In-use microbiological assessment of caffeine citrate 10 mg/mL oral solution

Julian Smith,¹ Chris Marks²

¹Lecturer in Pharmaceutics, University of South Wales, School of Applied Sciences, Pontypridd, CF37 4AT, UK
²Pharmacy Regional Quality Control Laboratory, Southmead Hospital, Bristol, UK

Correspondence to

Dr Julian Smith, JCS Pharma Consulting Ltd, Newport, Gwent NP18 2AB, UK; juliancsmith@outlook.com

Received 27 April 2017

Revised 3 October 2017

Accepted 10 October 2017

Published Online First

9 November 2017

EAHP statement 4: clinical pharmacy services.

ABSTRACT

Objectives This study was conducted to investigate the microbial contamination of caffeine citrate 10 mg/mL oral solution (CCOS) during a simulated in-use test in a clinical environment.

Methods A real-time in-use simulation study was conducted in a neonatal intensive care unit at a UK National Health Service hospital. Following the simulation, samples of the product were taken and assessed for microbiological contamination.

Results This study shows that CCOS does not comply with the European Pharmacopoeia (Ph Eur) Specification for Preservative Efficacy. However, it shows that the in-use contamination of the product in a clinical environment remained within the Ph Eur General Text (5.1.4) Specification for the Microbiological Quality of Non-Sterile Pharmaceutical Preparations.

Discussion There is a requirement for medicines to be developed and formulated specifically for paediatric use. This requires that excipients should be kept to a minimum. CCOS has been specifically developed to treat apnoea of prematurity in neonates. This product does not contain antimicrobial preservatives. It is produced as a terminally sterilised solution to enable an appropriate shelf-life. CCOS is currently marketed as a unit dose product, and once opened has an immediate-use, single-patient requirement. This gives CCOS an expensive unit cost. A suitable in-use shelf-life would reduce unit dose costs.

Conclusions The evidence from this study would suggest that CCOS, a product specifically formulated for use in neonates without antimicrobial preservatives, can safely be assigned a 7-day room temperature in-use shelf-life.

INTRODUCTION

The formulation of pharmaceutical dosage forms for young children, especially neonates, is a challenging process. It is essential that children's formulations contain as few harmful excipients as possible.¹ There is concern with paediatric oral solutions containing specific excipients such as antimicrobial preservatives and cosolvents.² One approach to reducing the number of excipients in oral solutions is to prepare them free from antimicrobial preservatives and sanitise or sterilise the solution during production. This action can reduce the product's in-use shelf-life by increasing the risk of microbiological contamination. The marketing of oral solutions as single-dose preparations will increase the overall cost. The potential microbial contamination of this type of preparation needs to be risk-assessed in a clinical situation to determine the effect this may have on the patient population.

This study was undertaken to investigate the consequences of storing preservative-free caffeine citrate 10 mg/mL oral solution (CCOS) after opening in a clinical ward environment on the microbial contamination of the solution.

METHODS AND MATERIALS

Materials

CCOS 5 mL bottles BN0042818 Exp 3/2018 were provided by Viridian Pharma and Martindale Pharma. Sterile 0.1% peptone water and all agar plates were purchased from Oxoid. Biosart 100 Monitor (0.45 µm pore size) was purchased from Sartorius. BioBall MultiShot 550 standard micro-organisms were purchased from bioMérieux.

CCOS is presented in a 6 mL clear type 1 glass screw cap phial. The closure system consists of a butyl rubber insert and a polybutylene terephthalate (Pocan) screw cap. Each bottle contains 5 mL of product. The closure is designed to enable sterilisation by autoclaving and is marketed as a single-use container. However, the rubber insert and screw cap can be replaced in their original condition after first opening. The list of excipients includes water for injections, sodium chloride and citric acid.

Preservative efficacy test

A test for efficacy of antimicrobial preservative in pharmaceutical products was carried out at International Laboratory Services, Derbyshire. The test was carried out according to the method in the European Pharmacopoeia (Ph Eur) General Text 5.1.3 'Efficacy of Antimicrobial Preservation'.³

