UNIVERSIDADE DA BEIRA INTERIOR
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Microbiological Quality Control of Non-sterile Compounded Medicines Prepared in Centro Hospitalar Cova da Beira

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Dissertação para obtenção do Grau de Mestre em
Ciências Biomédicas
(2º ciclo de estudos)

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Amostra Sem Valor

Eu sei que o meu desespero não interessa a ninguém.
Cada um tem o seu, pessoal e intransmissível:
com ele se entretém
e se julga intangível.

Eu sei que a Humanidade é mais gente do que eu,
sei que o Mundo é maior do que o bairro onde habito,
que o respirar de um só, mesmo que seja o meu,
não pesa num total que tende para infinito.

Eu sei que as dimensões impiedosas da Vida
ignoram todo o homem, dissolvem-no, e, contudo,
nesta insignificância, gratuita e desvalida,
Universo sou eu, com nebulosas e tudo.

António Gedeão, 1961

Dedication

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Resumo Alargado

A preparação de medicamentos manipulados, a par da indústria farmacêutica, constitui uma realidade de extrema importância na medida em que existem situações clínicas específicas para as quais esta prática surge como solução terapêutica. A não existência no mercado de determinadas fórmulas farmacêuticas obriga à adequação de produtos disponíveis, por ajuste de dose ou preparação de formas galénicas mais adequadas às necessidades dos doentes.

A preparação de medicamentos manipulados nas farmácias hospitalares e comunitárias é um fator importante na saúde pública, sendo necessário assegurar a qualidade e a segurança destes produtos. Estes medicamentos estão sujeitos a legislação específica e são preparados de acordo com os requisitos das Boas Práticas a Observar na Preparação de Medicamentos Manipulados que determinam a obrigatoriedade de testes de controlo de qualidade facilmente mensuráveis neste nível de produção, tais como caraterísticas organoléticas, verificação de volume ou massa dispensados, pH entre outros. O controlo de qualidade microbiológico exigido aos medicamentos industrializados, não é imposto aos medicamentos preparados à escala oficinal. Contudo, esta necessidade tem sido cada vez mais demonstrada devido a diversos problemas de saúde pública associados a medicamentos manipulados. O objetivo desta dissertação de mestrado foi avaliar a Qualidade Microbiológica dos medicamentos manipulados nos Serviços Farmacêuticos do Centro Hospitalar Cova da Beira, preparados durante o ano de 2014.

As amostras das formulações em estudo foram recolhidas em material estéril e processadas no prazo de 72h após a sua preparação, tendo sido posteriormente armazenadas nas condições preconizadas para cada formulação, ou seja, temperatura ambiente ou 2-8ºC. Para cada lote preparado nos Serviços Farmacêuticos do CHCB foram também recolhidas amostras para análise no término de validade. Os ensaios de Qualidade Microbiológica foram realizados de acordo com a metodologia da monografia 5.1.4 "*Microbiological Quality of Non-sterile pharmaceutical preparations and substances for pharmaceutical use*" da Farmacopeia Europeia 8.0, tendo sido realizadas contagens de aeróbios totais assim como de fungos/leveduras e confrontadas com as especificações da Farmacopeia Europeia 8.0 para as preparações orais aquosas e preparações de uso tópico ($\leq 2x10^2$ para aeróbios totais e $\leq 2x10^1$ para fungos)

De janeiro a dezembro de 2014, foram realizadas 421 análises de qualidade microbiológica, correspondendo a 27 formulações diferentes: 8 formulações de Preparações Intermédias, 11 preparações de Soluções/Suspensões de Uso Oral, 5 produtos de Aplicação Tópica e 3 Desinfetantes/Antissépticos. Todas as preparações apresentaram conformidade com a farmacopeia no momento da preparação. Contudo, 2 lotes da formulação "Solução Oral de Prednisolona 5mg/mL» e 1 lote de "Pomada de Nitroglicerina 0,25% e Cinchocaína 0,5%"

apresentaram contagens de microrganismos superiores aos limites estabelecidos pela Farmacopeia Europeia 8.0, no término da validade.

Os resultados põem em evidência a adequação dos procedimentos implementados nos Serviços Farmacêuticos do CHCB para garantir a qualidade microbiológica dos medicamentos manipulados. Para as formulações em que as quais se verificaram não conformes no término da validade, será necessário redefinir o prazo de validade estipulado, como na formulação "Pomada de Nitroglicerina 0,25% e Cinchocaína 0,5%" ou por outro lado, substituir algum excipiente atualmente utilizado na formulação por outro com uma maior estabilidade ao longo do tempo, como algum conservante, por exemplo. Neste sentido, os Serviços Farmacêuticos do CHCB procederam à substituição da formulação original de Prednisolona por uma formulação com parabenos, "Solução Oral de Prednisolona 5mg/mL com Parabenos», com o intuito de evitar o risco de contaminação durante o armazenamento. Após esta alteração, as análises da formulação com Parabenos demonstrou contagens conformes evidenciando a adequação do poder conservante dos Parabenos na estabilidade da formulação.

Concluindo, dentro das 421 análises de qualidade microbiológica apenas 3 evidenciaram resultados não conformes com as especificações da Farmacopeia Europeia 8.0. Este resultado põe em evidência a adequação das Boas Práticas de Manipulação implementadas nos Serviços Farmacêuticos do CHCB, assim como a eficácia dos medicamentos preparados, a segurança e saúde do doente.

De sublinhar que a metodologia aplicada no presente estudo tem um caráter preventivo de problemas de saúde pública. A implementação de um sistema de controlo de qualidade microbiológica de medicamentos manipulados é de todo vantajosa para o Sistema Nacional de Saúde na perspetiva da segurança do doente sendo evidente a pertinência do trabalho desenvolvido na minha dissertação.

Palavras-chave

Contaminação Microbiológica; Farmácia Hospitalar; Farmacopeia Europeia; Manipulação; Medicamentos Não estéreis; Preparações Oficinais; Qualidade Microbiológica; Segurança do Doente.

