

Stability Studies of Ampicillin Trihydrate in Suspensions and Acidic Aqueous Solutions

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Background: Ampicillin in trihydrate form is a β -lactamine antibiotic frequently used in therapy as suspensions and capsules. Because of the low stability only dry suspensions are sold, and also the low stability in acidic environment is source of unwanted side effects and bioavailability variations.

Aim: Our goal was to stabilize ampicillin so that a better stability can be obtained both in water suspensions and acidic solutions. This way misuse due to faulty preparation, side effects and bioavailability problems can be avoided.

Methods: In order to assess the changes in the ampicillin concentration high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) methods have been used.

Results: None of the tried excipients improved stability of ampicillin suspensions. In contrast cyclodextrins and magnesium salts of glutamic and aspartic acid greatly improved the stability of ampicillin acidic solutions. In high amounts cyclodextrins also change the decomposition kinetic of ampicillin, which is usually a first order kinetic process.

Conclusions: Cyclodextrins and magnesium salts of glutamic and aspartic acid have the potential to be used in ampicillin containing formulations in order to increase its stability, bioavailability and to reduce adverse effects.

Keywords: ampicillin, stability, auxiliary substances, chromatographic methods

Introduction

Approximately 60% of the antibiotics used today are β -lactamine antibiotics [1]. Stability is one of the major drawbacks concerning this class of antibiotics since β -lactamine cycle can be easily hydrolyzed [1].

Ampicillin in trihydrate form is a β -lactamine antibiotic frequently used in therapy as suspensions and capsules. Suspensions are conditioned in dry form and after preparation one week stability can be warranted. Orally administered, ampicillin has a relatively low bioavailability (60% administered on empty stomach and about 30% when administered with food) [2]. One explanation of this fact can be the low stability of ampicillin in acidic environment: pH 1–3 [1]. At these pH values β -lactamine cycle breaks and dimmers and trimmers are formed due to an electrophilic attack of the amino group in the secondary chain on the β -lactamine carbonyl of another ampicillin molecule. Because of these interactions adverse reactions can appear since the formed polymers have antigenic properties and also a low and variable bioavailability can be attained [3].

Associations with auxiliary substances (2-hydroxypropyl- β -cyclodextrins) was proved to be efficient in protecting the ampicillin molecule against degradation at low pH values [3,4].

The aim of this work was to study the effect of different auxiliary substances on degradation of ampicillin in suspensions and on the stability of ampicillin in acidic environment [3,4,5,6,7,8].

Methods used for ampicillin stability assessments were HPLC and TLC [9,10].

Material and methods

Reagents:

- ▶ Ampicillin trihydrate p.a. Applichem, Germany ;
- ▶ β -Cyclodextrin (β -CD) and 2-Hydroxypropyl- β -Cyclodextrin (2-HP- β -CD) analytical purity, from Molekula England;
- ▶ sodium citrate, citric acid, pharmaceutical purity, from Pernhofen Austria ;
- ▶ gum arabic, pharmaceutical purity;
- ▶ carboxymethyl cellulose sodium, pharmaceutical purity, Hercules Germania;
- ▶ sorbitol, pharmaceutical purity, Syral Franta;
- ▶ gradient grade HPLC solvents were purchased from Merck KgaA;
- ▶ food grade saccharose;
- ▶ Clark and Lubs buffer pH 1.2 (HCl - 1/5 M, KCl - 1/5 M, water), [4]
- ▶ Dihydrogen phosphate 0.2 M, analytical purity, from Merck KgaA;
- ▶ Acetone or analysis, analytical purity, from Merck KgaA;
- ▶ Acetonitrile, analytical purity, from Merck KgaA;
- ▶ Glacial acetic acid, analytical purity, from S.C. Nordic Invest SRL Cluj-Napoca ;
- ▶ Sodium bicarbonate, analytical purity, from S.C. Nordic Invest SRL Cluj-Napoca ;
- ▶ L-Glutamic acid hemimagnesium salt tetrahydrate, analytical purity, from Sigma Aldrich;
- ▶ DL-aspartic acid hemimagnesium salt tetrahydrate, pharmaceutical purity, from chemBlink;
- ▶ Silanized silica gel plate 60 F254 (Merck) of 0,25 mm;

- ▶ solutions for calibration curve were prepared by dissolving ampicillin trihydrate in mobile phase A (concentrations ranged between 5 and 120 µg/ml).

