Preservative Formulation and Effectiveness in Oral Solutions and Suspensions

Hang Guo
Drug Product Science & Technology Department

Chris Knutsen, PhD
Analytical & Bioanalytical Development Department

PDA Metro Meeting, Feb. 15, 2011
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
Sweeteners
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.

\[
\text{pH-pKa} = \log \frac{[\text{conjugate base}]}{[\text{acid}]}
\]

\[
\text{pH-pKa} = \log \frac{[\text{ionized}]}{[\text{unionized}]}
\]

\[
\text{pH-pKa} = \log \frac{[\text{ineffective P}]}{[\text{effective P}]}
\]
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
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

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

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
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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Duration</th>
<th>MP (% target)</th>
<th>PP (% target)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Initial</td>
<td>99.5</td>
<td>81.4</td>
</tr>
<tr>
<td>-20 C</td>
<td>2 wk</td>
<td>99.5</td>
<td>90.7</td>
</tr>
<tr>
<td>-20 C</td>
<td>4 wk</td>
<td>99.0</td>
<td>96.8</td>
</tr>
<tr>
<td>5 C</td>
<td>4 wk</td>
<td>99.0</td>
<td>95.4</td>
</tr>
<tr>
<td>5 C</td>
<td>13 wk</td>
<td>98.5</td>
<td>77.1</td>
</tr>
<tr>
<td>5 C</td>
<td>26 wk</td>
<td>98.5</td>
<td>96.4</td>
</tr>
</tbody>
</table>

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
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

<table>
<thead>
<tr>
<th>Organism</th>
<th>A</th>
<th>B</th>
<th>A</th>
<th>B</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. albicans</strong></td>
<td>5.7</td>
<td>5.7</td>
<td>3.7</td>
<td>2.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><strong>Z. rouxii</strong></td>
<td>5.7</td>
<td>5.7</td>
<td>3.6</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td><strong>A. niger</strong></td>
<td>5.5</td>
<td>5.6</td>
<td>2.8</td>
<td>&lt;1.0</td>
<td>2.2</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

**Log CFU/mL**

Quicker action against yeasts and mold.

A | Methylparaben (1.1 mg/mL)
B | Methylparaben (1.1 mg/mL) + 0.25 mg/mL ethylparaben
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 marcescens*, *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**

<table>
<thead>
<tr>
<th>Category</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Injections, other parenterals including emulsions, otic products, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles</td>
</tr>
<tr>
<td>2</td>
<td>Topically used products made with aqueous bases or vehicles, non-sterile nasal products and emulsions, including those applied to mucus membranes</td>
</tr>
<tr>
<td>3</td>
<td>Oral products other than antacids, made with aqueous bases or vehicles</td>
</tr>
<tr>
<td>4</td>
<td>Antacids made with an aqueous base</td>
</tr>
</tbody>
</table>
Basic Procedure

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

<table>
<thead>
<tr>
<th>Table Reference</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1.3.-1</td>
<td>Parenteral preparations, eye preparations, intrauterine preparations and intramammary preparations</td>
</tr>
<tr>
<td>5.1.3.-2</td>
<td>Ear preparations, nasal preparations, preparations for cutaneous application and preparations for inhalation</td>
</tr>
<tr>
<td>5..1.3.-3</td>
<td>Oral preparations, oromucosal preparations and rectal preparations</td>
</tr>
</tbody>
</table>
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.

10^6 CFU of Each of the challenge Organisms

Incubate Microbial Suspension

Sample at Day 7, 14, and 28
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±2.5°C 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. |
| Bacteria 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. |
| Bacteria 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. |
| Bacteria 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

<table>
<thead>
<tr>
<th></th>
<th>6 H</th>
<th>24 H</th>
<th>7 d</th>
<th>14 d</th>
<th>28 d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>NI</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>NI</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>NI</td>
</tr>
</tbody>
</table>

### Ear preparations, nasal preparations, preparations for cutaneous applications and preparations for inhalation

<table>
<thead>
<tr>
<th></th>
<th>2 d</th>
<th>7 d</th>
<th>14 d</th>
<th>28 d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>NI</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>NI</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>NI</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>NI</td>
</tr>
</tbody>
</table>