Abstract

The compounded medicines emerge as individualized therapeutic alternatives, which are justified because they constitute an adjusted response to the demands of a particular patient, complementing the therapeutic arsenal available by the pharmaceutical industry that fails to meet all the needs. The preparation of compounded medicines in hospital and community pharmacies is an important factor in public health and is necessary to ensure the quality and safety of these products. This need has been increasingly demonstrated due to various public health problems associated with the preparation of compounded medicines.

This work aimed to evaluate the quality of non-sterile formulations compounded at Centro Hospitalar Cova da Beira immediately after preparation and up to the defined expiration date.

Microbiological quality control tests were performed in accordance with the 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 analyzed immediatelly and reanalyzed after storage in the established conditions.

In the test period, 421 preparations were analysed corresponding to 27 different formulations, according to the route of administration: 8 intermediate preparations for oral use, 11 solutions/suspensions for oral use, 5 topical products and 3 disinfectants/antiseptics preparations. All preparations were in accordance with the pharmacopoeial specifications immediately after preparation. However, for the formulations «Prednisolone oral solution (5mg/mL)» and «Nitroglycerine and cinchocaine ointment (0.25%/0.5%)» the results of microbial counts exceeded the defined limits after storage up to the expiration date.

In conclusion, these results show that the compounding practices implemented at the Pharmacy Department of CHCB are able to assure the microbiological quality of compounded medicines. Moreover, with the evaluation of the microbiological quality of the preparations at the end of expiration date, it was possible to identify the need to reset the product period of validity (Nitroglycerin 0.25% (w/v) + Cinchocaine 0.5% (w/v) ointment) or to modify the formulation to assure the product microbiological protection (parabens addition to Prednisolone 5 mg/ml oral suspension).

Keywords

Compounding; European pharmacopoeia; Hospital pharmacies; Microbiological contamination; Microbiological quality; Non-sterile medicines; Officinal preparations; Patient safety.

Publications resulting from this work

- Oral presentation entitled "*Controlo de qualidade microbiológica de manipulados não estéreis produzidos no Centro Hospitalar Cova da Beira, EPE*", on 4th International Meeting on Quality and Patient Safety, Lisboa, 2014. (Abstract of Proceedings Book: **Annex 1**).
- Poster entitled "*Controlo de qualidade microbiológica de manipulados não estéreis produzidos no Centro Hospitalar Cova da Beira, EPE*", on 4th International Meeting on Quality and Patient Safety, Lisboa, 2014. (Abstract of Proceedings Book: **Annex 1**).
- Oral presentation entitled "*Metodologia implementada no controlo de qualidade microbiológica de manipulados não estéreis no Centro Hospitalar Cova da Beira*", on III Workshop de Qualidade em Saúde, Universidade da Beira Interior, Covilhã, 2014. (Brochure presentation: **Annex 2**).
- Oral presentation entitled "*Controlo de qualidade microbiológica de manipulados não estéreis: a experiência do Centro Hospitalar Cova da Beira*", on 7th Week APFH – 17th National Symposium. Lisboa, 2014. (abstract and certificate of best oral communication: **Annex 3**). Oral communication distinguished by APFH award 2014.
- Paper "*Microbiological quality control of non-sterile compounded products compounded in the Portuguese Hospital Center*" 2015 (submitted).
- Submission for the poster presentation entitled "*Controlo de qualidade microbiológica de manipulados não estéreis produzidos no Centro Hospitalar Cova da Beira, EPE* ". X CICS Annual Symposium 2015. University of Beira Interior. Covilha. 2015.

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List of Acronyms

AIM	Autorização de Introdução no Mercado
ATCC	American Type Culture Collection
BU	Beyond-Use Date
BU-AP	Beyond-Use Date – Ambulatory Patients
CFU	Colony Forming Unit
CHCB	Centro Hospitalar Cova da Beira
CM	Compounded Medicines
DL	Decree Law
FDA	U. S. Food and Drug Administration
FGP	Formulário Galénico Português
GCP	Good Compounding Practices
GMP	Good Manufacturing Practices
IA	Intrinsic Activity
INFARMED	Autoridade Nacional do Medicamento e Produtos de Saúde
ISPhC	International Society of Pharmaceutical Compounding
NECC	New England Compounding Center
PDA	Potato Dextrose Agar
PD-CHCB	Pharmacy Department of Centro Hospitalar Cova da Beira
Ph. Eur.	European Pharmacopoeia
SDA	Sabouraud Dextrose Agar
t0	After Preparation
TAMC	Total Aerobic Microbial Count
TSA	Tryptic Soy Agar
TYMC	Total Combined Yeast and Molds Count
UBI	Universidade da Beira Interior
USA	United States of America

Chapter 1

Introduction

As a consequence of pharmaceutical products industrialization, the exclusive small-scale production of Compounded Medicines (CM) has decreased over the years. However, the pharmaceutical industry is still unable to meet all therapeutic needs, especially conserning formulations that are not sustainable from an economic point of view. This frequently happens in medical specialties such as pediatrics, geriatrics, oncology, dermatology, among others. To meet this need the CM emerges as an important pharmaceutical activity in order to address therapeutic requirements of a particular patient, in accordance to its unique pathophysiological profile (1).

The use of specialized bibliographic sources, such as the Portuguese Galenic Formulary, (Formulário Galénico Português (FGP)) and the European Pharmacopoeia (Ph. Eur.), together with he the legislation implemented from 2004 (Ordinance No. 594/2004, of June 2, the Decree Law (DL) No. 90/2004 of April 20 and the DL No. 95/2004 of 22 April) and the Good Coumpounding Practices (GCP), specifically related to the preparation of CM, allow for the preparation of safe, effective, high quality and standardized medicines (1) at national level (2).

1 Compounded Medicines: definitions and historical perspective

The medicines prepared on a small scale in both community and hospital pharmacies, called CM, have been assuming a growing importance as therapeutics (3, 4).

