Microbiological quality control of non-sterile compounded medicines prepared in a Portuguese hospital centre

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ABSTRACT

Objectives This work aimed to evaluate the quality of non-sterile formulations compounded at Centro Hospitalar Cova da Beira (Covilhã, Portugal) immediately after preparation and up to the defined 'beyond-use date'.

Methods Microbiological quality control tests were performed in accordance with monograph 5.1.4 of the European Pharmacopoeia 8.0. Samples of compounded products were collected from January to December 2014 after preparation and were analysed immediately and reanalysed after storage under the established conditions, for each preparation.

Results In the test period, 392 preparations were analysed, corresponding to 24 different formulations (8 intermediate preparations, 11 oral solutions/suspensions and 5 topical preparations). All preparations were in accordance with the pharmacopoeia specifications immediately after preparation. However, for the formulations 'prednisolone oral solution (5 mg/mL)' and 'nitroglycerine and cinchocaine ointment (0.25%/0.5%)', the microbial counts of some batches exceeded the defined limits after storage up to the beyond-use date.

Conclusions These results show that the compounding practices implemented at this pharmacy department are able to ensure the microbiological quality of compounded products. This microbiological quality control methodology also allowed identification of the need to replace formulations shown not to be stable throughout the storage period. On the basis of these results, a monthly routine of microbiological control of a random sample of compounded medicines was established in order to ensure their quality and safety for use.

INTRODUCTION

Pharmaceutical compounding often represents the solution for therapeutic personalisation for particular populations (such as paediatric and geriatric patients) and specific therapeutic needs when commercial alternatives are not available.¹⁻⁴ These medicines are compounded in community and hospital pharmacies and are classified as 'officinal preparations' if their formula is inscribed in a formulary or pharmacopoeia, and 'magistral formulas' if the formula is detailed in a medical prescription.⁵ Compounded medicines are ruled by specific legislation that does not require Good Manufacturing Practices standards, which apply to industrialised medicines.⁵ However, standards for the production of compounded medicines, based

on recommendations from pharmacopoeias (such as the USA Pharmacopoeia (USP) or the European Pharmacopoeia (Ph.Eur.)) or national formularies, are available in several countries.^{1 2 6 7} The Portuguese Galenic Formulary (PGF) was first published in 2001 and represents the main bibliographic support for this important pharmaceutical activity in Portugal. It contains monographs on non-sterile compounded medicines and other compounded preparations and also the transcription of Portuguese/European legislation concerning compounding practices.⁶ These documents include recommendations on facilities, personnel, equipment, raw material, documentation, procedures and quality control that aim to ensure the quality, safety and traceability of compounded medicines. The quality control tests defined by the PGF for compounded preparations are those generally available in pharmacies and hospital settings such as appearance, odour, flavour, pH determination, and mass or volume determination. These tests do not include microbiological quality assessment of preparations according to pharmacopoeial standards, as defined for industrialised medicines.⁶ Most recently published, the Ph.Eur. 8.0 version of the 'Pharmaceutical Preparations' monograph states that measures should be taken to ensure the microbiological quality of both licensed and unlicensed preparations.⁷ However, since microbiological testing procedures are not usually accessible in community and hospital pharmacies, they are not routinely performed for quality control of compounded medicines.⁴

Compounded medicines have been associated with public health problems through microbiological contamination.^{2 8 9} While being particularly relevant for sterile preparations, reports on the microbial contamination of non-sterile pharmaceuticals have also been published.¹⁰ Concerns about the quality of non-sterile compounded drugs have been raised, especially because the majority of these preparations are prescribed for oral administration in frail patients and because non-compliant microbial burdens may promote organoleptic and drug-degradation processes, which may lead to decreased efficacy and safety.¹¹

The pharmacy department of Centro Hospitalar Cova da Beira, located in the city of Covilhã in the central region of Portugal, is certified according to the standard ISO 9001:2008, and has implemented several procedures to ensure the quality of compounded preparations: material and equipment cleaning and control; access restriction to

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compounding laboratory facilities; and specific compounding procedures. In order to evaluate the microbiological quality of non-sterile compounded medicines (and intermediate preparations used for compounding), a collaboration protocol was established between the hospital and an external laboratory. In this article, the results from microbiological testing of preparations compounded in this hospital centre from January to December 2014 are discussed.

METHODS

For validation of procedures, five collection type strains were included corresponding to three bacteria (*Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633) and two fungi (*Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16406), according to the specifications of Ph.Eur. 8.0.⁷

A neutralising solution was used to ensure that any preservative effect of the formulation was neutralised at the moment of microbial enumeration, allowing the existent microorganisms to be recovered and counted in agar medium. For this purpose, a mixture of neutralising compounds was prepared using commercial buffered peptone water, 30 g/L polysorbate 80, 3 g/L soy lecithin, 30 g/L saponins and 1 g/L octoxynol-9 (all from BDH Prolabo, Belgium).

