

INTRODUCTION

Spironolactone is used in pediatric patients to treat hypertension and nephrotic syndrom. In hospital pharmacies, compounded capsules are prepared. To reduce errors and facilitate administration, an oral suspension of spironolactone 5 mg/mL was formulated with 0.25% of xanthan gum in Inorpha® containing potassium sorbate (PS - antimicrobial preservative). (1)

To determine the microbiological storage conditions and shelf life of the spironolactone suspension and to assess the antimicrobial preservative efficacy of PS.

MATERIAL AND METHOD

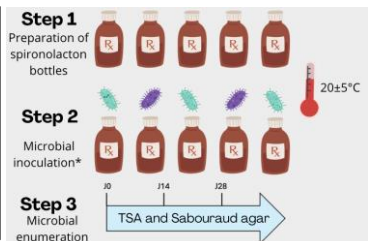
Method suitability

(Ph.Eur. Test 2.6.12/13)

Antimicrobial preservative effectiveness

(Ph.Eur. Test 5.1.3)

Microbial enumeration	Detection of specified <i>E.coli</i>
-3 dilutions tested (1:10, 1:25, 1:50) -Contamination with 5 MO (Ph.Eur): TYMC: <i>C.albicans</i> , <i>A.brassiliensis</i> TAMC: <i>S.aureus</i> , <i>P.aeruginosa</i> , <i>B.subtilis</i> -Surface inoculation and incubation: TSA (3 days at 30-35°C) and Sabouraud agar (5 days at 20-25°C)	-3 dilutions tested (1:10, 1:50, 1:100) -Contamination with <i>E.coli</i> then preincubation -Subculture in MacConkey broth and incubation: (72h at 42-44°C) -Surface inoculation and incubation: MacConkey agar (3 days at 30-35°C)
Preservative neutralization if:	
Difference in CFU< factor of 2 between the sample and positive control	Growth of <i>E.coli</i> detected in the sample and positive control



Expected log reduction
compared with day 0
(Non-sterile aqueous preparation
for oral administration)

Logarithmic reduction	D14	D28
Bacteria	3	NI
Mold / yeast	1	NI

*NI= No increase in the number of
viable MO in comparison to day 14

*In each bottle, one of the 5 MO of the Ph.Eur. Test 5.1.3
(*E.coli*, *S.aureus*, *P.aeruginosa*, *C.albicans*,
A.brassiliensis) was introduced

Microbiological stability study

(Ph.Eur. Test 5.1.4)

Ph.Eur. Limit of detection

Ph.Eur. limit	
TAMC (aerobic)	< 200 CFU/mL
TYMC (Yeast and mold)	< 20 CFU/mL
MO specified	Absence <i>E.coli</i>

M0.M1.M2.M3:

- Microbial enumeration
- *E.coli* research

RÉSULTS

Method suitability

- All dilutions tested were validated
- The lowest dilution was selected for the stability study (1:10 for the microbial enumeration and 1:10 for the detection of specified *E.coli*)

Microbiological stability study

Microbial enumeration results at M0_M1_M2_M3

	Mean CFU/mL							
	M0		M1		M2		M3	
	5°C	20°C	5°C	20°C	5°C	20°C	5°C	20°C
* <i>Kocuria rhizophila</i>								
** <i>Cladosporium cladosporioides</i>								
*** <i>Staphylococcus epidermidis</i>								
Closed bottles (n=3 batches)	0	TYMC= 17 **	0	0	TAMC= 17 ***	0	0	0
Opened bottles (n=3 batches)	TAMC= 17 *	0	0	0	0	TYMC = 117 **	0	0

- *E.coli* testing remained negative up to M3
- M1: Opened bottles at 20°C : TYMC > 20 CFU / mL

The study has been stopped in
the opened bottles under
ambient condition at M1

Antimicrobial preservative effectiveness

Microbial enumeration at day 0,14,28

Mean CFU (n=2)	D0	D14	D28
<i>E. coli</i>	10	0	0
<i>P.aeruginosa</i>	7	0	0
<i>S.aureus</i>	18	0	0
<i>C.albicans</i>	8	0	0
<i>A.brassiliensis</i>	9	0	0

- Logarithmic reduction respected
- No viable MO detected at day 14 and 28

CONCLUSION

20±5°C Microbiologically stable for 3 months before opening.
It is not microbiologically stable after opening.

5±3°C Microbiologically stable for 3 months both before
and after opening.



Antimicrobial preservation of PS is effective