Preparation of inocula

Tryptone soya agar (TSA) plates were inoculated from stock cultures of the following bacteria: *Pseudomonas aeruginosa* (National Collection of Industrial Food and Marine Bacteria (NCIMB) 8626), *Staphylococcus aureus* (National Collection of Type Cultures 10788) and *Escherichia coli* (NCIMB 8545). These cultures were incubated at 30°C–35°C for 18–24 hours. A sabouraud dextrose (SDX) agar plate was inoculated from the stock culture of *Candida albicans* (National Collection of Pathogenic Fungi 3179) and incubated at 20°C–25°C for 48 hours. An SDX agar plate was inoculated from the stock culture of *Aspergillus brasiliensis* (Institute for Microbiology and Immunology 149007) and incubated at 20°C–25°C for 7 days. The bacteria and yeast were harvested using 0.1% peptone water containing 0.9% sodium chloride to wash the surface growth from each plate into separate sterile universal bottles. The resultant suspensions

To cite: Smith J, Marks C. *Eur J Hosp Pharm* 2018;**25**:e130–e133.

were further diluted with the same liquid to reduce the count to approximately 1×10^8 colony-forming units (cfu)/mL. *A. brasiliensis* was harvested in an analogous manner with 0.9% saline containing 0.05% polysorbate 80. The suspensions were used immediately.

Inoculation of product

Aliquots of each microbial suspension were introduced into separate containers of the product to achieve a final concentration of 10^5 – 10^6 cfu/mL. The same volume of inoculum was simultaneously introduced into separate equivalent volumes of 0.1% peptone water containing 0.9% sodium chloride (bacteria and yeast) and saline/polysorbate 80 (mould) to be used as controls. The inoculated product was stored in the dark at 20°C–25°C.

Recovery of micro-organisms

Of the inoculated product 1 mL aliquots were removed at time 0, 14 and 28 days. Each was added to 9 mL of 0.1% peptone water containing the following as preservative inactivating agents: polysorbate 80 1.0%, lecithin 0.5%, Triton X-100 1.0% and sodium thiosulfate 1.0%. The control preparations were similarly sampled at time 0 to determine the viable counts of the cultures used and to confirm the suitability of the media used for their growth. Further dilutions were made as necessary in 0.1% peptone water containing 0.9% sodium chloride (bacteria and yeast) and saline/polysorbate 80 (mould). Of all dilutions 1 mL aliquots were incorporated in duplicate pour plates of the appropriate cooled molten agar. The pour plates were incubated at 30°C–35°C for 3 days for the bacteria and at 20°C–25°C for 5 days for yeast and mould. After incubation, the numbers of colonies on each plate were counted, and taking the dilution factor into account the number of cfu/mL of product was calculated.

Validation of recovery count

The suspensions of the test organisms were further diluted with 0.1% peptone water containing 0.9% sodium chloride (bacteria and yeast) and saline/polysorbate 80 (mould) to approximately 10^3 cfu/mL. Four petri dishes were used for each organism and 0.1 mL of the relevant suspension added to each plate. To the first set of plates 1 mL of product diluted 10-fold in recovery medium was added, to the second set 1 mL of product diluted 100-fold was added, to the third set 1 mL of product diluted 1000-fold was added, and the fourth set acted as a control having no product in them. The appropriate cooled molten agar was then added to the plates, which were incubated as described in the recovery of organisms. The plates were then examined for growth and the number of colonies present recorded on the raw data sheet.

In-use shelf-life assessment

Product storage and removal of doses

The venue for this study was the Neonatal Intensive Care Unit, Southmead Hospital, Bristol, UK. The management of samples and the taking of doses were undertaken by the pharmacy laboratory staff. The doses taken from containers being used in this study were discarded and were not given to patients. All containers were stored in the ward medicines cupboard for the duration of the study, but were physically segregated from normal ward stock so that samples being used in this study could not be used to treat patients. Doses were taken using nursing procedures and techniques in the clinical areas where medicines are normally dispensed.

An oral/enteral syringe was used to remove a 1 mL dose from each of the 10 bottles. The rubber insert and cap were replaced after opening. Three days and 5 days after opening, further 1 mL doses were removed from each bottle using a similar procedure. In total three 1 mL doses were dispensed from each container. Seven days after opening, the bottles were removed from the ward medicines cupboard and returned to the laboratory for microbiological assessment. The following week this was repeated with a second set of 10 bottles. A total of 20 bottles were sampled in the simulated in-use test over a period of 2 weeks.

The doses taken from the bottles at days 1, 3 and 5 were discarded. A further 1 mL sample was taken from the solution remaining in the bottles at day 7, and this was assessed for microbiological contamination as described below.

