Stability Studies of dosage forms – HPLC and microbiological control

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ABSTRACT

The realization of Stability Studies have a huge importance for public health once they have the information about the product quality regarding their respective time and storage conditions. The objective of the present work was to carry out the experimental execution of the Stability Studies of a new form of a β-lactam Extemporaneous Oral Suspension in order to submit for a market application.

The results have shown a stable dosage form with weak oscillations along the period of analysis, being only verified drug instability at accelerated degradation conditions, manifesting significant chances in the appearance and the content of Impurity F, a related substance of the active ingredient. At the commercial storage conditions, corresponding to the long term conditions (25ºC : 60% HR), changes were not verified along the period of analysis. Additionally, an estimation of the shelf life was extrapolated at these conditions and the outcome was 19 months.

The results of the developed dosage form were in agreement with the reference, pointing out that the pharmaceutical approach adopted at the development phase accomplishes the necessary quality required to a market application.

Keywords: Generic drug, Extemporaneous Oral Suspension, β-lactam, Stability Studies, shelf life

1. Introduction

The Guideline "Stability Studies of New Substances and Products", also known as Q1A, was the first guideline issued by the Ordando's Conference, describing the requirements for new applications into ICH boarders [1]. The Conference's main target becomes from the demand of standard conditions to support stability studies in distinct climatic zones, being these climatic zones, and their respective conditions, shown in Table 1 [2].

Table 1. Proposed revision in WHO classification system.

Climatic zone	Definition	Criteria (°C/hPa)	Testing conditions (°C/%RH)
I	Temperate climate	<15/<11	21/45
II	Subtropical and Mediterranean climate	>15-22/>11-18	25/60
III	Hot and dry climate	>22/<15	30/35
IVA	Hot and low humid climate	>22/>15-27	30/65
IVB	Hot and moderately humid climate	>22/>27-30	30/70
IVC	Hot and very humid climate	>22/>30	30/75

The purpose of a stability study lies in providing quality evidences of either an API or a product which differ under the influence of certain environmental factors, such as temperature, humidity and light. Moreover, the studies must be robust enough to support the transport, the storage and use. Regarding this, the guideline Q1A suggests the following storage conditions (Table 2.), in order to reproduce and measure the impact of these conditions on the product quality [3].

Table 2. – Storage condition and minimum time period cover by data at submission for each term of stability studies

Study	Storage condition	Minimum time period covered by data at submission
Long term*	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

Product

The β-lactam ring in the lactam-thiazolidine structure of penicillin and it is much more susceptible to nucleophillic attack than simple β-lactams. The penicillins show a fairly rigid structure due to the fusion between the β-lactam ring and the thiazolidine ring, resulting in a "V" shaped molecule [4].

The next reaccional scheme (Figure 1) shows the degradation routes of a generic β-lactam [5].

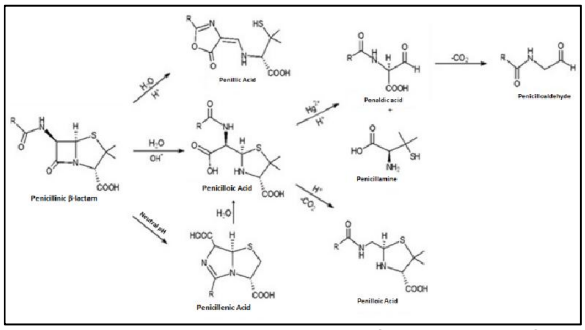

Figure 1. Typical routes of degradation of a generic β-lactam

As is figured above, in a neutral solution, penicillin forms penicilenic acid.

Accordingly, in an aqueous solution, it is expected that the hydrolysis rate increases with decreasing pH, making necessary the presence of buffer agents in order to provide a pH close to neutral, obtaining, in consequence, a great stability in solution.[6]

Illustrating with two structurally similar Penicillin G and Penicillin V, Penicillin V is much more stable in acidic environment. Assenmacher (1978) deduced that benzyl group of Penicillin G does not protect the β-lactam ring, which does not occur in Penicillin V, in which the phenoxy group protects β-lactam ring, and so, it shows a better stability than Penicillin G in acidic environments.[5]

Concerning its kinetic degradation in acid medium, it takes up this first order, with penillic and penilloic acids as the main degradation products at these conditions.[5,6,7]

As a suspension (particularly, an Extemporaneous Oral Suspension (EOS)), is noteworthy that, ideally, it should have the following characteristics:

- The sediment formed should not correspond to a compact body;
- Must occur a fast homogenization under moderate agitation;

- Its rheological properties must allow their suspended sediment particles to a speed that allows easy resuspendability or, preferably, that remain suspended.[8,9]

So, it is expected that, at the development stage, knowledge based approach would be adopted.