HPLC procedure [9]

- ▶ LiChroCART 250-4 LiChrospher 100 RP-18 (5 µm) Merck column;
- ▶ Mobile phase A: a mixture consisted of 0.5 ml diluted acetic acid, 50 ml solution of potassium dihydrogen phosphate 0.2 M and 50 ml of acetonitrile is diluted to 1000 ml with purified water;
- ▶ Mobile phase B: a mixture consisted of 0.5 ml diluted acetic acid, 50 ml solution of potassium dihydrogen phosphate 0.2 M and 400 ml of acetonitrile is diluted to 1000 ml with purified water;
- ▶ flow 1 ml/minute, mobile phase is formed of the mixture of 15 volumes B phase cu 85 volumes A phase;
- ▶ detection wavelength 254 nm;
- ▶ injection volume 20 µl.

Ampicillin suspensions stability experiment:

For better precision of sampling ampicillin suspensions were prepared in test tubes by measuring 60 mg of ampicillin trihydrate powder and the corresponding excipient and adding 2 ml of purified water. Following excipients were used: no excipient (1), 29.0 mg sodium citrate (2), 57.6 mg gum arabic (3), 5.0 mg carboxymethyl cellulose sodium (4), 109.0 mg magnesium glutamate (5), 97.4 mg magnesium aspartate (6), 162.0 mg β-Cyclodextrin (7), 162.0 mg 2-Hydroxypropyl-β-Cyclodextrin (8), 100.0 mg sodium citrate + 42 mg citric acid (9), 859.0 mg saccharose (10), 400.0 mg sorbitol (11).

Every sampling time a test tube was opened and the content was dissolved, in an ultrasonic bath, in 100 ml mobile phase A. After a 1:10 dilution 20 µl of sample was injected into the HPLC system.

Ampicillin solution stability experiment:

Ampicillin solutions were prepared in test tubes by measuring 10.0 mg of ampicillin trihydrate powder and the corresponding excipient and adding 4 ml of Clark-Lubs buffer. Following excipients were used: no excipient (1),

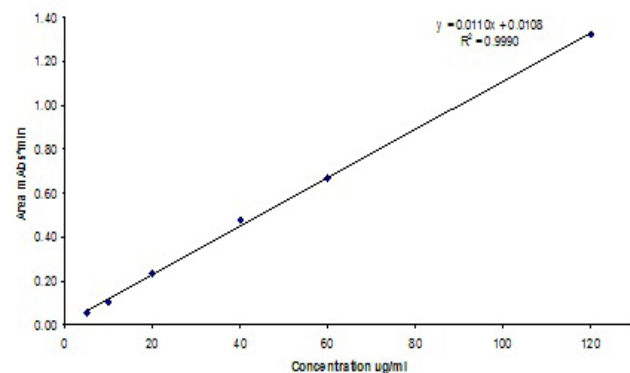


Fig. 1. Ampicillin calibration curve

27.9 mg 2-Hydroxypropyl-β-Cyclodextrin 1:1 molar ratio (2), 57.1 mg 2-Hydroxypropyl-β-Cyclodextrin 1:2 molar ratio (3), 137.5 mg 2-Hydroxypropyl-β-Cyclodextrin 1:5 molar ratio (4), 18.8 mg magnesium glutamate (5), 18.8 mg magnesium aspartate (6), 27.7 mg β-Cyclodextrin 1:1 molar ratio (7), 55.7 mg β-Cyclodextrin 1:2 molar ratio (8), 139.2 mg β-Cyclodextrin 1:5 molar ratio (9). At every sampling interval 40 µl ampicillin solutions were diluted with 960 µl mobile phase A and 20 µl were injected into the HPLC system.

