

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 gastroduodenal 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-hydroxypropyl- 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 + rabeprazole) [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-hydroxypropyl- 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].

An increase in amoxicillin stability in acidic environment is important for obtaining modified release pharmaceutical forms too [11].

Materials and methods

Reagents

- ▶ Amoxicillin trihydrate (Amox) 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;
- ▶ 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 (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 μ g/ml)
- ▶ iodine, pharmaceutical quality.

Table I. Rf values of samples analyzed by TLC

Sample series	Sample number Rf values								
	1	2	3	4	5	6	7	8	9
Series-I (chromatograms 1 and 2)- T0	0.63	0.95	0.63	0.64	0.64	0.64	0.64		
	0.83		0.83	0.91	0.91	0.91	0.91		
			0.95						
Series-II (chromatogram 3)- T0	0.61	0.94	0.64	0.65	0.94	0.65	0.61	0.95	0.96
	0.82		0.85	0.90		0.86	0.88		
Series-III (chromatogram 4)- T0	0.95	0.92	0.92	0.92	0.60	0.92	0.92	0.60	0.95
					0.86			0.92	
Series-III (chromatogram 5)- 24 hours	0.95	0.67	0.70	0.92	0.60	0.91	0.69	0.67	0.95
		0.92	0.92		0.88		0.91	0.91	
Series-III (chromatogram 6)- 48 hours	0.95	0.67	0.70	0.92	0.60	0.91	0.69	0.67	0.95
		0.92	0.92		0.88		0.91	0.91	
Series-IV (chromatogram 7)- T0	0.60	0.94	0.91	0.92	0.93	0.91	0.91	0.94	0.93
	0.90								
Series-IV (chromatogram 8)- 24 hours	0.64	0.91	0.70	0.69	0.91	0.90	0.71	0.93	0.93
	0.91		0.91	0.91			0.92		

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].

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) și β -CD 0.687 % (9).

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 μ l.

Development: over a path of 15 cm.

Drying: in air.

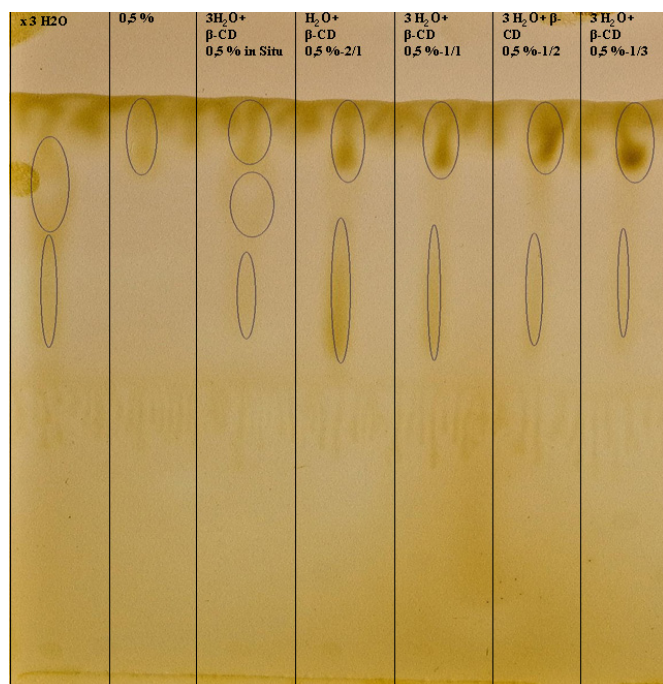


Fig. 1. Amoxicillin interaction with auxiliary substances Series 1, T0, spots visualized with iodine