In-use microbiological assessment

The microbiological assessment was undertaken at the Pharmacy Regional Quality Control Laboratory, Southmead Hospital, Bristol. This was performed in accordance with the Ph Eur General Text 5.1.4 and Method 2.6.12 and 2.6.13.⁴ All manipulations were carried out by trained staff in an unclassified room within a good manufacturing practice grade A working environment. Aseptic technique was used throughout the procedure. A negative control was performed during each working session using sterile 0.1% peptone water in place of the sample under test. For each sample, 1 mL was taken, diluted in 100 mL of sterile 0.1% peptone water and then filtered. The samples were filtered through a 0.45 µm pore size Sartorius Biosart 100 Monitor. Each sample was rinsed with a further 100 mL of sterile 0.1% peptone water. The density or concentration of micro-organisms was estimated by serial dilution of the concentrated solution to a suitable limit to achieve a total viable count (TVC) of between 10 and 100 cfu. For each bottle one sample was enumerated on TSA and others on SDX agar. TSA was incubated at 30°C–35°C and the SDX at 20°C–25°C; both media were incubated in accordance with Ph Eur Method 2.6.13.

Validation of in-use microbiological assessment

The microbiological assessment was validated by treating three test solutions to a similar filtration and enumeration process as the samples. The three test solutions tested were 'test solution', containing 10 µL BioBall solution (containing between 10 and 100 cfu) added to 100 mL sample solution (sample solution contains 1 mL of the CCOS aseptically added to 100 mL of 0.1% sterile peptone water); positive control, containing 10 µL BioBall solutions added to 100 mL of 0.1% sterile peptone water; and negative control, containing 100 mL 0.1% sterile peptone water. Each solution was filtered as described in the microbiological assessment method. For the test solution, an additional 100 mL of 0.1% sterile peptone water was passed through the filter as a rinse solution. The micro-organisms used were *A. brasiliensis* (NCTC 2275), *Bacillus subtilis* (NCTC10400), *C. albicans* (NCPF 3179), *S. aureus* (NCTC 10788) and *P. aeruginosa* (NCTC 12924) (which are the five British Pharmacopoeia micro-organisms), and *E. coli* (NCTC 12241) (which was included as *E. coli* has specific limits for oral solutions). All types of micro-organisms were plated on TSA, and *A. brasiliensis* and *C. albicans* were also plated on SDX agar. All TSA plates were incubated at 30°C–35°C and SDX plates were incubated at 20°C–25°C. All plates were incubated in accordance with Ph Eur Method 2.6.13.

Table 1 Preservative efficacy test recovery counts from CCOS at time 0

Organism	Mean cfu/mL sample after		
	0 hour	14 Days	28 Days
<i>Candida albicans</i>	8.2×10^5	3.7×10^3	8.2×10^3
<i>Aspergillus brasiliensis</i>	5.4×10^5	4.3×10^3	5.4×10^4
<i>Pseudomonas aeruginosa</i>	3.6×10^5	<50*	<50*
<i>Staphylococcus aureus</i>	6.1×10^5	<5	<5
<i>Escherichia coli</i>	2.2×10^5	<5	<5

*Inhibition was noted on validation plates for *P. aeruginosa* at the 10^{-1} dilution. Counts of less than 50 cfu/mL are therefore valid for this organism. No inhibition was noted on validation plates for the other organisms. Counts of less than 5 cfu/mL are therefore valid for these organisms.

CCOS, caffeine citrate 10 mg/mL oral solution; cfu, colony-forming unit.

RESULTS

Preservative efficacy test

The number of organisms recovered from the control solutions of 0.1% peptone water at time 0 showed that the test product CCOS does not inhibit the recovery of organisms and the test was valid. Table 1 shows the number of cfu/mL recovered from the CCOS test product and the reduction of micro-organisms relative to time.

In-use shelf-life assessment

Twenty bottles of CCOS were exposed to the in-use simulation in a clinical ward environment. From these 20 bottles, micro-organisms were recovered from only one bottle. Only 2 cfu of Gram-negative oxidase-positive rods was recovered from this bottle onto the TSA media. No cfu was recovered onto the SDX media.

Table 2 shows the numbers of cfu recovered from the validation test solutions. The micro-organism column details the test organisms used. The test solution cfu column records the number of organisms recovered from the inoculated test solution, and the positive control the number of organisms recovered from 0.1% sterile peptone water. The validation parameters for the microbiology assessment were that the organisms recovered from the test solution were within the range of half and twice the number of organisms recovered from the positive control. The negative control and environmental results also had to be within specification. Using these parameters, the results from the microbiological assessment were valid.

