Preservative Formulation and Effectiveness in Oral Solutions and Suspensions

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Outline

Formulating with Preservatives

- Excipients and preservatives
- Use of Parabens
- Regulatory concerns
- Formulation Scenarios

The Antimicrobial Effectiveness Test (AET)

- What is AET?
- AET Procedure and validation
- Interpretation of results
- Variability and Outsourcing
- AET in Product Development

Oral Liquids

Drug substances are formulated in Oral liquids including solutions, syrups, elixirs, and suspensions

They need to have protection against microbial growth

Oral Liquid Formulation Excipients

- Solvents / Co-solvents
- Solubilizers
- Preservatives
- Sweetners
- Surfactants
- Suspending Agents
- Antioxidants
- Flavoring Agents
- Buffering Agents

Why Preserve a Product?

- **For Non-sterile Dosage Forms**
  - To protect from microbiological growth or from microorganisms that are introduced during or subsequent to the manufacturing process.*
- **For Sterile Dosage Forms**
  - For products packaged in multi-dose containers, to inhibit growth of microorganisms that might be introduced from repeatedly withdrawing doses.*

*USP Chapter <51>

Formulation Considerations for Preservatives

Issues to consider

- Solubility
- Stability
- Taste/Palatability

Balance between the following factors:

- Drug stability and solubility vs. pH, storage temperature
- Preservative effectiveness and solubility in relation to pH of solution and storage temperature

Preservative Considerations

Activity against various microorganisms

pKa of preservative

pH of the product

Solubility of preservative (pH, temperature)

Stability of preservative (chemical, physical)

Suppliers/Cost/Regulatory limits/Safety

Preservative Effectiveness

Most acid preservatives are not effective above their pKa.

If the pH is higher than the pKa, more of the acid will be in the ionized form, thus potentially rendering the preservative ineffective.

pH-pKa = log [conjugate base]/[acid]

pH-pKa = log [ionized]/[unionized]

pH-pKa = log [ineffective P]/[effective P]

Partition Coefficient

Partition of preservative between organic and aqueous phases

Relevant to oral liquid systems where preservative may have better effect in one phase versus another

Effect of functional groups that can slightly increase (i.e. alkyl) or decrease (i.e. hydroxyl) the partition coefficient

Common Preservatives for Oral Formulations

- Benzoic acid and salts
- Sorbic acid and salts
- Parabens

Parabens

- Group of alkyl esters of p-hydroxybenzoic acid with an effective pH range of 4.0 to 8.0
- Most active against yeast, molds, and gram positive bacteria
- Antimicrobial activity decreases above pH 8 due to the formation of the phenolate anion (pKa=8.4)
- Parabens undergo hydrolysis in weak alkaline and strongly acidic solutions
- Parabens work more effectively in combinations

Paraben Properties

Paraben (R, alkyl group)	MW	Log P	Water Solubility (mg/mL)
Methyl	152.15	~1.95	~2.5
Ethyl	166.17	~2.47	~0.8
Propyl	180.20	~3.04	~0.4
Butyl	194.23	~3.57	~0.2

As alkyl chain length of the paraben ester group increases, antimicrobial activity increases but water solubility decreases and oil solubility increases

Estrogenic activity of parabens increases with length of alkyl group

Sweeteners

Examples of sugars include sucrose, fructose, glucose, maltose, lactose

Example of sugar alcohols/polyols include maltitol, lactitol, sorbitol

Reactivity of sugar (aldehyde/ketone group) is higher than that of polyol (hydroxyl group)

Reacting with residual reducing sugars may lead to Maillard browning reaction

Paraben Interactions

- Parabens can interact with Cyclodextrins
- Reduction in effectiveness in the presence of polysorbate 80
- Transesterification of methylparaben with sugars and polyols
- Sorption of parabens to various tubing materials

Toxicity

Sodium Benzoate

- Found to elicit non-immunological contact reactions including urticaria (skin rash)