TLC procedure [9,10]

- ▶ Plate: TLC silanised silicagel plate R
- ▶ Mobil phase: mix 10 volumes of acetone R and 90 volumes of 154 g/l solution of ammonium acetate previously adjusted to pH 5.0 with acetic acid R
- ▶ Application: 1 µl
- ▶ Development: over a path of 15 cm
- ▶ Drying: in air
- ▶ Detection: expose to iodine vapor until the spots appear and examine in daylight

Results

HPLC experiments

HPLC method performance check

Since the used method is a European Pharmacopoeia method a complete validation is not necessary for accepting the obtained results. Linearity and coefficients of variations were determined and acceptable results were obtained: coefficient of correlation $R = 0.9990$, residuals without correlation with concentrations, coefficients of variation under 5%.

Stability of suspensions according to the excipient used

Degradation of ampicillin without excipients was not significant at 7 days but significant at 16 days. Excipients used did not improve the stability of ampicillin; some of them (magnesium glutamate, magnesium aspartate, sorbitol and citrate buffer) even reduced its stability (Figure 2, Table I).

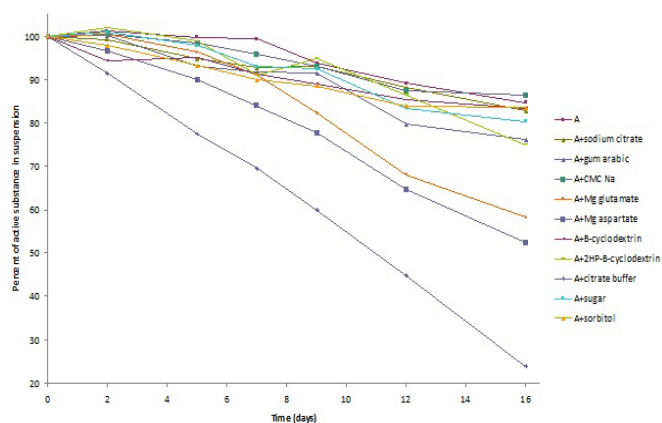


Fig. 2. Degradation curves of ampicillin (A) suspensions

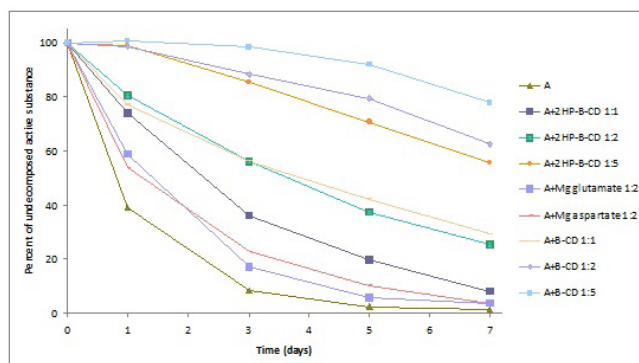


Fig. 3. Stability of ampicillin solutions at pH 1.2. Influence of excipients.

Stability of ampicillin solutions in acidic environment

All the used excipients increased the stability of ampicillin solutions. Best results were obtained with β -cyclodextrin in 1:2 and 1:5 mole ratio and 2-hydroxypropyl- β -cyclodextrin in 1:5 mole ratio. These excipients in the given mole ratios also modified the decomposition kinetics of ampicillin. The decomposition kinetic of ampicillin is a first order kinetic with a decomposition rate constant (k_d) of 0.91 days⁻¹ ($t_{half} = 0.76$ days, $R = 0.9995$).

Decomposition rate constant was calculated using the exponential equation that fitted every pair of results (time, concentration) and half life (t_{half}) was calculated using the equation $t_{half} = \ln 2/k_d$.