Sabouraud dextrose agar (SDA) and tryptone agar medium (TSA), used as culture media, were acquired from BDH Prolabo.

The compounded preparations tested in this work were prepared at the pharmacy department of Centro Hospitalar Cova da Beira, Covilhã, Portugal. These preparations were based on medical prescriptions (magistral formulas) or the PGF (official preparations) to be dispensed either to hospitalised or ambulatory patients. Samples of compounded preparations were collected from January to December 2014, immediately after preparation, using sterile material, and were stored under the recommended storage conditions defined for each formulation (room temperature or 2–8°C) and protected from light.

In total, 392 microbiological quality tests were performed on 24 different formulations, corresponding to 8 intermediate preparations (compounded preparations used to prepare compounded medicines), 11 oral solutions/suspensions and 5 topical application products. Table 1 describes the tested formulations and the number of batches tested for each condition.

Microbiological tests were performed within 72 h of preparation. Additional tests were performed in samples immediately after their established beyond-use date (expiry date defined for extemporaneous preparations).

The microbiological quality tests were performed according to the Ph.Eur. 8.0 (monograph 5.1.4: Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use).¹² For each formulation, the test was validated according to the procedure described in the monograph. Briefly, each different formulation was artificially inoculated with a microorganism-calibrated suspension, after incubation in culture medium and under aerobic conditions at the appropriate temperature. The recovery rate after artificial inoculation in a sample of each formulation was compared with the recovery rate of the control. Both the validation procedure and the quality tests were performed after dilution of the sample in a neutralising mixture (1:10). The method was considered validated when the number of colony-forming units (CFUs) counted in 1 mL of inoculated sample was at least 50% of that obtained in the control (neutralising solution inoculated with each microorganism). For recovery rates below 50%, a second dilution in neutraliser was performed, in the same proportion,

before inoculation with the testing microorganisms. In these cases, the microbiological quality test was performed using one and two dilutions of the product in the neutralising solution. The absence of toxicity of the neutraliser on these control microorganisms was confirmed by comparing the CFU counts recovered from the neutraliser and from buffered peptone water used as diluent, after inoculation under the same conditions.

The results were compared with the pharmacopoeial specifications for aqueous oral preparations and topical preparations ($\leq 2 \times 10^2$ for total aerobic organisms and $\leq 2 \times 10$ for fungi).

CFU count was performed in duplicate after sample incorporation into TSA (incubated at 30–35°C for 24–48 h) for bacteria and total aerobic organism counts and after sample incorporation into SDA (incubated at 20–25°C for 3–5 days) for yeasts and fungi. Results represent the mean CFU count.

RESULTS

For all preparations tested, conformity with the pharmacopoeial specifications was verified for microbiological quality immediately after preparation (t0) (figures 1–3).

For all intermediate preparations (ie, preparations that are further used to prepare solutions or suspensions for oral use), it was observed that CFU counts were within the defined range of Ph.Eur. 8.0 even after the established beyond-use date (figure 1). On the basis of these results, from September to December, only final preparations were analysed, since their quality would also reflect the microbiological quality of the intermediate preparations included in their preparation.

Regarding compounded solutions and suspensions for oral administration, it was observed that two out of 16 batches of prednisolone oral suspension (Pre) had CFU counts above the upper limit, while one batch had CFU counts close to the limit established by Ph.Eur. (fungi 2×10 CFU) 1 month after preparation (the defined beyond-use date) (figure 2). On the basis of these results, this formulation was replaced by a preserved formulation, with added parabens (PreP) in order to avoid the risk of contamination during storage. As shown in figure 2, in this new formulation, both t0 (immediately after preparation) and BU (beyond-use date/expiration date) samples tested showed CFU counts within the limits of Ph.Eur. 8.0, which was also observed for all other formulations tested in this group.

Concerning topical application products, a non-compliant batch of nitroglycerine 0.25%+cinchocaine 0.5% ointment was identified only after storage (BU) (figure 3). Since this result was observed for only one batch (out of 22), it was decided not to replace the formulation, although it was kept under surveillance in subsequent production batches, which were compliant with the specifications.

DISCUSSION

Despite being a long-established practice, compounding of medications in hospital and community pharmacies is still an essential pharmaceutical activity that allows customisation of therapeutics and is perceived by pharmacists as a highly specialised service.^{13–16} Quality control of these medicines is therefore an issue that needs to be studied and improved.