2. Materials and Methods

High-resolution chromatographic method (gradient) with UV detection - for the quantification of the active substance and degradation products content (internal method) was executed with an HPLC system VWR Hitachi Elite Lachrom (Quaternary pump L-2130; Auto-sampler L-2200; Oven L-2300; Detector DAD L-2455) with Data processor EZChromElite 3.3.2, and the mixing was abetted with an ultrasonic bath (Sonorex RK100 Bandelin),

For pH determination (based on Ph. Eur. 8th Edition chapter 2.2.3 Potentiometric determination of pH), a pH meter was used (744 pH Meter Metrohm). For water content determination, a Karl Fisher Titration was performed (based on Ph. Eur. 8th Edition, chapter 2.5.12 Water: semi-micro determination), with Karl Fischer apparatus (890 Titrando, 900 Touch control and 803 TI Stand Metrohm).

No descripition of samples and reference materials will be presented for confidentiality reasons.

3. Results and Discussion

3.1. Results

There will be presented three batch analysis (in 45 batches analyzed), one for each condition tested for 50 mg/200 mL (after reconstitution) preparation, which are shown in the following tables:

Table 3. – Stability Studies for batch C002, at long term conditions (25 °C/60 %HR)

Test	Specification	Time of analysis (months) and results		
		0	3	6
Description	White or off white powder, anise	*Complies*	*Complies*	*Complies*
pH value	5.0 – 7.5	6.2	6.2	6.2
Water Content (KF)	≤ 1.0 %	0.8	0.7	0.9
Assay (HPLC)	95.0 % - 105.0 %	102.1	100.6	101.1
Related Substances (HPLC)	Impurity D ≤ 4.0 %	0.41	0.47	0.42
	Impurity E ≤ 1.0 %	nd	0.1	0.14
	Impurity F ≤ 1.0 %	nd	0.05	nd
	Impurity B ≤ 1.0 %	nd	nd	nd
	Main unknown impurity ≤ 1.0 %	Below LOQ	0.07	0.11
Dissolution (HPLC)	Q = 90.0 % in 15 minutes	100.4	–	–
Microbiological Contamination				
TAMC	$\leq 10^3$ ufc / g	< 100 ufc / g	—	—
TYMC	$\leq 10^2$ ufc / g	< 100 ufc / g		
E. coli	Absent/ g	Absent/ g		

Table 4. – Stability Studies for batch C002, at intermediate conditions (30 °C/65 %HR)

Test	Specification	Time of analysis (months) and results		
		0	3	6
Description	White or off white powder, anise	*Complies*	*Complies*	*Complies*
pH value	5.0 – 7.5	6.2	6.1	6.2
Water Content (KF)	≤ 1.0 %	0.8	0.7	0.7
Assay (HPLC)	95.0 % - 105.0 %	102.1	100.7	98.9
Related Substances (HPLC)	Impurity D ≤ 4.0 %	0.41	0.47	0.49
	Impurity E ≤ 1.0 %	nd	0.19	0.18
	Impurity F ≤ 1.0 %	nd	0.2	0.23
	Impurity B ≤ 1.0 %	nd	nd	nd
	Main unknown impurity ≤ 1.0 %	Below LOQ	0.1	0.06
Dissolution (HPLC)	Q = 90.0 % in 15 minutes	100.4	–	–
Microbiological Contamination				
TAMC	$\leq 10^3$ ufc / g	< 100 ufc / g	—	—
TYMC	$\leq 10^2$ ufc / g	< 100 ufc / g		
E. coli	Absent/ g	Absent/ g		

Table 5. – Stability Studies for batch C002, at accelerated conditions (40 °C/75 %HR)