Parabens

- Estrogenic potential (animal data), breast cancer

Regulatory Considerations

21CFR211

- Excipient are also used in food and cosmetic industries

Excipient toxicity

- Genotoxicity, carcinogenicity

Patient population

- Pediatric (neonates, infants, toddlers, children, adolescents)

Scenario 1

- Compound “A” has a bitter taste and needed to be formulated as a pediatric oral solution
- The active reacted with reducing sugar impurities in sucrose
- Reformulation was necessary with a non-reducing sugar such as maltitol
- Upon reformulation with a maltitol, variability was seen with the preservative assay for propylparaben
- Propylparaben was not degrading (confirmed by HPLC analysis)
- Need to consider equilibrium solubility of parabens in maltitol

Preservative Assay in Maltitol Based Formulation

Condition	Duration	MP (% target)	PP (% target)
Initial	Initial	99.5	81.4
-20 C	2 wk	99.5	90.7
-20 C	4 wk	99.0	96.8
5 C	4 wk	99.0	95.4
5 C	13 wk	98.5	77.1
5 C	26 wk	98.5	96.4

Initial samples stored at 5C before analysis

Conclusion

- **Assessment of solubility showed parabens were above their saturation solubility at 5C**
- **Loss of parabens was due to precipitation at 5C**
- **A reduced level of parabens in the formulation avoided paraben precipitation**

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Fill Volume Effects

Fill Volume (mL)	Time (days)	MP (% target)	PP (% target)	Contact Area/Volume
30	30	93.9	88.8	1.67
90	30	93.9	94.0	1.09
150	30	93.5	95.0	0.98
210	30	93.6	95.6	0.93

Propylparaben (PP) loss most likely due to absorption, potentially because of higher log P of PP

Antimicrobial Effectiveness Test

AET demonstrates effectiveness of preservative in a product

- Antimicrobial Effectiveness Test (USP)
- Efficacy of Antimicrobial Preservation (EP)
- Preservation Effectiveness Test (JP)

Test organisms-bacteria, fungus, mold

Product requirements→typically 20-100mL

Scenario 2

Propylparaben has come under scrutiny due to its estrogenic activity and potential to affect fertility (animal data)

Regulatory authorities in the European Union have raised questions about its safety and use in formulations especially for pediatric population

Can ethylparaben be used in tandem with methylparaben in oral solutions to pass the AET for a proof of concept study?

AET Results, With and Without Ethylparaben

A	Methylparaben (1.1 mg/mL)
B	Methylparaben (1.1 mg/mL) + 0.25 mg/mL ethylparaben

	Day 0		Day 14		Day 28	
	Log CFU/mL					
Organism	A	B	A	B	A	B
C. albicans	5.7	5.7	3.7	2.0	<1.0	<1.0
Z. rouxii	5.7	5.7	3.6	<1.0	<1.0	<1.0
A. niger	5.5	5.6	2.8	<1.0	2.2	<1.0

Quicker action against yeasts and mold.

AET Considerations

Need to evaluate preservatives at reduced levels such that product will pass shelf life

- **Preservative level**
  - **Cover a range of concentrations below the optimal preservative concentration**
- **pH levels**
  - **One pH unit above/below product pH (based on drug solubility and stability) due to pH fluctuation**

The Antimicrobial Effectiveness Test

- What is the AET?
- AET Procedure and validation
- Interpretation of results
- Variability and Outsourcing
- AET in Product Development

What is the Antimicrobial Effectiveness Test?

- Compendial Test
- Not truly harmonized around the world
  - USP Chapter <51> “Antimicrobial Effectiveness Test”
  - EP Chapter 5.1.3 “Efficacy of Antimicrobial Preservation”
- Testing to confirm that the preservatives added in a formulation will work as expected over time.
- Used during formulation development and in stability programs.

What is the Antimicrobial Effectiveness Test?

- A developmental test in EU, may be release test in US
- Not ordinarily used for parenteral drugs, except for those that are preserved.
- Not a substitute for good GMP practices. - Preservation of a product is not the solution to microbial contamination issues!

