QUALIFICATION OF A STABILITY-INDICATING METHOD FOR THE DETECTION OF IMPURITIES IN HISTIDINE 117,20 MG / 3 ML AP-HP

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INTRODUCTION

DRDP-AGEPS → Developpement of drugs for orphan diseases not covered by the competitive industry (ex: The Histidine/Copper kit forMenkes disease)

Menkes Desease

The Histidine/Copper kit AP-HP
(a hospital preparation)
1 Copper inj. Ampoule CuCl (6mg/mL)
1 Histidine in ampoule (117,2mg/mL)
Shelf life : 24 months

OBJECTIVES

- ❑ Demonstrate the stability indicator nature of the degradation products (DP) research method of histidine ampoules.
- ❑ Identify the pathways of Histidine degradation and generate DP of the active substance (Histidine).
- ❑ Demonstrate the benefit of inerting in the production of Histidine ampoules: selection of a PDD representative of the degradation, the evolution of which is evaluated with a predictive method of stability.

METHODS

❖ Forced degradation under the following conditions:

Stress	Experimental conditions	Storage conditions		Sampling time (days)
Thermal	Amber glass vials Heat chamber	-	90°C	1, 2
Photolytic	Transparent glass vials	UV-Vis	25°C	8, 13, 20
Oxidation	Amber glass vials Heat chamber	0,1 to 3% H_2O_2	60°C	1
		1 to 5% MMPP	60°C	1
Hydrolysis	Amber glass vials Heat chamber	1M NaOH	60°C	8, 29, 57
		1M HCl	60°C	8, 29, 57

Table 1: Degradation conditions of the Histidine solution (without inerting) prepared according to the formulation of the hospital preparation

❖ Detection of impurities by reversed phase HPLC-PDA and identification by LC-MS

❖ Justification for the inerting of Histidine vials

The experimental plan using the stability prediction software based on the Arrhenius law (ASAPPrime®).

- T0: Day 0 (4 samples)
- 45°C: Day 12 (2), Day 15 (2), Day 18 (3), Day 21 (3)
- 55°C: Day 5 (2), Day 8 (2), Day 13 (2), Day 21 (3)
- 65°C: Day 2 (2), Day 4 (2), Day 8 (2), Day 17 (3)
- 75°C: Day 1 (2), Day 2 (2), Day 4 (2), Day 7 (3)
- 90°C: Day 1 (4), Day 2 (5)

x 2 series → Non inerted / Inerted

- Evaluation of the prospective shelf life for each set.

RESULTS

FORCED DEGRADATION

Stress factors	Control	Thermal	Molecular oxidation	Radical oxidation	Photolitic	Alkaline Hydrolysis	Acid Hydrolysis
Stress Conditions	Not Applicable	90 °C	3% H_2O_2 60 °C	5% MMPP 60 °C	UV-VIS light 25 °C	1M NaOH 60 °C	1M HCl 60 °C
Aspect	Colorless	Colorless to yellow	Yellow to brown	Yellow to brown + precipitate	Colorless to yellow	Colorless to yellow	Colorless to yellow
Sampling time (Days)	0	2	1	1	21	57	57
Histidine (RT = 5,80 min)	100%	97,96%	94,96%	95,74%	98,69%	97,21%	98,91%
Peak 2 (RRT = 0,62)	0%	0,52%	3,83%	1,27%	0,86%	0,54%	0,43%
Peak 4 (RRT = 2,86)	0%	0,65%	0,47%	2,03%	1,31%	1,34%	0,46%
Sum of peaks	0	15	35	23	31	13	11

Table 2: Examples of results in percentage of normalized area of Histidine DP under various stress factors. (Peak 1 RRT = 0.43, Peak 2 RRT = 0.62, Peak 3 RRT = 2.00, and Peak 4 RRT = 2.80)

Stress condition	Degradation level
H_2O_2 Oxidation	+++
MMPP Oxidation	+++
Photolytic	++
Thermal	+
Alkaline	+
Acid	+

- ➢ Histidine degradation is observable through changes in the solution's color, (Colorless -> Yellow -> Brown).
- ➢ The chromatographic method allows for the separation of a large number of DPs highlighting the method's stability-indicating nature (Figure 3).
- ➢ Oxidation is identified as the primary degradation pathway of histidine

IDENTIFICATION OF A DP THROUGH MS COUPLING

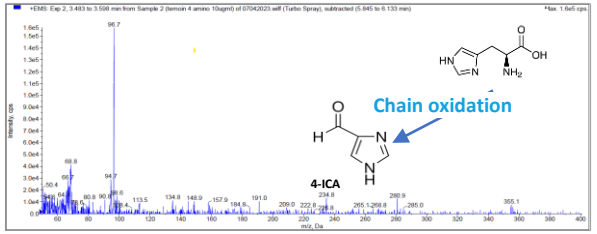


Figure 1: ESI+ MS Scan of 4-Imidazolecarboxaldehyde (4-ICA), specificity of the MRM transition 97/69.

