PERIODICO di MINERALOGIA Volume 90, No. 2, 2021

Studies regarding the stability of pharmaceutical formulations realized through association of beta-lactamic antibiotics
1. Stability of an oral suspension powder containing cefadroxil and cefixim

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Abstract

Third generation cephalosporins are highly active against the majority of Gram-negative bacteria, producers or non-producers of beta-lactamase, but less active against penicillinase-producing staphylococci.

There are known therapeutical schemes in which polymicrobian infections are treated simultaneously with different antibiotics with complementary antimicrobian spectrum, such as association of ampicillin with cloxacillin, in which every component is prepared and administered separately – fact that implies high costs related to formulation and treatment.

By associating in a single pharmaceutical product two beta-lactamic resistant antibiotics, we can extend the action spectrum of the antimicrobian drugs that are used in the treatment of severe infections caused by staphylococci or other Gram-positive bacteria, together with Gram-negative bacteria. This kind of association may be realized by: association of a third generation cephalosporin (cefixime, ceftibuten, cefprozil, cefpodoxim-proxetil, cefetamet-pivoxil etc) with a first generation cephalosporin (cefadroxil, cefaclor, cefalexine etc) [1].

This study presents the research accomplished in order to evaluate the stability of a multi-dose powder used for an oral suspension realized through association of 250 mg of cefadroxil + 100 mg of cefixime/5 ml reconstituted suspension. The powder is packed in vials containing granulated powder used to obtain 60 ml of suspension.

Keywords: cefadroxil, cefixime, antibiotics, beta-lactam, stability, oral suspension powder, association, synergy.

Introduction

Cefadroxil and cefixime are cephalosporins currently used in therapy. In vitro studies showed that by associating cefadroxil and cefixime, a new extended spectrum antibiotic is obtained, active both against Gram-positive bacteria and Gram-negative bacteria, in which the two components are acting additive or synergic as regards the antibacterian action (table 1).
In order to orally administrate this association, different pharmaceutical formulations have been realized: capsules, tablets, single and multidose powders for oral suspension.

In comparison with tablets and capsules, the powder for oral suspension shows higher biodisponibility and is easier to administrate in children and elder people, the most vulnerable age categories.

The qualitative composition of the powder is the following: cefadroxil monohydrate, cefixime trihydrate, xantan gum, sugar, sodium benzoate, flavour, polysorbat 80, color agent FD & C Yellow no. 6.

Table 1 [1]. MICs for cefadroxil, cefixime and the association cefadroxil+cefixime (2:1)

Crt. NO.	Antibiotic	Test microorganism/number of tested strains					
		Staphylococcus aureus/77		*Escherichia coli/95*		*Klebsiella/20*	
		MIC_{50} μg/ml	MIC_{90} μg/ml	MIC_{50} μg/ml	MIC_{90} μg/ml	MIC_{50} μg/ml	MIC_{90} μg/ml
1.	cefadroxil	0,6	5,0	8,0	≥8,0	8,0	≥8,0
2.	cefixime	1,0	≥8,0	0,06	0,5	0,06	0,125
3.	cefadroxil +cefixime (2:1)	0,5	2,0	0,125	0,65	0,06	0,25

Materials and method

Cefadroxil monohydrate is produced by Dobfar (Italy) and has the following main quality characteristics:

- content: 989 micrograms/mg
- water: 5.15 %
- appearance: almost white crystalline powder
- pH = 4.9

Cefixime is produced by Orchid (India) and has the following main quality characteristics:

- content: 982 micrograms/mg
- water: 10.5%
- appearance: light-yellow crystalline powder
- pH = 3.3

The declared stability period for both antibiotics is of minimum 3 years.
The excipients were provided by commercial sources and qualitatively comply with European Pharmacopoeia, 3rd edition.

The powder has been tested from the point of view of ”long-term” stability at 24ºC and of short-term stability (“accelerate stability”) at 40ºC in the said package (brown glass vials with plastic cap and seal). The vials were kept in isothermal conditions in rooms with controlled humidity: relative humidity of 60% for the vials kept at 24ºC and relative humidity of 75% for the vials kept at 40ºC.

At the same time, the stability of the reconstituted suspension has been studied in order to evaluate the administration period.

