Short Communication

Thomas Carpentier, Eve Maillard, Mathilde Royer, Lina Mustapha and Frédéric Marçon*

Antimicrobial preservation efficacy of liquid glucose and liquid maltitol syrups with and without 0.1% sorbic acid

<https://doi.org/10.1515/ptph-2021-0007>

Received August 20, 2021; accepted December 16, 2021;

published online December 31, 2021

Keywords: drug compounding; drug contamination; preservatives; sorbic acid; sweetening agents.**Abstract**

Objectives: Amongst paediatric pharmaceutical forms, syrups offer advantages such as ease of administration and good palatability. They also exhibited microbial self-preservation properties that may be useful to enhance shelf life of liquid formulation. The objective of our works is to test the self-preservation efficacy of maltitol and glucose syrup without or with sorbic acid as described in the European pharmacopoeia.

Methods: The European Pharmacopoeia test of antimicrobial preservation efficacy was performed on liquid glucose syrup and liquid maltitol syrup with and without 0.1% sorbic acid.

Results: Unpreserved glucose and maltitol syrups did not meet the European Pharmacopoeia acceptance criteria for antimicrobial preservative efficacy due to the regrowth of *Aspergillus brasiliensis* on day 28 whereas glucose and maltitol syrups with 0.1% sorbic acid pass the test.

Conclusions: The addition of a preservative (sorbic acid) in glucose and maltitol syrups allows the validation of the antimicrobial preservative efficacy test of the European Pharmacopoeia. Further tests are needed to see if preservative efficacy is maintained despite dilutions or in the presence of active pharmaceutical ingredients.

Introduction

Among the paediatric oral forms, liquid forms offers advantages such as their ease of dosage and administration and their acceptability [1] however, the presence of water can reduce the physico-chemical and microbiological stability compared to dry forms (e.g. powder, tablets).

Preservatives can be used to improve the antimicrobial stability of liquid forms although care must be taken not to introduce excipients that are toxic to children. For instance, parabens (e.g. methylparabens, ethylparabens, propylparabens) are well-known preservatives that may be associated with hyperbilirubinemia, hypersensitivity reactions and oestrogenic effects in children, which justify daily dose limits [2]; benzoic acid may also be associated with kernicterus and hypersensitivity reactions in children [3]. Among preservatives, sorbic acid may be preferred for paediatric formulation due to its low toxicity [4]. It is generally recognised as safe, producing only irritant or allergic reactions when applied to the skin. And sorbic acid is used in foods and pharmaceuticals at concentrations ranging from 0.05 to 0.2% and its acceptable daily intake has been set at up to 25 mg/kg of bodyweight in adults [4].

It's also possible to improve the overall microbial stability of formulations by adjusting its pH, water activity, and the nutrients contents of formulations [5]. For instance high osmolarity (low water activity) of syrups allows for self-preservation and thus limits the addition of preservatives [6]. Indeed, syrups are liquid forms containing a high concentration of soluble carbohydrates. And, by increasing the viscosity of the medium and with their sweetening properties they may be useful to hide the bitterness of an active pharmaceutical ingredient (API) and therefore increase patient compliance [7]. Traditionally, the simple syrup based on sucrose is used, but low caloric and/or non-cariogenic polyols

***Corresponding author: Frédéric Marçon**, Centre Hospitalier Universitaire d'Amiens, 1 Rue du Professeur Christian Cabrol, 80054 Amiens, France; and Laboratoire AGIR – UR4294, Université de Picardie Jules Verne, Amiens, France, Phone: +33 3 22 08 71 40, E-mail: Marcon.frederic@chu-amiens.fr. <https://orcid.org/0000-0002-4071-6623>

Thomas Carpentier, Eve Maillard, Mathilde Royer and Lina Mustapha, Centre Hospitalier Universitaire d'Amiens, Amiens, France, E-mail: Carpentier.Thomas@chu-amiens.fr (T. Carpentier), eve.maillard@free.fr (E. Maillard), Royer.Mathilde@chu-amiens.fr (M. Royer), Mustapha.Lina@chu-amiens.fr (L. Mustapha)

syrups (e.g. sorbitol syrups, maltitol syrups, glucose syrups) may be preferred for paediatric formulations [8, 9].

As no data were found in the literature regarding self-preservation efficacy of liquid maltitol syrups (composed of maltitol, sorbitol and hydrogenated oligo- and polysaccharides) and liquid glucose syrups (composed of glucose and maltodextrins) regarding a pharmaceutical use we would like to study it with and without the addition of sorbic acid to see if they can be a good starting point for the formulation of paediatric drugs.