Pharmaceutical compounding is the preparation of custom-made medications. Compounding encompass a triad that includes the patient, practitioner and a pharmacist. According to the Pharmacopoeia, a CM is a preparation that includes formulations prepared according to the clinicians' instructions and other compendial formularies (5).

The U. S. Food and Drug Administration (FDA) defines traditional pharmacy compounding as the combining, mixing, or altering of ingredients to create a customized medication for an individual patient (6). Pharmaceutical compounding plays a valuable role in providing access to medications for individuals with unique medical needs, which cannot be met with a commercially available product. For instance, a prescriber may request a pharmacist to compound a suspension for a pediatric or geriatric patient unable to swallow a medication in its commercially available form. In pharmacy compounding, an individualized medicine is prepared at the request of a prescriber on a small scale (7).

According to the national authority of medicines and health products, INFARMED in Portugal, CM are any magistral formulas or officinal formulations, prepared and dispensed under the responsibility of a pharmacist (8).

These definitions highlight the intent of compounding to prepare a small quantity medication based on a practitioner's prescription. Industrial pharmaceutical manufacturing, by contrast, is related to the large scale production of medication, under Good Manuacturing Practices (GMP) without regard to a specific patient, population or prescription, and requires regulatory approval use (5).

By definition, as established in DL No. 90/2004 of April 20 and DL No. 95/2004 of April 22 a officinal preparation is a medicine prepared according to compendial indications of a pharmacopoeia or formulary, in the officinal pharmacies or in the hospital pharmacy services, intended to be dispensed directly to patients assisted by the pharmacy or service. On the other hand, a magistral formula is a CM prepared in according to a medical prescription and intended for a particular patient. These medications can be the subject of prior preparation, provided they are included in the list approved by the INFARMED, that are presented as multidose preparations and are distributed in packaging for single dose (8).

Pharmacy compounding is a vital service that helps many people and serves an important public health need for patients who cannot be treated with other medication. In fact, it is the pharmaceutical art and science of preparing personalized medications for patients. CM results from the mixture of individual ingredients in defined concentrations and vehiculed in excipients to achieve a specific dosage form that meets the patient needs. Commercially available dosage forms may sometimes be modified to better fit the dosing individual needs. (9).

The personalized therapy of patients by prescribing magistral formula is, nowadays, a primary reason for CM use, being this activity a key part of Hospital Pharmacies (4, 10). By establishing its quantitative and qualitative composition is possible to adjust the therapy to specific pathophysiological profile of each patient, addressing some aspects such as: age, sex, general physical condition, metabolism and disease in a patient specific (10). The CM are prepared in accordance with good practices to be followed in the preparation of CM in pharmacy and hospital officinal, which falls on eight key areas: personnel, premises and equipment, documentation, raw materials, packaging materials, compounding, quality control and labeling (8) The magistral pharmacy, aiming at the compounding of medicines, is an integral part of the pharmaceutical practice. As stated earlier, this activity of the pharmacist is intrinsically focused on the patient. In each case, the prepared product should reflect in all its aspects, the specific patient needs, including not only the aspects related to the disease itself (active ingredient or association of active substances and respective dose), but also other patient situations and conditions that must be considered (allergy, diabetes,

enzymatic deficiencies, renal or hepatic failure, among others), the age of the patient (specific medicines to pediatric and geriatric use), the existence or not of an impaired oral capacity / the patient's dexterity to properly self-administer the medication, the patient preferences with regard to dosage forms and their organoleptic characteristics (in particular as regards color, taste and aroma), etc. This is emphasized by the International Society of Magistral Pharmacy (International Society of Pharmaceutical Compounding - ISPhC), founded in 2004, whose mission is to promote the CM around the world to meet the needs of the patients (3, 11).

Despite this important mission, during the last decades and due to the industrial advancement, CM were forgotten. This was also consequence of the existence of a loose and ambiguous legislative framework, with a national formulary of outdated content with a lack of concepts and quality standards essential to ensure the safety and efficacy of the medicine (12, 13).

If on one hand, the constraints of the practice of quality compounding, turned down the implementation of these medicines, on the other, the lack of alternatives in the industrialized therapeutic imposed their reappearance (12, 13).

DL No. 95/2004, of 22 April established laws and regulations and proceeded the review of the technical and legal framework applicable to CM, covering the officinal and magistral preparations. This new framework aimed at strengthening the public health safeguards in the use of these products through its best rating and this is directly dependent of the greater quality assurance, safety, effectiveness and credibility of these (13-16).

In Portugal, as in most countries, CM have great relevance to pharmacists (3). According to the DL No. 95/2004 of 22 April, the prescription of CM is the doctor's responsibility, and the supervision of the preparation and dispensing of CM is the pharmacist´s responsibility. The pharmacist is responsible for the approval of the prescribed galenic formula, assuring that it is the appropriated choice based on pharmacotherapeutic and galenic characteristics, that CM preparation was made according to the GCP and ensuring its quality. The law allows the pharmacist to prepare CM on their own officinal workspace (1, 16).

1.1 Advantages and disadvantages of using Compounded Medicines

The use of CM in therapeutics is considered advantageous due to its patient personalized approach but also due to economic and quality reasons as CM are prepared in exact amounts and for a specific patient, unnecessary waste and costs are avoided. In what concerns to CM quality this is guaranteed as it obey to strict legislation and GCP standards(14).

CM allows for the association of unavailable active substances in the industrialized medicinal products, when such strategies are justified from the pharmacotherapeutic point of view. This factor, in addition to facilitate the treatment, as the doctor prescribes only a single drug to be compounded and administered, also contributes to greater adherence by the patient. This is frequently requested for dermatology, oncology and pain management for chronically ill patients (1).

The filling of therapeutic niches not commercialized by the pharmaceutical industry is another area where CM are very important. These active substances (called orphans) with proven therapeutic utility are not commercialized as the pharmaceutical products are discontinued by industry or fail to be introduced on the market (AIM-Autorização de Introdução no Mercado) (3). On the other hand, for patients allergic to certain industrial ingredients (preservatives, antioxidants, colorants, flavorings / odors, glucose or even lactose), it becomes useful to prepare personalized medicines, free of these excipients (17).