The powder long-term stability studies extended over a period of 12 months. In order to assess the stability term of the powder, an isothermal, long-term procedure has been used, based mainly on the degradation rate of the active substances. By interpolating the experimental data, a mathematical model of the degradation process of the two active substances has been obtained (in dry state and, respectively, in suspension).
Based on the mathematical model, the storage period has been assessed using the graphic method; the storage (stability) period has been obtained by intersecting the curve described by the recession equation with the line corresponding to the minimal specification described in the pharmacopoeia. Practice has shown that the storage period is real, exceeding by 1-2 years the effective experimental period. [2].

Analytical determinations have been performed every 3 months.

The stability test conducted under “accelerated aging” conditions have been performed used an isothermal procedure, on a period of 6 months at 40ºC; measurements have been performed every 3 months. In this case, also, the storage (stability) period has been graphically determined, using the recession equations.

At the same time, the stability of the reconstituted suspension has been studied, at 5 C and at 24ºC, during 7 days. The acceptance conditions have taken into account the provisions of USP 26 [3-4].

A HPLC method has been used for the quantitative measurements of the two antibiotics, in the following conditions:

- HPLC, Agilent type
- Lichrosorb RP 18 (250 x 4.6 i.d.) (10 micrometers) chromatographic column
- Mobile phase: acetonytril: buffer pH 5 = 40:960 (v/v)
- Solvent: phosphated buffer pH 5
- UV detector, wave length = 230 nm
- Mobile phase flow: 1.5 ml/min
- Injected volume: 10 microliter
- Column temperature: room temperature

Acceptance conditions for the powder:

- antibiotic content: 90 – 120 % of the declared content
- pH = 4 – 5
- powder appearance: light-yellow to yellow.

Results and discussion

The results of the studies are shown bellow.

1 Powder stability study

1.1 Long term stability study

The results of long-term test are shown in the following table:

Table 2. Qualitative parameters of the powder containing 250 mg of cefadroxil + 100 mg cefixime/5 ml reconstituted suspension (average values for 3 vials)

Time, months	Powder appear-ance	Content in active substance				pH
		cefadroxil		cefixime		
		g/vial	% of the declared quant.	g/vial	% of the declared quant.	
0	Light yellow	3,08	102,66	1,013	101,3	4,54
3	Light yellow	3,015	100,5	0,9966	99,66	4,58
6	Light yellow	2,99	99,66	0,983	98,3	4,55
9	Light yellow	2,94	98	0,986	98,6	4,66
12	Yellow	2,93	97,6	0,963	96,3	4,63

During the 12 months conservation period in vials at 24ºC, the antibiotic content decreases by approx. 5%. PH modifications are not significant and the color does not modify considerably, being well-masked by the color agent.

Due to the fact that the main quantitative parameter is the content of every active substance, modeling of the powder degradation process has been performed taking into consideration the modification of the content of the active substances.

Table 3. In-time modification of the cefadroxil and cefixime content (average values for 3 vials)

time, months	0	3	6	9	12
Cefadroxil content, (% of declared content)	102,66	100,5	99,66	98	97,6
Cefixime content, (% of declared content)	101,3	99,66	98,3	98,6	96,3

The experimental data have been used to assess the degree of the degradation of the two antibiotics in isothermal conditions, in order to estimate the storage period of the powder.

In-time variations of the cephalosporin content (as percentage of the declared value on the vial) have been graphically represented, thus obtaining the degradation curves (fig. 1)