Microbiological quality of non-sterile compounded medicines is particularly important to ensure both the safety and efficacy of these preparations. High microbial burdens may promote serious infections, especially in immunocompromised patients, while microbial metabolites may lead to changes in pH and degradation of drugs, rendering them less effective or even toxic due to the formation of degradation products.^{4 11} Since

Table 1 Tested formulations, route of administration and number of batches tested for each condition

Formulation	Type of preparation	Storage temperature defined	Batches t0	Batches BU	Total batches prepared	Percentage of preparations tested within this period
Simple syrup (SS)	IP	RT	13	13	21	62
Simple syrup with parabens (SSP)	IP	RT	1	1	2	50
Sodium bicarbonate 1.4% aqueous solution (SBI)	IP	RT	31	31	99	31
Parabens concentrated solution (PCo)	IP	RT	1	1	1	100
Methylcellulose gel 1% (MG 1%)	IP	RT	1	1	2	50
Citric acid 25% aqueous solution (CA 25%)	IP	2–8°C	1	1	2	50
Vehicle for oral solutions and suspensions (V)	IP	RT	7	7	14	50
Banana flavouring aqueous solution 10%	IP	2–8°C	2	2	2	100
Trimethoprim 1% oral suspension (T)	OU	2–8°C	12	12	15	80
Prednisolone 5% oral suspension (Pre)	OU	RT	16	16	38	68
Prednisolone 5% oral suspension preserved with parabens (PreP)	OU	RT	5	5	7	71
Nystatin oral suspension (Nis)	OU	RT	39	39	98	40
Chloral hydrate 10% syrup (CH)	OU	2–8°C	23	23	30	77
Amiodarone 0.5% oral suspension (A 0.5%)	OU	2–8°C	2	2	2	100
Propranolol HCl 0.1% oral suspension (Prop 0.1%)	OU	2–8°C	1	1	1	100
Nitrofurantoin 5% oral suspension (N)	OU	RT	3	3	3	100
Ursodeoxycholic acid 4% oral suspension (UA 4%)	OU	2–8°C	1	1	5	20
Ursodeoxycholic acid 5% oral suspension (UA 5%)	OU	2–8°C	2	2	4	50
Omeprazole 0.4% oral suspension (O)	OU	2–8°C	4	4	7	57
Nitroglycerine 0.25%+cinchocaine 0.5% ointment (N/C)	TA	RT	22	22	42	52
Potassium permanganate 0.01% aqueous solution (PP 0.01%)	TA	RT	2	2	4	50
Fusidic acid 2%+betamethasone 0.1% ointment (F/B)	TA	RT	2	2	3	67
Betamethasone 0.1%+salicylated vaseline 2% ointment (B/SV 2%)	TA	RT	4	4	7	57
Betamethasone 0.1%+salicylated vaseline 5% ointment (B/SV 5%)	TA	RT	1	1	2	50

Concentrations are presented as % w/v for liquid preparations and w/w for semi-solid preparations.

Batches BU, number of batches analysed after the beyond-use date (expiration date); Batches t0, number of batches analysed immediately after preparation; IP, intermediate preparation; OU, oral use; RT, room temperature; TA, topical application.

microbiological tests are not usually available to pharmacies, this factor is commonly disregarded for compounded medicines.

The results of this study show 100% compliance with the requirements of the European Pharmacopeia concerning microbiological quality immediately after preparation, indicating the adequacy of the compounding procedures used in this hospital pharmacy. Although compounded medicines do not undergo the normal procedures of licensing involved in industrial manufacture, compliance with the specific recommendations for their preparation is essential to minimise the risk of product contamination, incorrect labelling and concentration of active substances.^{7–16} The official recommendations available in Portugal focus on eight key areas: personnel; facilities and equipment; documentation; raw materials; packaging material; compounding; quality control and labelling.^{6–17–18} For each of these areas, specific recommendations are essential for the quality of the final preparations, particularly those that are associated with hygiene: restriction of access to compounding areas, use of protective equipment during preparation, adequate cleaning and organisation of facilities and materials used in preparations, verification of the quality control of final packaging, among many others. In

Centro Hospitalar Cova da Beira, these recommendations have been studied, implemented and established in detailed written procedures focusing on cleaning (including the type of product to be used in cleaning of compounding material), the correct procedures to be used while compounding, and the authorised personnel to undertake this task. The positive results obtained in this study indicate that these efforts are associated with the microbiological quality of final preparations.