The pharmaceutical industry typically produces a limited range of pharmaceutical forms to treat systemic diseases, the active substances which commonly exist as solid oral form (capsule or tablet) and / or injectable pharmaceutical forms. The technical difficulties which, in some cases, preclude the obtaining of liquid medicines for oral administration with extended shelf-life, have contributed to the absence of such pharmaceutical forms (18).Thus, coumpounding make possible the preparation and dispensing of various pharmaceutical forms. This aspect is particularly important when the oral route is impaired and patients have difficulties in swallowing. Coumpounding allow the preparation of pharmaceutical forms as oral solutions or suspensions, suppositories or sublingual tablets to be administered in an appropriated way (19-21).

According to the literature, in the preparation of CM the most mentioned disadvantages by hospitals were the difficulties in obtaining raw material of pharmacopoeial grade, as well as the lack of time available to prepare the formulations, the need for equipment calibration as well as the need for compliance with all GCP standards (1).

In community pharmacies, the noted disadvantages were the difficulties in obtaining raw materials and the economic disadvantages underlying the waste of raw material and costs associated with production (2). This can be explained by the quality requirements, since, according to Good Practices, the raw materials must meet the requirements of the monograph and should preferably be purchased from suppliers authorized by INFARMED (22). Currently it is difficult to identify suppliers that provide these raw materials in time and have small product quantities available to avoid waste (1).

2 Microbiological Quality

The Pharmaceutical Microbiology is responsible to address product quality, focusing on product development and methods, production and stability, assuring the patient safety (23).

In fact microbiological contamination becomes a public health problem when it results in undesirable effects when using pharmaceutical products (23). Thus, a critical field of the pharmaceutical microbiology is the microbiological quality control, specifically the study of microbial contaminants associated with the production of pharmaceuticals. This regards both sterile and non-sterile pharmaceutical products, and therefore, the pharmaceutical microbiology is involved: in understanding the probability of increased contamination of the product, finding ways to minimize such contamination; in developing methods for detecting contamination; and understanding the severity of these effects. For this, it is necessary to quality control tests defined for CM are those generally available in pharmacies/hospital settings such as appearance, odor, flavor, pH determination and mass or volume determination (24). These tests do not include the microbiological quality assessment of preparations according to pharmacopoeial standards, as defined for industrialized medicines (25).

2.1 Microbiological quality of pharmaceutical preparations and substances for pharmaceutical use

Compounding, packaging and storage of pharmaceutical preparations should be conducted to ensure, during the established period of use, a satisfactory microbiological quality, which is evaluated by conducting quality control and microbiological stability studies as described in Ph. Eur. 8.0. The microbiological quality of the preparations is defined as meeting the criteria established in 5.1.4 monograph of the Ph. Eur. 8.0 "*Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use*"(25, 26).

Microbiological stability is understood as the capacity that medicines have to maintain within specified limits, its sterility or resistance to microbial growth. When antimicrobial agents are present, they should retain its effectiveness within the prescribed limits (26, 27).

The monograph 5.1.4 of the Ph. Eur 8.0 provides tests for the quantitative determination of total aerobic microbial count (TAMC) and Total Yeast/Moulds Count (TYMC) that might be present in pharmaceutical ingredients and finished products. These methods are not applicable to products containing viable microorganisms as active ingredients. Alternative procedures may be used, but must show to be equivalent to Pharmacopoeial methods. All aspects of the test are conducted under conditions designed to limit extrinsic contaminants from personnel, environment, reagents, or glassware. Antimicrobial activities inherent in the test sample must be removed or neutralized, and the applied method must be non-inhibitory

to microbial growth through demonstration of adequate recovery for representative microorganisms in validation testing. Microbial recovery is enumerated by one of three described methods: 1) Membrane Filtration, 2) Plate Count (pour-plate or spread-plate techniques), or 3) Most Probable Number The Most Probable Number method is reserved for TAMC in low bioburden samples, and is not suitable for the estimation of fungal recovery (25, 26).

The suitability test is conducted to demonstrate the applicability of the method for detection of microbial contamination in the test product. Validation testing is usually performed prior to product testing using a panel of five representative microorganisms as indicators. The microorganisms are used in the validation procedures, including: *Staphylococcus aureus* (Gram-positive coccus), *Pseudomonas aeruginosa* (non-fermentative Gram-negative bacillus), *Bacillus subtilis* (spore-forming Gram-positive bacillus), *Candida albicans* (yeast), *Aspergillus brasiliensis* (mold). Concurrent validation and product testing are possible based on the product history, and must be performed prior to product release. The compositions of required diluents and media are described in the monograph 2.6.12 ("Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests") of the Ph. Eur 8.0 (26, 28).

The microbial limits recommended in Ph. Eur. – Monograph 2.6.12 for total aerobic microbial count (TAMC $<10^3$ CFU/g), total combined yeasts and molds count (TYMC $< 10^2$ CFU/g) and tests for absence of the specified organisms by route of administration are shown in **Table 1 (28)**.

Table 1 European Pharmacopoeia Acceptance Criteria for Microbiological Quality of Non-sterile Dosage Forms (26).

Route of Administration	TAMC (CFU/g or CFU/mL)	TYMC (CFU/g or CFU/mL)	Specified microorganisms
Oral (non-aqueous)	10^3	10^2	*Escherichia coli*
Oral (aqueous)	10^2	10^1	*Escherichia coli*
Rectal	10^3	10^2	None designated
Oromucosal	10^2	10^1	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Gingival	10^2	10^1	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Cutaneous	10^2	10^1	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Nasal	10^2	10^1	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Auricular	10^2	10^1	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Vaginal	10^2	10^1	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Candida albicans*
Transdermal Patch (medicine matrix, adhesive layer and backing)	10^2	10^1	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Inhalation	10^2	10^1	*Staphylococcus aureus* *Pseudomonas aeruginosa* Bile-tolerant Gram-negative bacteria
Pharmaceutical substances	10^3	10^2	None designated

The microbiological test methods are highly variable and must be validated with a limit of detection as close as possible to the indicated acceptance criteria (26). The list of microorganisms in **Table 1** is not exhaustive. The significance of other microorganisms recovered should be evaluated in terms of route of administration, the nature of the product (e.g., growth promotion properties), the method of application, the intended recipient

(neonates, infants, debilitated conditions, etc.), the use of immunosuppressive agents, and the presence of disease or organ damage (29).