### Oral Preparations, oromucosal preparations and rectal preparations

<table>
<thead>
<tr>
<th></th>
<th>14 d</th>
<th>28 d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>NI</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>NI</td>
</tr>
</tbody>
</table>

NR = No Recovery  
NI = No Increase
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.
Acknowledgements

Divyakant Desai, Robert Garmise, Peter Timmins
Venkatramana Rao, Mark Bolgar, Karen Burke
Leticia Quinones
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, ophthalmic, 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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>Gram positive cocci</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Gram negative rod</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Gram negative rod</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>Fungus (yeast)</td>
</tr>
<tr>
<td><em>Z. rouxii</em></td>
<td>Fungus (yeast)</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>Fungus (mold)</td>
</tr>
</tbody>
</table>
Injections, other parenterals including emulsions, otic, sterile nasal products made with aqueous bases or vehicles

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP/JP 7, 14 and 28 days</td>
<td>EP 6 and 24 hours</td>
</tr>
<tr>
<td>USP/JP 2, 7, 14, and 28 days</td>
<td>EP 2 log reduction at 7 days</td>
</tr>
</tbody>
</table>

**Bacteria**
- 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 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

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>USP/JP</strong></td>
<td><strong>EP</strong></td>
</tr>
<tr>
<td><strong>USP/JP</strong></td>
<td><strong>EP</strong></td>
</tr>
<tr>
<td><strong>14 and 28 days</strong></td>
<td><strong>2, 7, 14, and 28 days</strong></td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td><strong>Fungus</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Fungus</strong></td>
</tr>
<tr>
<td>Not less than 2 log reduction from initial count at 14 days and no increase from 14 to 28 days</td>
<td>No increase from initial count at 14 days and 28 days</td>
</tr>
<tr>
<td>2 log reduction from initial count at 2 days, 3 log reduction at 7 days with no increase at 28 days</td>
<td>3 log reduction at 14 days and no increase at 28 days</td>
</tr>
<tr>
<td>2 log reduction at 14 days and no increase at 28 days</td>
<td>1 log reduction at 14 days and no increase at 28 days</td>
</tr>
</tbody>
</table>
Oral products other than antacids made with aqueous bases or vehicles

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 and 28 days</td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td>Fungus</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td>Fungus</td>
</tr>
<tr>
<td>Not less than 1 log reduction from initial count at 14 days and no increase from 14 days to 28 days</td>
<td>No increase from initial count at 14 days and 28 days</td>
</tr>
</tbody>
</table>
## Category 4

**Antacids made with an aqueous base**

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 and 28 days</td>
<td>Bacteria Fungus</td>
</tr>
<tr>
<td></td>
<td><strong>No increase from the initial calculated count at 14 days and 28 days</strong></td>
</tr>
</tbody>
</table>
Taste Masking

<table>
<thead>
<tr>
<th>Basic tastes found on tongue:</th>
<th>Sweet, Salty, Sour, Bitter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masking agents:</td>
<td>Vanilla, Orange, Cherry, Bubble Gum, Berries, Mints</td>
</tr>
<tr>
<td>Taste masking techniques:</td>
<td>Sweetening agents, viscosity modification, microencapsulation</td>
</tr>
</tbody>
</table>
Reference Articles

Sugar Preservative Interaction

Paraben Sorption
Reference Articles

Oral Liquid Formulations


Preservatives and Microbiology Testing Related

- Martinez, Microbial bioburden on oral solid dosage forms, Pharm. Tech., Feb. 2002, 58-70
- Sutton et al, Development of the antimicrobial effectiveness test as USP chapter <51>, PDA J. Pharm. Sci. and Tech., Vol.56, No.6, 2002, 300-311
- Charnock et al, Combining esters of para-hydroxy benzoic acid (parabens) to achieve increased antimicrobial activity, J. Clin Pharm. And Ther., 2007, 32, 567-572

Safety

- Soni et al, Safety assessment of esters of p-hydroxybenzoic acid (parabens), Food and Chemical Tox., 2005, 43, 985-1015