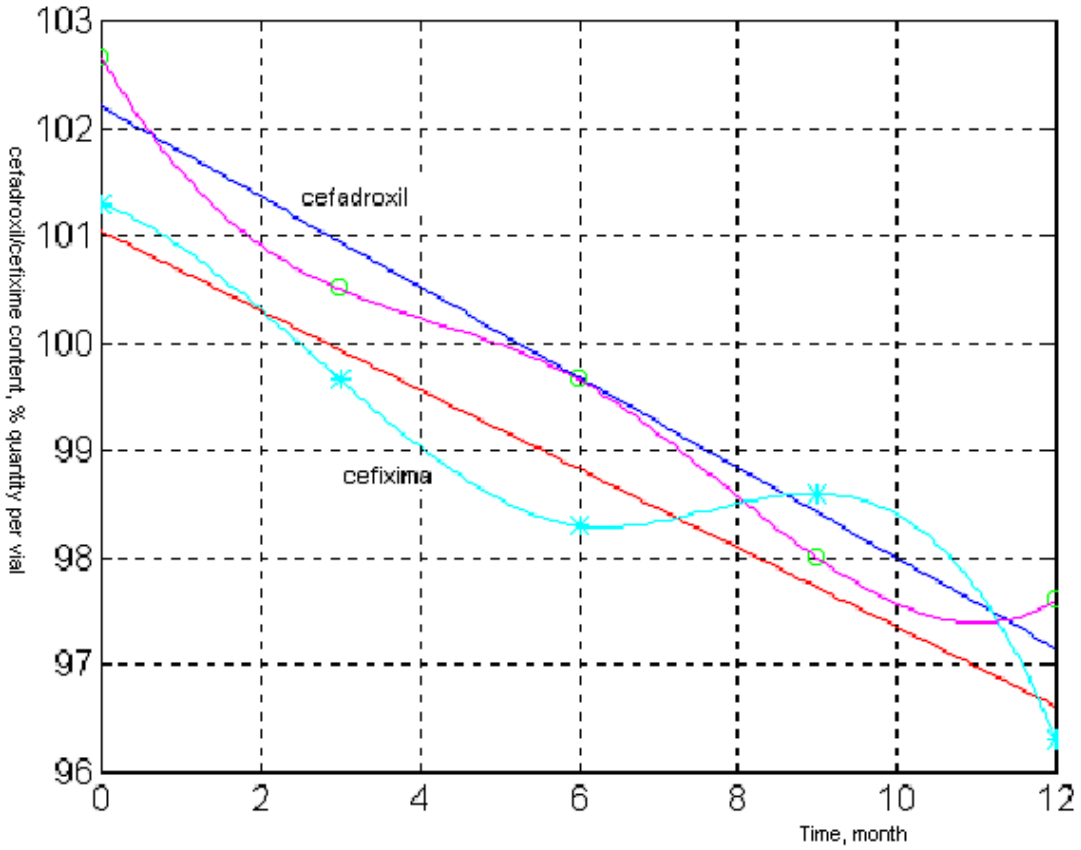

Figure 1. Degradation of the active substances from powder; storage conditions: 25 C, during 12; representation of regression equations

By interpolating the experimental data the following regression equations have been obtained, describing a 0 degree kinetic degradation of the two antibiotics (correlation

coefficient 0.9765 for the degradation equation of cefadroxil and, respectively, 0.9510 for cefixime).

- In-time degradation equation for cefadroxil:

C_1= –0,4207 t+102,208;

- In-time degradation equation for cefixime:

C_2= –0,3687 t+101,044;

In which C1 = the content of cefadroxil/vial (as percentage of the declared value/vial) C2 = the content of cefixime/vial (as percentage of the declared value/vial), and t = time, in months.

The graphical assessment of the stability period has been performed by intersecting the curves described by the regression equations with the line corresponding to the minimal accepted content of substance inscribed on the vial, respectively 90% of the declared content (figure 2).