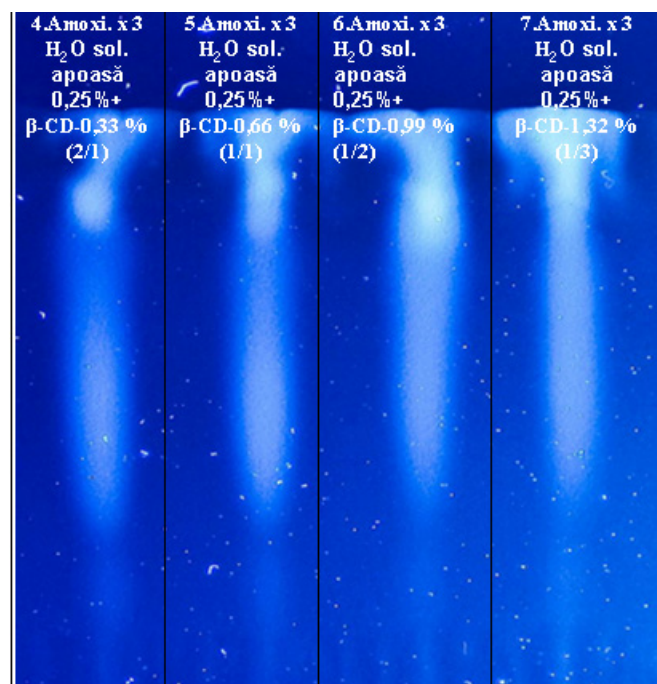


Fig. 2. Amoxicillin interaction with auxiliary substances Series 1, T0, spots visualized under UV light

Tabel II. Influence of auxiliary substances on the parameters of ampicillin decomposition process

Excipient	no excipient	2-HP-BCD 1:1	2-HP-BCD 1:2	2-HP-BCD 1:5	Mg-Glu 1:2	Mg-Asp 1:2	BCD 1:1	BCD 1:2	BCD 1:5
k_d (days ⁻¹)	1.329	0.488	0.308	0.147	0.821	0.907	0.273	0.142	0.101
t_{half} (days)	0.52	1.42	2.25	4.71	0.84	0.76	2.53	4.88	6.86
R	> 0.999	0.9889	0.9765	0.9705	> 0.99	> 0.99	0.9713	0.9740	> 0.99

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;

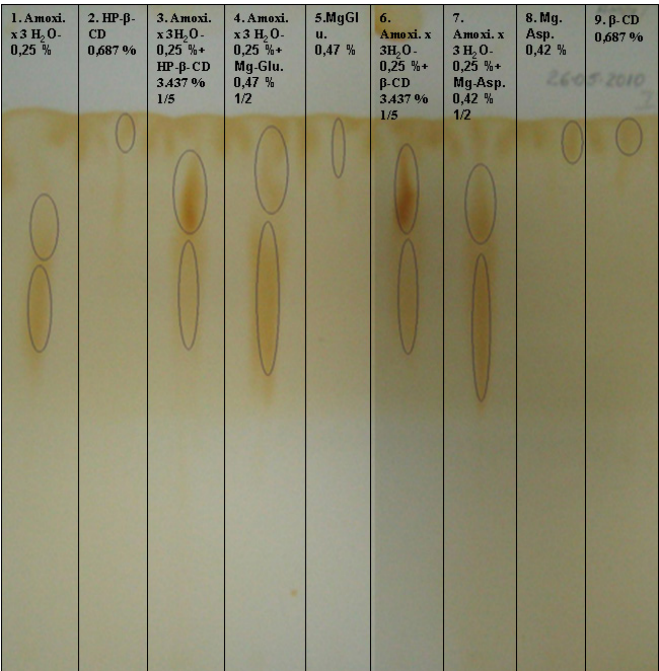


Fig. 3. Amoxicillin interaction with auxiliary substances Series II, T0, spots visualized with iodine

- ▶ flow : 1 ml/min, mobile phase consists of a mixture of mobile phase B with mobile phase A in a ratio of 98:8;
- ▶ detection wavelength: 254 nm;
- ▶ injected volume 20 μl.

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

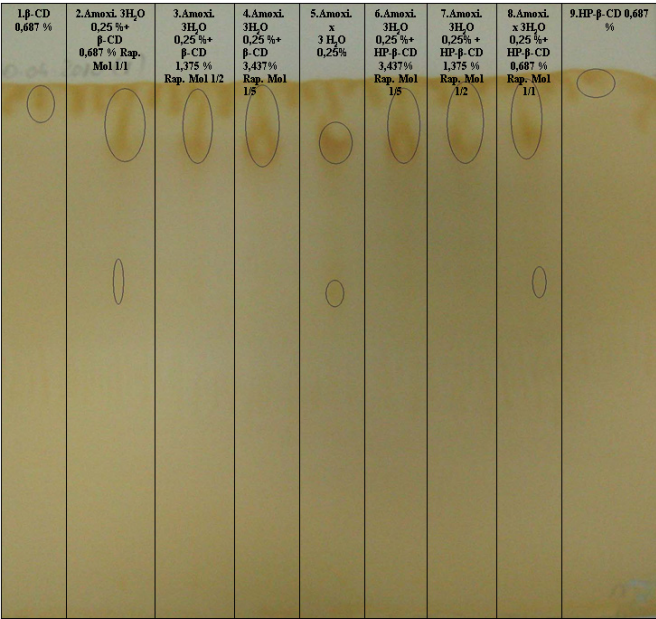


Fig. 4. Amoxicillin interaction with auxiliary substances Series III, T0, spots visualized with iodine

