Effectiveness of Antimicrobial Preservation of Extemporaneous Diluted Simple Syrup Vehicles for Pediatrics

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OBJECTIVES Extemporaneous or magistral formulation of active pharmaceutical ingredients using traditional compounding techniques is a common practice when no commercial form is available for pediatrics. For this vulnerable group of patients, the formulation must be prepared with the minimum quantity and lowest proportion of excipients approved for pediatrics, avoiding the use of preservatives. Often the vehicles used for these preparations are dilutions of simple syrup with water. The objective of this study is to assess the effectiveness of antimicrobial preservation in simple syrup diluted with aqua conservans (conserved water), without propylene glycol or with a reduced proportion of parabens.

METHODS The European Pharmacopoeia test of efficacy of antimicrobial preservation was applied to 5 trial vehicles prepared with simple syrup diluted with water.

RESULTS Simple syrup is stable during 14 days. Vehicles prepared with simple syrup diluted with purified water did not meet the microbiological quality criteria, but when they are diluted with water that incorporates propylene glycol and parabens (aqua conservans), then they meet the criteria. In addition, if the water is prepared with parabens and without propylene glycol, the criteria for the dilution are met. Nevertheless, if the dilution is done with water prepared with an insufficient proportion of parabens to act as preservatives, the dilution does not meet the pharmacopoeia microbiological criteria.

CONCLUSIONS Dilution of simple syrup (50:50 v/v) to prepare a vehicle for extemporaneous or magistral preparation is microbiologically safe when water with methylparaben and propylparaben is used in a proportion of 0.08% and 0.02% (w/w), respectively, avoiding the use of propylene glycol as a solvent and thus its toxic effects in pediatrics.

ABBREVIATIONS API, active pharmaceutical ingredient; CM, growth of countless microorganisms; NI, no increase

KEYWORDS antimicrobial preservation; conserved water; parabens; propylene glycol; simple syrup

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Introduction

The lack or scarcity of a marketed active pharmaceutical ingredient (API) at pediatric doses is the main reason to formulate or at worst reformulate adult dosage forms of APIs. This practice gives rise to off-label or unlicensed use.¹ On formulating these APIs in liquid form for oral administration it is common practice to use syrup as vehicle. In general, there are 3 types of syrups: simple syrup containing only sucrose and purified water, flavoring syrup containing flavored substances, and medicinal syrups to which other therapeutic compounds have been added.² These syrups are used to enhance palatability or to increase viscosity of the formulation and also to create adequate osmotic pressure to inhibit microbial contamination (>60% w/w).³ At present the availability of marketed suspending vehicles such as Ora (Perrigo, Dublin, Ireland) products simplifies the

compounding of oral syrups. These products have a high cost and complex composition owing to their constituent suspending agents, preservatives, sweeteners, and buffers.⁴ This makes them non-ideal candidates for pediatric formulation, where the number and quantity of excipients in a formulation should be the minimum necessary to support product quality.⁵ Therefore, at least in pediatrics, it is necessary to use simple vehicles that can be prepared by using traditional compounding techniques.⁶

In pediatrics, simple syrup is often diluted with carriers such as water, or other excipients for different purposes, such as to reduce the amount of sucrose administered or adjust the viscosity of the final preparation.⁷ The influence of dilution on the efficacy of antimicrobial preservation is insufficiently studied in the literature and should be further studied, since it

Table 1. Composition of Each Vehicle Studied % (w/w)

No.	Vehicle	Sucrose (%)	Methylparaben (%)	Propylparaben (%)	Propylene Glycol (%)
1	Simple syrup	64	–	–	–
2	Simple syrup: purified water (50:50 v/v)	32	–	–	–
3	Simple syrup: aqua conservans (50:50 v/v)	32	0.04	0.01	0.45
4	Simple syrup: aqua conservans without propylene glycol (50:50 v/v)	32	0.04	0.01	–
5	Simple syrup: aqua conservans diluted without propylene glycol (50:50 v/v)	32	0.008	0.005	–

is a habitual practice in the preparation of non-sterile multidose formulations.⁸ In some countries the Pharmacopoeias or the National Formularies of Pharmaceutical Compounding include a monography of water used in compounding,^{9,10} named *aqua conservans*¹¹ (conserved water), that is prepared with a hydroxybenzoates (parabens) solution.^{12,13} The propylene glycol habitually used as drug solvent in this hydroxybenzoate solution is toxic at least for infants, since it can accumulate and cause lactic acidosis, central nervous system depression, coma, hypoglycemia, seizures, and hemolysis.¹⁴ As European Medicines Agency¹⁵ indicates however, owing to insufficient clinical evidence of comparable effects in humans, continued use of parabens as antimicrobial preservatives appears to be justified, particularly in the case of pediatric formulations. Nevertheless, the concentration should be at the lowest feasible level (0.015% and 0.01% for methylparaben and propylparaben, respectively).¹⁵ For these reasons, quantities added to the water must be just sufficient for the desired preservative effect, without being in excess.

