillin trihydrate, ethylcellulose, stearic acid, and magnesium stearate. For formulations which did not contain ethylcellulose, all ingredients were passed through a 20 mesh sieve, then mixed together and stirred well. For formulations which contain ethylcellulose, stearic acid was melted and mixed with a 1% (w/w) ethanol solution of ethylcellulose at 50 - 60°C, then mixed with amoxicillin and dried at 37°C over night. The dried material was then mixed with magnesium stearate. Tablets were produced by placing an appropriate amount of the mixture, containing 750 mg amoxicillin as the trihydrate

form, between two oval concave punches in a 0.750" × 0.378" oval shaped die, and compressing with a load of 8000 pounds force on a Carver Laboratory Press (Fred S. Inc., Summit, NJ).

Dissolution tests

In vitro dissolution of each formulation was performed at least in duplicate using the USP dissolution paddle method apparatus (Hanson Research Corp. Northridge, CA) at 50 rpm and 37°C. Each formulation was pretreated with simulated gastric fluid without pepsin (pH 1.4 ± 0.1) for 2 hours, then was transferred into simulated intestinal fluid without pancreatin (pH 7.4 ± 0.1). Dissolution samples were collected at 1, and 2 hours (in gastric fluid), and 3, 4, 6, 8, 12, and 24 hours (in intestinal fluid) with replacement of equal volume , equal temperature media. The effect of varying percentages of ethylcellulose and stearic acid, the effect of purity of the stearic acid, and the effect of compression pressure were studied. All dissolution samples were assayed at 274 nm using an HP 8452A Diode Array spectrophotometer (Hewlett-Packard company). The percentage of amoxicillin released is the amount of amoxicillin released divided by the amount of amoxicillin in the tablet (750mg), multiplied by 100.

Standard curves

Standard solutions were prepared with concentrations of amoxicillin in a range of 0.02 - 0.5 mg/ml. Then the standard curves for intestinal and gastric fluid were saved in the disk. Calibration the spectrophotometer before each assay.

RESULTS AND DISCUSSION

Figure 2.1 shows dissolution profiles of amoxicillin in formulations which contain different percentages of ethylcellulose. The formulations are summarized in Table 2.1. From Figure 2.1 (a) in intestinal fluid only, Figure 2.1 (b) in gastric fluid followed by intestinal fluid, it can be seen that release rates decrease with increasing percentage of ethylcellulose. The formulation with only 0.16% ethylcellulose gave an approximately zero order release over 24 hours when allowed to dissolve in intestinal fluid only.

Table 2.2 summarizes formulations tested which did not contain any ethylcellulose. The dissolution profiles of amoxicillin from these formulations containing only stearic acid are shown in Figure 2.2 (a) in intestinal fluid only, Figure 2.2 (b) in gastric fluid followed by intestinal fluid. The release rate decreased with increasing percentage of stearic acid in the formulation.

$T_{50\%}$ (time to release 50% of drug) of amoxicillin from the different formulations containing different percentages of stearic acid are compared in Figure 2.3. As the percentage stearic acid increased, the $T_{50\%}$ of amoxicillin increased for dissolution in intestinal fluid only. The $T_{50\%}$ was 3.2, 5.6, and 8.4 hours for formulations containing 7.8, 14.5, and 20.2% stearic acid, respectively. However, with 2 hours gastric fluid pretreatment, amoxicillin dissolutions were much more rapid due to the higher solubility in lower pH value, the $T_{50\%}$ was 1.4, 1.5, and 2.2 hours for formulations containing 7.8, 14.5, and 20.2% stearic acid, respectively.

Table 2.1 Formulations which contain fixed amount of stearic acid and different ethylcellulose concentrations

Ingredients	Formulations[a]		
	#1	#2	#3
Amoxicillin trihydate (g)	5.74	5.74	5.74
Magnesium stearate (g)	0.18	0.18	0.18
Stearic acid (95%, g)	0.50	0.50	0.50
Ethylcellulose[b] ($g_{solution}$)	1.00	2.50	4.00

[a] Stearic acid (95%) and ethylcellulose ethanol solution are combined in a beaker, stirred at 50-60°C to melt the stearic acid, then amoxicillin is added, and mixed for 10 min. The product is dried at 37°C over night, mixed with magnesium stearate, and tableted.

[b] Ethylcellulose (viscosity 45) dissolved in ethanol solution (1%, w/w).

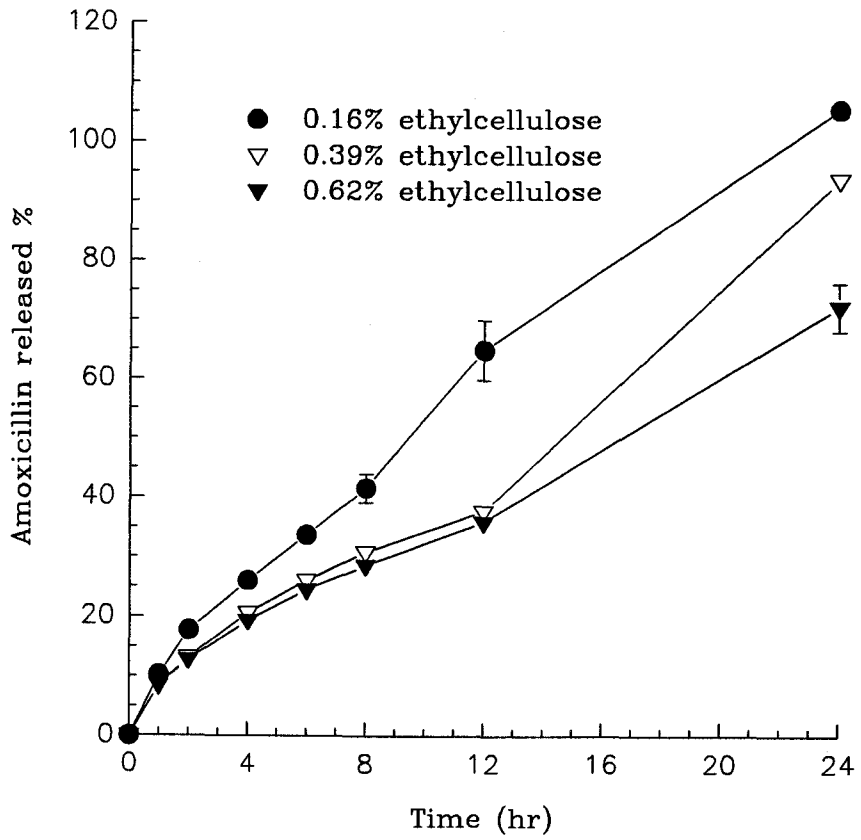

Figure 2.1a Effect of ethylcellulose on the dissolution profiles of amoxicillin in simulated intestinal fluid only. Each data point represents the mean ± standard deviation of four replications, except where the standard deviation is too small to show.

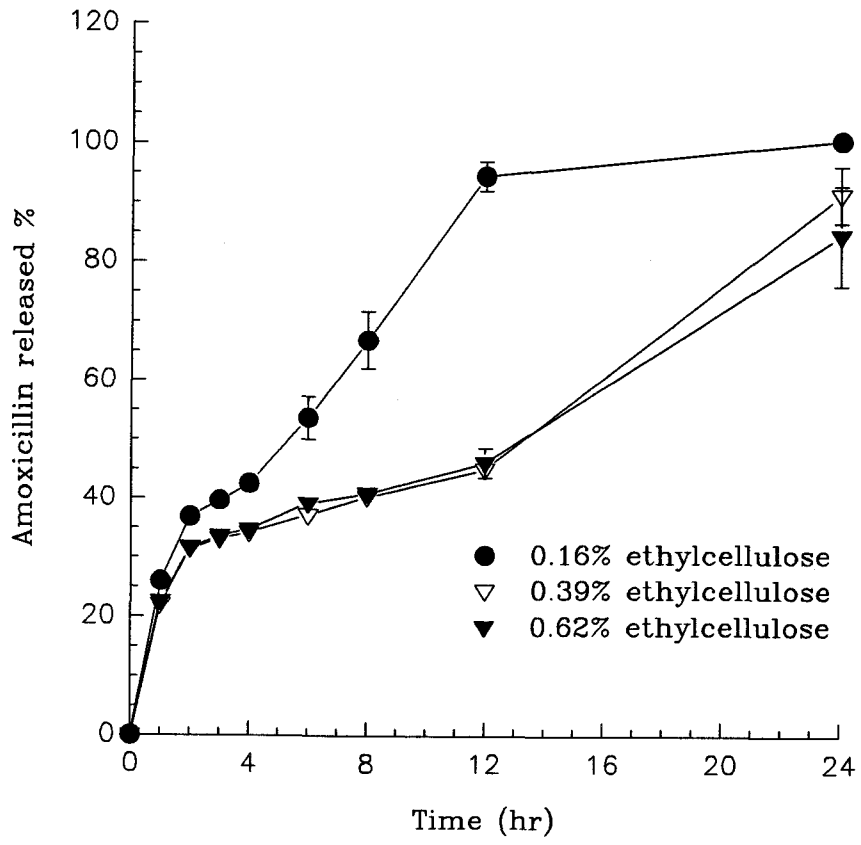

Figure 2.1b Effect of ethylcellulose on the dissolution profiles of amoxicillin with 2 hours simulated gastric fluid pretreatment. Each data point represents the mean ± standard deviation of two replications, except where the standard deviation is too small to show.

Table 2.2 Formulations which contain different amount of stearic acid

Ingredients	Formulations		
	#1	#2	#3
Amoxicillin trihydate (g)	5.74	5.74	5.74
Magnesium stearate (g)	0.18	0.18	0.18
Stearic acid (95%, g)	1.50 (20.2%)	1.00 (14.5%)	0.50 (7.8%)

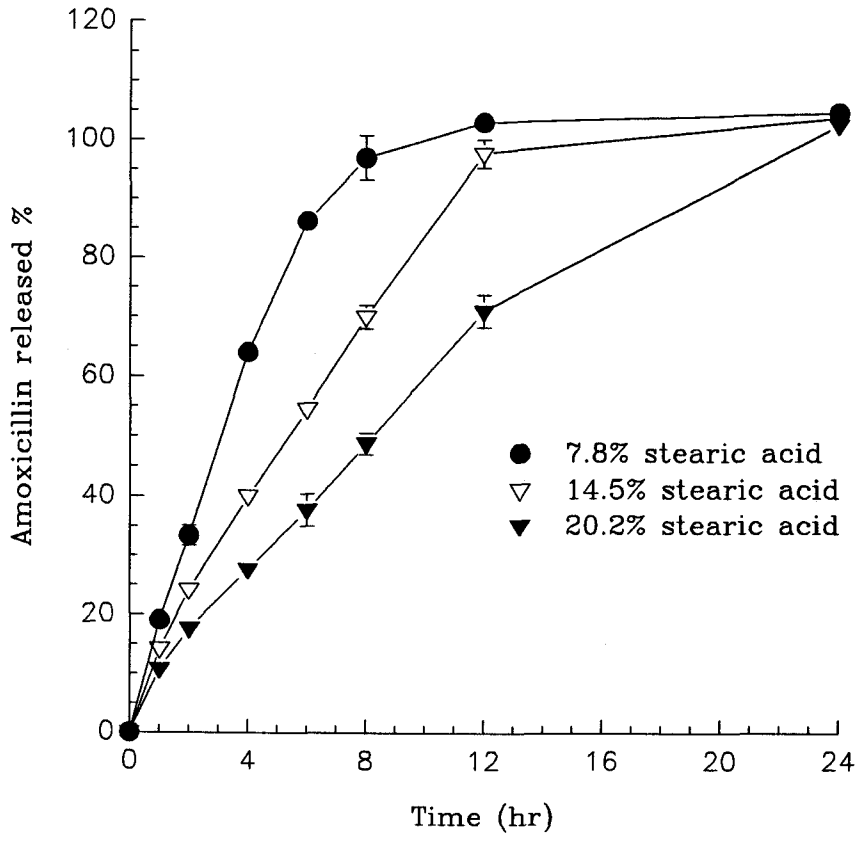

Figure 2.2a Effect of stearic acid on the dissolution profiles of amoxicillin in simulated intestinal fluid only. Each data point represents the mean ± standard deviation for four replications, except where the standard deviation is too small to show.

