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The stability of pharmaceutical formulations achieved by combining beta-lactamic antibiotics

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Abstract

Isoxazolyl penicillins (Cloxacillin, dicloxacillin, flucloxacillin and oxacillin) are very effective antibiotics in treatment of infections caused by Staphylococcus spp. Cloxacillin, dicloxacillin and oxacillin have nearly identical spectrums of activity and can be considered therapeutically equivalent when comparing in vitro activity. These penicillinase-resistant penicillins have a narrower spectrum of activity than natural penicillin. Their antimicrobial efficacy is aimed directly against penicillinase-producing strains of gram positive cocci, particularly staphylococcal species.

On the other hand, third generation cephalosporins are very effective against a large number of gram negative bacteria (including beta-lactamase - producing strains) and have poor activity against Staphylococcal species.

Ceftriaxone Sodium is a semisynthetic broad spectrum third generation cephalosporin antibiotic for intravenous and intramuscular administration.

Mixing in one pharmaceutical form oxacilline sodium and Ceftriaxone Sodium (or other third generation cephalosporin) we have obtained a new antibiotic which had a very good antibacterial activity.

In this paper we performed stability studies of a new parenteral antibiotic: an association of an third generation cephalosporin (ceftriaxone) with one isoxazolyl penicillin (oxacillin).

Keywords: ceftriaxone, oxacillin, antibiotic, beta-lactams, stability, powder for injection, association, synergism.

Introduction

Oxacillin and ceftriaxone are beta-lactam antibiotics widely used in therapy. These drugs are available on the market as powders for injection. The usual doses are: ceftriaxone 250 mg, 500 mg and 1g vial formulation, and for oxacillin 500 mg, 1g and 2g vial formulation.

Previous studies reveal that association of oxacillin with ceftriaxone yielded a new antibiotic with a wide spectrum of activity against gram positive and gram negative bacteria. (table 1.) [1]. We prepared mixture of oxacillin and ceftriaxone as follows: 500 mg oxacillin + 250 mg ceftriaxone and 1000 mg oxacillin + 500 mg ceftriaxone. In this paper are presented our research regarding stability studies of the new products.

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Table 1.

No	Microorganism	C.M.I., μg/ml		
		oxacillin Na	Ceftriaxone Na	oxacillin Na: ceftriaxone Na = 2:1
1.	*Streptococcus pneumoniae*	*0,05*	*0,03*	*0,02*
2.	*Staphylococcus aureus (12)*	*0,25*	*1,0*	*0,2*
3.	*Staphylococcus aureus (2)R*	*0,5*	*1,0*	*0,1*
4.	*Haemophylus influenzae*	*>64*	*0,06*	*0,02*
5.	*Escherichia coli*	*>64*	*0,12*	*0,4*
6.	*Proteus sp.*	*>64*	*0,04*	*0,02*

Materials and methods

Oxacillin sodium is made by Aurobindo, India and has the following parameters:

- Assay 100.9%
- Impurity (A,B, C, D, E) <0.1%; sum=0.1%
- Water: 9.6%
- White crystalline powder
- pH 7.1

ceftriaxone is supplied by High Tech, Korea an has the following parameters:

- assay: 860.11 μg/mg
- water content: 4.56%
- White crystalline powder
- pH 6.17

The powder mixture was dosed in 20 ml amber vials closed tight with rubber stopper and Al cap. The powder was analyzed for performing the stability studies in dry form. After that we perform stability studies of antibiotic in solution as well using suitable solvents (so called "in use" stability testing). For this purpose the vials were kept 12 months at room temperature (25 ± 2ºC) and 6 month at 40ºC (accelerated stability studies). The solutions in suitable solvents were kept for few days at room temperature and in the refrigerator (2-5ºC)[6, 7].

For determination of the stability period of the powder in dry state we used the isotherm procedure on long term, based mainly on degradation rate of active substances. In this purpose we have determined the active substances content and related substances at three month intervals. Tests were performed using 5 vials for one analyze and considering the mean values. Interpolating the experimental values yield the mathematical model of the two antibiotics degradation in dry state. The adequacy of mathematical model was assessed using Fischer test for a confidence interval of 95%.