This study assesses the effectiveness of antimicrobial conservation in vehicles prepared with diluted simple syrup. For this dilution, purified water and aqua conservans were used, and in the latter, the proportion of propylene glycol and parabens used as excipients was eliminated or reduced, respectively.

Materials and Methods

Vehicles. We studied 5 vehicles (Table 1). Vehicle 1 was simple syrup and the others were dilutions of it with other solutions in the proportion 50:50 (v/v). The simple syrup was prepared as a solution of 64% sucrose w/w in purified water, which is roughly equivalent to 85% w/v.¹⁶ A concentrated solution of hydroxybenzoates was prepared by mixing 8 g of methyl p-hydroxybenzoate (methylparaben) and 2 g of propyl p-hydroxybenzoate (propylparaben) with propylene glycol qs 100 g.¹² The aqua conservans was made up of 1 g of this solution in purified water qs 100 mL.¹¹ In vehicle 4, the hydroxybenzoates were diluted at the same concentration as in the aqua conservans, without the addition of propylene

glycol. Finally, vehicle 5 was prepared by using the lowest proportion of each hydroxybenzoate recommended for oral solutions and suspensions (0.015% methyl p-hydroxybenzoate and 0.01% propyl p-hydroxybenzoate),¹⁵ without the addition of propylene glycol.

All excipients were prepared from pharmacopoeia-grade raw materials, provided by Acofarma (Madrid, Spain). The culture media was prepared from commercial media (Scharlab, Barcelona, Spain).

Efficacy of Antimicrobial Preservation. In this study the European Pharmacopoeia test of efficacy of antimicrobial preservation¹⁷ was applied, which is stricter than the antimicrobial effectiveness test of United States Pharmacopoeia.¹⁸ This test must show that the formulation provides adequate protection against adverse effects due to contamination or microbial growth during storage and use. The test consists of deliberate contamination of the preparation in the final container with a prescribed inoculum of suitable microorganisms, conservation of the inoculated preparation at a set temperature, withdrawing samples from the container at specified time intervals, and counting microorganisms in the samples taken. The preservative properties of the preparation are adequate if a significant decrease or no increase in the number of microorganisms occurs in the inoculated preparation after the prescribed times and temperatures. The acceptance criteria vary depending on the type of preparation (parenteral, ophthalmic, intrauterine, intramammary, otic, nasal, cutaneous, inhaled, oral, or rectal) and the degree of protection required.

The microorganisms used were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus brasiliensis*, *Escherichia coli* (for oral administration vehicles), and *Zygosaccharomyces rouxii* (for oral administration vehicles with high sugar content), all of which were obtained from the Spanish Type Culture Collection in Valencia, Spain. The inoculum for each microorganism was prepared on the surface of soybean casein digest agar for bacteria, or Sabouraud-dextrose agar without the addition of antibiotics for fungi. Incubation is at 30°C to 35°C for 18 to 24 hours in the case of bacteria, at 20°C to 25°C for 48 hours

Table 2. Test Results for the 5 Vehicles Studied^{*,†‡}

Organism	Incubation time (days)	<i>S aureus</i>		<i>P aeruginosa</i>		<i>C albicans</i>		<i>A brasiliensis</i>		<i>E coli</i>		<i>Z rouxii</i>	
		14	28	14	28	14	28	14	28	14	28	14	28
Sample No.	1	6.0	NI	5.0	NI	4.0	NI	3.0	-1.0	5.0	NI	4.0	NI
	2	0.2	-0.3	-1.1	0.3	-1.3	0.2	-0.6	-0.6	0.7	CM	-2.1	CM
	3	6.0	NI	5.0	NI	2.0	2.0	2.0	NI	5.0	NI	4.0	NI
	4	6.0	NI	5.0	NI	4.0	NI	2.0	NI	5.0	NI	4.0	NI
	5	6.0	NI	6.0	NI	4.0	NI	1.0	1.0	-1.0	2.0	4.0	NI

CM, growth of countless microorganisms; NI, no increase in number of viable microorganism as compared to the previous reading

* Positive numbers: log reduction in the inoculated dose of microorganisms after different incubation times (14 and 28 days).