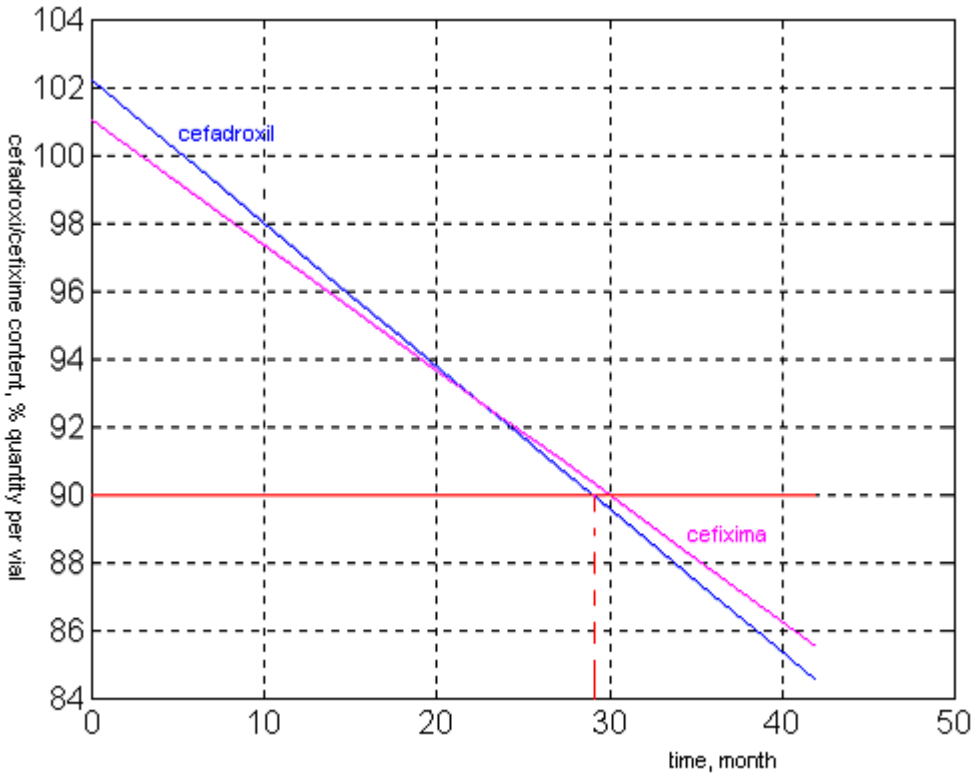

Figure 2. Graphic assessment of the powder storage period

This graphic allows assessment of the stability period of the powder as being of 29 months (approx. 2.4 years). It cam be said that the degradation is similar for the two antibiotics, in the above-mentioned storage conditions.

1.2 Stability of the suspendable powder in isothermal conditions of "accelerated aging"

The results obtained in storage conditions according to the isothermal procedure, i.e. 6 months at 40ºC) are shown in the following table:

Table 4. Qualitative parameters of suspendable powder at 40ºC (average values for 3 vials)

Time, months	Powder appearance	Content in active substance				pH
		cefadroxil		cefixime		
		g/vial	% of declared quant.	g/vial	% of declared quant.	
0	Light yellow	3,08	102,66	1,013	101,3	4,54
3	yellow	2,98	99,33	0,966	96,6	4,78
6	Brown yellow	2,78	92,66	0,903	90,3	5,05

The table shows that, although after 6 month the content of the active substances is still within the acceptance limits, the fact that a pronounced modification of the color to yellow-brown and a modification of the pH beyond the permitted limits have lead to the conclusion that the product cannot be stored at temperatures higher than 25ºC. In such conditions, both cephalosporins suffer accentuated degradation even in the absence of light, a reason for which the quantity of active substance/vial is 10% lower for cefadroxil and 11% lower for cefixime.

2. Stability of the reconstituted suspension

The stability of the aqueous reconstituted suspension has been studied in order to establish the storage (stability) period, according to the storage temperature. The stability of the suspension has been monitored during 10 days at 2 - 8ºC, in the absence of light, and at room temperature (24ºC) in the presence of natural night.

Both chemical and organoleptic parameters have been measured.

In-time variation of the quality parameters of the reconstituted suspension is shown in table 5.

Acceptance conditions for the reconstituted suspension are as follows:

- minimum cefadroxil content: 2.7 g/vial
- minimum cefixime content: 0.9/vial
- pH 4-5
- suspension color: light-yellow to yellow

Table 5. Variation of quality parameters of the suspension containing 250 mg of cefadroxil and 100 mg cefixime/5 ml suspension, during 10 days in the refrigerator (2 – 8ºC), and at room temperature (25ºC).

Time, days	Cefadroxil content, g/vial		Cefixime content, g/vial		pH	
	2-8°C	24°C	2-8°C	24°C	2-8°C	24°C
0	3,08		1,013		4,54	
1	3,05	3,02	1,0	0,988	4,55	4,57
2	3,03	3,015	0,988	0,965	4,55	4,55
3	3,03	3,02	0,984	0,962	4,57	4,59

4	2,97	2,94	0,981	0,941	4,56	4,78
5	2,94	2,85	0,967	0,923	4,59	4,75
6	2,89	2,81	0,933	0,913	4,72	4,77
7	2,88	2,78	0,915	0,877	4,85	5,05
8	2,73	2,66	0,910	-	4,95	-
9	2,71	-	0,887	-	5,1	-
10	2,59	-	-	-	-	-

- qualitative parameters of the powder used to reconstitute the aqueous oral suspension.

-parameters outside the acceptance limits

Data from the table allow the following interpretation:

- cefadroxil in suspension is stable during 9 days in the refrigerator and 7 days at room temperature
- cefixime in suspension is stable during 8 days in refrigerator and 6 days at room temperature
- the pH stability period is of 8 days in a refrigerator and 6 times at room temperature.

The study shows that the suspension can be administered during 6 days if kept at 25ºC, and 8 days if kept in the refrigerator.