Based on mathematical model we assessed the stability period using a graphic method [2], intersecting the curves described by degradation equations of active substances, (respective the increase in degradation products content) with the limits described by pharmacopoeia.

The admissibility conditions were established in accordance with main international pharmacopoeias. [3,5]

Determinations of the active substances were performed using a HPLC system Agilent, model 1100. The analytical methods we used were in accordance with USP 26. [3, 4]

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Admissibility conditions for powders:

- ceftriaxone: 90-115%
- oxacillin: 90-115%
- related substances: max 1% each, sum < 5%
- pH=6 – 8.5
- appearance: white or almost white crystalline powder

Results and discussions

Stability studies
Long-term stability studies
Results were presented in table 2

Table 2. Stability studies on a mixture 500 mg oxacillin+250 mg ceftriaxone (as sodium salts)

Parameter	Time, months				
	initial	3	6	9	12
Ceftriaxone content, mg/vial.	253,5	250,5	248,0	248,07	246,1
Oxacillin content mg/vial.	499,25	496,65	493,8	490,45	486,85
Related substances: • individual • sum	max.0,45% 1,45	max.0,47% 1,66	max.0,49% 2,36	max.0,55% 2,68	max.0,55% 3,05
pH	6,88	6,89	6,76	6,70	6,71
Appearance	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow

After 12 month at room temperature, ceftriaxone content had a 3% drop, and oxacillin content drop almost 2.5%. pH has been stable, and the color of the mixture remain the same.

As it was already mentioned, modeling the degradation process was made considering the variation in active substances content, related substances content in time (table 3.)

Table 3. Variation in Ceftriaxone and oxacillin content in time

Time, month	0	3	6	9	12
Content in ceftriaxone, (% from declared content)	101,4	100,2	99,2	99,23	98,43
Content in oxacillin (% from declared content)	99,85	99,33	98,76	98,09	97,37
Content in related substances, % (as sum)	1,45	1,66	2,36	2,68	3,05

Data obtained experimentally were used for the evaluation of the order of reaction of degradation of the two antibiotics in isotherm conditions and for the estimation of the preservation of powder at 25ºC. The degradation graphics in picture 1 were obtained in a graphic representation of the variations in time for the antibiotic concentration (as percent from the quantity declared on the vial). Figure 1.

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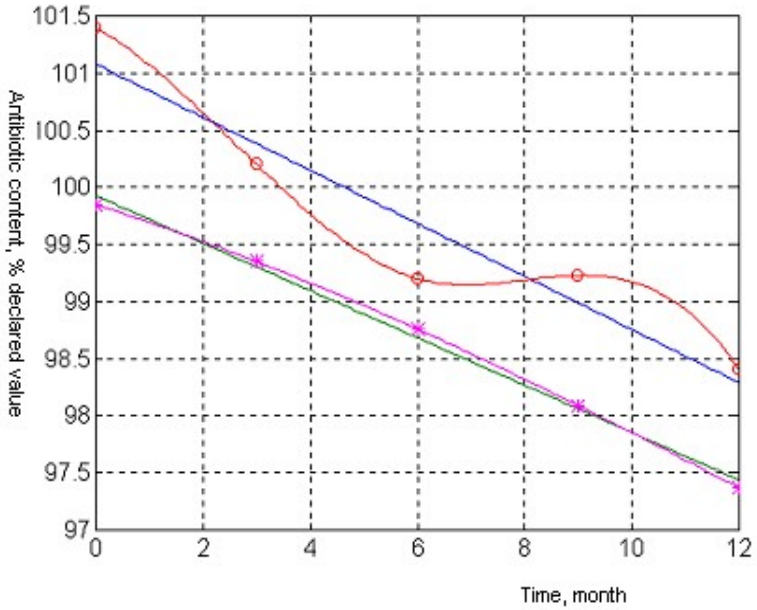

Figure 1. The degradation of active substances from powder under preservations conditions at 25ºC for 12 months and the representation of regression equations.