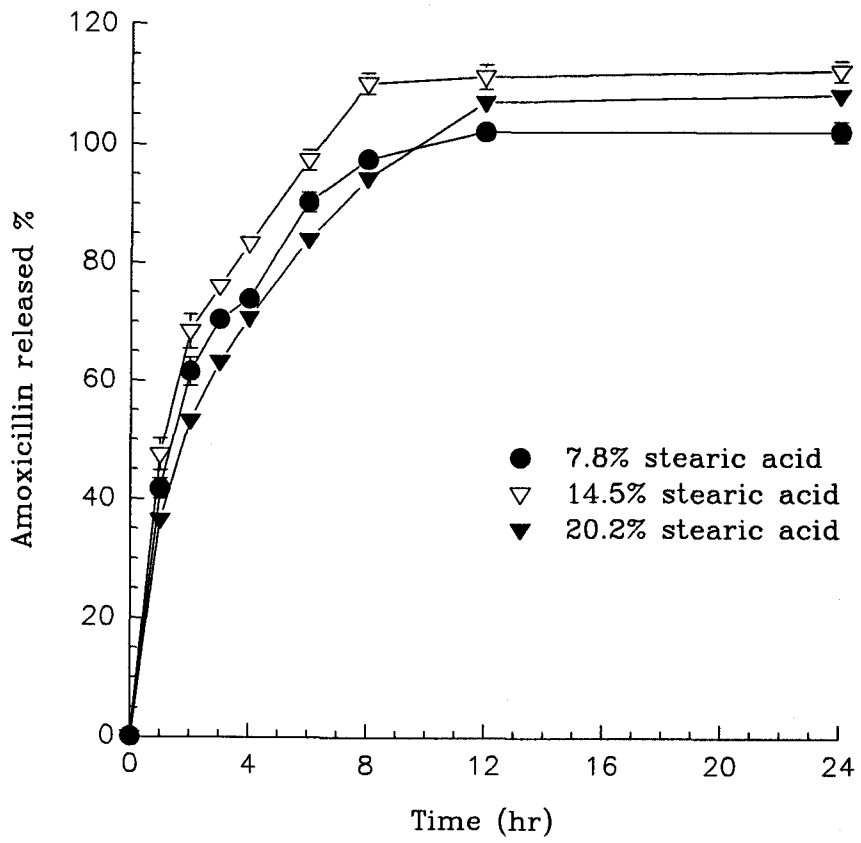

Figure 2.2b Effect of stearic acid on the dissolution profiles of amoxicillin with 2 hours simulated gastric fluid pretreatment. Each data point represents the mean ± standard deviation for two replications and six replications for formulation containing 14.5% stearic acid, except where the standard deviation is too small to show.

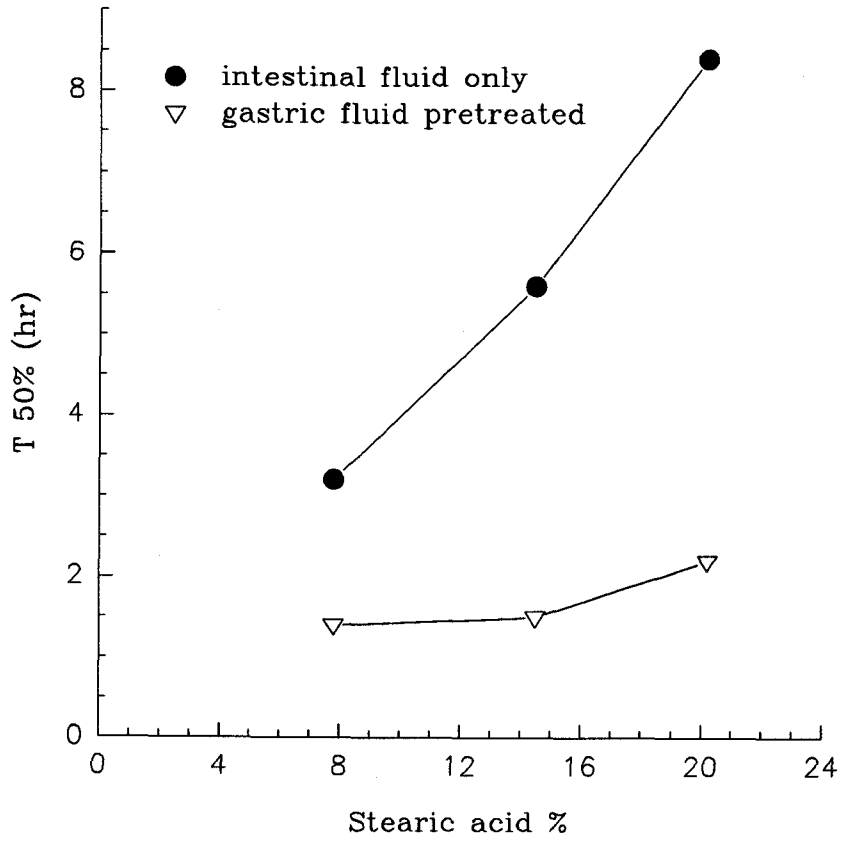

Figure 2.3 Time for 50% amoxicillin dissolved as a function of percentage stearic acid.

Dissolution of a sustained release tablet containing 14.5% stearic acid compared with conventional immediate release capsules in 2 hours gastric fluid followed by intestinal fluid is shown in Figure 2.4. The sustained release formulation gives a slower release patten with release being nearly complete at 8 hours.

The purity of stearic acid is a factor which may change the release rate of drug. Formulations containing 95% stearic acid mixed with 95% myristic acid are summarized in Table 2.3. The dissolution profiles for amoxicillin from these formulations are shown in Figure 2.5 (a) in intestinal fluid only and Figure 2.5 (b) in gastric fluid followed by intestinal fluid. Figure 2.5 shows that the formulation which contains equal proportions of stearic acid and myristic acid gave the fastest release rate. There is little difference in the dissolution profiles among those formulations which contain 50/50, 80/20 stearic acid and myristic acid, 100% stearic acid, and stearic acid USP. These results suggest that small differences in stearic acid purity will not dramatically affect amoxicillin dissolution.

Figure 2.6 shows that compression pressure had an effect on amoxicillin dissolution. As tableting pressure increases, dissolution decreases. A compression load of 8,000 pounds force was chosen for the tablets in this study.

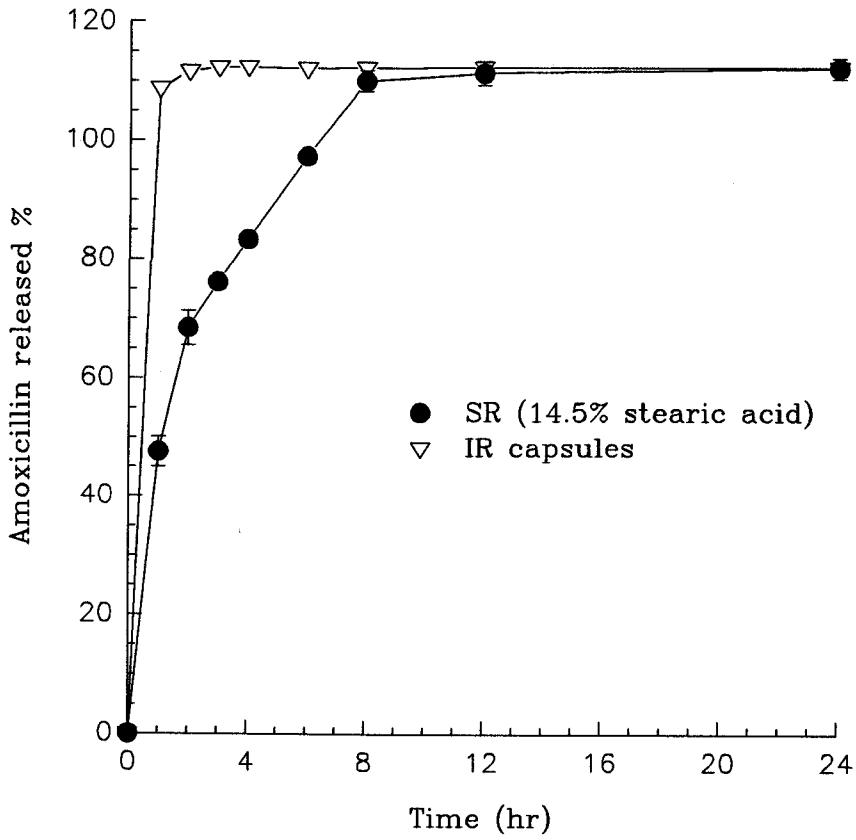

Figure 2.4 Comparison of a sustained release (SR) tablet with commercially available immediate release (IR) capsules. Dissolution profiles of amoxicillin with 2 hours simulated gastric fluid pretreatment. Each data point represents the mean ± standard deviation, two replications for IR and six replications for SR, except where the standard deviation is too small to show.

Table 2.3 Formulations which contain stearic acid of differing purities

Ingredients	Formulations			
	#1	#2	#3	#4
Amoxicillin trihydate (g)	5.74	5.74	5.74	5.74
Magnesium stearate (g)	0.18	0.18	0.18	0.18
Stearic acid (g)	1.00	1.00	1.00	1.00
Ratio[a] (w/w)	100[b]	80[b]:20[c]	50[b]:50[c]	100[d]
Melting point	67-69°C	61.5-62.5°C	49-51°C	54°C

[a] Ratio of stearic acid and myristic acid. Weigh 95% stearic acid and 95% myristic acid (w/w), mix together, heat until melted, cool to room temperature, powder, and pass through a 20 mesh sieve.

[b] 95% stearic acid.

[c] 95% myristic acid.

[d] USP stearic acid (mixture of stearic acid and palmitic acid).

