Improvements of Oxacillin Stability in a pH = 1.2 Acidic Environment

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Introduction: Oxacillin sodium is a semisynthetic penicillin used in therapy against Staphylococcus species. It is orally administered as capsules. Because of the low stability of oxacillin in stomach acid, a low bioavailability is recorded during oral administration (30%). The **aim** of this study was to improve, by using some auxiliary substances, the stability of oxacillin in acidic environment.
Methods: The improvement of oxacillin stability was measured by high performance liquid chromatography in the presence of β-cyclodextrin, 2-HP-β-cyclodextrin, magnesium glutamate and magnesium aspartate.
Results: Cyclodextrins significantly improved the stability of oxacillin in acidic environment. Glutamate and aspartate showed no effect on this regard. First order decomposition kinetics of oxacillin was modified by cyclodextrins.
Conclusions: Significant improvement of oxacillin stability and possible pharmacokinetics can be achieved by using cyclodextrins.

Keywords: oxacillin, stability, decomposition, cyclodextrins, glutamate, aspartate

Introduction

Oxacillin is a narrow spectrum semisynthetic penicillin, resistant to penicillinase. Since it is resistant to penicillinase enzymes, such as that produced by Staphylococcus aureus, it is widely used clinically to treat infections with penicillin-resistant Staphylococcus aureus. Capsules of 250 and 500 mg are available [1].

Because oxacillin is highly unstable in acidic environment it is decomposed by gastric acid leading to a low bioavailability (30%) [2].

The instability in acidic environment is exhibited also by other penicillins.

Methods used to increase the stability of penicillins in acidic environment are published in scientific literature for ampicillin [3], amoxicillin [3], phenoxymethylpenicillin [4]. These methods use auxiliary substances as: β-cyclodextrin [5], 2-HP-β-cyclodextrin [3,4], magnesium glutamate [6] and magnesium aspartate [6] in different mole ratios.

Among other penicillin derivatives, the increase of oxacillin stability in acidic environment is less studied.

The purpose of this study is to increase the stability of oxacillin in acidic environment using auxiliary substances that were proved to be efficient in the case of other penicillins.

Materials and methods

Reagents

- Oxacillin sodium monohydrate (Oxa) p.a. Applichem, Germany;
- β-Cyclodextrin (β-CD) and 2-Hydroxypropyl-β-Cyclodextrin (2-HP-β-CD) analytical purity, from Molekula England;
- 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;
- Acetonitrile, HPLC gradient grade, from Merck KgaA;
- L-Glutamic acid hemimagnesium salt tetrahydrate (Mg-Glu), analytical purity, from Sigma Aldrich;
- DL-aspartic acid hemimagnesium salt tetrahydrate (Mg-Asp), pharmaceutical purity, from chemBlink;
- solutions for calibration curve were prepared by dissolving Oxa in acetonitrile free mobile phase A (concentrations ranged between 1 and 125 μg/ml).

The stability of oxacillin was studied in a pH = 1.2 acidic environment. This was done by obtaining an amoxicillin solution in Clark-Labs buffer [4], adding an auxiliary substance and measuring at different time intervals the concentration of the non-decomposed active substance by an HPLC technique [7,8].

The auxiliary substance was weighted in 5 ml polypropylene test tubes and dissolved in 4 ml of a solution containing 0.625 mg/ml oxacillin sodium monohydrate.

The following excipients quantities and mole ratios were used: no excipient (1); 6.42 mg β-cyclodextrin mole ratio 1/1 (2); 12.87 mg β-cyclodextrin mole ratio 1/2 (3); 19.27 mg β-cyclodextrin mole ratio 1/3 (4); 32.12 mg β-cyclodextrin mole ratio 1/5 (5); 44.97 mg β-cyclodextrin mole ratio 1/7 (6); 4.4 mg magnesium glutamate (7); 4.4 mg magnesium aspartate (8); 6.75 mg 2-HP-β-cyclodextrin mole ratio 1/1 (9); 13.5 mg 2-HP-β-cyclodextrin mole ratio 1/2 (10); 20.25 mg 2-HP-β-cyclodextrin mole ratio 1/3 (11); 33.75 mg 2-HP-β-cyclodextrin mole ratio 1/5 (12); 47.25 mg 2-HP-β-cyclodextrin mole ratio 1/7 (13).