As a result of interpolating the data obtained experimentally, the following regression equations were obtained:

- Degradation equation in time for ceftriaxone

C_1= –0,2323 t+102,08; corelation coefficient =0,9574)

- Degradation equation in time for oxacilline

C_2= –0,2073 t+99,928; (corelation coefficient =0,9975)

- Equation describing the growth of related substances content.

C_3= 0,1407 t + 1,396; (correlation coefficient =0,9879)

Where C1= ceftriaxone content /vial (as % from the declared quantity per vial)
C2 = oxacillin content /vial (as % from the declared quantity per vial)
C3 = related substances content, % (as sum)
T = time in months.
The above equations define a line, which shows that antibiotic degradation appears after an order 0 kinetic.
The graphic evaluation of stability period of the powder was achieved considering the minimum value of time (in months) that satisfies all conditions:

- Time where both antibiotics have a minimum 90% from the declared content / vial (obtained by intersecting the lines described by the regression equations with the line corresponding to the minimum admitted content /vial (picture 2a)

Time where the level of related substances is under the 5% limit (obtained by intersecting the line described by the equation giving the growth of related substances content with the line indicating the maximum admitted content of 5%. (picture 2b).

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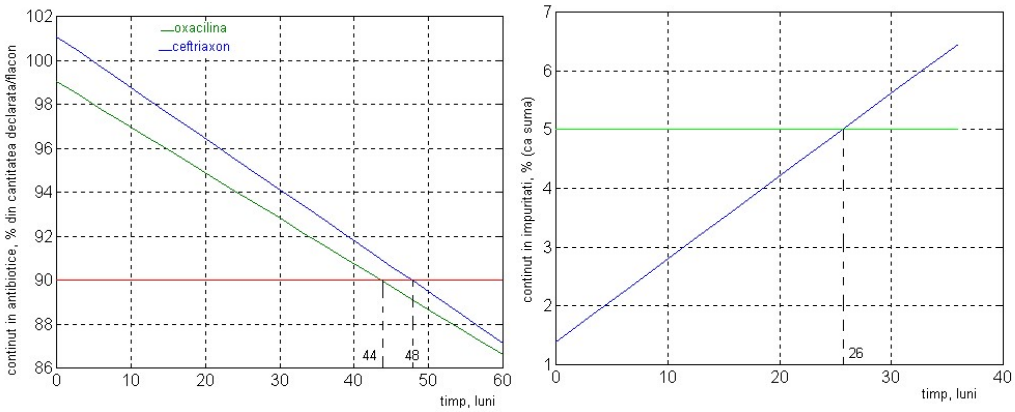

Figure 2. The graphic evaluation of stability period for the powder mixture.
As shown in picture 2, the content of active substances remains within limits foe about 4 years, but the content of related substances overcomes the 5% limit after 26 months. Consequently, we can evaluate the powder's stability period to 26 months (2 years).

1.2. Accelerated stability studies of the powder in isotherm conditions

In table 4 are shown the results obtained under powder preservation conditions according to the isotherm procedure at 40ºC for a period of 6 months.

After 6 months the content of active substances is still within limits, while the level of related substances and the pH overcome it. Also we notice a strong change in the powder's color to yellow-brown. Therefore it was proved that the product cannot be preserved at temperatures higher than 25°C.

Table 4. Qualitative parameters of powder for injection at 40°C (medium value per 5 vials)

Parameter	Time, month		
	initial	3 months	6 months
Content in ceftriaxone, mg/vial % from the declared content/vial	 253,5 101,4	 243,6 97,44	 235,5 94,22
Content in oxacillin, mg/vial % from the declared content/vial	 499,25 99,85	 481,9 96,38	 472,5 94,5
Related substances: • individual • sum	 max.0,45% 1,45	 max.1,47% 4,93	 max. 3,85% 10,22
pH	6,88	6,23	10,2
Powder aspect	Slightly yellow	Slightly yellow	Yellow-brown

2. "In use" stability studies

We performed stability "in use" studies with suitable solvents in order to establish the period of use depending on the nature of solvent and temperature where the solution is preserved. The solutions were prepared by adding 3,6ml solvent to a vial containing 250 mg ceftriaxone and 500 mg oxacillin.