Test	Specification	Time of analysis (months) and results		
		0	3	6
Description	White or off white powder, anise	*Complies*	*Complies*	*Complies*
pH value	5.0 – 7.5	6.2	6.2	6.1
Water Content (KF)	≤ 1.0 %	0.8	0.7	0.9
Assay (HPLC)	95.0 % - 105.0 %	102.1	97.4	97.1
Related Substances (HPLC)	Impurity D ≤ 4.0 %	0.41	0.8	1.02
	Impurity E ≤ 1.0 %	nd	0.97	0.48
	Impurity F ≤ 1.0 %	nd	0.86	0.98
	Impurity B ≤ 1.0 %	nd	nd	nd
	Main unknown impurity ≤ 1.0 %	Below LOQ	0.23	0.41
Dissolution (HPLC)	Q = 90.0 % in 15 minutes	100.4	—	97.7
Microbiological Contamination				
TAMC	≤ 10^3 ufc / g	< 100 ufc / g	—	< 100 ufc / g
TYMC	≤ 10^2 ufc / g	< 100 ufc / g		< 100 ufc / g
E. coli	Absent/ g	Absent/ g		Absent/ g

Table 6. – Content of Impurity F along time, for each preparation, and their respective kinetic constants, for intermediate conditions of stability studies.

Dosage (mg/mL)*/ Apresentation da EOS (mL)	Batch	ln_0	$ln_{t=3}$	$ln_{t=6}$	k (s^{-1})
50/125	C001	-3.00	-1.61	-1.56	0.240
	C002	-3.00	-1.90	-1.77	0.200
	C003	-3.00	-1.90	-1.66	0.220
50/200	C001	-3.00	-1.56	-1.24	0.290
	C002	-3.00	-1.61	-1.47	0.250
	C003	-3.00	-1.61	-1.56	0.240
100/60	C001	-3.00	-1.47	-1.11	0.310
	C002	-3.00	-1.90	-1.66	0.220
	C003	-3.00	-1.97	-1.83	0.190
100/125	C001	-3.00	-1.66	-1.11	0.310
	C002	-3.00	-1.90	-1.83	0.190
	C003	-3.00	-1.90	-1.77	0.200
100/200	C001	-3.00	-1.56	-1.47	0.250
	C002	-3.00	-1.71	-1.77	0.200
	C003	-3.00	-1.71	-1.66	0.220

** Oral suspension concentration, expressed in mg/mL of active substance, after reconstution*

Determination of kinetic parameters

For the characterization of the degradation profile, in order to determine the shelf life period, the approach adopted was based on the formation of Impurity F, regarding that any other impurity, as well as the active substance, does not meet the requirements, *i.e.*, a significance change was not verified, with the exception of Impurity E, although this impurity did not show an increased rate along the test period.

A first order kinetics follows the exposed behavior[10] :

$$\frac{dC}{dt} = k.C \qquad (1)$$

$$\int_0^t \frac{1}{C} dC = \int_0^t k\, dt \qquad (2)$$

$$ln\left(\frac{C_t}{C_0}\right) = k(t - t_0) \qquad (3)$$

Based on the above formula, the data was treated, being obtained the following results:

Table 7. – Content of Impurity F along time, for each preparation, and their respective kinetic contants, for accelerated conditions.

Dosage (mg/mL)*/ Apresentation da EOS (mL)	Batch	$ln_{t=0}$	$ln_{t=3}$	$ln_{t=6}$	k (s^{-1})
50/125	C001	-3.00	0.00	0.250	0.540
	C002	-3.00	-0.25	-0.220	0.460
	C003	-3.00	-0.27	-0.140	0.480
50/200	C001	-3.00	-0.0400	0.300	0.550
	C002	-3.00	-0.150	-0.0200	0.500
	C003	-3.00	-0.200	-0.0600	0.490
100/60	C001	-3.00	0.170	0.390	0.560
	C002	-3.00	-0.220	-0.130	0.480
	C003	-3.00	-0.240	-0.140	0.480
100/125	C001	-3.00	-0.0100	0.370	0.560
	C002	-3.00	-0.260	-0.150	0.470
	C003	-3.00	-0.300	-0.150	0.470
100/200	C001	-3.00	-0.050	0.380	0.560
	C002	-3.00	-0.120	-0.110	0.480
	C003	-3.00	-0.150	-0.0500	0.490