Materials and methods

Materials

Following syrups were studied, the maltitol syrup (Lycasin® 80/55 Maltitol Syrup, Roquette, France) and the glucose syrup (Glucose Syrup 4779, Roquette, France). Sorbic acid (Inresa, France) has been added in these syrups at its usual concentration of 0.1% (m/v) in the final product.

The Lycasin® 80/55 Maltitol Syrup is mainly composed of D-Maltitol [50–55]% with 80 referring to the percent (m/m) of dry substance and 55 to the dextrose equivalent of the syrup. Its pH ranges from 5.0 to 7.5. This syrup meets the liquid maltitol monograph of the pharmacopoeia (Ph. Eur. 07/2019:1236).

The Glucose Syrup 4779 is mainly composed of dextrose with 47 referring to the dextrose equivalent and 79 to the percent (m/m) of dry substance of the syrup. Its pH ranges from 4.0 to 6.0. This syrup meets the liquid glucose monograph of the pharmacopoeia (Ph. Eur. 01/2017:1330).

Efficacy of antimicrobial preservation

The European Pharmacopoeia test of antimicrobial preservation efficacy was performed [10].

The microorganism strains (BioBall®, Biomérieux SA, France) of *Staphylococcus aureus* (ATCC 6538; NCTC 10788; NCIMB 9518; CIP 4.83), *Pseudomonas aeruginosa* (ATCC 9027; NCIMB 8626; CIP 82.118), *Candida albicans* (ATCC 10231; NCPF 3179; IP 48. 72) and *Aspergillus brasiliensis* (ATCC 16404; IMI 149007; IP 1431.83) were used. They were revived or grown using casein soya beans digest agar (TSA3, Biomérieux SA, France) for bacteria and Sabouraud-dextrose agar (SDA-LI, Merck Life Science, France) for fungi incubated at 30 °C for 18–24 h for bacteria and at 20 °C for 48 h (for *C. albicans*) to seven days (for *A. brasiliensis*).

Colony visual aspect, and *C. albicans*, *P. aeruginosa*, *S. aureus* odour were checked by experienced technician and pharmacists.

Afterwards, 10⁸ CFU/mL suspensions of these different microorganisms were prepared in 0.9% sodium chloride using the MacFarland method [11]. *A. brasiliensis* suspension was obtained using 0.5 g/L of polysorbate 80 to avoid spore sedimentation as recommended by the European pharmacopoeia. Concentrations of these initial inoculum were controlled by plate sedimentation. Briefly, 1/10th serial dilutions of these suspensions were performed to produce 10³, 10² and 10¹ CFU/mL suspensions and 100 µL of these suspensions were plated on appropriate agar medium and incubated at the appropriate temperature depending on the germ as described above.

Syrups with and without sorbic acid were placed in 50 mL borosilicate glass bottles closed with GL32 caps. They were then inoculated with 200 µL of these 10⁸ CFU/mL suspensions to obtain a final concentration of 10⁶ CFU/mL in syrups. The dilutions produced by inoculation were considered negligible as volumes of inoculum did not exceed 1% of the volume of inoculated syrups (final volume 20 mL). The eight inoculated syrups produced were incubated at 20 °C (protected from light) for 28 days. On days 0, 14 and 28, 1/10th serial dilution were performed with 1 mL of each syrup to count viable germs as described for the control of initial inoculum. Syrups were gently shaken after inoculation and before each manipulation and plates with 30–300 UFC were selected to count viable germs.

All plating were duplicated. Syrup initial contamination or unwanted contamination of inoculated syrups was checked visually when counting germs on plates.

Results

All inoculum's concentrations were in accordance with expected results (~10⁸ CFU/mL) as well as all count at day 0 (~10⁶ CFU/mL). Both unpreserved syrups (glucose and maltitol) meet pharmacopoeia acceptance criteria on day 14 with a log reduction greater than three for bacteria and greater than one for fungi on day 14. However, they both failed to meet acceptance criteria on day 28 with a growth of *A. brasiliensis* being observed when compared to day 14.

When adding 0.1% sorbic acid, both preserved syrups finally meet the European Pharmacopoeia acceptance criteria on days 14 and 28 (Table 1).

No external or initial contamination was observed during this study with colonies having the same visual aspect and odour than initial strains.

Discussion

It has been previously described that with the efficacy of antimicrobial preservation test inoculum simple syrup was microbiologically stable for 14 days [6] which corresponds to the results we found with both maltitol and glucose unpreserved syrups. It may be therefore interesting to start compounding unlicensed paediatric drugs with these unpreserved syrups if you are looking for a 15-days stability without adding preservatives though the efficacy of antimicrobial preservation remains to be performed in the presence of the selected API.