Acceptance criteria is applied to individual results or the average of replicate counts in colony-forming units per gram or mL of the product (CFU/g or CFU/mL). The maximum acceptable range for microbial enumeration is 2 times the limit. For example, results for a TAMC ranging from 5–20 CFU/mL would meet the specification of 10 CFU/mL (29).

The microbial limit for non-sterile products must be within an acceptable range that does not cause health hazards to intended patient groups or diminish product stability (26)

3 Microbiological Control of Non-Sterile Compounded Medicines

The target of CM are populations with very specific needs and without adequate response in the pharmaceutical industry. Thus, the preparation of CM is an important factor in public health and the assurance of quality and safety of these products is absolutely necessary. Thus, this kind of medicines is highly regulated in relation to its prescription, preparation and dispensing (14).

The preparation of CM in community and hospital pharmacies must meet standards that ensure the quality of the products produced. Good Practice Note on the Preparation of CM in Community and Hospital Pharmacy, the FGP and Ph. Eur. are part of the mandatory library of hospital and community pharmacies, and bring excellent support to the preparation of medicines, ensuring the standardization of produced medicines, their safety, efficacy and quality (2). However, these rules refer only to methods that are accessible to an officinal level (as organoleptic characteristics, the volume or mass dispensed check, pH, mass uniformity, among others) so does not include microbiological quality control that is required to manufactured products.

CM have been associated with public health problems due to microbiological contamination. While being particularly relevant for sterile preparations, these reports also raise questions on the quality of non-sterile CM especially considering that the majority of these preparations are prescribed for oral administration in frail patients.

3.1 Bibliographic sources to support the preparation of Compounded Medicines and Legal Framework

In hospitals, the pharmaceutical assistance is an essential part of the health care processes at all levels of complexity. It is a priority that the activities of pharmaceutical services should be

performed in order to ensure effectiveness and safety in the process of use of medicines and other health products (30).

It is essential that any hospital´s pharmaceutical services adopt the GCP in order to unify rules and procedures, with activity indicators, quality and safety. Good practices include the management of human resources and setting functions, management of economic resources, preparation and control of pharmaceuticals, medicine-delivery systems and medicines information centers (31).

In any pharmaceutical preparation, it remains the requirement for safety and efficacy. For this, a proper structure must exist as well as a system of procedures ensuring a "Quality System in Preparation of Pharmaceutical Formulations" (13). In fact, although not subjected to a process of AIM, the compounded are prepared according to GCP, which focus on eight key areas as mentioned above: personnel; facilities and equipment; documentation; materials; packaging material; compounding; quality control and labeling (8). These were designed to minimize the risk of any pharmaceutical production, such as unexpected contamination of products, incorrect labels and erroneous concentration of active substance, among others, which cannot be eliminated by the final product quality control. These actions ensure that products are consistently produced and controlled according to quality standards, resulting in a safe, effective and appropriate medicine for their intended use and required in the prescription (32).

Since 2001, the FGP is a working tool, tailored to the needs of contemporary therapy, which, nowadays, is properly used in community pharmacies and hospital. In addition to having contributed to increase the quality of CM, the FGP gathered scattered formulas and others that, despite being usually prescribed and prepared, were not published or had been the subject of proper studies. This also includes extracted preparations of domestic and foreign forms, preparations entered in the Portuguese Pharmacopoeia 9.0 and pharmacopoeia of other countries. The content of FGP spans not only the standards set by the GCP objectively for the pharmaceutical practice, but also the monographs and all matters related to CM: legislation, recommendations and technical information, etc (25).

The legislative framework that regulates CM was also restructured in 2005, swith subsequent modernization concepts, broadening of scope, clarification of responsibilities / competencies and standardization processes. This new regulatory framework is aims the strengthening of the public health safeguards in the use of these products by ensuring its quality, safety, effectiveness and credibility (8).

The Portuguese Pharmacopoeia 9.0 is elaborated in accordance with the Ph. Eur, published under the auspices of the Council of Europe, of which Portugal is a permanent member. The Pharmacopoeia consists of a codex of standards and methods to ensure in a certain political

and geographic space, the quality assurance of medicines for human and veterinary use, establishing through its monographs the requirements to be met by drugs, raw materials, other substances for pharmaceutical use and analytical methods to use in its characterization, dosage, among others. The mission of the Ph. Eur is to participate in the protection of public health, through the development of common recognized specifications, used by health professionals and in general, for all of those who are involved in the quality of the product. They must be of appropriate quality as they constitute for both patient and the consumer, one of the fundamental guarantees regarding the safe use of medicines. Its existence makes free movement of medicines in Europe, easier, and is also a guarantee of quality for exported medicines in Europe (2).

3.2 Microbiological Quality Control: Manufactured and Compounded Medicines

There are significant differences between CM and industrialized medicines (7). Pharmaceutical compounding is distinct from pharmaceutical manufacturing, in which products can be mass produced without a specific prescription while the formulations produced by the pharmaceutical industry are produced on an industrial scale under conditions of production, packaging and distribution defined by law (33).

Another important difference is that the majority of CM are not clinically tested for safety and efficacy, nor is bioequivalence testing conducted as is required for industrialized products (7).

The type and extent of quality control testing required for approved products in pharmaceutical industry is greater than the testing done on CM. Quality control tests defined for CM are those generally available in pharmacies/hospital settings such as organoleptic characteristics: appearance, odor, flavor, pH determination and mass or volume determination (34). These tests do not include the microbiological quality assessment of preparations according to pharmacopoeial standards, as defined for industrialized medicines (25)

Another major difference between CM and industrialized medicines is that compounding pharmacies are exempt from the federal GMP regulations that are obligatory for all approved pharmaceutical manufacturers (7).