** Oral suspension concentration, expressed in mg/mL of active substance, after reconstitution*

A statiscal treatment has been applied, in the interest of getting representative parameters, selecting the reaction rate constants that were within the Confidence Interval:

Table 8. – Mean kinetic constants, standard deviation and confidence interval for accelerated and intermediate conditions

Condições (ºC / % HR)	k_{mean}	σ	n
25 / 60			
30 / 65	2.38E-01	3.95E-02	15
40 / 75	5.05E-01	3.68E-02	15

Determination of the shelf-life period

With regard to shelf life period, it is necessary to take into account the limit of specification, and calculate when it is achieved. Thus, for a first order kinetics [11],

$$ln\left(\frac{C_{t.l.}}{C_0}\right) = k(t_{t.l.} - t_0) \qquad (4)$$

$$t_{t.l.} = \frac{ln(C_{t.l}) - ln(C_0)}{k} + t_0 \qquad (5)$$

$$t_{t.l.} = \frac{ln(C_{t.l.}) - ln(C_0)}{a.e^{\frac{-E_a}{R.T}}} + t_0 \qquad (6)$$

Whereas $C_{t.l.}$ represents the acceptance threshold concentration (1.0 % for impurities) and $t_{t.l.}$ the moment which this concentration is achieved. The results are showed on Table 9:

Table 9. – Mean kinetic constants and their respective temperatures, to determinate the drug product degradation profile, based on Arrhenius profile

Conditions (ºC / % HR)	*k_{mean}*	*$Ln(k_{mean})$*	*1/T (K^{-1})*
25 / 60			3.35E-03
30 / 65	0.236	-1.44	3.30E-03
40 / 75	0.492	-0.71	3.19E-03

Performing a linearization beetwen ln(k) and 1/T, are obtained the Arrhenius parameters of the β-lactamic EOS (Table 10.).

Table 10. – Arrhenius Parameters for the β-lactamic EOS studied

Parameters of Arrhenius equation	
$-E_a/R$	-6957
ln(a)	21.51
a	2.19E+09
E_a (cal/mol)	3501

Once obtained the kinetic parameters, it was possible to determine the shelf life period, for each condition studied.

Table 11 – Shelf Life Period, in months, for each term of stability studies conducted

Conditions (ºC / % HR)	*Shelf life period (months)*
25 / 60	19
30 / 65	13
40 / 75	6

3.2. Discussion

In long term and intermediate studies no significant change was verified, then, no objection will be done for these conditions.

In accelerated conditions, significant changes were detected in the appearance and Impurity F content, which are discussed in detail below, as well as the other tests conducted.

Appearance

Changing in appearance (color) of the pharmaceutical preparation was observed in the majority of batches tested at 40 °C/75 %RH, which identifies the presence of significant changes (as

defined in guideline Q1A).These changes are potentially associated with the degradation of the active substance or the excipients used. From the results of assay and related substances, are obtained a noticeable degradation when performing accelerated stability tests.

Regarding excipients, degradation may fall in flavorings and/or sugars, due to, in its composition, the pharmaceutical formulation has 4 main groups [12,13]:

- Polymers with suspending functions- which are stable under the tested conditions, changing the aspect just for temperatures above 100 °C.
- Ionic solids with sliding and absorbent (of moisture) - have stability in a wide range of temperature.
- Sugars used as masking agent -when reducing sugars are part of the formula (as happens in the dosage form), occurs Maillard reaction at high temperatures, which forms yellowish products.
- Flavoring agents – organic compounds with typical high degrees of volatility. Not effecting directly the aspect, the heat applied on system can change the flavor, decreasing its intensity as a result of its volatility, a fact that was not observed in sensory analysis performed to the drug product.

Observing the factors above, it is expected that the change in appearance was due to degradation of the sugar, through its exposure to high temperatures.