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For this purpose we studied the stability of solutions for 10 days in the fridge (2-8°C) in the absence of light and at the room temperature (24°C) and natural light.

Research aimed chemical parameters, aspect and smell.

Table 5 shows the variation in time of the solution's quality parameters.

Table 5. The variation in time of the solution's quality parameters containing 250 mg ceftriaxone and 500 mg oxacillin / vial.

Time, days	Content of ceftriaxone, % from the declared quantity/vial		Content of oxacillin, % from the declared quantity/vial		Related substances content, % (as sum)		pH	
	4°C	24°C	4°C	24°C	4°C	24°C	4°C	24°C
Water for injections								
1	101,4	101,4	99,85	99,85	1,45	1,45	6,88	6,88
2	99,23	98,9	99,24	98,8	2,75	3,87	6,78	6,72
3	97,87	94,3	96,33	95,55	3,21	4,68	6,73	6,31
4	95,5	89,77	93,55	92,44	4,66	5,52	6,25	5,89
5	92,44	-	90,13	89,23	5,3	-	6,16	-
6	90,3	-	87,33	-	-	-	6,08	-
7	89,82	-	-	-	-	-	5,78	-
solution 0,9% NaCl								
1	101,4	101,4	99,85	99,85	1,45	1,45	6,88	6,88
2	99,44	98,77	99,52	98,76	2,86	3,56	6,78	6,49
3	97,67	94,55	96,35	95,62	3,51	4,93	6,73	6,22
4	95,34	89,87	93,66	92,66	4,53	5,82	6,25	5,67
5	92,56	-	90,22	89,02	5,75	-	6,16	-
6	90,45	-	88,67	-	-	-	6,08	-
7	89,70	-	-	-	-	-	5,78	-
solution 5% dextrose in water								
1	101,4	101,4	99,85	99,85	1,45	1,45	6,88	6,88
2	97,92	92,45	96,33	92,4	2,85	4,22	6,68	6,45
3	91,75	88,77	91,34	86,6	4,76	5,63	6,55	6,04
4	89,56	-	89,55	-	5,12	-	6,47	5,23
5	-	-	-	-	-	-	6,0	-
6	-	-	-	-	-	-	5,67	-
1% lidocaine solution								
1	101,4	101,4	99,85	99,85	1,45	1,45	6,88	6,88
2	97,35	89,56	91,44	88,12	3,21	5,55	6,44	5,25
3	90,44	-	87,65	-	4,53	-	5,65	-
4	89,25	-	-	-	5,87	-	-	-

Analyzing the data from the above table we emphasize the following stability of the prepared solutions:

Table 6. "In use" solution stability

Nr. crt.	Solvent	stability, days	
		4°C	24°C
1.	Water for injections	4	3
2.	Isotonic solution NaCl	4	3
3.	5% dextrose solution	3	2
4.	1% lidocaine	2	1

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Conclusions

- There was achieved an association of isoxazolyl penicillins with third generation cephalosporin (1:2 in ratio) in a unique pharmaceutical form for injection.
- There was studied the stability of the mixture of oxacillin both as dry state and solution, using adequate solvents.
- Research proved that the mixture of oxacillin and ceftriaxone is stable preserved at temperatures under 25°C for a period of minimum 2 years.
- There was established the kinetics of active substances degradation in the above mentioned preservation conditions.
- The stability of the "in use" solutions is 1 to 3 days preserved at room temperature and 1 to 4 days preserved in the fridge, according the nature of the used solvent.
- The mixture of antibiotics allows the treatment of a large range of bacteria infections.

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