Basic Procedure

- **Use specific ATCC microorganisms (or additional sources for EP)**
  - ***Escherichia coli (required for USP, recommended for oral products for EP)***
  - ***Pseudomonas aeruginosa***
  - ***Staphylococcus aureus***
  - ***Candida albicans***
  - ***Aspergillus brasiliensis***

Basic Procedure

- **Additional Organisms**
  - *Zygosaccharomyces rouxii* (for EP for products with high sugar concentrations
  - Environmental isolates
  - Per EP:

    "…designated microorganisms are supplemented, where appropriate, by other strains or species that may represent likely contaminants to the preparation."

    - For a parenteral, you might want to consider challenging with organisms associated with nosocomial infections.

Basic Procedure

- **Examples**
  - Resistant organism in cosmetic formulation
  - Bacillus
  - Nosocomial Organisms
    - ***Serratia marscens*, *Candida albicans*, *Streptococcus*, *Staphylococcus aureus***

Aside: FDA and other HA's are now asking for hold time studies on non-preserved drug preparations

Basic Procedure

- Determine what the product is:
- EP and USP have different Categories:
- USP

Category	Product Description
1	Injections, other parenterals including emulsions, otic products, sterile nasal products, and opthalmic products made with aqueous bases or vehicles
2	Topically used products made with aqueous bases or vehicles, non-sterile nasal products and emulsions, including those applied to mucus membranes
3	Oral products other than antacids, made with aqueous bases or vehicles
4	Antacids made with an aqueous base

Basic Procedure

- Determine what the product is:
- EP and USP have different Categories:
- EP

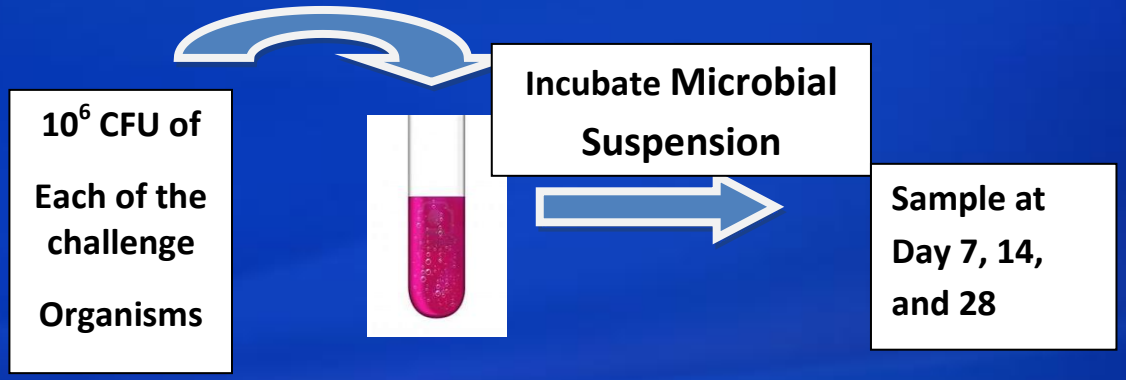
Table Reference	Product Description
5.1.3.-1	Parenteral preparations, eye preparations, intrauterine preparations and intramammary preparations
5.1.3.-2	Ear preparations, nasal preparations, preparations for cutaneous application and preparations for inhalation
5..1.3.-3	Oral preparations, oromucosal preparations and rectal preparations

Basic Procedure

- Separate containers for each organism to be tested, including appropriate controls
  - Alternatively, dispense aliquots into sterile containers which can be protected from light.
- Prepare the cultures to be used. You have to demonstrate that the inocula have the right levels of microorganisms.
- The cultures must be freshly prepared

Basic Procedure

- Inoculate the products individually with the specific organism, 1 organism per aliquot
- The concentration of organisms should achieve, in general, between 10^5 to 10^6 cfu/mL.



Basic Procedure

- Perform inoculum recovery to assure the original inoculation level and to estimate the concentration of organisms in the challenged products.
- For EP, perform time 0 recovery
- Store products, protected from light at 22.5±2.5ºC for the time specified in the tables.
- At the test time, remove aliquots and perform plate counts.

