Improvements of Amoxicillin Stability in Acidic Environment

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Background: Helicobacter pylori is a gram negative bacteria responsible for a series of gastrointestinal diseases: gastric and gastro-duodenal ulcers. Usually used in combinations with other drugs, amoxicillin is effective against this germ. Amoxicillin has better stability than other penicillins in solutions with pH between 4 and 7, but stability is decreased at low pH values (gastric acidity).

Aim: Our goal was to improve amoxicillin’s stability by using auxiliary substances such β-cyclodextrin (β-CD), 2-hydroxypropil-β-cyclodextrin (2-HP-β-CD), magnesium glutamate and magnesium aspartate.

Methods: Influence of these excipients on amoxicillin stability was assessed at pH value of 1.2 and also in weakly alkaline environment. High pressure liquid chromatography and thin layer chromatography were used to quantitate these influences.

Results: All the studied excipients improved the stability of amoxicillin, best results being recorded when amoxicillin was associated with cyclodextrins in a mole ratio of 1:5.

Conclusions: Poor stability of amoxicillin in acidic environments can be overcome by using cyclodextrins and magnesium salts of glutamic and aspartic acids.

Keywords: amoxicillin, stability, auxiliary substances, chromatographic methods

Introduction

Amoxicillin associated with a proton pump inhibitor as omeprazole [1, 2] or in triple therapy (amoxicillin + metronidazole + rameprazole) [3] is efficient in the treatment of hyperacidic gastritis and gastroduodenal ulcers when Helicobacter pylori is present.

Amoxicillin in trihydrate form is used as capsules or pediatric suspensions. It has a better bioavailability than ampicillin [4]. Amoxicillin is stable in aqueous suspensions (pH 4-7) for a week [5]. Stability is greatly reduced in highly acidic environment (stomach) and also in weakly alkaline ones [1, 2].

Cyclodextrins (β-cyclodextrin (β-CD), 2-hydroxypropil-β-cyclodextrin (2-HP-β-CD)) have the ability to form inclusion complexes with many drugs. This way solubility, stability and bioavailability of such drugs can be improved. In highly acidic environment two types of inclusion complexes were found using a mole ratio of 1:1 [1,2,6]. In these complexes penam or phenyl groups were included in cyclodextrins hydrophobic cavity. When amoxicillin: cyclodextrin mole ratio is increased both complexes can coexist [1,2,6]. Importance for amoxicillin stability has the complex where penam cycle is included in the cyclodextrin cavity [1,2,6].

Magnesium glutamate and aspartate were also used for improving the stability of β-lactam antibiotics [7].

Our goal was to study the influence of the above mentioned auxiliary substances on the stability of amoxicillin in weakly alkaline and highly acidic environments. Methods that were used are: high performance liquid chromatography (HPLC) [8,10] and thin layer chromatography (TLC) [8, 9, 10].

Materials and methods

Reagents

- Amoxicillin trihydrate (Amox) p.a. Applichem, Germany;
- β-Cyclodextrin (β-CD) and 2-Hydroxypropil-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, analytical purity, 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;
- Silanized silica gel plate 60 F254 (Merck) of 0,25 mm;
- Sodium bicarbonate, analytical purity, from S.C. Nordic Invest SRL Cluj-Napoca;
- Acetonitrile, analytical purity, 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;
- Glacial acetic acid, analytical purity, from S.C. Nordic Invest SRL Cluj-Napoca;
- Acetonitrile, analytical purity, from S.C. Nordic Invest SRL Cluj-Napoca;
- 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;
- Silanized silica gel plate 60 F254 (Merck) of 0,25 mm;
- solutions for calibration curve were prepared by dissolving amoxicillin trihydrate in mobile phase A (concentrations ranged between 5 and 120 μg/ml).

An increase in amoxicillin stability in acidic environment is important for obtaining modified release pharmaceutical forms too [11].
Improvements of Amoxicillin Stability in Acidic Environment

Thin layer chromatography (TLC) procedure:
Amoxicillin concentration was 0.25% and two types of studies were made:

1. Samples were dissolved in 4.2% sodium bicarbonate. Spots were visualized under UV light and iodine coloration [8].

Two series of samples were made:
- series I: Amox (1); β-CD 0.5% (2); Amox + β-CD 0.5% in situ (3); Amox + β-CD 2:1 (4); Amox + β-CD 1:1 (5); Amox + β-CD 1:2 (6); Amox + β-CD 1:3 (7);
- series II: Amox (1); 2-HP-β-CD 0.687% (2); Amox + 2-HP-β-CD 1:5 (3); Amox + Mg-Glu 1:2 (4); Mg-Glu 0.47% (5); Amox + β-CD 1:5 (6); Amox + Mg-Asp 1:2 (7); Mg-Asp 0.42% (8), β-CD 0.687% (9);

2. Samples were dissolved in Clark-Lubs buffer and were analyzed immediately after preparation, after 24 hours and after 48 hours. Spots were visualized by iodine coloration [1, 2].