However, the active ingredient degradation products could also show yellowish colour. Chromatographic analysis (associated with photodiode array detector) of batches allowed the verification that degradation productsare not compatible with the formation of yellow coloration (absorption of degradation products is restricted to the ultra-violet region).

pH

By potentiometric analysis no significant changes were observed, being the lowest value obtained 6.0. The slight decrease observed suggests that acidic degradation products were formed (which correspond to degradation products of the active substance). This analysis is corroborated by evaluating the results obtained for the determination of the active substance and its related substances.

Water Content

No changes of water content in relation to its initial content. These results suggest a suitable isolation container system.

Assay and Related Substances

Regarding the assay, it was found an average variation of 4.0% between the initial assay and the performed at 6 months.

Concerning Impurity B, was not verified an increase in its content. It is known that this product is only formed under acid conditions, and the range of pH 6.0 does not appear close for its formation. The Impurity D has a variation within the acceptable threshold, with the particularity of presenting a 4.0% limit, whereas the other related

substances have a 1.0% acceptance threshold. Its initial concentration was superior to the others, due to the fact that it is an inherent impurity to the manufacturing process of the active substance, as evidenced in the certificate of analysis. It is known that impurity D is formed at neutral and acidic pH conditions, whereby its increase was expectable.

A situation that seems an anomalous situation is the increasing content of Impurity E up to 3 months of analysis, followed by a decline in the next 3 months. However, the literature shows the occurrence of a direct conversion of impurity E in F, thereby explaining the negative variation observed [5].

In Impurity F significant changes were detected, consistent with the literature, being an impurity that forms in a wider range of pH, between the acidic and neutral pH.

Regarding the data obtained, is not possible to define precisely the shelf life period. As exposed in the Guideline Q1A (R2), is necessary conclude the long-term stability tests to set a definitive shelf life. Nevertheless, in guideline Q1A (R2), if significant changes are expected, is suggested the conduction of stability tests over than 3 periods, to obtain more representative results.

Regarding the mathematical data treatment, (the Arrhenius curve), it has been determined by the data associated with the three storage conditions studied, though no changes have been observed in long-term stability, and, therefore, the kinetic constant for this condition was not predictable. As a result, the obtained estimate was not based on these data. However, the extrapolation for long-term conditions indicates a shelf life of 19 months, for long term conditions (25 °C of Temperature and 75 % of Relative Humidity). The reference medicine of the drug product has a shelf life of 24 months, suggesting that is a good shelf life estimative.

The kinetics of formation of Impurity F, is 1st order, which is in agreement with the literature.

Other conclusions are related to the buffering system applied, since the pH value for the worst case scenario (accelerated stability studies) never decreases beyond 6.0. Thus, it confirms the presence of an optimum buffering system, being observed minimal degradation of the active substance, since the degradation profile is closely correlated with pH value.

The water content did not change in the conditions studied, and so that, is assured that the type of container (type III amber glass) used in the primary packaging is suitable for the package intend. This primary packaging, associated with the respective closing system (polyethylene screw cap with an internal sealant), provides the necessary isolation required by authorities,

Another factor that was considered, and investigated, was the contribution of the headspace on the degradation profile, concluding that no increased degradation occurs in performances that have more air volume available. This can be explained by inactivity of the gas contained in the product (molecular nitrogen), and for not occurring gas transfers (or their occurrence were not significant), likewise do not take place water transfer, between the outside and the inside of container.

4. Conclusions

The stability tests conducted in the first six months of the new pharmaceutical form corroborate the pharmaceutical options adopted at the development stage of the generic drug.

The reference medicine, applied in the product development, it has a shelf life of 24 months, coupled with restrictions on storage conditions: "keep the temperature not exceeding 25 ° C."

Unsatisfactory results were observed for the generic drug in conditions of accelerated stability, indicate the need to restrict the product's storage temperature, in accordance with the reference product applies.

The product of this study was stable in the intermediate and long-term stability studies, suggesting that it will remaining stable within the desired shelf life (24 months) when stored at or below 25 °C. This fact is corroborated by the estimated shelf life (19 months) based on data obtained at 25 °C /60 %RH and 30 °C/65 %RH storage conditions.

Regarding this, the data obtained up to date anticipate that the drug product presents the same shelf life and storage conditions to the applicable reference medicine, which will allow it regulatory approval and subsequent commercialization.

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