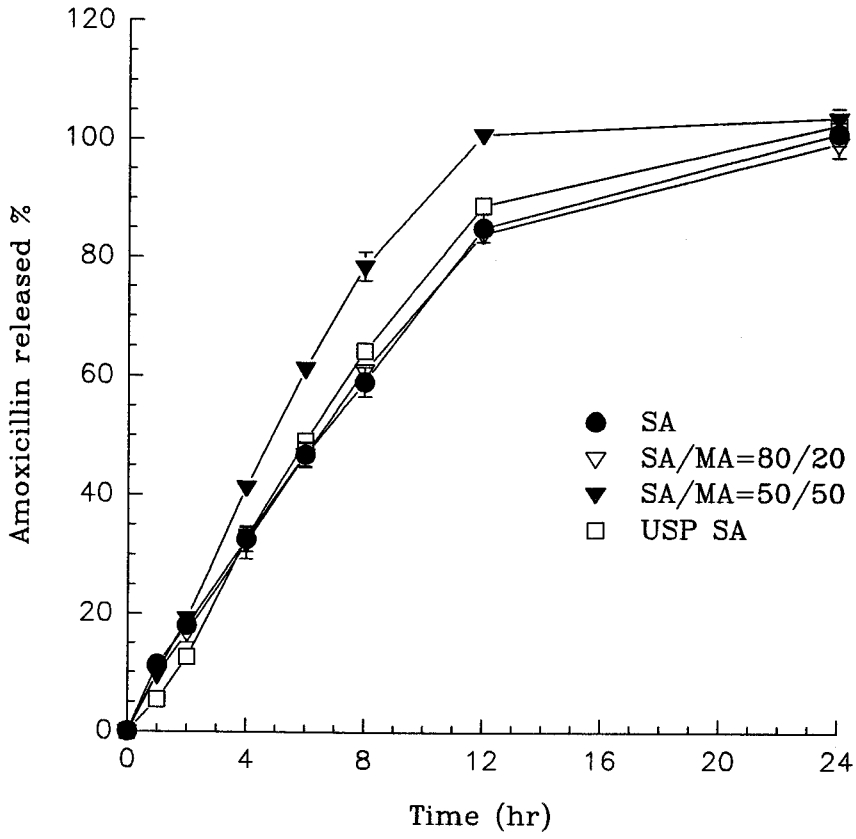

Figure 2.5a Effect of the purity of 95% stearic acid (SA) mixed with 95% myristic acid (MA) on dissolution profiles of amoxicillin in simulated intestinal fluid only. Each data point represents the mean ± standard deviation for two replications, expect where the standard deviation is too small to show.

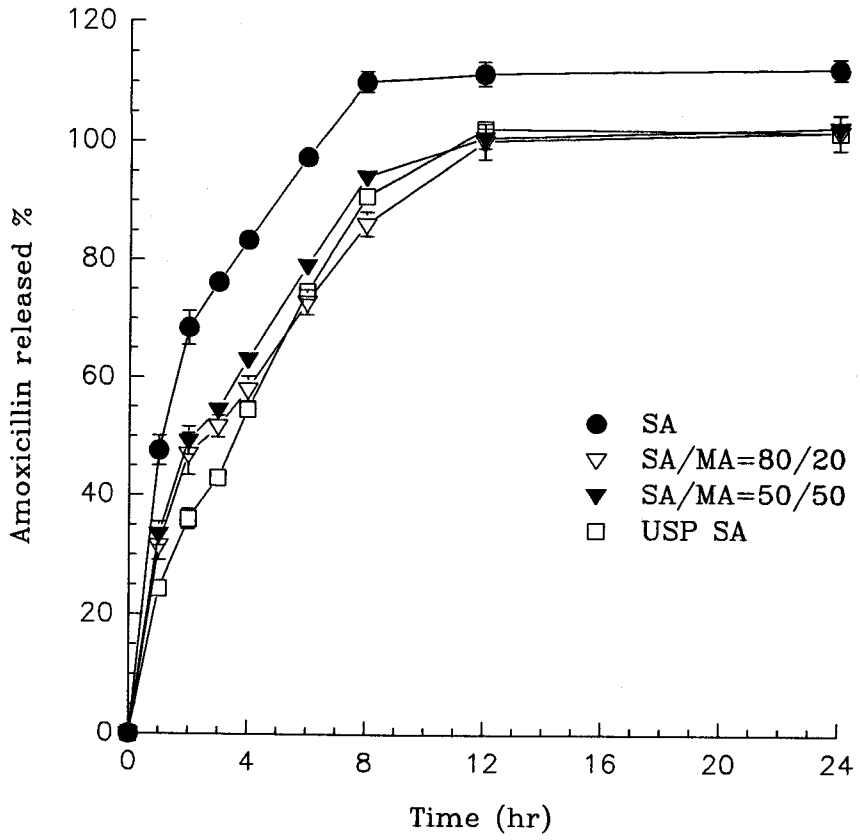

Figure 2.5b Effect of the purity of 95% stearic acid (SA) mixed with 95% myristic acid (MA) on dissolution profiles of amoxicillin with 2 hours simulated gastric fluid pretreatment. Each data point represents the mean ± standard deviation for two replications, except where the standard deviation is too small to show.

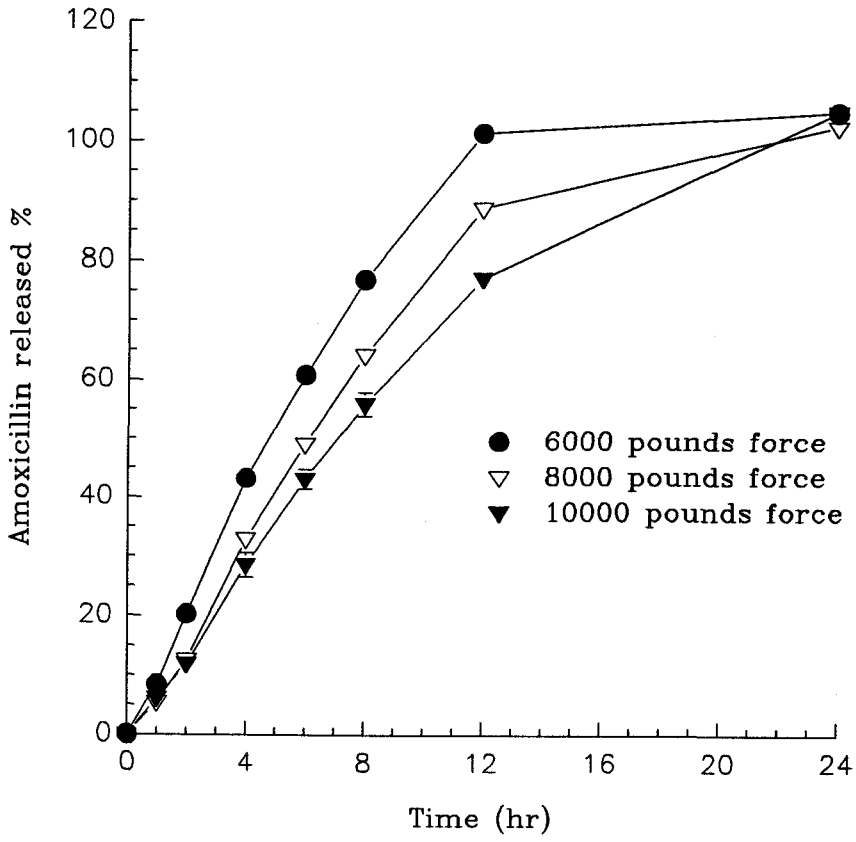

Figure 2.6 Effect of compression pressure on drug mixture containing 14.5% USP stearic acid. Dissolution profiles of amoxicillin in simulated intestinal fluid only. Each data point represents the mean ± standard deviation for two replications, except where the standard deviation is too small to show.

CONCLUSION

The release rates for drug compressed into tablets are affected by many parameters. The percentage of stearic acid in a tablet formulation is a key factor for controlling the release of amoxicillin in the sustained release dosage forms tested. Of the several formulations tested which contain different percentages of stearic acid, the best formulation contained 14.5% stearic acid and gave the desired controlled release over a 8 hour period *in vitro*.

The purity of the stearic acid had some effects on drug release, but a small impurity will not dramatically affect amoxicillin dissolution. Tableting pressure also had some effects on amoxicillin dissolution. As tableting pressure increased, the rate of dissolution decreased.

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8. United States Patent. Patent number: 5,149,542 (Example 2).

CHAPTER 3
DETERMINATION OF AMOXICILLIN IN URINE BY HPLC AND EVALUATION OF SUSTAINED RELEASE AMOXICILLIN IN HUMAN SUBJECT

ABSTRACT

The stability of amoxicillin in urine was studied. After pH adjustment, amoxicillin was sufficiently stable in urine for up to 24 hours to determine drug concentrations with an HPLC autoinjector.

An HPLC analytical procedure for determination of amoxicillin concentrations in human urine using reversed-phase column (C18, 5 μm), with UV detection at 229 nm, is described. Optimal elution conditions were determined by studying several variables: pH, buffer concentration, and ratio of mobile phase components. A mobile phase consisting of 5% (v/v) methanol in 0.005 M potassium dihydrogen phosphate buffer (pH = 4.80 $\pm$ 0.05) at a flow rate of 1.2 ml/min provided good resolution of amoxicillin and 4-acetamidophenol (APAP, internal standard) peaks with a retention time of less than 24 minutes. The method is simple, rapid, and reliable.

Immediate release amoxicillin capsules, suspension, and sustained release amoxicillin tablets were evaluated in one human subject. There were two sustained release tablets, SR(A) and SR(B), containing 7.8 and 14.5% stearic acid, respectively. The desired sustained release patten was not shown *in vivo* even though these SR formulations showed sustained release *in vitro*. A relative bioavailability from these formulations of 30 - 40% was found, when compared to the immediate release

capsules. Results of oral administration of amoxicillin suspension in repeated small doses at frequent times to mimic sustained release input support the presence of an absorption window for amoxicillin.

INTRODUCTION

Quantification of antibiotics were originally performed using microbiological techniques. This guarantees determination of the microbiologically active principles, including active metabolites. Disadvantages of such techniques are non-selectivity (i.e. active metabolites are co-determined), relatively long analysis times, and low precision of the results. The relative standard deviation is generally about 15% (1, 2). Recently, many chemical assays based on HPLC methods have been introduced offering rapid, selective, and precise methods of determination for this class of compounds (3, 4, 5, 6, 7, 8, 9, 10). All these methods have particular merits and demerits in respect to sensitivity, selectivity, specificity, and convenience.

High drug concentrations in urine are possible with low blood concentrations because the volume of urine is much less than the total body volume of distribution. Therefore, the sensitivity of the HPLC assay method was not a great concern for this study. The purpose of this study was to develop a simple and rapid technique for the determination of amoxicillin in urine to evaluate the relative bioavailability of the sustained release amoxicillin formulations.

Immediate release capsules, suspension and sustained release amoxicillin SR(A) and SR(B) were given to a single human subject. The desired sustained release patten was not found. The relative bioavailabilities of the sustained release formulations were quite low, only 30 - 40% compared to a commercially available immediate release capsule.

MATERIALS AND METHODS

Materials

Amoxicillin capsules (Novopharm Inc., Schaumburg, IL 60173 USA) and amoxicillin suspension (Warner Chicott labs, Morris plains, NJ 07950 USA) were purchased from The Oregon State University Student Healthy Center pharmacy. Amoxicillin trihydrate USP, compacted, Mfg. lot No. 6453-XS, was kindly supplied by Biocraft Laboratories, Inc. Potassium dihydrogen phosphate, disodium hydrogen phosphate, citric acid, and HPLC grade methanol were purchased from Mallinckrodt Specialty Chemicals Company (Paris, Kentucky 40361, USA). Perchloric acid (concentrated) was purchased from J. T. Baker Chemical Company (Phillipsburg, NJ 08865, USA). 4-Acetamidophenol (APAP) was purchased from Sigma Chemical Company (ST. Louis, MO 63178, USA). HPLC columns (C18, particle size 5 μm, pore size 100 Å) were purchased from Rainin Instrument Co., Inc. (Woburn, MA 01801, USA)

The solution for protein precipitation was 0.33 N perchloric acid solution. The pH of the solution was below 2. The solution for internal standard was 1 mg/ml APAP stored refrigerated in foil-covered glass to exclude light. The solution for pH adjustments was 100 ml of 0.5 M disodium hydrogen phosphate and 350 ml of deionized water adjusted with 1 M citric acid to pH 4.85 before addition of water to 500 ml. The mobile phase was 5% methanol in 0.005 M potassium dihydrogen

phosphate buffer (pH = 4.80 ± 0.05). Before use, the mobile phase was degassed 20 minutes by stirring under vacuum.