Basic Procedure

- At the test time, remove aliquots and perform plate counts.

Perform 10-fold serial Dilutions

Plate dilutions to determine number of survivors

Calculate the log reduction

Basic Procedure

- Determine the $\log_{10}$ of the concentration of the organisms remaining in the samples and compare the results to the required results from the tables in the individual chapters.
- Note that the requirements are different, depending on the class of product.
- Note also that ***no increase*** is defined as ***not more than 0.5 $\log_{10}$ increase in the counts***.

Interpretation of Results

- Results are interpreted vs the relevant compendia
- USP

Category 1	
Bacteria	Not less than 1.0 log reduction from the initial calculated count, at 7 days. Not less than 3.0 log reduction from the initial count at 14 days. No increase from the count at 14 days to the count at 28 days.
Yeast and Mold	No increase from the initial count calculated at 7, 14 and 28 days
Category 2	
Bacteria	Not less than 2.0 log reduction from the initial calculated count, at 14 days. No increase from the count at 14 days to the count at 28 days.
Yeast and Mold	No increase from the initial count calculated at 14 and 28 days
Category 3	
Bacteria	Not less than 1.0 log reduction from the initial calculated count, at 14 days and no increase from the count at 14 days to the count at 28 days.
Yeast and Mold	No increase from the initial count calculated at 14 and 28 days
Category 4	
Bacteria, Yeast and Mold	No increase from the initial calculated count at 14 and 28 days.

Interpretation of Results

		6 H	24 H	7 d	14 d	28 d
Bacteria	A	2	3	-	-	NR
	B	-	1	3	-	NI
Fungi	A	-	-	2	-	NI
	B	-	-	-	1	NI

Ear preparations, nasal preparations, preparations for cutaneous applications and preparations for inhalation					
		Log Reduction			
		2 d	7 d	14 d	28 d
Bacteria	A	2	3	-	NI
	B	-	1	3	NI
Fungi	A	-	-	2	NI
	B	-	-	1	NI

Oral Preparations, oromucosal preparations and rectal preparations		
	Log Reduction	
	14 d	28 d
Bacteria	3	NI
Fungi	1	NI

NR = No Recovery
NI = No Increase

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Validation

- Must be able show inactivation of the preservative by demonstrating recovery of organisms in presence of the preservative.
- Inactivation may be done by
  - Use of neutralizers
  - Dilution
- For all of you in Parenteral operations, think Bacteriostasis/Fungistasis

Validation

- **The neutralizer (inactivating agent) must have the following properties:**
  - **Not have inhibitory effects on the microorganisms**
  - **Should completely overcome the activity of the preservative**
  - **If it inactivates the preservative by combining with it, the resultant product must not be toxic to the microorganisms.**

Validation

- The following must be shown:

- Neutralizer Efficacy –The neutralizer effectiveness demonstrated

- Neutralizer Toxicity – The neutralizer is not, itself, toxic to the microorganisms.

- The challenge cfu should not be less than 70% of the viable count.

Sources of Variability

- The source of the microorganisms
  - ATCC
  - Various other culture collections
- Growth and harvesting of cultures
  - Liquid vs agar cultures
  - Composition of recovery buffers
  - Composition of neutralizers
- Plate counting rules, and training
- Mathematical transformations

Sources of Variability

- If you are contracting this work out, please make sure that your contract lab
  - has a real knowledge of how to perform this test
  - although it is only a short test in the compendia, it is not a simple test.
  - is well aware of the changes in the compendia
  - has all the proper controls in place
  - has documentation in control

AET as Part of Product Development

- Part of Pre-clinical Development
- Consideration of preservative must balance toxicity and regulatory considerations with effective preservation
- Use AET to define concentration where preservative is no longer effective.