Figure 4 shows the changes in the decomposition kinetics of ampicillin when cyclodextrins are used in higher ratio than ampicillin. The equation that fits the results ($R=0.9963$, residuals lower than 2%) is

$$\% \text{ of active substance} = e^{a + \frac{b}{\text{Time} + c}}$$

Table I. Losses of ampicillin in suspensions; influence of the excipient

No.	Excipient used	Loss at 7 days %	Loss at 16 days %
1	No excipient	0.54	15.33
2	Sodium citrate	7.26	17.25
3	Gum arabic	8.26	23.85
4	CMC Na	4.16	13.49
5	Magnesium glutamate	9.03	41.79
6	Magnesium aspartate	16.03	47.55
7	β -cyclodextrin	8.78	16.57
8	2-hydroxypropyl- β -cyclodextrin	8.88	25.00
9	Citrate buffer	30.60	76.12
10	Saccharose	6.98	19.73
11	Sorbitol	10.12	16.39

Table II. Influence of the excipients on ampicillin decomposition kinetics (when first order kinetic is employed)

Excipient	No excipient	2-HP- β -CD 1:1	2-HP- β -CD 1:2	Magnesium glutamate 1:2	Magnesium aspartate 1:2	β -CD 1:1
k_d (days ⁻¹)	0.911 \pm 0.035	0.335 \pm 0.011	0.195 \pm 0.003	0.561 \pm 0.022	0.523 \pm 0.043	0.175 \pm 0.011
t_{half} (days)	0.76	2.06	3.55	1.23	1.32	3.96
R	0.9995	0.9992	0.9996	0.9993	0.9968	0.9950

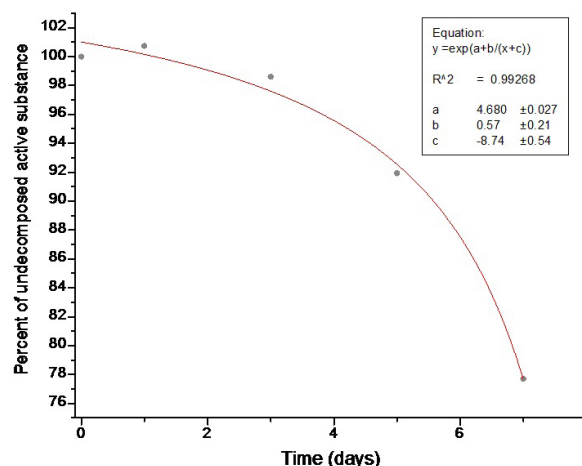


Fig. 4. β -cyclodextrin (1:5) modified decomposition kinetics of ampicillin in acidic solution

TLC experiments

TLC experiments showed that there is an interaction between cyclodextrins and ampicillin resulting in an increased stability of ampicillin. In acidic environment the spot characteristic to ampicillin ($R_f = 0.88$), disappears after 1 days being replaced by another spot that has a smaller $R_f = 0.63$. In the presence of cyclodextrins the intensity of the decomposition spot is much lower and the ampicillin spot remains clearly visible. Higher concentrations of cyclodextrin have higher protective effect (Figure 5 and 6).

Discussions

HPLC experiments

Stability of suspensions according to the excipient used

Degradation of ampicillin followed a formal zero order decomposition kinetic (Figure 2). This behavior was observed probably due to the fact that dissolution of ampicillin takes place more slowly than the decomposition.

Stability of ampicillin suspensions was followed for 16 days (more than twice the warranty period) [6]. Interestingly, no stability increase was observed for the auxiliary substances that were tried during this study (Figure 2). Some of the studied excipients are often used in ampicillin suspensions: saccharose, sorbitol, citric acid, citrate buffer, CMC-Na, gum arabic [6,7,8].