The same study also found that simple syrup failed to meet European Pharmacopoeia acceptance criteria with *A. brasiliensis* at day 28 which also corresponds to the results we found with both maltitol and glucose unpreserved syrups. Indeed, *A. brasiliensis* is a xerophilic species, which can live in a water-poor environment. It can germinate in an

Table 1: Antimicrobial preservative efficacy test results for glucose and maltitol syrups with and without preservatives.

		Without sorbic acid						With 0.1% (v/m) sorbic acid					
		Day 0			Day 14			Day 0			Day 14		
		Count, UFC/mL	Count, UFC/mL	Reduction, log	Count, UFC/mL	Reduction, log	Count, UFC/mL	Count, UFC/mL	Count, UFC/mL	Reduction, log	Count, UFC/mL	Reduction, log	Count, UFC/mL
<i>Staphylococcus aureus</i>	Liquid glucose	Sample 1	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	> 6
		Sample 2	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	> 6
Liquid maltitol	Sample 1	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	1 × 10 ⁶	<1 × 10 ¹	<1 × 10 ¹	> 6	<1 × 10 ¹	> 6	> 6
	Sample 2	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	1 × 10 ⁶	<1 × 10 ¹	<1 × 10 ¹	> 6	<1 × 10 ¹	> 6	> 6
<i>Pseudomonas aeruginosa</i>	Liquid glucose	Sample 1	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	> 6
	Sample 2	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	1 × 10 ⁶	1 × 10 ¹	1 × 10 ¹	5	<1 × 10 ¹	> 6	> 6
Liquid maltitol	Sample 1	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	1 × 10 ⁶	<1 × 10 ¹	<1 × 10 ¹	> 6	<1 × 10 ¹	> 6	> 6
	Sample 2	1 × 10 ⁶	1 × 10 ¹	5	<1 × 10 ¹	> 6	1 × 10 ⁶	<1 × 10 ¹	<1 × 10 ¹	> 6	<1 × 10 ¹	> 6	> 6
Liquid glucose	Sample 1	1 × 10 ⁶	2 × 10 ²	3.7	2 × 10 ²	3.7	1 × 10 ⁶	3 × 10 ³	3 × 10 ³	2.5	1 × 10 ³	3	3
	Sample 2	1 × 10 ⁶	2 × 10 ²	3.7	2 × 10 ²	3.7	1 × 10 ⁶	3 × 10 ³	3 × 10 ³	2.5	1 × 10 ³	3	3
Liquid maltitol	Sample 1	1 × 10 ⁶	1 × 10 ²	4	1 × 10 ²	4	1 × 10 ⁶	1 × 10 ²	1 × 10 ²	4	8 × 10 ¹	4.1	4.1
	Sample 2	1 × 10 ⁶	1 × 10 ²	4	1 × 10 ²	4	1 × 10 ⁶	1 × 10 ²	1 × 10 ²	4	5 × 10 ¹	4.3	4.3
Liquid glucose	Sample 1	1 × 10 ⁶	1 × 10 ⁴	2	3 × 10 ⁵	0.5*	1 × 10 ⁶	1 × 10 ⁶	1 × 10 ⁶	2	1 × 10 ⁶	2	2
	Sample 2	1 × 10 ⁶	1 × 10 ⁴	2	3 × 10 ⁵	0.5*	1 × 10 ⁶	1 × 10 ⁶	1 × 10 ⁶	2	1 × 10 ⁶	2	2
Liquid maltitol	Sample 1	1 × 10 ⁶	1 × 10 ³	3	2 × 10 ⁴	1.7*	1 × 10 ⁶	1 × 10 ⁶	1 × 10 ³	3	1 × 10 ³	3	3
	Sample 2	1 × 10 ⁶	1 × 10 ³	3	2 × 10 ⁴	1.7*	1 × 10 ⁶	1 × 10 ³	1 × 10 ³	3	1 × 10 ³	3	3

*Indicate an increase in comparison to the previous count. UFC, unit forming colony.

environment with a very low water activity [12] which explains why osmotic pressure alone cannot inhibit the growth of *Aspergillus* in selected syrups. A study on the contamination of maple syrups [13] also found that *Aspergillus* species were common contaminants during packaging at the minimum recommended reheating temperatures, which illustrates the ability of *Aspergillus* to grow in unpreserved syrups at room temperature. When stored at 4 °C for 12 months, no growth was observed in maple syrups. While our studies did not explore the effect of temperature on the efficacy of antimicrobial preservation of unpreserved syrups, this should be done on final formulation to see if unpreserved syrups could meet acceptance criteria thus limiting the use of preservatives.