Apparatus

The HPLC system consisted of a Waters Associates (Milford, MA, USA) a model 6000A solvent delivery system, a WISP 712 autoinjector, and a model 441 UV absorbance detector. A 4.6 × 250.0 mm C18 column with a guard column (Microsorb-MV, 5 μm, 100 Å, Rainin Instrument Co., Inc., Mack Road, Woburn, MA 01801, USA) were used to separate the analytes. Data were recorded on a strip chart recorder (Linear Instruments Corp., Irvine, CA).

Sample preparation

Method by Vree, et al. (1): 20 μl urine and 30 μl 4-Acetamidophenol (APAP) (1 mg/ml) were mixed with 0.5 ml of perchloric acid (0.33 N) on a Vortex mixer. 100 μl were injected onto the high performance liquid chromatograph.

Method by Carlqvist and Westerlund (2): 20 μl urine and 30 μl APAP (1 mg/ml) were mixed with 0.5 ml of pH adjustment solution (pH 4.85) on a Vortex mixer. 100 μl were injected onto the high performance liquid chromatograph.

Standard curve

Standard solutions in urine were prepared with amoxicillin concentrations in a range of 0.2 to 2.0 mg/ml. Due to the instability of the amoxicillin in urine, the standard solutions had to be prepared fresh daily before assay. A standard calibration curve was constructed by plotting the ratio of amoxicillin peak height to internal standard peak height versus known amoxicillin concentration. There were no detectable peaks from vehicles or solvents.

In vivo study protocol

One human subject (32 years old female, healthy, and free of any medications) participated in the study. Subject fasted at least 12 hours before taking the drug. For each trial, the subject had at least a one day wash-out period. The half life of amoxicillin is relatively short, 1.05 hr (11), and the data show no accumulation from the sustained release products. 750 mg of amoxicillin were administered with each dose. The full volume of urine produced during the study period was collected and measured for all time points except time 0, which was used as a blank for analysis. A small portion of each urine sample was prepared as described and analyzed for amoxicillin concentration by HPLC with UV detector at 229 nm. Urine samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, and 12 hours.

RESULTS AND DISCUSSION

Stability study

Amoxicillin is an amphoteric compound. The pK_a values of the COOH, NH_2, and OH groups are 2.4, 7.4, and 9.6, respectively; thus the drug has a slightly acid character. A 0.2% (m/v) solution of the drug in CO_2 free water has a pH of 3.5 - 5.5. Owing to its strongly polar character, amoxicillin is relatively soluble in water. The solubility at pH 4 - 8 varies from 4.2 g/l to 9.0 g/l. Amoxicillin is unstable in the strongly acid solutions required for the drug to be present in the unionized form (pH below 2.4) (12).

Amoxicillin is rather unstable in biological fluids (2). A urine sample containing 20.0 mg/ml lose 53.6% of drug at 37°C during a 24 hour period (Figure 3.1). Higher concentrations of drug in urine seemed to be more unstable than lower concentration. The reason is probably due to the tendency of aminopenicillins to polymerize at high concentrations (13). Studies of the stability of the drug in urine at physiological and ambient temperatures indicated the importance of rapid handling of specimens (2). For this experiment, all samples were pH adjusted within 30 minutes and analyzed within 8 hours after sample collection.

The method by Vree, et al. (1) is very simple and rapid. It is very useful for an immediate assay after a single sample is collected. The suitability of the method for the assay of large numbers of samples is limited since amoxicillin degrades over

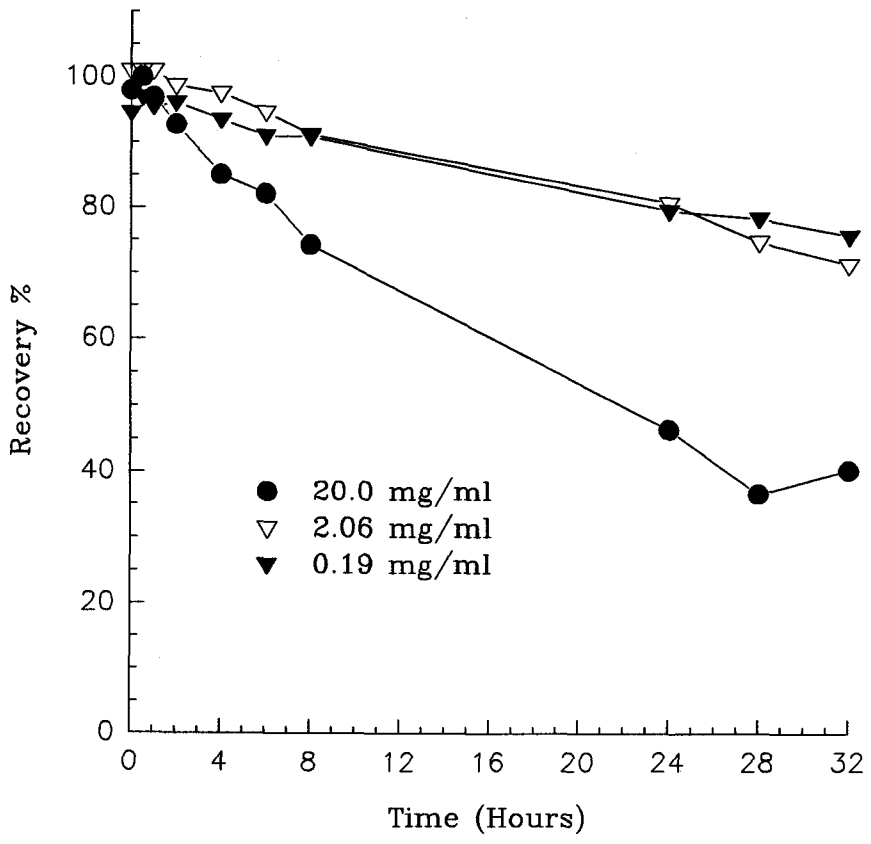

Figure 3.1 Stability of amoxicillin in urine at 37°C. Aliquot of the sample were taken from a thermostatted water bath and immediately frozen by a dry ice-ethanol mixture and stored at -70°C before analysis by HPLC (2).

time. Figure 3.2 shows that the peak height ratio of amoxicillin and APAP in urine decreased over a 20 hour period, due to amoxicillin degradation.

Thus, the Carlqvist and Westerlund method (2), which adjusts the pH of urine samples to overcome the instability of amoxicillin in body fluids, was used. Amphoteric penicillins often have their optimal stability at the isoelectric point. For amoxicillin, the isoelectric point is pH 4.8. The addition of 0.5 ml pH adjustment solution to 20 μl of urine provided conditions that kept amoxicillin intact for at least 24 hours at room temperature. This is an adequate time to perform the assays with an automated injector. Figure 3.3 shows the peak height ratios of amoxicillin and APAP in urine over a 48 hour period. The urine samples were stable up to 24 hours.

HPLC analysis

The volume ratio of methanol in potassium dihydrogen phosphate buffer had a significant effect on the retention time of amoxicillin (see Table 3.1). The higher the percentage of methanol, the shorter the retention time of the amoxicillin. Excellent resolution and retention times were obtained with 5% (v/v) methanol in 0.005 M potassium dihydrogen phosphate buffer (pH = 4.80 $\pm$ 0.05).

The analysis and recording parameters as shown in Table 3.2 provided excellent conditions for measurement of amoxicillin and APAP peak heights. Under the conditions described, amoxicillin and internal standard were clearly separated and eluted within 24 minutes. Mean retention times for amoxicillin and APAP were 6.5,

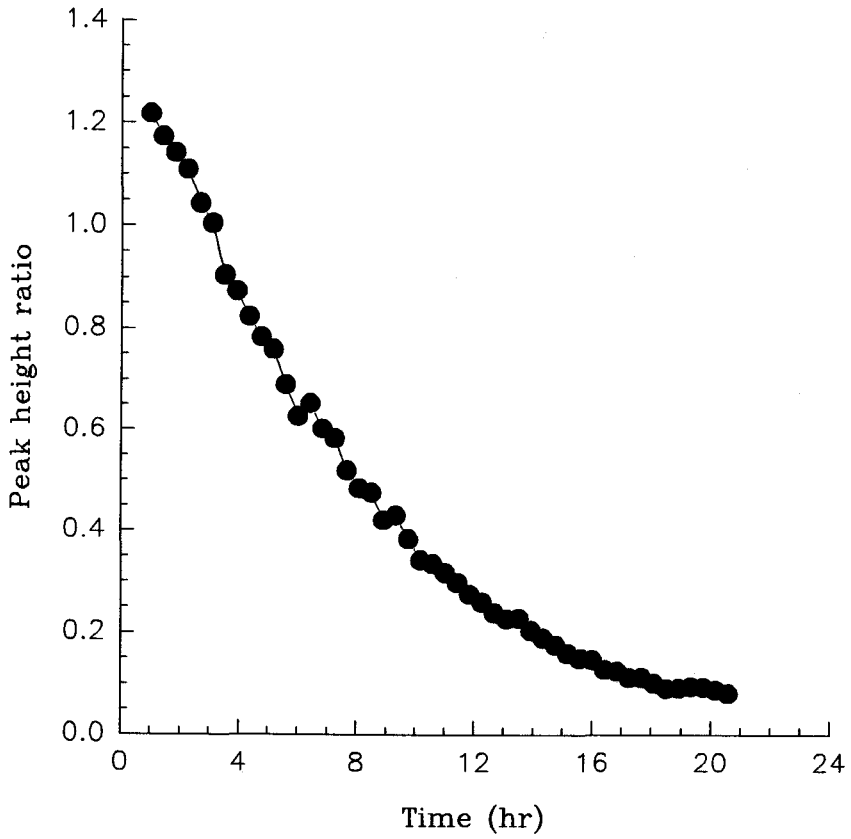

Figure 3.2 Stability of amoxicillin at room temperature during a 20 hour period. Samples were prepared by the method of Vree, et al. (1).

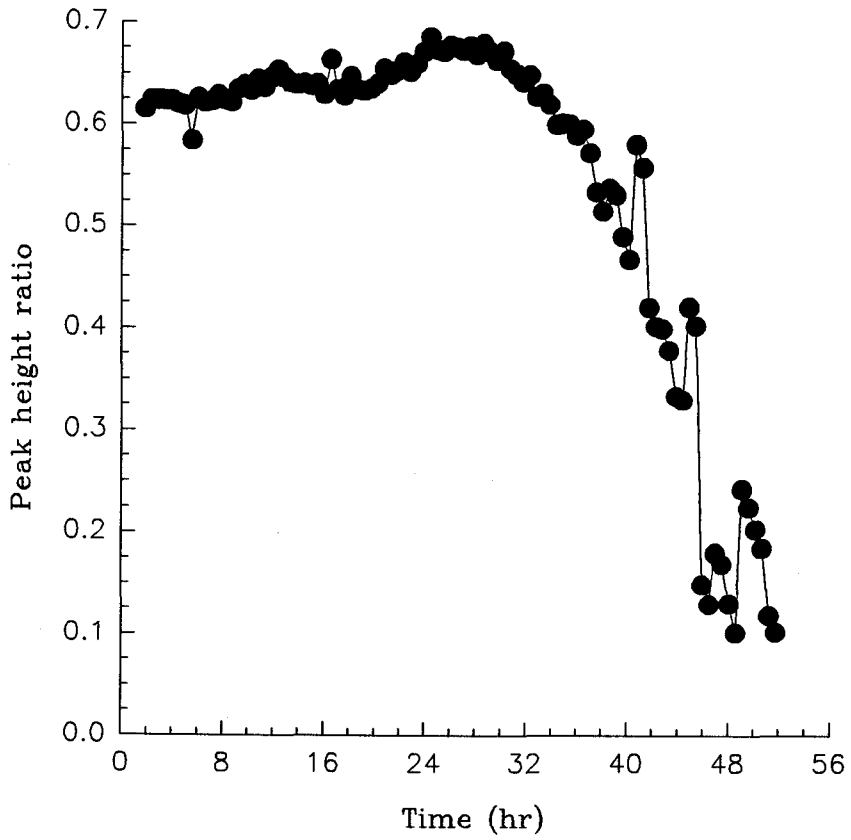

Figure 3.3 Stability of amoxicillin at room temperature during a 48 hour period. Samples were prepared by the method of Carlqvist and Westerlund (2).