There were 4 excipients that lowered the stability of ampicillin: magnesium glutamate, magnesium aspartate, sorbitol and citrate buffer with losses at 7 days of: 9.03, 16.03, 10.12 and 30.6% respectively. It is interesting to note that

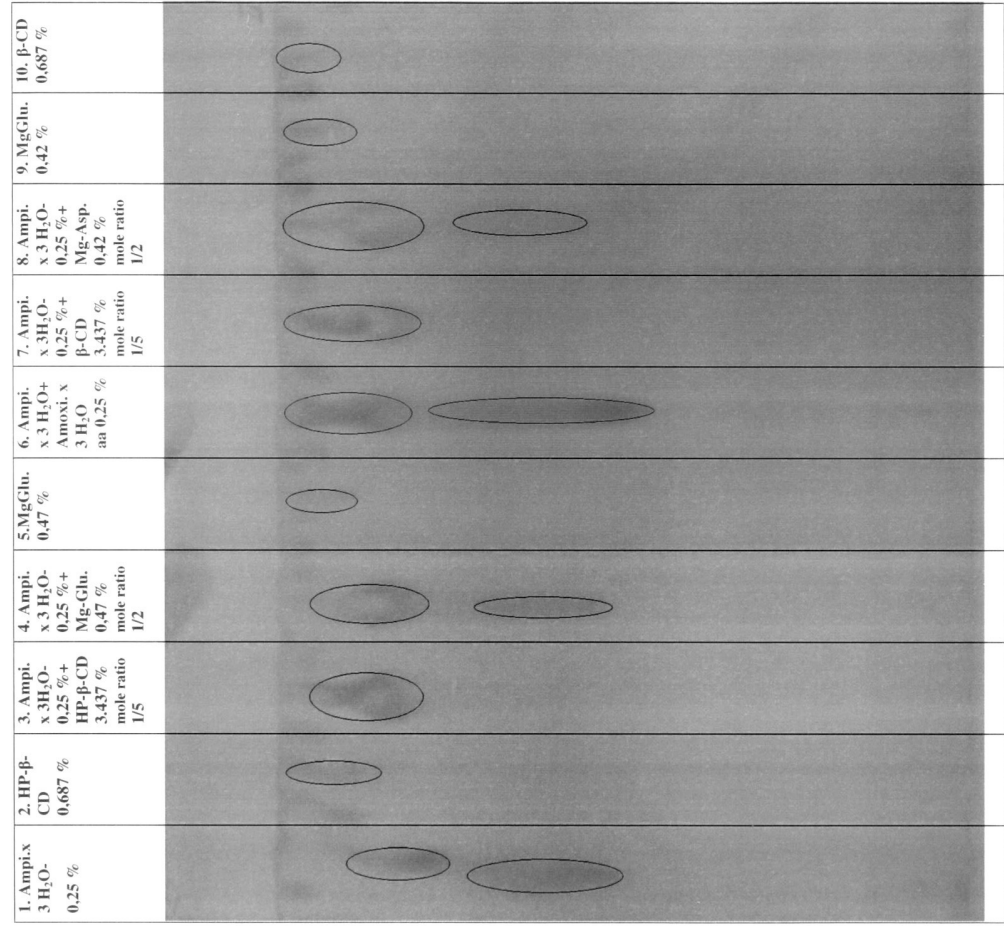


Fig. 6. Excipients influence on ampicillin stability (pH = 1.2)