Table I. Rf values of samples analyzed by TLC

<table>
<thead>
<tr>
<th>Sample series</th>
<th>Sample number</th>
<th>Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>1</td>
<td>0.63</td>
</tr>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>2</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>3</td>
<td>0.63</td>
</tr>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>4</td>
<td>0.64</td>
</tr>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>5</td>
<td>0.64</td>
</tr>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>6</td>
<td>0.64</td>
</tr>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>7</td>
<td>0.64</td>
</tr>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>8</td>
<td>0.91</td>
</tr>
<tr>
<td>Series-I (chromatograms 1 and 2)- T0</td>
<td>9</td>
<td>0.91</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>1</td>
<td>0.61</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>2</td>
<td>0.94</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>3</td>
<td>0.64</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>4</td>
<td>0.65</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>5</td>
<td>0.94</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>6</td>
<td>0.65</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>7</td>
<td>0.65</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>8</td>
<td>0.91</td>
</tr>
<tr>
<td>Series-II (chromatogram 3)- T0</td>
<td>9</td>
<td>0.91</td>
</tr>
<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>2</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>3</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>4</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>5</td>
<td>0.95</td>
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<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>6</td>
<td>0.95</td>
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<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>7</td>
<td>0.95</td>
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<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>8</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-III (chromatogram 4)- T0</td>
<td>9</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>2</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>3</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>4</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>5</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>6</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>7</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>8</td>
<td>0.95</td>
</tr>
<tr>
<td>Series-IV (chromatogram 7)- T0</td>
<td>9</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Plate: TLC silanised silicagel plate R 20 × 20 cm;
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.

Two series of samples were made:
- series III: β-CD 0.687% (1); Amox + β-CD 1:1 (2); Amox + β-CD 1:2 (3); Amox + β-CD 1:5 (4); Amox (5); Amox + 2-HP-β-CD 1:5 (6); Amox + 2-HP-β-CD 1:2 (7); Amox + 2-HP-β-CD 1:5 (8); 2-HP-β-CD 0.687% (9);
- series IV: Amox (1); 2 HP-β-CD 0.687% (2); Amox + 2 HP-β-CD 1:5 (3); Amox + Mg-Glu 1:2 (4); Mg-Glu 0.47% (5); Amox + β-CD 1:5 (6); Amox + Mg-Asp 1:2 (7); Mg-Asp 0.42% (8) and β-CD 0.687% (9).

Plate: TLC silanised silicagel plate R 20 × 20 cm;
Mobile 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

High pressure liquid chromatography (HPLC) procedure
In a series of test tubes 10 mg of Amox were weighted together with the auxiliary substance. The mixture was dissolved in 4 ml Clark-Lubs buffer.

The following excipients were used: no excipient (1); 27.5 mg 2-HP-β-CD, Amox: auxiliary molar ratio 1:1 (2); 55.0 mg 2-HP-β-CD, 1:2 (3); 137.4 mg 2-HP-β-CD 1:5 (4); 18.8 mg Mg-Glu (5); 18.8 mg Mg-Asp (6); 27.4 mg β-CD, 1:1 (7); 54.9 mg β-CD, 1:2 (8); 137.4 mg β-CD, 1:5 (9). At every analyzed time interval 40 μl of sample solution was mixed with 960 μl of mobile phase A, followed by injection of 20 μl mixture.

- LiChroCART 250-4 LiChrospher 100 RP-18 (5 μm) Merck column;
- pH = 5 buffer solution: 250 ml potassium dihydrogen phosphate 0.2 M solution, adjusted to pH = 5 with diluted sodium hydroxide solution R, is diluted to 1000 ml with water;
- mobile phase A: acetonitrile R and pH = 5 buffer in ration of 1/99 V/V;
- mobile phase B: acetonitrile R and pH = 5 buffer in ratio of 20/80 V/V;

Results

TLC experiment
The obtained results are summarized in Table I and Figures 1–8.

HPLC experiment
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 (Figure 9): coefficient of correlation R = 0.9990, residuals (lower than 10%) without correlation with concentrations, coefficients of variation under 5% (Figure 9, 10).

All auxiliary substances improved stability of amoxicillin in acidic environment. Best results were recorded for β-CD 1:5, β-CD 1:2 and 2-HP-β-CD 1:5. Amoxicillin half-life is increased by 12 fold in the presence of β-CD 1:5 (Table II). Amoxicillin decay is a first order kinetic process with a decomposition rate constant of 1.329 days⁻¹. It can be seen that cyclodextrins but not Mg-Glu and Mg-Asp have the tendency to change the kinetic of