Table 3.1 Effect of volume ratio of methanol in potassium dihydrogen phosphate buffer on the retention time of amoxicillin and APAP

Volume ratio[a]	Retention time (minutes)	
	Amoxicillin[b]	APAP[c]
100 : 400	3.29	NA[d]
75 : 425	3.76	NA
50 : 450	4.74	NA
25 : 475	6.50	19.79

[a] Volume ratio of methanol and 0.005 M KH_2PO_4 buffer.
[b] 0.04 mg/ml amoxicillin aqueous solution.
[c] 1 mg/ml APAP aqueous solution.
[d] Not measured.

Table 3.2 Optimal parameters for HPLC analysis with UV detector

Mobile phase = 5% methanol in 0.005 M KH_2PO_4 buffer
Flow rate = 1.2 ml / min
Wavelength = 229 nm
Sensitivity = 0.5 AUFS
Chart speed = 10 cm / hr

and 19.8 minutes, respectively. Figure 3.4 shows an average standard curve for amoxicillin from nine standard curves produced over a four month period. Slope, intercept, and correlation coefficient were 0.81309, -0.0104, and 0.999, respectively.

In vivo study

Amoxicillin is excreted 50 - 70% unchanged in the urine. Most of the drug is excreted within 2 hours of dosing, but effective concentrations against susceptible organisms remain in the urine up to 8 hours after administration (14).

Figure 3.5 shows the cumulative amount of drug excreted in urine versus time. Figure 3.6 shows the urinary excretion rate of amoxicillin with time. The final percentages of drug recovered from urine were 63.3, 19.0, and 25.2% for immediate release capsules, SR(A), and SR(B), respectively. The relative bioavailability for the sustained release formulations were rather low, only 30 - 40% compare to immediate release capsules. These results are consistent with other studies (15, 16, 17, 18, 19, 20, 21).

There are a number of investigations into the absorption process of aminopenicillins (22, 23, 24, 25). The absorption mechanisms of amino β-lactam antibiotics are complicated and there is controversy about the extent to which carrier-mediated transport systems participate in the absorption process. Absorption has been proposed to occur mainly in the upper small intestine, which is consistent

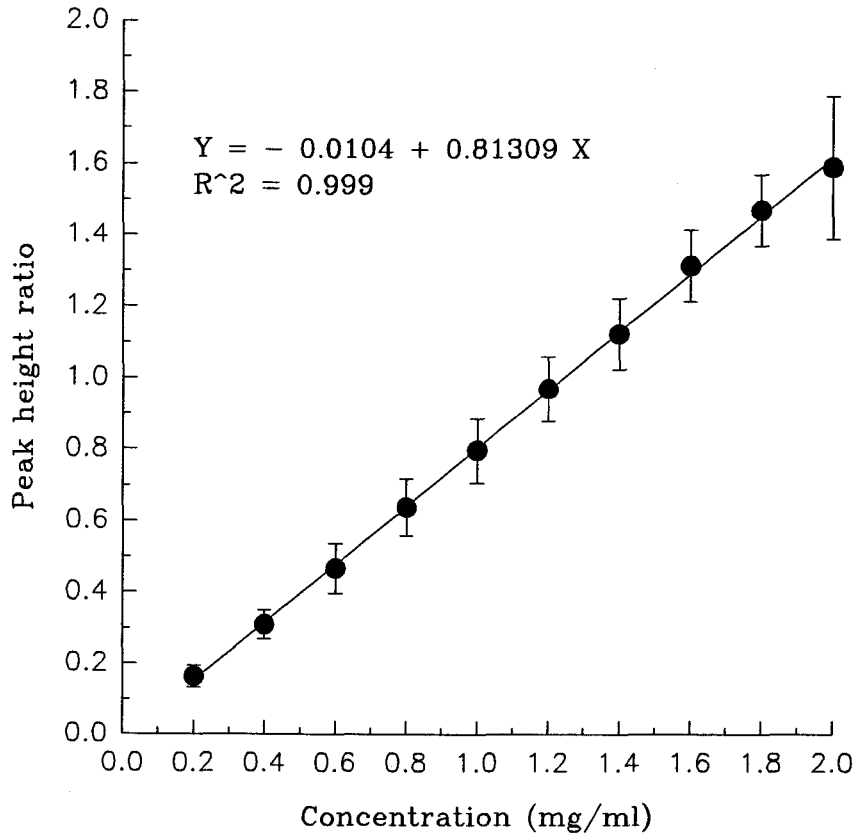

Figure 3.4 An average standard calibration curve for amoxicillin concentration (0.2 to 2.0 mg /ml) versus peak height ratio of amoxicillin to APAP (internal standard).

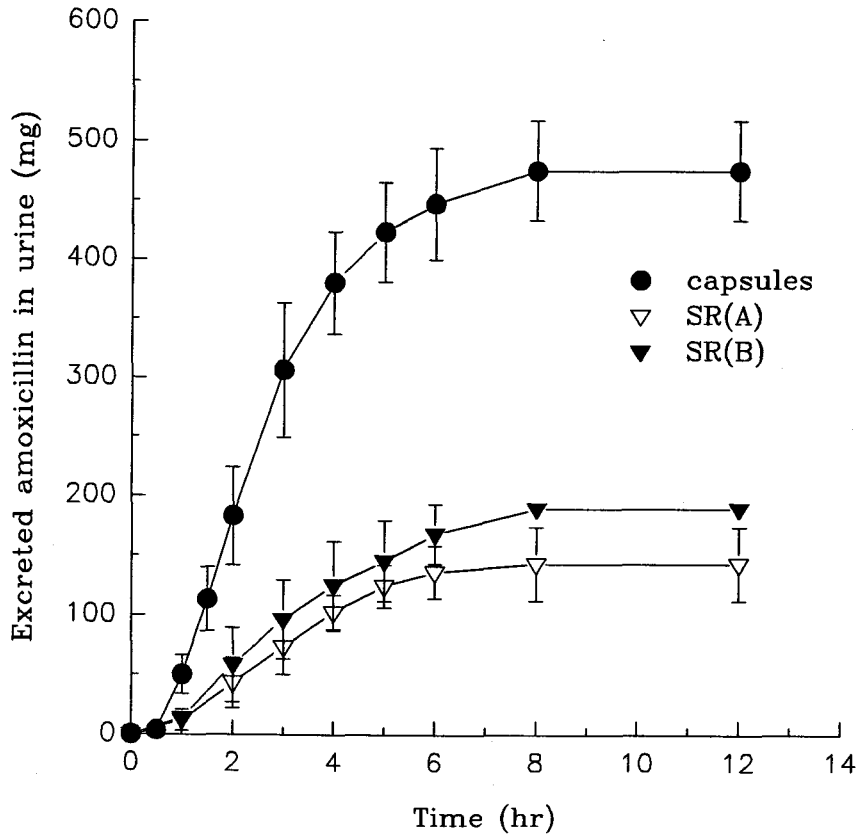

Figure 3.5 Comparison of amoxicillin excreted in urine from capsules, SR(A), and SR(B). Each data point represent the mean ± standard deviation for two trials, except where the standard deviation is too small to show.

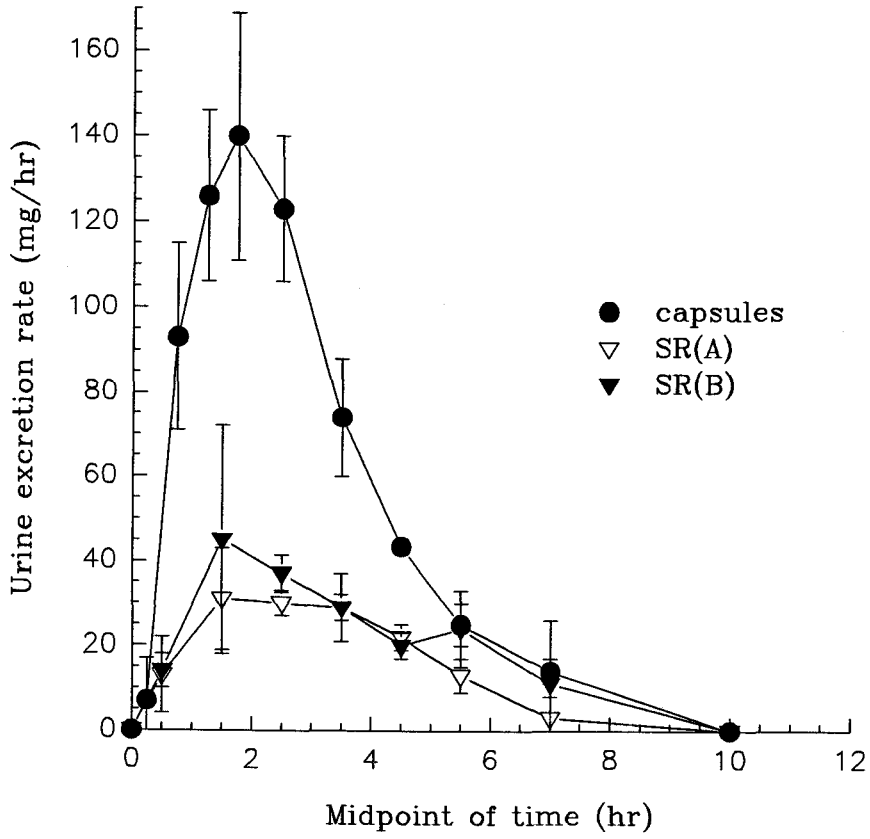

Figure 3.6 Comparison of urinary excretion rates of amoxicillin from capsules, SR(A), and SR(B). Each data point represent the mean ± standard deviation for two trials, except where the standard deviation is too small to show.

with the results reported herein. Thus, absorption would occur only in a narrow zone, an absorption window.

If this is true, an obvious way to design oral formulations for optimal bioavailability is to maximize the release rate of amoxicillin from solid dosage forms in the upper small intestine. The best way to provide amoxicillin would be as an already dissolved form. Hespe, et al. (21) compared an effervescent tablet and a commercial reference formulation. The effervescent tablet performs best by showing the highest AUC and C_{max} values.

One approach to evaluate oral sustained release input is to calculate the amount of drug released every hour *in vitro* for the sustained release formulation during an 8 hour period of dissolution. The subject may then take the same amount of drug in suspension form at the same rate to minimize the formulation effect. The resulting excretion profile from this method should correlate closely to the desired excretion profile for sustained release oral input. Table 3.3 shows the amount of amoxicillin released from a sustained release tablet *in vitro*. Figure 3.7 shows that the excretion profile for the desired sustained release by intermittently dosed suspension is much higher than that of the observed sustained release formulation. The decreased excretion might be due to an absorption window. No absorption occurred after the sustained release tablet had passed the absorption window.