AET as Part of Product Development

- As the development progresses, you will want to consider stability of your preservative system.
  - Recommend that you don't wait too long
- Consider doing "in-use" stability
  - Test (AET) at the end of the "shelf life" for an opened package
- Although the FDA only requires validation for Phase 1, it doesn't make sense not to do it all along.
  - Don't want to make decisions based on bad data ☹

Conclusions

- Formulation of oral solutions requires consideration of multiple factors
- Preservative selection needs to balance stability, solubility, pH range, AET requirements, safety.
- AET has multiple sources of variability, requires careful planning to design the experiments.
- AET test is critical part of development of oral solutions/suspension and pharmacopeia provide different requirements for the various formulation types.
- When contracting out, you need understand the experience and capabilities of the contract laboratory.

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References & Additional Information

Preservatives

Preservatives are substances added to dosage forms to protect them from microbiological growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process

- **But not a substitute for cGMP**

Some dosage forms that require preservatives include injectables, nasal, opthalmic, topical and oral products made with aqueous bases/vehicles

Preservatives are commonly used in food, cosmetic, and pharmaceutical industries to prevent microbial growth from contaminating finished products

- **Facial creams, deodorants, processed foods, drug products**

Microorganisms Classification

Microorganism	Class
S. aureus	Gram positive cocci
P. aerug	Gram negative rod
E. coli	Gram negative rod
C. albicans	Fungus (yeast)
Z. rouxii	Fungus (yeast)
A. niger	Fungus (mold)

Category 1

Injections, other parenterals including emulsions, otic, sterile nasal products made with aqueous bases or vehicles

Time Interval		Acceptance Criteria					
USP/JP	**EP**	**USP/JP**		**EP**			
7, 14 and 28 days	**6 and 24 hours**	**Bacteria**	**Fungus**	**Bacteria**		**Fungus**	
				Criteria A	**Criteria B**	**Criteria A**	**Criteria B**
	2, 7, 14, and 28 days	Not less than 1 log reduction from initial count at 14 days, not less than 3 log reduction from initial count at 14 days and no increase from 14 to 28 days	No increase from initial count at 7, 14 days and 28 days	2 log reduction at 6 hours, 3 log reduction at 24 hours, no recovery at 28 days	1 log reduction at 24 hours, 3 log reduction at 7 days, no increase on the 28 days	2 log reduction at 7 days and no increase at 28 days	1 log reduction at 14 days, no increase on the 28 days

Category 2

Topically used products made with aqueous bases or vehicles, non-sterile nasal products and emulsions, including those applied to mucous membranes

Time Interval		Acceptance Criteria					
USP/JP	EP	USP/JP		EP			
14 and 28 days	2, 7, 14, and 28 days	Bacteria	Fungus	Bacteria		Fungus	
				Criteria A	Criteria B	Criteria A	Criteria B
		Not less than 2 log reduction from initial count at 14 days and no increase from 14 to 28 days	No increase from initial count at 14 days and 28 days	2 log reduction from initial count at 2 days, 3 log reduction at 7 days with no increase at 28 days	3 log reduction at 14 days and no increase at 28 days	2 log reduction at 14 days and no increase at 28 days	1 log reduction at 14 days and no increase at 28 days

Category 3

Oral products other than antacids made with aqueous bases or vehicles

Time Interval	Acceptance Criteria			
USP/EP/JP	USP/JP		EP	
14 and 28 days	Bacteria	Fungus	Bacteria	Fungus
	Not less than 1 log reduction from initial count at 14 days and no increase from 14 days to 28 days	No increase from initial count at 14 days and 28 days	3 log reduction from initial count at 14 days with no increase at 28 days	1 log reduction from initial count at 14 days with no increase at 28 days

Category 4

Antacids made with an aqueous base

Time Interval	Acceptance Criteria			
USP/EP/JP	USP/JP		EP	
14 and 28 days	Bacteria	Fungus	Bacteria	Fungus
	No increase from the initial calculated count at 14 days and 28 days		N/A	

Taste Masking

Basic tastes found on tongue: Sweet, Salty, Sour, Bitter

Masking agents: Vanilla, Orange, Cherry, Bubble Gum, Berries, Mints

Taste masking techniques: Sweetening agents, viscosity modification, microencapsulation

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