DISCUSSION

The formulation of pharmaceutical dosage forms in young children, especially neonates, is a challenging process. It is essential that children's formulations should contain as few harmful excipients as possible.¹ There is concern with excipients such as antimicrobial preservatives and solvents.²

The Ph Eur Preservative Efficacy Test provides information on the capability of a pharmaceutical product to limit microbial contamination and proliferation. If an oral solution is being developed and does not have adequate antimicrobial activity, antimicrobial preservatives can be added to achieve this specification. Antimicrobial preservatives are normally added to prevent microbial proliferation arising under in-use conditions, but for paediatric formulations this may not be desirable. The use of these substances should be avoided in general and especially when considering formulations for the paediatric population.⁵

A method of ensuring that preservative-free aqueous oral preparations are not contaminated with micro-organisms during unopened storage is to sanitise or sterilise it in the original container. This approach raises a question about what in-use shelf-life should be recommended. If the advice for these solutions is that they can only be used as unit dose (single-use, single-patient) containers, then the cost may become the limiting factor. If preservative-free oral liquids are to be assigned an in-use shelf-life, then risk factors must be considered and systems put in place, such as suitable containers, product handling and appropriate ingredients, to reduce these risks. A suitable validated in-use shelf-life should be assigned to ensure the expected quality of the product is maintained throughout its period of use.⁶ It would be essential to minimise risk of cross-contamination between patients using suitable techniques such as restricting a single bottle to an individual patient or to use the entire bottle on multiple patients at one dosing interval using unique dosing syringes.

CCOS does not contain antimicrobial preservatives. However, the product pH range and specification are not conducive to proliferation of micro-organisms. The PET was undertaken to determine if CCOS inherently complies to the Specification for Preservative Efficacy without the addition of antimicrobial preservatives. The results in table 1 show that CCOS conforms to the Ph Eur specifications for preservative efficacy for *P. aeruginosa*, *S. aureus* and *E. coli*, but does not comply for the yeast, *C. albicans*, or fungi, *A. brasiliensis*. However, the results show that CCOS is bacteriostatic for these organisms over a 28-day period and the one log reduction specification is achieved at the 14-day

Table 2 Results from validation of microbiological assessment

Micro-organism	Agar type	Test solution cfu	Positive control cfu (c)	Lower limit (c÷2)	Higher limit (c×2)	Pass/Fail*
<i>Aspergillus brasiliensis</i>	TSA	37	23	12	46	Pass
<i>A. brasiliensis</i>	SDX	50	47	24	94	Pass
<i>Escherichia coli</i>	TSA	34	24	12	48	Pass
<i>Bacillus subtilis</i>	TSA	50	37	19	74	Pass
<i>Candida albicans</i>	TSA	38	32	16	64	Pass
<i>C. albicans</i>	SDX	42	39	20	78	Pass
<i>Staphylococcus aureus</i>	TSA	38	53	27	106	Pass
<i>Pseudomonas aeruginosa</i>	TSA	41	36	18	72	Pass

*Validation passes if the number of colonies from the test solution does not differ by a factor greater than 2 from the number of colonies from the positive control solution.

Notes: Negative controls and environmental monitoring comply.

The system has been shown to be suitable when (1) between 10 and 100 cfu of each type of micro-organism are present in the positive controls; (2) there is no interfering growth in the sample; (3) there is no growth on the negative control; and (4) there is no growth on the settle plate.

cfu, colony-forming units; TSA, tryptone soya agar; SDX, sabouraud dextrose agar.

time interval. This means that the product may be susceptible to microbiological proliferation should the product be contaminated in-use. However, although CCOS does not comply to the Ph Eur specification at 28 days, micro-organisms do not actively proliferate in CCOS and the risk of microbiological contamination over shorter periods will be small. These PET results show that CCOS may be suitable for a short, less than 14 days, in-use shelf-life based on the Ph Eur specifications for microbiological quality for an oral solution.

CCOS remained within the Ph Eur specifications for microbiological quality for an oral solution throughout the period of this in-use assessment. The in-use test was undertaken in a clinical area and would represent the exact environment CCOS would be exposed to in a real in-use situation. Microbiological contamination was only recovered from 1 of the 20 containers subject to the in-use challenge. This container only demonstrated a total aerobic microbial count of 2 cfu/mL. This is well within the Ph Eur specification of 200 cfu/mL. The results in [table 2](#) demonstrate that this testing method is valid for the TVC of CCOS.