At every sampling time 200 μl sample was mixed with 800 ml acetonitrile free mobile phase A, followed by injection of 30 μl mixture into the HPLC system.

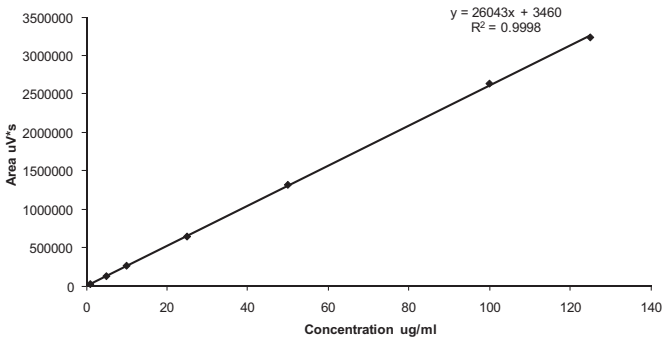

Fig. 1. Oxacillin calibration curve

HPLC method

For measuring the concentrations of oxacillin in the samples a HPLC method officinal in the European Pharmacopoeia was used [7].

- LiChroCART 250-4 LiChrospher 100 RP-18 (5 μm) Merck column;
- mobile phase: acetonitrile 250 ml + 750 ml of a 2.7 g/L dihydrogen phosphate solution with a pH brought to 5 with diluted sodium hydroxide;
- flow: 1 ml/min;
- detection wavelength: 225 nm;
- injected volume 30 μl.

Results

Method performance check

Since the used method is officinal in the European Pharmacopoeia [7] method a complete validation is not necessary for accepting the obtained results. However, in order to ensure an optimal functioning of the system, linearity and specificity of the method were checked.

A 1–125 μg/ml concentration range was used for the calibration curve. The range was chosen in order to ensure a quantification of at least 1% of the starting concentration. In this way the concentration of the active substance can be measured until 99% decomposition. Good specificity and linearity (coefficient of correlation R = 0.9998, residuals with values lower than 10%, no correlation between residuals and concentration) were obtained (Figure 1).

Cyclodextrins used in different mole ratios significantly increased the stability of oxacillin in acidic environment (Figure 2).

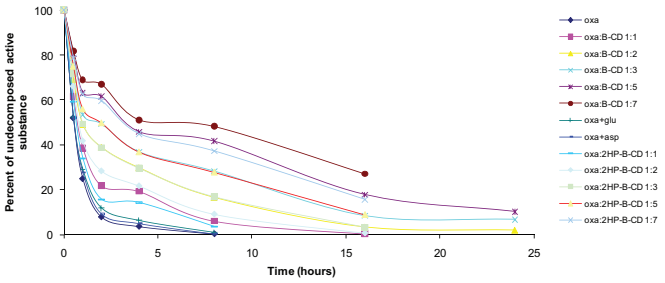

Fig. 2. The influence of auxiliary substances on oxacillin stability

By calculating the percent of the active substance one can see that increasing the cyclodextrin concentration the protective effect increases too.

Figure 3 shows the percents of oxacillin left at different time intervals and the relation between this percent and the oxacillin/cyclodextrin mole ratio. An asymptotic correlation between concentration of cyclodextrin and the stability in the acidic medium can be observed. Higher the concentration of cyclodextrin higher the protective effect is. This protective effect increase has a limit; over this limit increasing cyclodextrin concentration no further benefits are obtained.

Similar results were obtained for 2-HP-β-cyclodextrin (Figure 4). The protective effect of this cyclodextrin is somewhat lower that that of β-cyclodextrin.

It is interesting to note that the presence of cyclodextrins changed the kinetic of the decomposition process of oxacillin. Because of this, it was not possible to calculate a decomposition rate constant and a half life of oxacillin in the presence of cyclodextrins. The shape of the curve obtained by plotting the percent of the not decomposed oxacillin as a function of time suggests an exponential correlation. Neither first nor second order kinetics does not offer a good correlation. For a first order kinetic process the coefficient of correlation R is higher than 0.96 but the residuals are to high.

Without cyclodextrins the decomposition process of oxacillin (Figure 4) is described perfectly by a first order kinetic process (R = 0.9991, most of residuals under 2% without correlation with the time). The decomposition rate constant (kd) was computed using the first order kinetic process equation, and the half life was computed using the equation: $t_{1/2} = \ln 2/kd$.