Another difference is that compounding pharmacies are not obliged to report adverse events to the regulator (INFARMED, in Portugal), whereas adverse event reporting is mandatory for manufacturers of medications implementing the market. Thus, adverse events associated with CM may be difficult to detect, particularly if the affected patients are widely scattered in different geographic areas (7).

When it comes to CM it is said that they have a certain period of use (the “beyond use date”), which in fact corresponds to the "Expiration Date" of the medicine, where the time considered, is that of the specific treatment for the patient to whom the CM is directed. Thus, the period of use is the interval of time in which the expected CM maintains its characteristics. The referred interval of time is estimated based on general guidelines, references or real-time stability studies who set certain conditions (35). In industrialized products, on the other hand, the expiration date is longer and is set before the medicament is implemented on the market, after all stability tests are correctly performed.

Table 2 it can be seen by comparison the main differences between CM and manufactured preparations.

Table 2 Key differentiating factors between compounded medicines and manufactured products which result in different forms of quality control (7, 25, 26, 33, 34).

	Compounded Medicines	**Manufacturing Products**
Production	Officinal community pharmacies and / or Hospital	In series by the Pharmaceutical Industry
Scale	Small	Large
Dosages and concentrations of Active Principle	Customized	Standardized
Stability Studies	Not Mandatory	Required
Quality control	Directed to the final product	In all production stages
Microbiological Quality Control	It is not required.	Required
Expiration date	Shorter period according to length of treatment for which the patient was prescribed	Longer period it has stabilizers, preservatives and other supporting in its constitution
Labelling	Customized	Standardized
Informative Buletin	Generally Not included	Included
Regulatory Body	INFARMED	INFARMED and GMP
Bibliography	FGP and GCP	Ph. Eur. 8.0

3.3 Adverse Events Related to Microbial Contamination of Compounded Medicines

Contamination of pharmaceuticals with microorganisms may lead to adverse effects on the therapeutic properties of the medicines, and may potentially cause injuries to intended

recipients. Cases of contaminated sterile and non-sterile products have been reported in increasing numbers, and often associated with the presence of objectionable microorganisms (29).

In the USA (United States of America), the FDA became aware of 55 product quality problems associated with CM between 1990 and 2001. The agency therefore conducted a limited survey of 29 different CM sourced from 12 compounding pharmacies, testing 8 different products of various dosage types (oral, injectable, topical, among others) against established quality standards. Ten out of 29 samples (34 %) failed quality testing, mostly for sub-standard potency ranging from 59 to 89 % of the target dose (7).

A 2011 outbreak of *Serratia marcescens* bacteremia, which infected 19 patients at six Alabama hospitals, 9 of whom died, was caused by contaminated total parenteral nutrition bags from a compounding pharmacy. This case made evident the need for compliance with the rules listed in the pharmacopoeia and the need for better regulation and supervision in the preparation of CM. In the same year, a repackaged intravitreal injection of Bevacizumab® (used off-label to treat macular degeneration) caused a cluster of eye infections in Florida. Investigators traced *Streptococcus* infections from multiple eye clinics to one pharmacy, which dispensed the preservative-free product in single-use syringes. Twelve patients were infected, and some lost all of their remaining vision. A later publication cited 5 more patients being blinded in the Los Angeles area, and 4 patients in Nashville acquired similar infections from the compounded version (7).

In September 2012, a cluster of patients in Tennessee contracted fungal meningitis several weeks after receiving an epidural injection of methylprednisolone acetate, which had been compounded by the New England Compounding Center (NECC) in Massachusetts. The steroid had been injected into roughly 14.000 patients in more than 20 states. Over 500 cases of meningitis were confirmed, and dozens of patients died. Several different fungal species were identified in clinical specimens from the meningitis patients. Testing by FDA confirmed the presence of visible contamination and fungus in unopened vials of drug. A subsequent FDA inspection stated that there was no evidence that the process NECC used to sterilize the drugs was effective, and no corrective actions were taken to locate and remove the bacteria and mold from the facility (7).

The 2012 meningitis outbreak was not a unique event. In 2001, five patients were infected with bacterial meningitis, and three died after receiving Betamethasone injections contaminated with *Serratia* bacteria, which had been compounded by a pharmacy in California (7). In 2002, four women contracted meningitis, and one died, from a Steroid injection contaminated with the fungus *Exophiala dermatitidis*, which had been compounded by a pharmacy in South Carolina (36).

A review of the FDA enforcement reports during 2004–2011 revealed that approximately 75% of non-sterile product recalls were in fact due to contaminated over-the-counter or personal care products. The majority of these recalls were attributed to the following: presence of objectionable organisms (72%), contamination levels exceeding microbial limits (15%), sterility or microbial diagnostic kit errors (7%), failed microbiological tests (5%), manufacturing deficiencies (1%) (29).

In a recent unpublished UK survey it was noted that 54% of 112 pediatric extemporaneous formulations had inadequate data on shelf life. It is important that pharmacists accept responsibility for the quality of the formulation, stability of the product, and the quality of ingredients used (37). These serious adverse events drew attention to CM.

In 2012-2013 six congressional referenced hearings were held to understand the factors that lead to these adverse events and ways to prevent such incidents in the future. The safety of CM has been a concern of Congress for over two decades due to the expansion of pharmaceutical compounding. Potential safety risks for CM include problems with *potency* (the dosage is inaccurate, either too strong or too weak), *purity* (the drug contains other chemicals that could be harmful) and c*ontamination* (the drug is contaminated with a bacteria, fungus, molds or virus) (17).