The in-use study has shown that CCOS easily maintains the Ph Eur specifications for microbiological quality for an oral solution throughout the 7 days of this in-use assessment. The PET results demonstrate that micro-organisms do not proliferate in this product for a period of 14 days. Therefore, the results presented here demonstrate that microbiological CCOS can be safely assigned an in-use shelf-life of 7 days at room temperature. The microbiological quality of this product is maintained within the specifications required of a standard oral solution during the in-use period.

The strength of this study is that it simulated a real in-use situation. Limitations include difficulties in controlling the environment, the staff taking doses were pharmacy staff rather than nursing staff, the relatively small number of containers sampled and the limitation of only using one clinical area. Pharmacy staff were used to take doses so that the study did not encroach on nursing staff time and as the product was being used in circumstances not described in the Summary of Product Characteristics to ensure patients did not receive doses of CCOS being used in this study. The pharmacy staff used normal nursing procedures to remove doses and the impact this had on the study was considered minimal. However, there is an expectation that pharmacy staff who have been trained in aseptic technique are going to be less likely than nursing staff to contaminate oral solutions. The study was run at room temperature to provide a worst-case scenario for proliferation of micro-organisms. This risk of proliferation could be further reduced by storing CCOS in a ward refrigerator. This study did not investigate the potential of chemical product cross-contamination; however, using good nursing techniques, this should be minimal and no greater than the risk to any other oral solution.

This study demonstrates that the risk to the neonatal population of using CCOS with an in-use shelf-life of 7 days is minimal. Risk factors to consider when assessing in-use shelf-life of products such as this must include the immune status of the patients, the aseptic technique of the staff handling the product, the number of doses to be removed from the bottle, the equipment used to remove doses and the cleanliness of the environment where doses are being prepared. All these factors can increase

the likelihood of microbiological contamination of the product, especially by bacteria which can grow or form spores in an environment with an absence of nutrients, such as *Pseudomonas* or *Bacillus* species. Benefits of CCOS to be considered is that the product has a small 5 mL volume limiting the number of doses that can be taken, the diameter of the neck is small at 20 mm, the product does not actively support microbiological proliferation and the insert provides a good seal that is easily replaced. This result would benefit from further studies in a controlled patient situation, using nursing staff to withdraw doses in multiple clinical situations.

CONCLUSIONS

The data provided demonstrate that a sterile solution of CCOS (Viridian Pharma brand) once opened can be assigned an in-use shelf-life of 7 days at room temperature. This recommendation falls within the 2 weeks of in-use shelf-life for unpreserved oral solutions recommended by national guidelines.⁷

What this paper adds

What is already known on this subject

- Specifications for in-use testing of oral solutions.
- Risks of in-use microbiological contamination of unpreserved oral solutions.
- Risks of using oral solutions containing antimicrobial preservatives in neonates.

What this study adds

- An assessment of the level of microbiological contamination that does occur when unpreserved oral solutions are used in an actual clinical situation.
- Proposal of an in-use shelf-life for preservative-free oral solution.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

© European Association of Hospital Pharmacists (unless otherwise stated in the text of the article) 2018. All rights reserved. No commercial use is permitted unless otherwise expressly granted.

REFERENCES

- 1 van Riet-Nales DA, Schobben AF, Vromans H, *et al*. Safe and effective pharmacotherapy in infants and preschool children: importance of formulation aspects. *Arch Dis Child* 2016;101:662–9.
- 2 Graham S, Turner M. European study of neonatal exposure to excipients (ESNEE). *Infant* 2011;7:196–9.
- 3 Council of Europe. European Pharmacopoeia: General Text 5.1.3. *Efficacy of antimicrobial preservation*. 8th edn. Strasbourg, 2016.
- 4 Council of Europe. European Pharmacopoeia: General Text 5.1.4. *Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use*. 8th Ed. Strasbourg, 2016.
- 5 CHMP. The guideline on excipients in the dossier for application for marketing authorisation of a medicinal product. (EMA/CHMP/QWP/ 396951/2006) http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003382.pdf (accessed 19 Nov 2016).
- 6 British Pharmacopoeia Commission. Appendix SC V B. Preservative-free Unlicensed Medicines. *British pharmacopoeia 2017*. V. London: TSO: British Pharmacopoeia Commission, 2017.
- 7 Touw D, Vigneron J. Chapter 22 Stability in Practical Pharmaceutics. In: Bouwman-Boer Y, Fenton-May V, Le Brun P, eds. *An international guideline for the preparation, care and use of medicinal products*: Springer, 2015:p457.