<table>
<thead>
<tr>
<th>Excipient</th>
<th>no excipient</th>
<th>2-HP-β-CD 1:1</th>
<th>2-HP-β-CD 1:2</th>
<th>2-HP-β-CD 1:5</th>
<th>Mg-Glu 1:2</th>
<th>Mg-Asp 1:2</th>
<th>BCD 1:1</th>
<th>BCD 1:2</th>
<th>BCD 1:5</th>
</tr>
</thead>
<tbody>
<tr>
<td>k&lt;sub&gt;d&lt;/sub&gt; (days⁻¹)</td>
<td>1.329</td>
<td>0.488</td>
<td>0.308</td>
<td>0.147</td>
<td>0.821</td>
<td>0.907</td>
<td>0.273</td>
<td>0.142</td>
<td>0.101</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (days)</td>
<td>0.52</td>
<td>1.42</td>
<td>2.25</td>
<td>4.71</td>
<td>0.84</td>
<td>0.76</td>
<td>2.53</td>
<td>4.88</td>
<td>6.86</td>
</tr>
<tr>
<td>R</td>
<td>&gt; 0.999</td>
<td>0.9889</td>
<td>0.9765</td>
<td>0.9705</td>
<td>&gt; 0.99</td>
<td>&gt; 0.99</td>
<td>0.9713</td>
<td>0.9740</td>
<td>&gt; 0.99</td>
</tr>
</tbody>
</table>
amoxicillin decomposition: lower the values of the coefficient of correlation (R) and increase the values of residuals. Decomposition rate constant (kd) was computed using the exponential equation that fitted every pair of time – concentration values. Half-life was computed using the formula: \( t_{1/2} = \ln(2)/kd \). (Table II).

**Discussions**

**TLC experiment**

Series I chromatograms (Figures 3 and 4):
- there are 3 spots of interest in the chromatograms of this series: amoxicillin Rf 0.83, \( \beta \)-CD Rf 0.95 and decomposition products of amoxicillin Rf 0.63;
- sample 3, made by in situ mixture, shows that there are no interaction between components since all three spots are present;
- samples 4, 5, 6, 7 show that there is an interaction between components: changes in the Rf values of amoxicillin and decreased intensity of the decomposition product spot.

Series II chromatograms (Figure 5):
- the following spots are present: degradation product of amoxicillin Rf 0.61 [1], amoxicillin Rf 0.82, and in the case of samples 2, 5, 8 and 9 spots with Rf values of 0.94, 0.94, 0.95, 0.96 that correspond to the excipients;
- samples 4, 5, 6, 7 show that there is an interaction between components: changes in the Rf values of amoxicillin and decreased intensity of the decomposition product spot.

**Fig. 5. Amoxicillin interaction with auxiliary substances Series III, 24 hours after preparation, spots visualized with iodine**

**Fig. 6. Amoxicillin interaction with auxiliary substances Series III, 48 hours after preparation, spots visualized with iodine**

**Fig. 7. Amoxicillin interaction with auxiliary substances Series IV, T0, spots visualized with iodine**

**Fig. 8. Amoxicillin interaction with auxiliary substances Series III, 24 hours after preparation, spots visualized with iodine**
changes in Rf values of amoxicillin in samples 3, 4, 6 and 7 show that there is an interaction between components [1,2].

Series III chromatograms (Figures 4, 5 and 6):
- spots recorded for analyzed substances are: amoxicillin Rf 0.86, degradation product of amoxicillin Rf 0.60, cyclodextrins Rf 0.95;
- changes in Rf value of amoxicillin in samples 2, 3, 4, 6 and 7 show that there is an interaction between the components [1,2];
- the spot of the decomposition product of amoxicillin is reduced in diameter and is less intense when auxiliary substances are present. This phenomenon is more visible after 24 and 48 hours showing that in the presence of auxiliary substances stability of amoxicillin is greatly enhanced.

Series IV chromatograms (Figures 7 and 8):
- the following spots are present: degradation product of amoxicillin Rf 0.60, amoxicillin Rf 0.90, and in the case of samples 2, 5, 8 and 9 spots with Rf values of 0.94, 0.93, 0.94, 0.93 that correspond to the excipients;
- changes in the Rf values of amoxicillin in samples 3, 4, 6 and 7 show that there are interactions between amoxicillin and auxiliary substances [1]. In the case of sample 6 same observations can be made after 24 hours.

HPLC experiments
HPLC analyzed regarding the stability of amoxicillin in acidic environment showed a marked increase of stability when cyclodextrins and magnesium salts of glutamic and aspartic acid are present. Similar experiments published so far [1,2,6,7] tend to confirm our findings.
Even a tendency of cyclodextrins to change the kinetics of amoxicillin decomposition was present, formal first order kinetics was able to fit all results obtained in this study [12].

Conclusions
TLC experiments results lead us to the following conclusions:
- amoxicillin is unstable in highly acidic environment (pH 1.2)
- interactions between amoxicillin and used excipients are more noticeable in acidic environment
- HPLC analysis proved that associations with cyclodextrins or magnesium salts of amino acids greatly improve amoxicillin stability. Improvement of stability can be observed even after one week after solutions were prepared.
- best increase of stability can be attained by using solutions of Amox: β-CD 1:5 and Amox:2-HP-β-CD 1:5;
- since amoxicillin stability depends on the integrity of penam cycle, we can conclude that better stability means that there must be an inclusion of this cycle in cyclodextrin cavity in certain conditions.
- HPLC experiments permit to quantitate observations made by TLC regarding amoxicillin stability.

References