† Negative numbers: increase in microorganism count after initial time of incubation.

‡ To meet the European Pharmacopoeia criteria for oral preparations, the antimicrobial activity of the vehicles studied must result in a 3 log reduction (equal or more) in the inoculated dose of bacteria after 14 days and NI as compared to the previous reading at 28 days. For fungi, these criteria change to 1 log reduction (equal or more) at 14 days and NI as compared to the previous reading at 28 days.

with *C albicans* and *Z rouxii*, and at 20°C to 25 °C for 1 week or until good sporulation is achieved with *A brasiliensis*. A minimum subculturing was sometimes required. A sterile liquid suspension containing sodium chloride 9 g/L was used to collect bacterial, *C albicans*, and *Z rouxii* cultures. For *A brasiliensis* the sterile liquid suspension must also contain 0.5 g/L polysorbate 80. Enough liquid should be used to reduce the suspended microbial count to about 10⁸ organisms per milliliter. Subsequently, appropriate samples were removed from each suspension (0.1 mL from serial dilutions) and the number of colony-forming units per milliliter was determined in each suspension by plate count. This value was used to determine the inoculum and the reference values used in the assay. The suspensions should be used immediately.

The test then began with inoculation of the studied vehicles. Inoculate is done in the vehicles' final package with each of the test microorganisms in order to obtain an inoculum of 10⁵ to 10⁶ organisms per milliliter or per gram of preparation. The volume of the inoculum suspension did not exceed 1% of the volume of the product. It was thoroughly mixed to ensure homogeneous distribution. The inoculated product was maintained at 20°C to 25°C, and protected from light. At time zero and at suitable intervals, depending on the type of product (e.g., oral preparations at 14 and 28 days), a sample of each package (1 mL) is removed and the number of viable microorganisms determined by plate count. Results are the average of duplicate readings.

Results

To meet the European Pharmacopoeia criteria for oral preparations, the antimicrobial activity of a preservative must result in a 3 log reduction in the inoculated dose of bacteria after 14 days and no increase as compared to the previous reading at 28 days. For fungi, these criteria change to 1 log reduction at 14 days and no increase as compared to the previous reading at 28 days.

Table 2 shows the results of the assays. As can be seen, simple syrup (vehicle 1) was microbiologically stable during 15 days (a log reduction above 3 was detected at 14 days for every microorganism). *A brasiliensis* growth was detected at 28 days (a negative log reduction at 28 days). When this vehicle was diluted with purified water (vehicle 2), at each sampled time, the quality criteria were not met (log reduction was less than the desired one or an increase in growth was detected). Vehicle 3, simple syrup diluted with aqua conservans, met the quality criteria (log reduction was above 3 or 1 for bacteria or fungi, respectively, at 14 days and no increase at 28 days) owing to its containing propylene glycol and parabens in a final proportion of 0.04% and 0.01% w/w for methylparaben and propylparaben, respectively. Vehicle 4, aqua conservans without propylene glycol, met the microbiological quality criteria too. When vehicle 5 was assayed, in which aqua conservans was prepared without propylene glycol and lower proportions of parabens, bacterial growth (*E coli*) was detected at 14 days, and vehicle 5 did not meet quality criteria.

Discussion

Simple syrup (vehicle 1) was microbiologically stable during its declared validity period of 15 days¹⁶ even though fungal growth was later detected. The quality criteria for vehicle 2 were not met since it lacked preservatives, except sucrose itself in too low a proportion (32% w/w) to prevent microbial contamination (<60% w/w).³ The proportions of parabens in vehicle 3 (above or equal to the minimum proportion recommended, 0.015% and 0.01% for methylparaben and propylparaben, respectively¹⁵) prevent the growth of the inoculated microorganism. With the aim of eliminating the propylene glycol and reducing the parabens' proportions to the minimum, vehicles 4 and 5 were assayed. Vehicle 4, in which aqua conservans is prepared without propylene glycol to prevent its toxic action,

met the microbiological quality criteria. Therefore, the elimination of propylene glycol is possible when the aqua conservans is prepared with parabens above the lowest feasible level, in the final proportion (equal to vehicle 3). On the other hand, when vehicle 5 was assayed, prepared with aqua conservans without propylene glycol and the lower quantity of parabens recommended as preservatives for oral liquid formulations, bacterial growth was detected at 14 days. This minimum quantity of parabens in vehicle 5 is evidently insufficient to protect it from microbial contamination; indeed the final proportions (0.008% and 0.005% for methylparaben and propylparaben, respectively) in the diluted syrup were less than those recommended.

