Study of the microbiological stability and preservative efficiency of a 2 mg/mL hydrochlorothiazide oral suspension in Inorpha®

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GERPAC EUROPEAN SOCIETY OF HOSPITAL PHARMACEUTICAL TECHNOLOGIES
COM24-36383

Introduction

Hydrochlorothiazide is a diuretic used in pediatrics for which no suitable commercial form exists.
We plan to prepare a 2 mg/mL oral suspension in Inorpha®, which physico-chemical stability was determined from the literature (3 months at 4 ± 2°C[1] and 2 months at 20°C[2]).

Objective

Assessing the microbiological stability and antimicrobial preservation efficacy of the 2 mg/mL Hydrochlorothiazide preparation in Inorpha®.

Material and methods

Suitability of the test method (Test 2.6.12/13 Ph.Eur.)

Investigation of the dilution neutralizing potassium sorbate (Ph. Eur. Essai 2.6.12.-2)

Microbiological Enumeration (Ph.Eur. 2.6.12)

Contamination by *P.aeruginosa, S.aureus, B.subtilis, C.albicans* et *A.brasiliensis*			
Tested dilutions	1/10th	1/20th	1/50th

➢ Inoculation of contaminated samples, positive control (5 MO) and negative control (T) –> surface-spread method.

✓ Expected result : less than a factor of 2 difference between the positive control and the diluted sample.

Test for specified micro-organisms (Ph.Eur 2.6.13)

Contamination by *E.coli*				
Dilutions	1/10th	1/100th	1/200th	1/1000th

➢ Detection of E. coli in contaminated samples, positive control, and negative control by:


Pre-incubation


Selection


Subculture (Gélose McConkey)

✓ Expected result: Identification of the dilution that allows the detection of E. coli.

Microbiological stability study (Test 5.1.4 Ph.Eur.)

Determination of the hydrochlorothiazide preparation stability period (n=6 batchs)

Study Conditions

Stability	Lots	Conservation 25 ± 2°C	Conservation 4 ± 2°C
Before opening (Unopened bottles)	Batchs 1-3	12 Bottles	12 Bottles
After opening (Opened at Day 0)	Batchs 4-6	3 Bottles	3 Bottles

Daily Sampling by oral syringe.

Study progress

Microbiological enumeration and detection of *E. coli* using the dilution validated by the applicability test (1/10th)

J0 → J21 → M2 → M3

✓ Expected results for a non-sterile aqueous oral preparation :

	European Pharmacopoeia limits (Test 5.1.4.-1)
Aerobic germs	< 200 CFU/mL
Mould and yeast	< 20 CFU/mL
Specified microorganisms	Absence of *E.coli*

Antimicrobial preservation efficacy (Test 5.1.3 Ph.Eur.)

Evaluation of the potassium sorbate efficacy at the concentration present in Inorpha®

1) 5 bottles of 2 mg/mL hydrochlorothiazide oral suspension in Inorpha®.

2) Microbial suspensions: Use of Bioballs (Biomerieux®) corresponding to the 5 strains recommended by the Ph. Eur. (Test 5.1.3)

3) Contamination of each bottle with one of the 5 microorganisms.

Study progress

Microbiological enumeration by surface-spread method		
Day 0	**Day 14**	**Day 28**

Storage of bottles at room temperature.

✓ Expected results(Essai 5.1.3.-3 Ph.Eur.) :

	Logarithmic reductions Day 14	Logarithmic reductions Day 28
Bacterias	3	No increase
Yeasts	1	No increase

Results

Suitability of the test method (Test 2.6.12/13 Ph.Eur.)

Microbiological enumeration :
Validation of all dilutions
Test for specified microorganisms :
Detection of *E.coli* in all dilutions tested

➢ Choice of the smallest validated dilution: 1/10th

Antimicrobial preservation efficacy (Essai 5.1.3 Ph.Eur.)

	Average CFU (n= 2 géloses) Day 0	Day 14	Day 28
S. aureus	111	0	0
P.aeruginosa	45	0	0
E.coli	74	0	0
C.albicans	64	0	0
A.brasiliensis	31	0	0

➢ Potassium sorbate effective at the concentration present in Inorpha®.

Microbiological stability study (Essai 5.1.4 Ph.Eur.)

	Average CFU/mL							
Conservation	☀				❄			
Test day	J0	J21	M2	M3	J0	J21	M2	M3
Before opening	0	0	0	0	33*	0	0	0
After opening	17*	0	0	0	0	0	0	0

* Identification by MALDI-TOF
TAMC : *B.subtilis*

➢ Oral suspension stable 3 months at 25 and 4°C, before and after opening.

Conclusion

The oral suspension is stable for 3 months under both storage conditions. It leads to set the shelf life at 3 months before and 2 months after opening for bottles stored at 4±2°C.

1. Mise au point d'une suspension buvable d'hydrochlorothiazide, Dr ROBIN Hélène, Thèse d'exercice, Poitiers 2014
2. STABILITÉ D'UNE SUSPENSION D'HYDROCHLOROTHIAZIDE À 2MG/ML, Laure NAPOLY, Poster APHIF 2014