Preserved maltitol and glucose syrups meet acceptance criteria on day 28, with no regrowth of *A. brasiliensis* being observed. It is well known that sorbic acid is effective on fungi and on *A. brasiliensis* [14]. Therefore it seems interesting to use it rather than parabens if this species is incriminated in the failure of the antimicrobial preservation test as previously observed with simple syrups [6] or observed in our study, as it looks safer for compounding paediatric drugs.

To go further it would be interesting to study the impact of a dilution in water of syrups as dilution of simple syrup, even preserved with parabens, has previously been described as problematic [6, 15]. From an industrial point of view other strains should also be tested such as *Zygosaccharomyces rouxii* (recommended by European pharmacopoeia) and other osmophilic yeast such as *Saccharomyces cerevisiae* or *Saccharomyces mellis* [16].

Our data suggest both maltitol and glucose syrup could be used as a starting point to paediatric drug formulation notably for unlicensed drug compounding. They represent a non-cariogenic alternative to simple syrup or an alternative to commercial suspending agents. From a microbiological point of view, they are stable for at least 14 days at ambient temperature without the need to add a preservative although the impact of the selected API remains to be explored. Longer microbiological shelf life required the use of sorbic acid at a concentration of 0.1% to pass the European Pharmacopoeia test of antimicrobial preservation efficacy.

Acknowledgments: Roquette for the supply of syrup samples.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Not applicable.

Ethical approval: Not applicable.

References

1. Mistry P, Batchelor H. Evidence of acceptability of oral paediatric medicines: a review. *J Pharm Pharmacol* 2017;69: 361–76.
2. European Medicines Agency. Reflection paper on the use of methyl- and propylparaben as excipients in human medicinal products for oral use; 2015. https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-use-methyl-propylparaben-excipients-human-medicinal-products-oral-use_en.pdf [Cited 9 Aug 2021].
3. Nair B. Final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate. *Int J Toxicol* 2001; 20(3 Suppl):23–50.
4. Walker R. Toxicology of sorbic acid and sorbates. *Food Addit Contam* 1990;7:671–6.
5. Denyer SP, Baird RM. Guide to microbiological control in pharmaceuticals and medical devices, 2nd ed. Boca Raton Fla: CRC Press; 2006.
6. Santoveña-Estévez A, Suárez-González J, Vera M, González-Martín C, Soriano M, Fariña JB. Effectiveness of antimicrobial preservation of extemporaneous diluted simple syrup vehicles for pediatrics. *J Pediatr Pharmacol Therapeut* 2018; 23:405–9.
7. Walsh J, Cram A, Woertz K, Breitreutz J, Winzenburg G, Turner R, et al. Playing hide and seek with poorly tasting paediatric medicines: do not forget the excipients. *Adv Drug Deliv Rev* 2014; 73:14–33.
8. Al Humaid J. Sweetener content and cariogenic potential of pediatric oral medications: a literature. *Int J Health Sci* 2018;12: 75–82.
9. Grenby TH, Mistry M. Properties of maltodextrins and glucose syrups in experiments in vitro and in the diets of laboratory animals, relating to dental health. *Br J Nutr* 2000;84: 565–74.
10. Council of Europe, European Directorate for the Quality of Medicines & Health Care. Efficacy of antimicrobial preservation. In: Council of Europe, European Directorate for the Quality of Medicines & Health Care, editor. *European pharmacopoeia*. 10.6. Strasbourg: Council of Europe; 2021.
11. Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial susceptibility testing protocols. Boca Raton, FL: CRC Press; 2007.
12. Pitt JJ, Hocking AD. Fungi and food spoilage, 3rd ed. New York, NY: Springer US; 2009.
13. Whalen ML, Morselli MF. Fungi associated with pure maple syrup packed at the minimum recommended reheating temperature 1. *J Food Protect* 1984;47:688–9.
14. Plumridge A, Hesse SJA, Watson AJ, Lowe KC, Stratford M, Archer DB. The weak acid preservative sorbic acid inhibits conidial germination and mycelial growth of *Aspergillus niger* through intracellular acidification. *Appl Environ Microbiol* 2004;70: 3506–11.
15. Ghulam A, Keen K, Tuleu C, Wong IC-K, Long PF. Poor preservation efficacy vs. quality and safety of pediatric extemporaneous liquids. *Ann Pharmacother* 2007;41:857–60.
16. International Commission on Microbiological Specifications for Foods. Sugar, syrups and honey. In: International Commission on Microbiological Specifications for Foods, editor. *Micro-organisms in foods*. Boston, MA: Springer US; 1998:418–39 pp.