Figure 3.8 and Figure 3.9 indicate that bioavailabilities for an equal dose of amoxicillin in commercially available capsules, commercially available suspension,

Table 3.3 Amount of amoxicillin released *in vitro* from an oral sustained release tablet SR(B)

Time (hr)	Drug released[a] (%)	Amount[b] (mg)	Dose[c] (mg)
0	0	0	315
1	42	315	142.5
2	61	457.5	52.5
3	68	510	40
4	74	550	51.25
5			51.25
6	87	652.5	32.5
7			32.5
8	~ 100	750	32.5

[a] Average percentage release of drug from six replication.

[b] Amount of amoxicillin = 750 × average percentage release of drug.

[c] Drug taken as a suspension form (5 mg/ml) every hour to provide oral input equal to the amount released by a sustained release tablet.

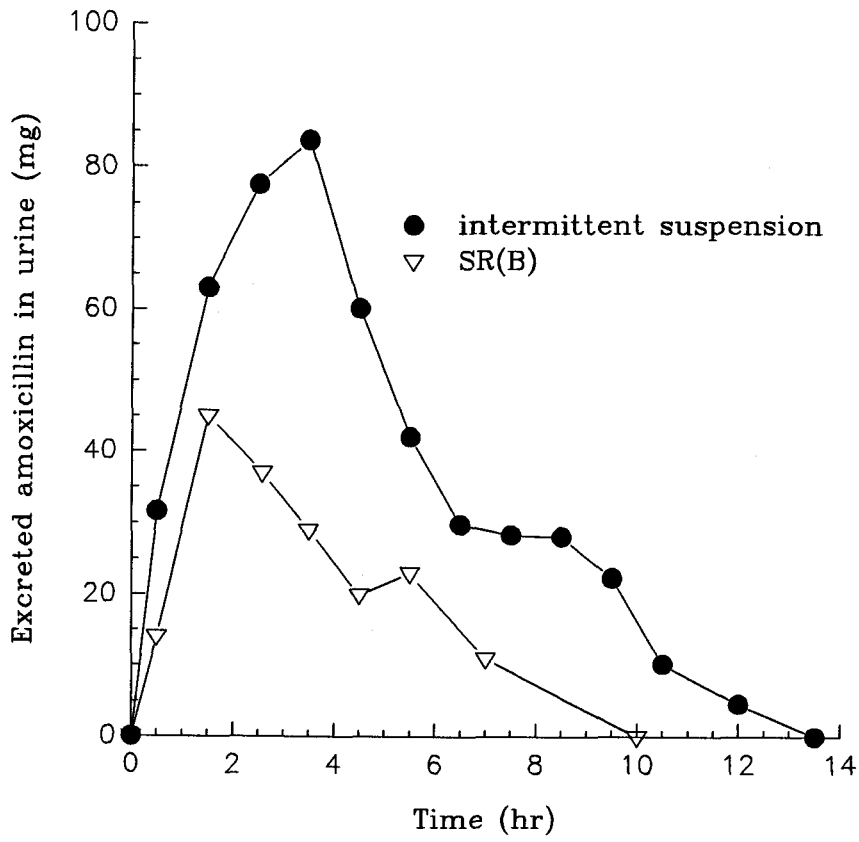

Figure 3.7 Comparison of urinary excretion rates of amoxicillin from SR(B) and intermittent dosing of suspension.

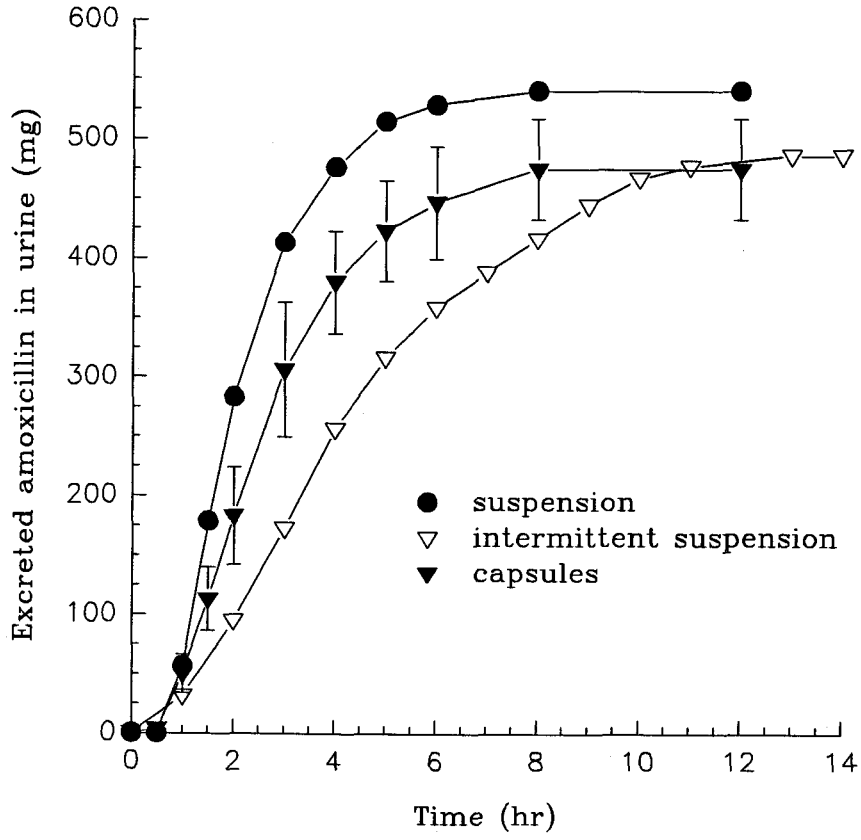

Figure 3.8 Comparison of amoxicillin excreted in urine from single dose suspension, intermittent dosing of suspension, and capsules.

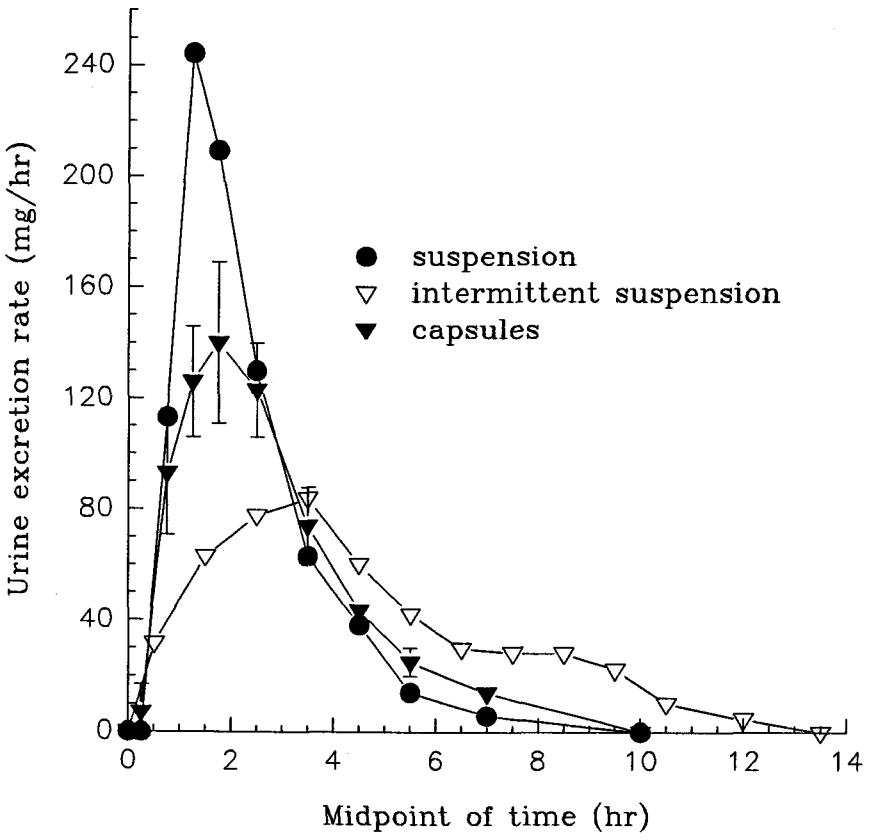

Figure 3.9 Comparison of the urinary excretion rates of amoxicillin from single dose suspension, intermittent dosing of suspension, and capsules.

and commercially available suspension but dosed intermittently are approximately the same. The difference among the bioavailabilities are within 15%.

In order to make a sustained release dosage form for a drug which has an absorption window, it is necessary to find a way to retain the drug at or above the absorption window. Although amoxicillin is reported to be acid stable, the stability is maximum at pH 4.8. The drug degrades in gastric fluid (pH=1.4 $\pm$ 0.1). Figure 2.10 shows that the concentration of amoxicillin decreases over time in gastric fluid. The degradation half-life is 8.08 hours. Thus, if a controlled release dosage form containing amoxicillin is designed to remain in the stomach over 12 hours, up to about 65% of the drug may degrade. This information in conjunction with the presence of an absorption window suggests that it will be very difficult to develop a fully bioavailable oral controlled release dosage form for amoxicillin.

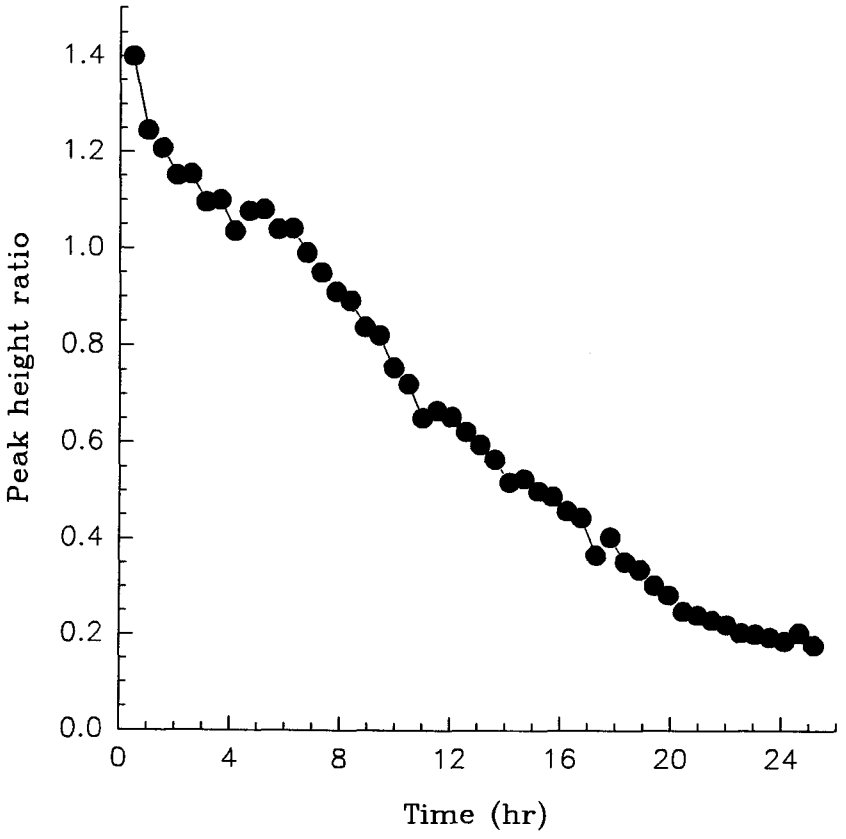

Figure 3.10 Stability of amoxicillin in gastric fluid (pH = 1.4 ± 0.1) at room temperature during a 24 hour period.