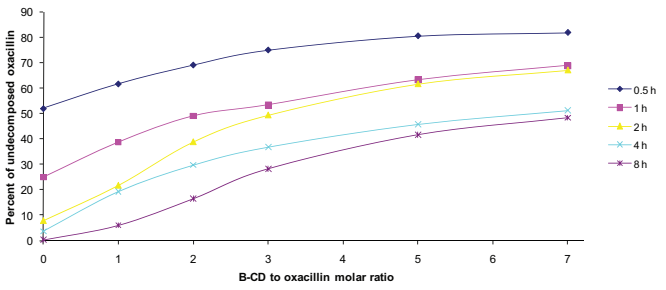

Fig. 3. Protective effect of β-cyclodextrin; correlation of this effect with oxacillin/cyclodextrin mole ratio

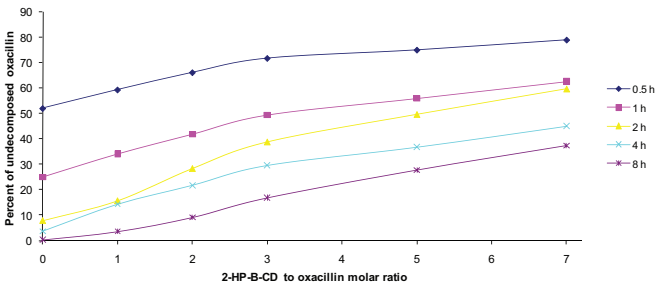

Fig. 4. Protective effect of 2-HP-β-cyclodextrin; correlation of this effect with oxacillin/cyclodextrin mole ratio

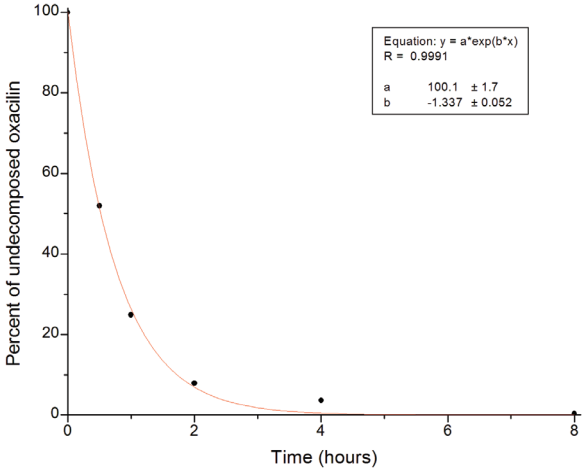

Fig. 5. Oxacillin decomposition process, t½ = 31 minutes, decomposition rate constant kd = 1.33 h^{-1}, (R >0.999, residuals under 2%).

Discussions

HPLC analysis showed that a significant increase of oxacillin stability in acidic environment can be obtained by using cyclodextrins. The effect is similar to that described for other penicillins [3–5]. In a few hours oxacillin completely decomposes in acidic environment, but in the presence of cyclodextrins more than 50% of oxacillin remains not decomposed in the same period of time. This stability increase can be clinically important since it can lead to higher bioavailability and decreased interindividual variations.

Magnesium glutamate and magnesium aspartate did not bring any changes in oxacillin concentrations or oxacillin kinetics during the decomposition process. This observation is interesting since there are penicillins where magnesium glutamate and magnesium aspartate significantly increased their stability [6,9].

Conclusions

This work proves that cyclodextrins are able to significantly increase the stability of oxacillin in an acidic environment with a pH of 1.2.

Unexpectedly no improvements in oxacillin stability can be attained by using magnesium salts of amino acids (glutamate and aspartate).

Between the increase of stability in acidic environment and the concentration of cyclodextrin there is an asymptotic correlation, which means that there is a limit of cyclodextrin concentration above which no further protective effect can be obtained. Between the two studied cyclodextrins there is no important difference in the stability increase.

Decomposition of oxacillin is due to the opening of the β-lactam ring. A protective effect of cyclodextrins can be explained by an inclusion of the penam ring in the cavity of cyclodextrins. This finding is suggested by the literature data too [3–5].

Increasing the stability of oxacillin can be certainly a clinical advantage for this product.

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