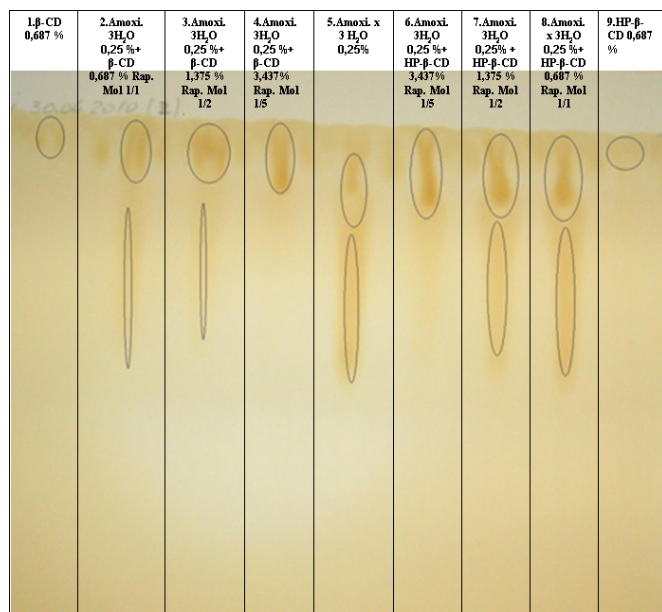


Fig. 5. Amoxicillin interaction with auxiliary substances Series III, 24 hours after preparation, spots visualized with iodine

amoxicillin decomposition: lower the values of the coefficient of correlation (R) and increase the values of residuals. Decomposition rate constant (k_d) was computed using the exponential equation that fitted every pair of time – concentration values. Half-life was computed using the formula: $t_{1/2} = \ln 2/k_d$. (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 R_f 0.83, β -CD R_f 0.95 and decomposition products of amoxicillin R_f 0.63;

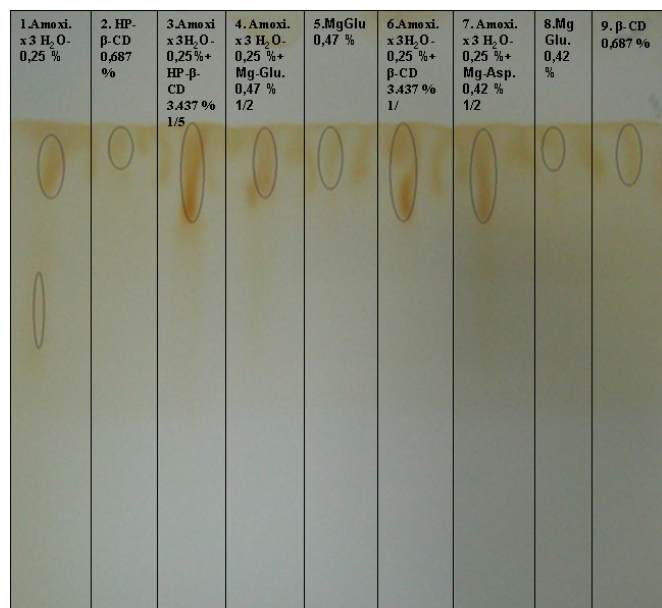


Fig. 7. Amoxicillin interaction with auxiliary substances Series IV, T0, spots visualized with iodine

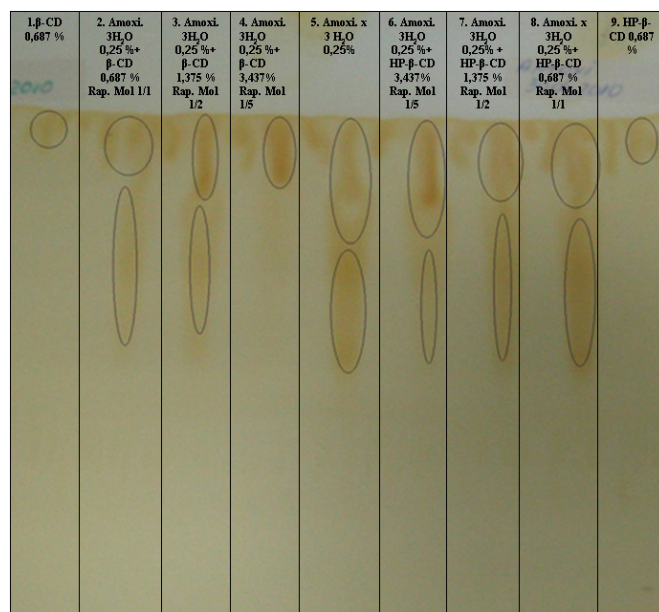


Fig. 6. Amoxicillin interaction with auxiliary substances Series III, 48 hours after preparation, spots visualized with iodine