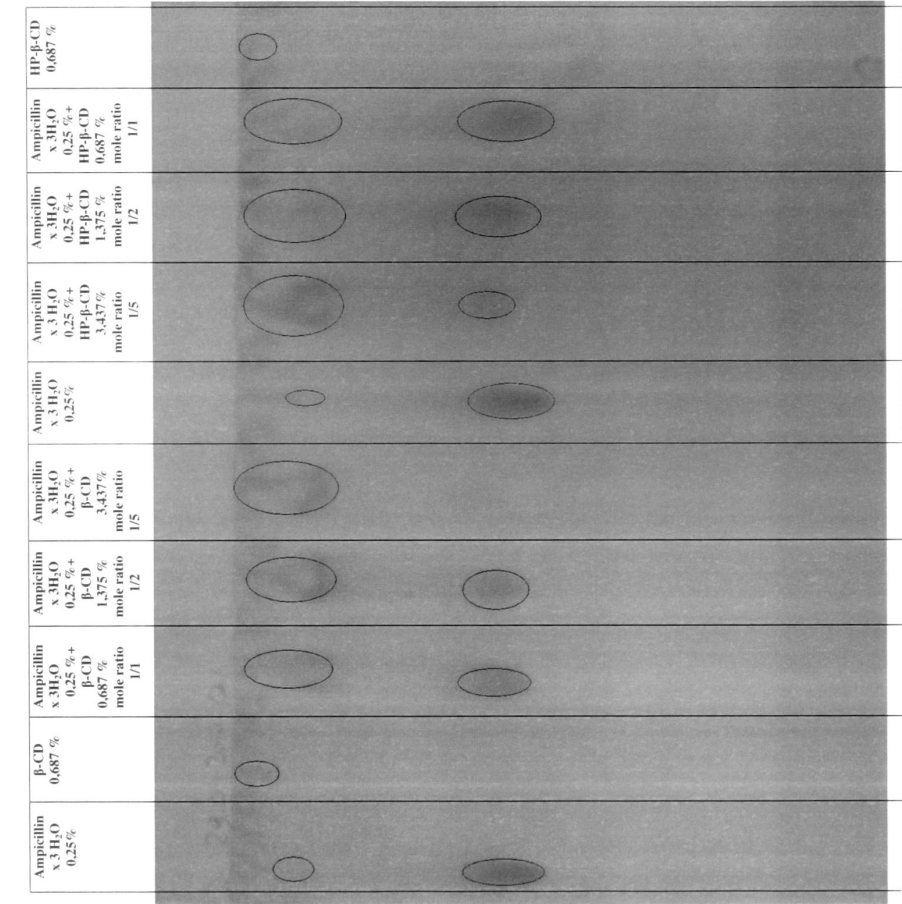


Fig. 5. Cyclodextrin influence on ampicillin stability (pH = 1.2).

sorbitol and citrate buffer are used in some ampicillin suspensions [7,8].

After 16 days of degradation there were only 4 excipients that were not worsen significantly the stability of ampicillin: CMC-Na, sorbitol, β -cyclodextrin, sodium citrate. It is worth to mention that no significant increase of stability was recorded for any excipient. Decrease of concentration in the ampicillin suspension without excipients was 15.32% (Table I).

Stability of ampicillin solutions in acidic environment

In contrast to suspensions where stability is decreased by the added excipients, in acidic environment there are possibilities to increase stability [3,4]. Previously published works showed that in acidic environment ampicillin is stabilized by 2-hydroxypropyl- β -cyclodextrin [3,4], and penicillin G is stabilized by magnesium glutamate [5]. Our work confirmed the published results and showed that β -cyclodextrin, magnesium glutamate and magnesium aspartate also protect ampicillin against decomposition in acidic environment.

Formula that fits results where shift in the decomposition kinetic was observed suggests that there might be an autocatalytic phenomenon, or other process, that speeds up the decomposition reaction with time. It means that cyclodextrins protect the substance from decomposition only for a certain amount of time after which the protective effect decreases. However at 7 days cyclodextrin protected ampicillin solutions had tens of times more unchanged ampicillin than non protected solution had.

TLC experiments

TLC experiments yielded similar results to that obtained with the HPLC technique and the same concentration dependent protective effect was recorded for cyclodextrins, magnesium glutamate and magnesium aspartate [figure 5,6].

Conclusions

Ampicillin if not dissolved is stable in contact with water for 7 days but left for longer periods of time concentra-

tion is steadily decreasing, and that is the reason why, for the time being, only dry suspensions are used in therapy (dry suspensions need to be prepared by the patient and are prone to preparation errors compared to the ready to use suspensions). None of the tested excipients permitted the formulation of a stable suspension that can be conditioned in a ready to use form. It worth to mention that some of the excipients used in preparation might even worsen the stability of ampicillin suspensions, thus their use should be reconsidered (citrate buffer).

Solutions of ampicillin in acidic environment were very unstable but there were excipients that successfully increased the stability in this environment. These excipients regarding the concentration in which were used were able to even change the kinetics of ampicillin decomposition. Because some drawbacks of ampicillin use are caused by decomposition in acidic environment, using stabilizing excipients such as cyclodextrins might improve therapeutic response and reduce side effects.

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