Importantly, this work has allowed identification of the need to redefine the expiration date of preparations or change them for more stable formulations, which would not have been recognised in normal conditions. The fact that non-compliant batches were identified for prednisolone oral suspension after the beyond-use date stimulated an important discussion on the adequacy of that specific formulation. Since intermediate preparations used in this formula were compliant with the microbiological pharmacopoeial requirements at both test time points, it is possible that some degree of contamination may have arisen from the tablets used in these preparations which, although within the limits in the dried dosage form, may have proliferated in the liquid form during storage. It was confirmed that, for the two compounded batches

Figure 1 Microbiological quality results of intermediate preparations used for oral suspensions/solutions. Bars represent the number of batches classified as 'compliant' or 'non-compliant' according to the specifications of European Pharmacopoeia 8.0 for aqueous oral preparations (SS, simple syrup; SSP, simple syrup with parabens; SBI, sodium bicarbonate 1.4% aqueous solution; PCo, parabens concentrated solution; MG 1%, methylcellulose gel 1%; CA 25%, citric acid 25% aqueous solution; V, vehicle for oral solutions and suspensions; BF, banana flavouring aqueous solution 10%). BU, analysed after the beyond-use date; t0, analysed immediately after preparation.

that were not compliant at the beyond-use date, different batches of prednisolone tablets were used. Also contamination during the compounding procedure cannot be excluded, although it is less probable since it was not observed in any other oral preparations compounded on the same days.

The replacement of this formulation for a paraben-containing formula resulted in a more stable preparation and therefore helped to improve the safety of this oral suspension, which is mainly used in children.

For topical preparations, only one non-compliant batch among all those studied was identified after the beyond-use date. Although the microbiological quality of these topical preparations may be thought to be less critical than that of the oral ones, it should be noted that many of them are intended to be applied to damaged skin—for example, 'nitroglycerine and

cinchocaine ointment', which is prescribed for the treatment of anal fissures. Therefore their quality control may actually avoid serious adverse effects on the health of the patient.

All preparations included in this study are classified as 'extemporaneous preparations' according to the Ph.Eur., since they are intended to be supplied immediately after preparation as opposed to the 'stock preparations', which are prepared in advance and stored until a prescription is received.⁷ If these were regarded as 'stock preparations', their characterisation and quality control could be improved, since they would be prepared in advance and released only when accepted on the basis of the received quality control results. Obviously, this strategy would only be applicable to preparations used on a regular and predictable basis. On the basis of these results, the administration board decided to maintain microbiological quality assessment of compounded medicines in a

Figure 2 Microbiological quality results of compounded oral solutions and suspensions. Bars represent the number of batches classified as 'compliant' or 'non-compliant' according to the specifications of European Pharmacopoeia 8.0 for aqueous oral preparations (T, trimethoprim 1% oral suspension; Pre, prednisolone 5% oral suspension; PreP, prednisolone 5% oral suspension preserved with parabens; Nis, nystatin oral suspension; CH, chloral hydrate 10% syrup; A 0.5%, amiodarone 0.5% oral suspension; Prop 0.1%, propranolol HCl 0.1% oral suspension; N, nitrofurantoin 5% oral suspension; UA 4%, ursodeoxycholic acid 4% oral suspension; UA 5%, ursodeoxycholic acid 5% oral suspension; O, omeprazole 0.4% oral suspension). BU, analysed after the beyond-use date; t0, analysed immediately after preparation.

Figure 3 Microbiological quality results of compounded topical application formulations. Bars represent the number of batches classified as 'compliant' or 'non-compliant' according to the specifications of European Pharmacopoeia 8.0 for topical application (N/C, nitroglycerine 0.25%+cinchocaine 0.5% ointment; PP 0.01%, potassium permanganate 0.01% aqueous solution; F/B, fusidic acid 2%+betamethasone 0.1% ointment; B/SV 2%, betamethasone 0.1%+salicylated vaseline 2% ointment; B/SV 5%, betamethasone 0.1%+salicylated vaseline 5% ointment). BU, analysed after the beyond-use date; t0, analysed immediately after preparation.

random sample of non-sterile final formulations, therefore certifying the quality of customised preparations dispensed for special populations and specific clinical conditions.

CONCLUSIONS

This 1-year study demonstrated the microbiological quality of non-sterile compounded medicines prepared at a Portuguese hospital pharmacy, where specific compounding procedures are implemented according to current standards of good practice. Moreover, the study of preparations after storage allowed identification of the need to either redefine the beyond-use date or improve specific formulations with preservatives.

These results show that microbiological quality control represents an important strategy to improve the safety of compounded medicines. Furthermore, the results may help other pharmacists working in hospital and community pharmacies to increase and ensure the quality of compounded medicines through the implementation of adequate compounding procedures, such as those described here.

What this paper adds

What is already known on this subject

- Compounded medicines are prepared in hospital and community pharmacies to fulfil patients' therapeutic needs. Microbiological quality control tests for these preparations are not usually accessible to community and hospital pharmacies.
- Very few data are available on implementation of methodologies concerning microbiological quality control of compounded medicines.

What this study adds

- This study describes the implementation of a microbiological quality control procedure for non-sterile compounded medicines to ensure their quality and verify their microbiological stability over the storage period.
- These results highlight the importance and feasibility of microbiological quality control tests on compounded medicines in a hospital setting.

Competing interests None declared.

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