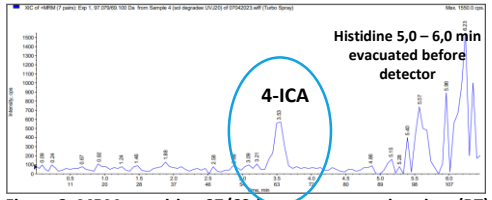


Figure 2: MRM transition 97/69 found at a retention time (RT) of 3.5 minutes in a histidine solution degraded under light for 20 days.

The LC-UV analysis revealed that 4-ICA elutes at the same retention time as Peak 2 . (Figure 3)
The identification of Peak 2 was confirmed through LC-MS coupling. (Figures 1 and 2)

➢ The peak at 3.5 minutes is indeed 4-ICA.

It is planned to replicate the methodology with an **oxo-histidine** reference, another degradation product mentioned in the literature [1,2]

DEGRADATION PREDICTION

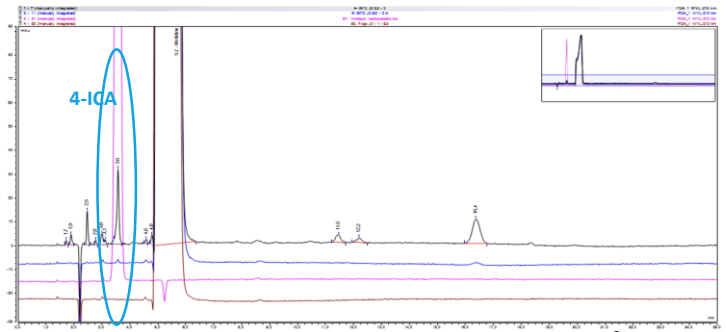
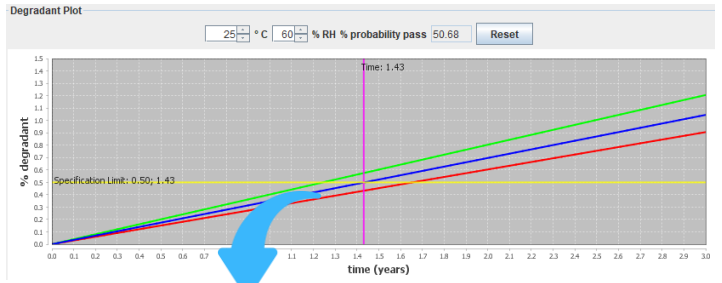


Figure 3: Chromatograms of histidine solutions degraded over 2 days at 90°C, inerted (in blue) and inerted (in black) undegraded histidine solution at Day 0 (in brown), 4-ICA reference (in pink).



Stability prediction = **1,43 years**

The degradation in the inerted set was very low suggesting a shelf life significantly greater than 24 months *= The Histidine/Copper kit shelf life*

- ➢ This comparative study demonstrates the effectiveness of inerting in the histidine injectable ampoule manufacturing process

CONCLUSION

The method used in stability studies allows for the visualization and separation of various DPs obtained through forced degradation. The identification of 4-imidazolecarboxaldehyde has provided a representative indicator of histidine solution degradation. The ASAPPrime® program has enabled the prediction of the stability of the histidine formula in less than a month and justified the use of an inerting process for histidine vials.
This study illustrates the analytical methodologies employed in the drug development process.

[1] Mason B, McCracken M, Bures E, Kerwin B. Oxidation of free l-histidine by tert-butylhydroperoxide. Pharm Res. 2010;27(3):447–56
[2] Méndez-Hurtado J, López R, Suárez D, Menéndez MI. Theoretical study of the oxidation of histidine by singlet oxygen. Chemistry. 2012 Jul 2;18(27):8437-47.