CONCLUSION

A sustained release formulation of amoxicillin has been formulated and tested. The desired release rate *in vitro* was achieved but the bioavailability is rather low due to an absorption window. The results suggest that a fully bioavailable oral controlled release dosage form of amoxicillin is not possible unless a way to found to protect the drug from gastric degradation and to retain the dosage form in the upper small intestine (in the area of the absorption widow).

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APPENDICES

APPENDIX A
PROFILES OF AMOXICILLIN EXCRETED IN URINE AND THE EXCRETION RATE

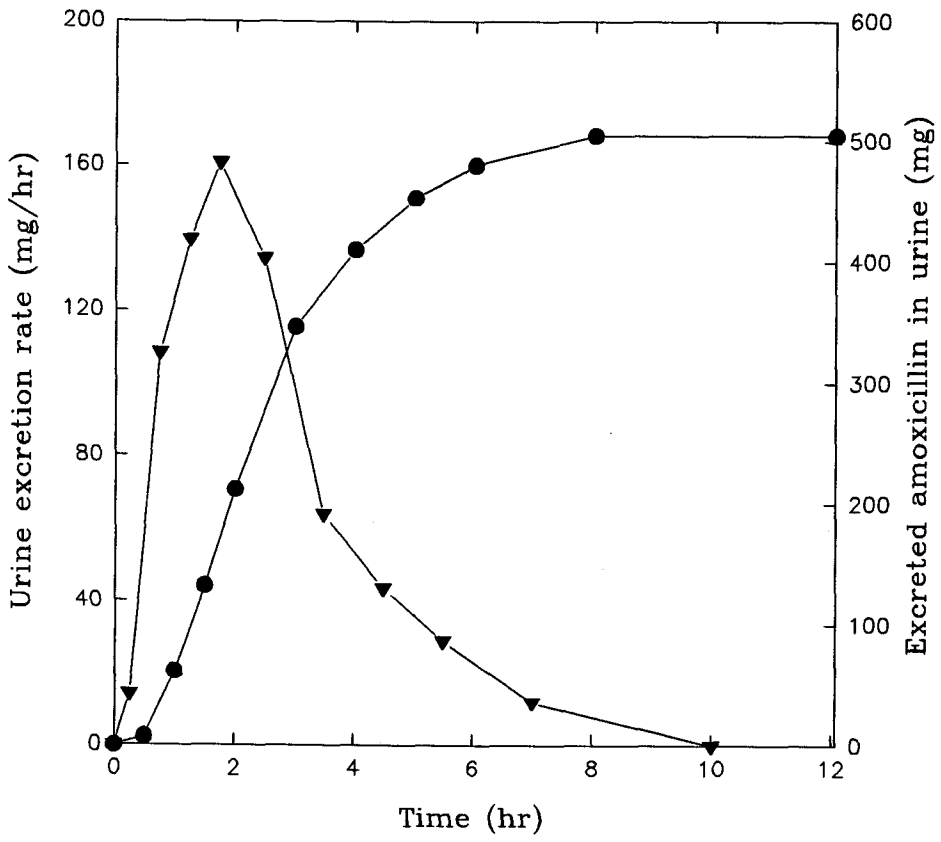

Figure A.1 Profiles of amoxicillin excreted in urine (solid circle) and the excretion rate (triangle) for capsules for trial 1.

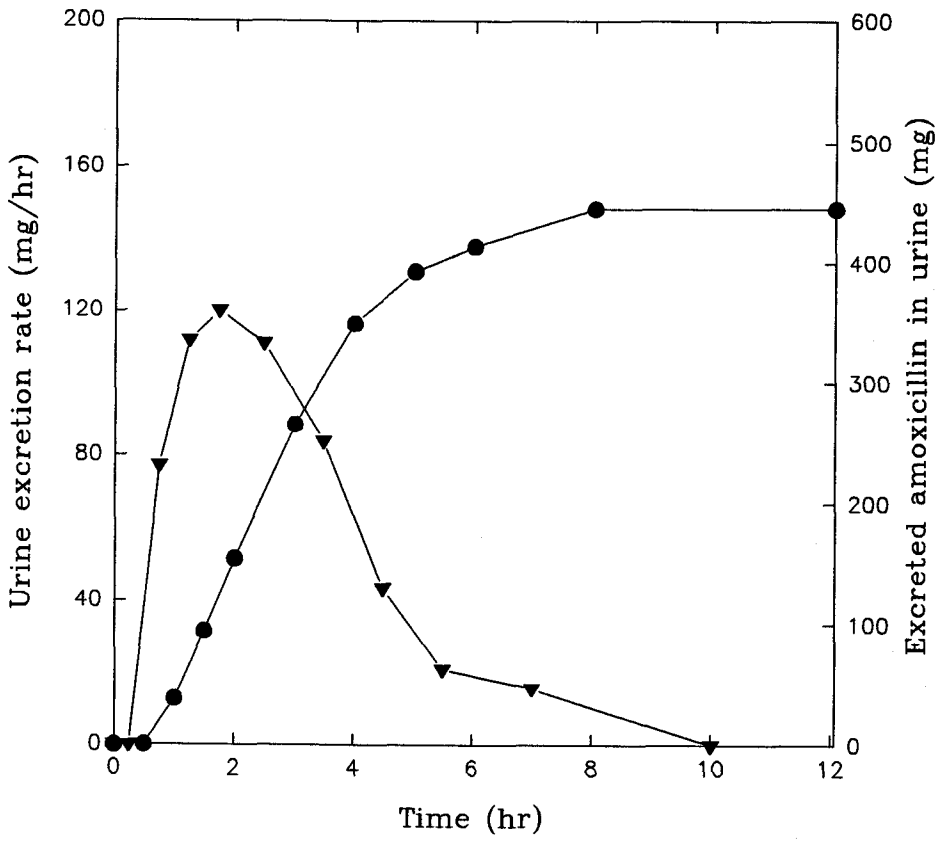

Figure A.2 Profiles of amoxicillin excreted in urine (solid circle) and the excretion rate (triangle) for capsules for trial 2.

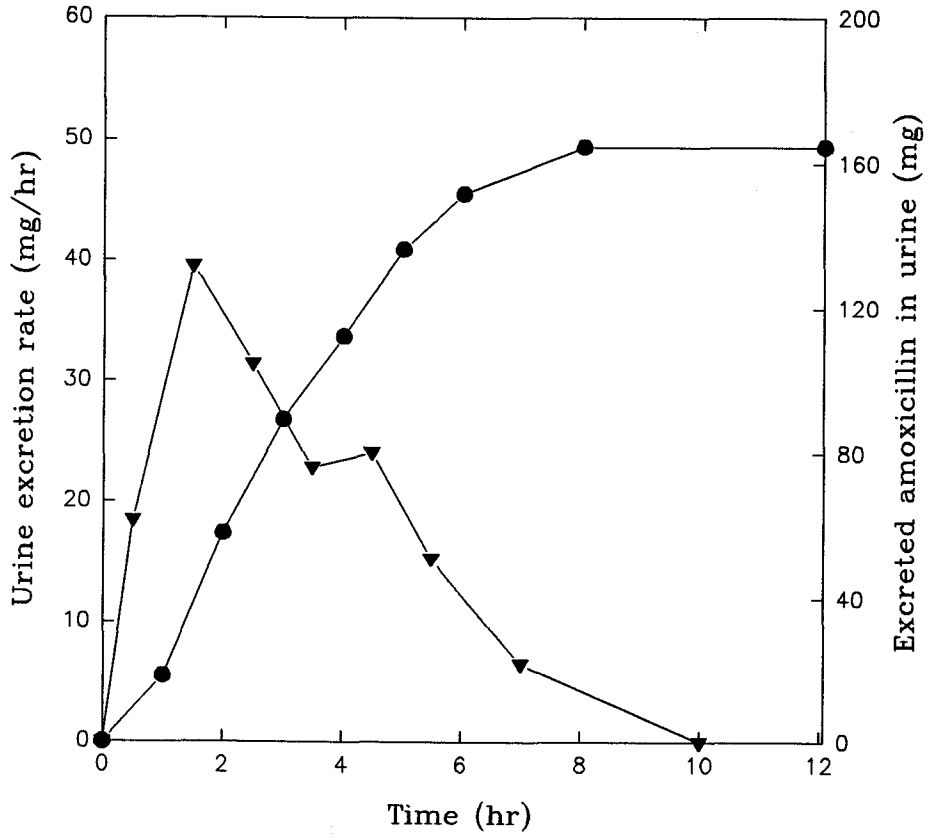

Figure A.3 Profiles of amoxicillin excreted in urine (solid circle) and the excretion rate (triangle) for sustained release tablet containing 7.8% stearic acid for trial 1.

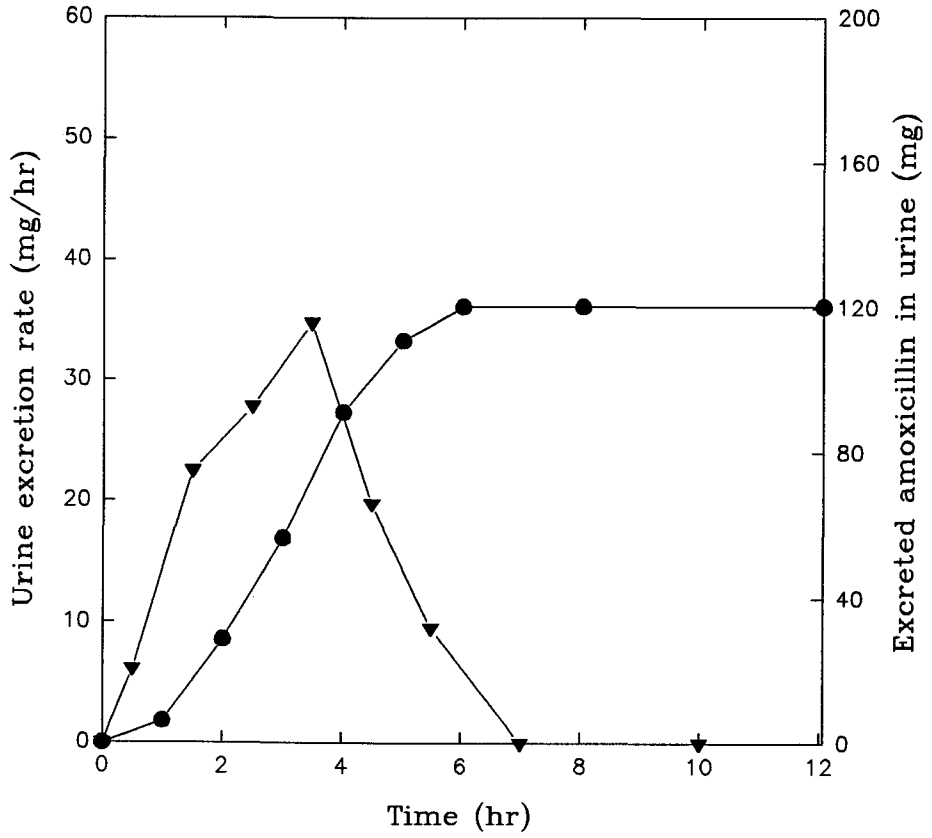

Figure A.4 Profiles of amoxicillin excreted in urine (solid circle) and the excretion rate (triangle) for sustained release tablet containing 7.8% stearic acid for trial 2.