3.4 Importance of microbiological quality as guarantee and safety parameter for the patient

There is ample evidence that improperly compounded sterile and non-sterile preparations injure and kill patients. Several state boards of pharmacy have significantly revised their pharmacy practice acts by expanding regulatory provisions to include the supervision regarding the control of the microbiological quality of non-sterile products in which it is assumed the presence of limited bioburden, the purpose of analysis is to confirm the absence of pathogens, as well as, if necessary, determine the number of viable microorganisms. The high microbial load should be considered to the extent that may compromise the stability of the product, with consequent loss of therapeutic efficacy, degradation of the active ingredient, or by altering the fundamental physical parameters for its activity, such as pH. In addition, changes in physical and chemical properties of non-sterile preparations may also affect its therapeutic action, compromising the bioavailability of the product and the acceptance thereof by the patient (38).

The implementation of GCP, which standardize rules and procedures, as well as the implementation of FGP and the adoption of the standards listed in the Ph. Eur. 8.0 contribute to improve the microbiological quality of magistral pharmaceutical products leading to increase the quality, efficacy and safety of use of the CM by the patient, found naturally in a fragile situation.

4 Aims

This study was carried out to study the microbiological quality of non-sterile CM prepared in the Pharmacy Department of Centro Hospitalar Cova da Beira (PD-CHCB).

This thesis has two specific aims:

1) To assess the Microbiological Quality of non-sterile CM in CHCB prepared during 2014 (Hospital Center accredited by the Joint Commision International since 2010);

2) To infer on the adequacy of the procedures implemented in the PD-CHCB and specifically concerning non-sterile compounding (certified ISO 9001 since 2011).

Chapter 2

Materials and Methods

2.1 Study Protocol

The tested CM in this work were prepared at the PD-CHCB. These preparations, to be dispensed either to hospitalized or ambulatory patients, were based on medical prescription (magistral formulas) or the FGP (officinal preparations). Tested formulations and respective route of administration are described in **Table 3**. (1, 10).

During 2014, from January to December, samples were collected in sterile equipment and processed within 72 hours after preparation thereof, having been stored in the recommended conditions for each formulation (room temperature or 2-8 ° C).

For each batch samples were collected for method validation as well as samples after preparation (t0) and close to the beyond-use (BU) date. In the last 3 months of study, samples taken at the ambulatory patients of the PD-CHCB were analyzed (BU-AP). Samples considered for this analysis were samples whose batch had been also analyzed to t0 and BU.

Table 3 Compounded medicines tested in this work and respective route of administration.

Formulations	Route of Administration
Simple Syrup (SS)	Intermediate Preparations for Oral Administration
Simple Syrup with Parabens (SSP)	
Sodium Bicarbonate 1,4% (w/v) aqueous solution (SBi)	
Parabens Concentrated solution (PCo)	
Methylcellulose Gel 1% (w/v) (MG 1%)	
Citric Acid 25% (w/v) aqueous solution (CA 25%)	
Vehicle for Oral Solutions and Suspensions (V)	
Banana Flavoring aqueous solution 10% (w/v) (BF)	
Trimethoprim 10 mg/mL oral suspension (T)	Oral Use
Prednisolone 5 mg/mL oral suspension (Pre)	
Prednisolone 5mg/mL oral suspension preserved with Parabens (PreP)	
Nistatine oral suspension (Nis)	
Chloral Hydrate 10% (w/v) syrup (CH)	
Amiodarone 0.5% (w/v) oral suspension (A 0.5%)	
Propranolol HCl 0.1% (w/v) oral suspension (Prop 0.1%)	
Nitrofurantoine 5 mg/mL oral suspension (N)	
Nitroglycerin 0.25% (w/v) + Cinchocaine 0.5% (w/v) ointment (N/C)	Topical Application
Potassium Permanganate 0.01% (w/v) aqueous solution (PP0.01%)	
Fusidic acid 2% (w/v) + Betamethasone 0.1% (w/v) ointment (F/B)	
Betamethasone 0.1% (w/v) + Salicilated Vaseline 2% (w/v) ointment (B/SV 2%)	
Betamethasone 0.1% (w/v) + Salicilated Vaseline 5% (w/v) ointment (B/SV 5%)	
Acetic Acid 3% (w/v) aqueous solution (AA 3%)	Disinfectant / Antiseptic
Colloidal Silver 2% (w/v) aqueous solution (CS 2%)	
Iodine 5% (w/v) aqueous solution (I 5%)	

2.2 Raw materials and Microorganisms

Three different culture media prepared according to manufacturer´s instructions were used in this study: Sabouraud Dextrose Agar (SDA), Tryptic Soy Agar (TSA) and culture medium Potato Dextrose Agar (PDA), were acquired to Prolabo (BDH Prolabo®, Belgium) (26).

One diluent solution (pH = 7) (29) was used in the preparation of microrganisms suspensions and prepared according to the manufacturer's instructions: Buffered Peptone Water (BDH Prolabo®, Belgium) (26).

A neutralizer solution was used to assure that any preservative effect of the formulation was neutralized previously to the microbial enumeration allowing the existent microorganisms to be recovered and counted in medium agar. For this purpose a mixture of neutralizing compounds was prepared using commercial Buffered Peptone Water, Polysorbate 80 (30 g/L), Soy Lecithin (3 g/L), Saponins (30 g/L) and Octoxynol-9 (1 g/L), were acquired to Prolabo (BDH Prolabo®, Belgium) (26).

For test validation 5 collection type strains were included, corresponding to 3 bacteria (*S. aureus* ATCC 6538 , *P. aeruginosa* ATCC 9027, *B. subtilis* ATCC 6633) and 2 fungi (*C. albicans* ATCC 10231, *A. brasiliensis* ATCC 16406), according to the specifications of the Ph. Eur. 8.0 (26).

2.3 Method Validation

Upon receipt of a new untested formulation, the method was validated in order to guarantee the effectiveness and non-toxicity of the neutralizer applied to the samples. Briefly, from microrganisms stock cultures, it was prepared a culture for each microorganism under the conditions defined in **Table 4**, in mid-specific agar culture plate. From this plate, a second culture was prepared, being this the work culture used to prepare the suspensions (26).

Table 4 Incubation conditions, culture means, absorbance, times and temperatures of the reference microorganisms under the conditions for the preparation of the study (26).