The degradation of the active substances in suspension follows a grade 2 kinetics for cefadroxil (figure 3), and, respectively, a linear one for cefixime (figure 4).

For cefadroxil:

At 5°C: $C_1=0,0044\ t^2 – 0,0026\ t + 3,0658$

At 25°C $C_1= 0,0042\ t^2 – 0,0162\ t + 3,0679$

For cefixime:

At 5°C: $C_2= –0,0139\ t + 1,0204$ (correlation coefficient = 0,9753)

At 25°C $C_2= – 0,0175\ t + 1,0092$ (correlation coefficient = 0,988).

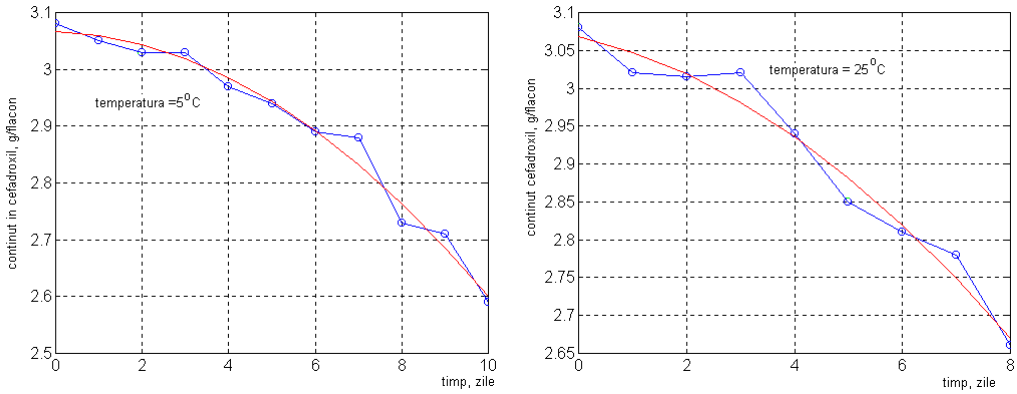

Figure 3. Degradation of cefadroxil suspension at 5 C and, respectively, at 25 C

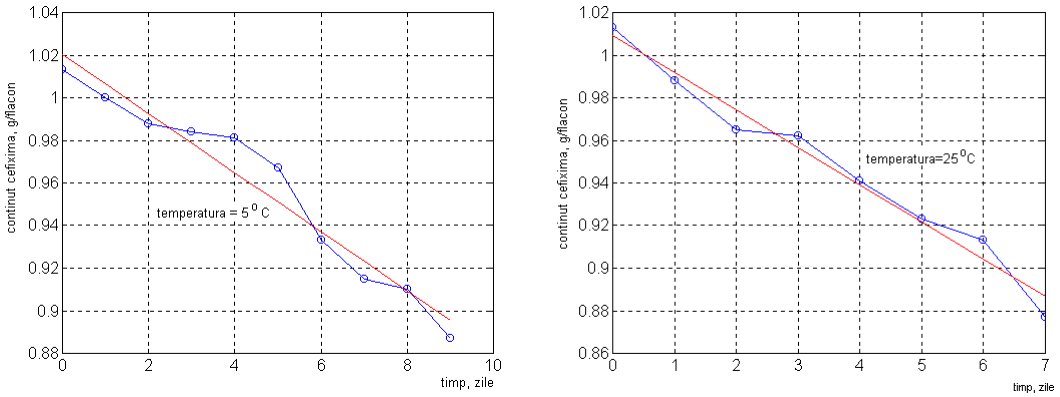

Figure 4. Degradation of cefixime suspension at 5 C and, respectively, at 25ºC

Conclusions

- Cefadroxil (first generation cephalosporin) and cefixime (third generation cephalosporin) have been associated in a unique pharmaceutical formulation;
- The stability of powder for oral suspension containing 250 mg cefadroxil and 100 mg cefixime/5 ml has been studied, due to its higher bioavailability in comparison with other oral formulations;
- The studies showed that the product is stable when kept under 25ºC for a minimum period of 2 years;
- The kinetics of the degradation of the active substances has been determined (in the said storage conditions);
- The stability of the reconstituted suspension (at 5ºC and, respectively, at 25ºC) has been studied and the kinetic equations that describe the degradation of the active substances have been established;
- The administration period for the reconstituted suspension has been shown as being of 6 days at room temperature and, respectively, 8 days if the product is kept in the refrigerator.

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