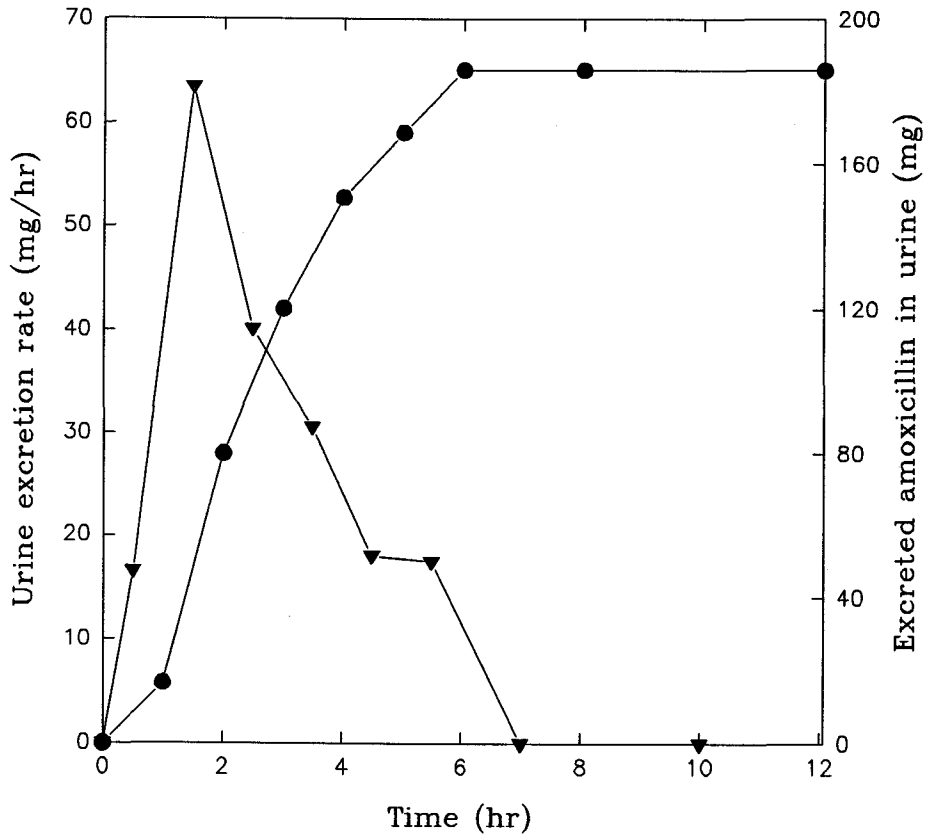

Figure A.5 Profiles of amoxicillin excreted in urine (solid circle) and the excretion rate (triangle) for sustained release tablet containing 14.5% stearic acid for trial 1.

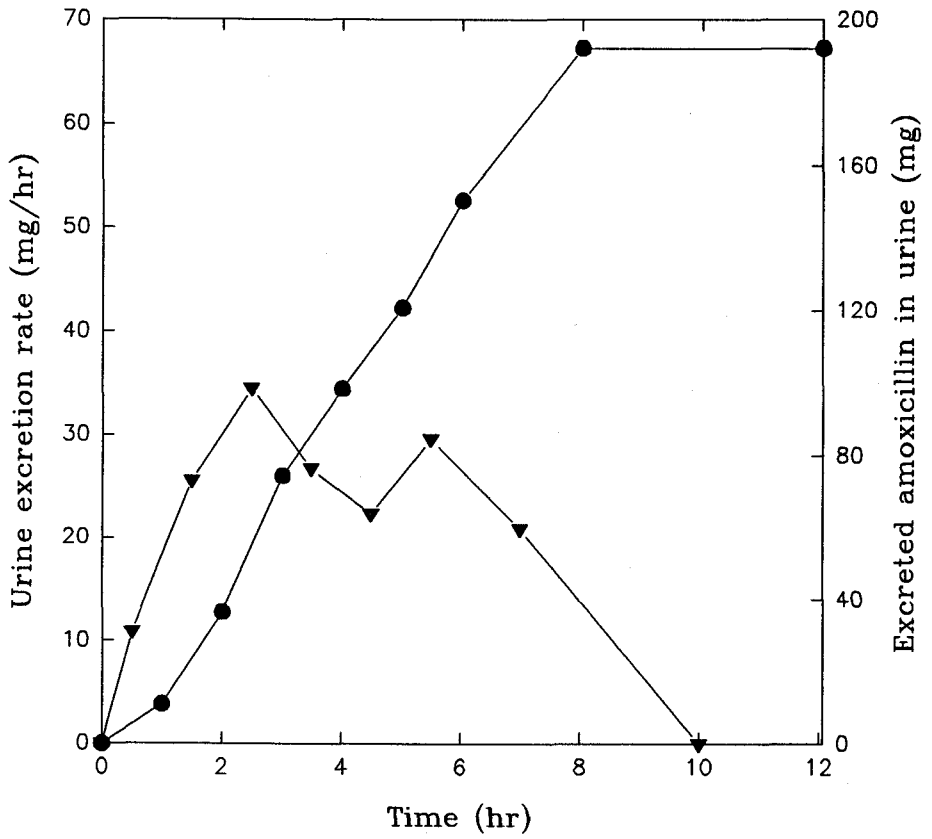

Figure A.6 Profiles of amoxicillin excreted in urine (solid circle) and the excretion rate (triangle) for sustained release tablet containing 14.5% stearic acid for trial 2.

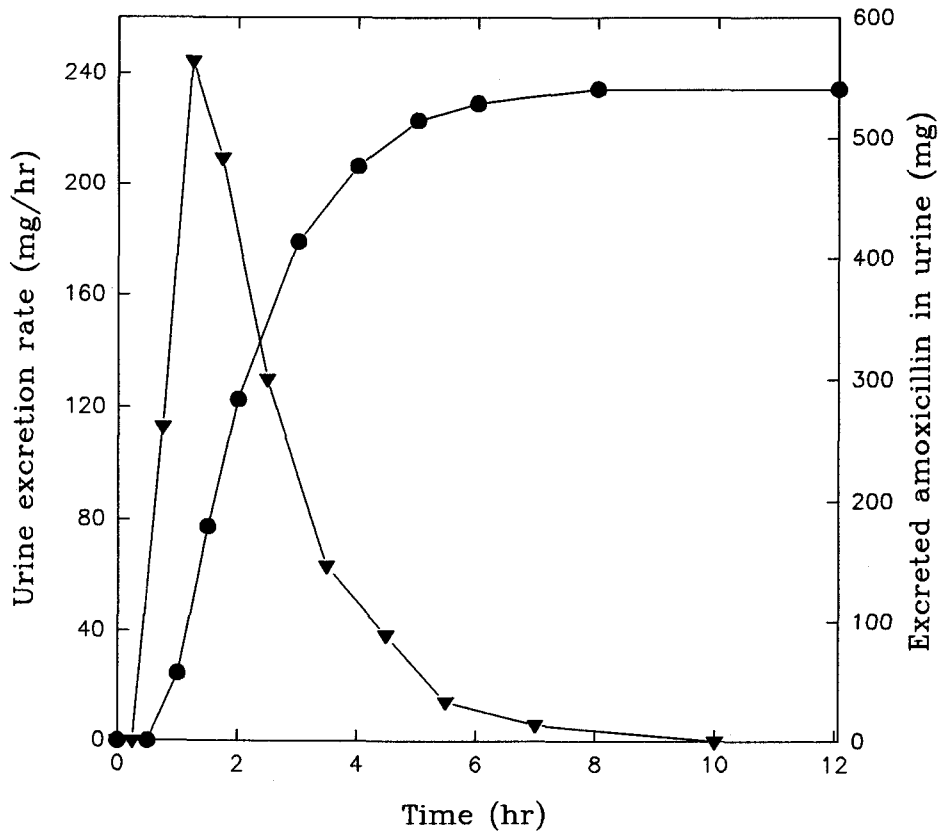

Figure A.7 Profiles of amoxicillin excreted in urine (solid circle) and the excretion rate (triangle) for single dosing of suspension.

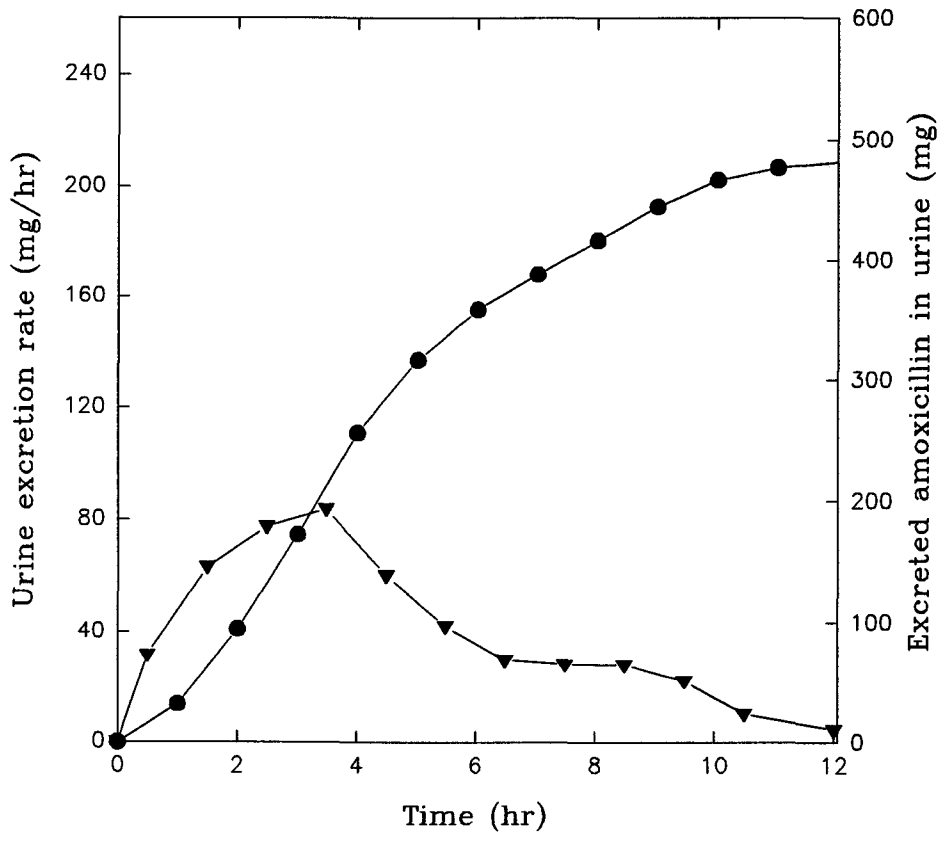

Figure A.8 Profiles of amoxicillin excreted in urine (solid circle) and the excretion rate (triangle) for intermittent dosing of suspension.

APPENDIX B
PREPARATION OF SOLUTIONS

1. Simulated gastric fluid

Dissolve 6.0 g of NaCl in 21 ml concentrated HCl. Add deionized water to 3000 ml. Adjust pH to 1.4 $\pm$ 0.1.

2. Simulated intestinal fluid

Dissolve 20.4 g of potassium phosphate monobasic (KH_2PO_4) in about 2000 ml deionized water. Add 570 ml of 0.2 N NaOH. Mix and adjust resulting solution with 0.2 N NaOH to pH 7.4 $\pm$ 0.1. Add deionized water to 3000 ml.

3. Solution of pH adjustment

Add 100 ml of 0.5 M disodium hydrogen phosphate to 350 ml of deionized water. Adjust pH to 4.85 with 1 M citric acid. Dilute with deionized water to 500 ml.

4. HPLC mobile phase

Add 200 ml methanol in 3800 ml 0.005 M potassium dihydrogen phosphate solution. Degas 20 minutes by stirring under vacuum.