- ▶ 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 R_f 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 R_f 0.61 [1], amoxicillin R_f 0.82, and in the case of samples 2, 5, 8 and 9 spots with R_f values of 0.94, 0.94, 0.95, 0.96 that correspond to the excipients;

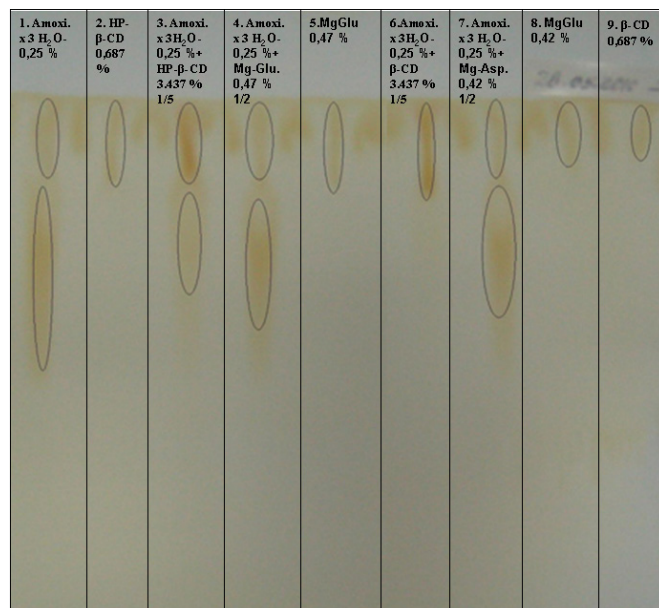


Fig. 8. Amoxicillin interaction with auxiliary substances Series III, 24 hours after preparation, spots visualized with iodine

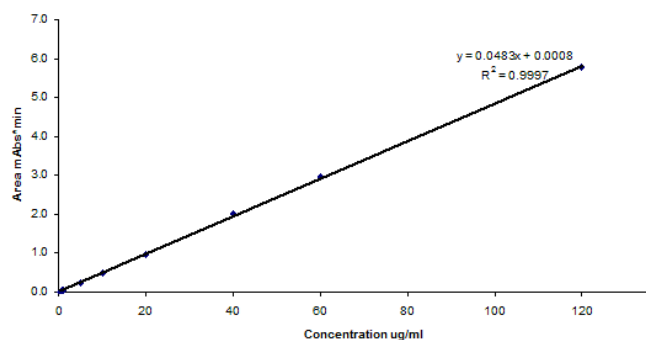


Fig. 9. Amoxicillin calibration curve

- ▶ changes in R_f 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 R_f 0.86, degradation product of amoxicillin R_f 0.60, cyclodextrins R_f 0.95;
- ▶ changes in R_f 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 R_f 0.60, amoxicillin R_f 0.90, and in the case of samples 2, 5, 8 and 9 spots with R_f values of 0.94, 0.93, 0.94, 0.93 that correspond to the excipients;
- ▶ changes in the R_f 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 is 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)

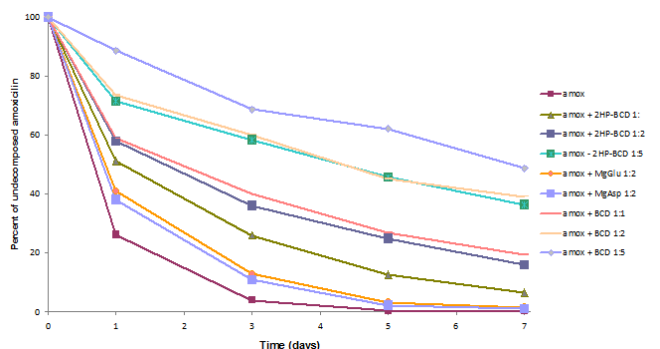


Fig. 10. Influence of auxiliary substances on amoxicillin stability

- ▶ 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- α -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.

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