For all the above, when APIs are formulated with simple syrup diluted 50:50 v/v with aqua conservans (vehicle 3) or with aqua conservans without propylene glycol as solvent (vehicle 4), these vehicles are able to inhibit microbial growth with and without the use of propylene glycol, respectively, which is not recommended for pediatrics owing to its toxic effects. Thus, less toxic formulations can be used to administer APIs to this vulnerable group. But, if it is used, simple syrup diluted 50:50 v/v with purified water (vehicle 2) or dilute aqua conservans prepared with the lowest feasible proportion of hydroxybenzoates recommended to exert a preservative effect without propylene glycol (vehicle 5) is not able to inhibit growth if these vehicles become contaminated by microorganisms before or during API administration.

Taking into account that this study is made as the Pharmacopoeia test indicates, and the vehicles are tested without the API incorporation and are incubated not during the real administration of the doses, the microorganism contamination can be greater if adequate hygienic measures are not considered.

In conclusion, when diluted simple syrup is necessary to use in the formulation of an API in pediatrics, it is possible to use water with parabens at adequate proportions without being in excess to assure the effectiveness of its antimicrobial preservation and without propylene glycol used as solvent. Thus, if during the oral administration of the formulation, it is contaminated, the preservatives will be able to inhibit their growth.

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REFERENCES

1. Standing JF, Tuleu C. Paediatric formulations getting to the heart of the problem. *Int J Pharm*. 2005;300(1–2):56–66.
2. UNC Eshelman School of Pharmacy. Syrups. In: *The Pharmaceutics and Compounding Laboratory*. 2018.
3. Rowe RC, Sheskey PJ, Quinn ME. *Handbook of Pharmaceutical Excipients*. 6th ed. London, England: PhP; 2009.
4. Musko M, Sznitowska. Use of compounded dispersing media for extemporaneous pediatric syrups with candesartan cilexetil and valsartan. *Acta Pharm*. 2014;64(4):463–474.
5. Essential Medicines and Health Products Information Portal. Development of Paediatric Medicines: Points to Consider in Formulation. *WHO Technical Report Series*, No. 970, Annex 5; 2012. <http://apps.who.int/medicinedocs/en/d/Js19833en/>. Accessed March 19, 2018.
6. Brion F, Nunn AJ, Rieutord A. Extemporaneous (magistral) preparation of oral medicines for children in European hospitals. *Acta Paediatr*. 2003;92(4):486–490.
7. Ghulam A, Keen K, Tuleu C, et al. Poor preservation efficacy versus quality and safety of pediatric extemporaneous liquids. *Ann Pharmacother*. 2007;41(5):857–860.
8. Cabañas MJ, Cañete C, González SX, et al. Microbiological quality of pediatric oral liquid formulations. *Farm Hosp*. 2016;40(1): 427–435.
9. *Czech Pharmacopoeia*. Prague, Czech Republic: Grada; 2009.
10. *Formulario Nacional*. Madrid, España; 2014.
11. Agua conservante. *Acofarma*. <http://www.acofarma.com/es/formulacion-magistral/faq-s-tecnicas/142-como-se-prepara-el-agua-conservans>. Accessed March 19, 2018.
12. Solución concentrada de hidroxibenzoatos. *Acofarma*. <http://www.formulacionmagistral.org/blog/elaboracion/solucion-conservante-de-hidroxibenzoatos/>. Accessed March 19, 2018.
13. Formulation in Pharmacy Practice. Compound hydroxybenzoate solution. *Australian Pharmaceutical Formulary and Handbook*. 16th ed. <http://www.fshealth.gov.za/sites/DWoods/Backpages/Preservatives.HTML>. Accessed March 19, 2018.

14. Lim TY, Poole RL, Pageler NM. Propylene glycol toxicity in children. *J Pediatr Pharmacol Ther.* 2014;19(4):277–282.
15. European Medicines Agency. Reflection paper on the use of methyl- and propylparaben as excipients in human medicinal products for oral use. October 22, 2015. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/11/WC500196733.pdf. Accessed March 19, 2018.
16. Syrup. In: *The United States Pharmacopeial Convention*, ed. USP 30 NF 25. Baltimore, MD: 2006;1233–1234.
17. Efficacy of antimicrobial preservation. In: *European Pharmacopoeia*, ed. 8th ed. 5.1.3. 01/2011:50103
18. Antimicrobial effectiveness testing <51>. US Pharmacopeia. USP 29 NF 24. http://www.pharmacopeia.cn/v29240/usp29nf24s0_c51.html. Accessed March 19, 2018.