	Microorganisms	Incubation conditions of means of culture	CFU / mL	Absorbance 600 nm	Incubation conditions of the plates under test
Bacteria	*S. aureus* ATCC 6538	TSA 32.5 ºC ± 2.5 ºC during 18 a 24 h	1 x10^7 - 1 x10^8 CFU/mL	0.08	TSA 32.5ºC ± 2.5 ºC during 24h to 48 h
	P. aeruginosa ATCC 9027			0.075	
	B. subtilis ATCC 6633			0.150	
Fungi	*C. albicans* ATCC 10231	SDA 22.5 ºC ± 2.5 ºC during 2-3 days	1 x10^6 - 1 x10^7 CFU/mL	0.750	SDA; 22.5 ºC ± 2.5 ºC during 3 a 5 days
	A. brasiliensis ATCC 16406	PDA 22.5 ºC ± 2.5 ºC during 5-7 days	1 x10^6 - 1 x10^7 CFU/mL	1.3	PDA; 22.5 ºC ± 2.5 ºC during 3 a 5 days

To prepare the inoculum suspensions of bacteria and fungi it was added 9 mL of sterile diluent in a falcon sterile 15 ml tube and resuspended a sample from working culture with the aid of a sterile loop and by gently scraping it against the walls of the tube plunging it slightly into the thinner. The tube was shaken on a vortex mixer to homogenize the suspension and adjusted to the absorbance of the suspension using a diluent solution and by taking into account the values shown in **Table 4**.

From the initial suspension, 1:10 dilutions were made in diluent until the dilution of 10^{-4} for bacteria to 10^{-3} for fungi. The suspension was used within 2 hours after preparation.

Thus moved 1 g preparation to be tested into a tube containing 9 ml of neutralizer (everytime the effectiveness of the neutralizing wasn't demonstrated, a second dilution, 1:100, was performed from this) and left at room temperature for 30 ± 15 minutes. Subsequently 5 tubes were prepared, one for each test organism and inoculated with 100 µL of the suspension of each reference microorganism. A control was prepared by adding 100 µL of the suspension of each microorganism to 10 mL of neutralizer. All the preparations were vortexed and proceeded to inoculate 1 ml of each in duplicate for incorporation into culture media and incubation conditions suitable for each microorganism defined in **Table 4** above. The results of CFU are presented as the mean value of duplicate plates.

The method was considered validated when the number of CFU counted in 1 mL of inoculated sample was at least 50% of that obtained in the control (neutralizer solution inoculated with each microorganism) (26). For recovering rates below 50%, a second dilution in neutralizer was performed, in the same proportion, before inoculation with the testing microorganisms. Due to the nature of the formulations in study is plausible that the active principle inherent to these preparations has an intrinsic effect that potentiates the inhibition of microbiological contamination. Thus microorganisms are automatically inhibited by the intrinsic activity (IA) of the test formulation (28).

The absence of toxicity of the neutralizer upon these control microorganisms was confirmed by comparing the CFU counts recovered from the neutralizer and from buffered peptone water used as diluent, after inoculation in the same conditions (26).

2.4 Microbiological Quality of Compounded Medicines

In a sterile 15 mL tube was placed 1 g of the sample collected in the PD-CHCB and 9 ml of neutralizer was added. The methodology implemented in this microbiological quality study was adapted accordingly to the quantity of sample available for analysis, as instead of analyzing 10g sample in 90ml of neutralizing the proportion was reduced as described.The

mixture was left for 30 ± 15 minutes at room temperature in order to promote the preservative neutralization. All the preparations were vortexed and carried to the injection of 1 mL of in duplicate by incorporating the appropriate culture medium and incubation conditions: TSA for bacteria incubated aerobically at 32.5ºC ± 2.5ºC for 48 hours and SDA for fungi / yeast to 22.5ºC ± 2.5ºC aerobically for 3-5 days (26).

After the incubation period under the conditions and times mentioned above, the CFU count per plate was carried out, in duplicate. The results of CFU are presented as the mean value of duplicate plates and were compared with the Ph. Eur. 8.0 specifications for aqueous oral preparations and topical preparations (≤ $2x10^2$ for TAMC and ≤$2x10^1$ for TYMC), depending on the type of CM tested (26).

2.5 Descriptive Analysis

Subsequent to counting the number of CFU per plate, was performed to compare the duplicate calculating an average number of CFU per plate between formulations. This quantitative analysis of the number of CFU per plate allows to compare the stability of CM under review t0, to the BU and BU-AP.

Data were entered into Microsoft Excel software, proceeding to the construction of tables and figures.

Chapter 3

Results and Discussion

3.1 Method Validation Testing

The method validation tests were always conducted whenever new formulations for analysis, were received in a total of 27 different formulations studied.

After performing the assay the microbial counts corresponding to the number of microrganisms recovered from inoculated samples were compared to the control groups. Results show that the neutralizing system used (described in Chapter 2 and recommended by the Ph. Eur. 8.0) was suitable for the inactivation of the preservative action in most of the formulations under study. In some cases, a further dilution was necessary to inactive the preservative of the formulation (26). In **Table 5** it is possible to observe descriptively the dilutions for the tested formulations which have been validated.

Table 5 Description of the dilutions necessary to validate the procedure for each formulation.

Formulations		*S.aureus* ATCC 6538	*P. aeruginosa* ATCC 9027	*B. subtilis* ATCC 6633	*C. albicans ATCC 10231*	*A. brasiliensis* ATCC 16406
Intermediate Preparations for Oral Administration	SS	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	SSP	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	BiS	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	CoP	10^{-1}	10^{-2}	10^{-1}	10^{-2}	10^{-2}
	GM 1%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	CA 25%	10^{-1}	10^{-2}	10^{-2}	10^{-1}	10^{-1}
	V	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	BE	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
Oral Use	T	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	Pre	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	PreP	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	Nis	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-2}
	CH	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	A 0.5%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	Prop 0.1%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	N	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	UA 4%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	(A 5%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	O	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
Topical application	N/C	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	PP0.01%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	F/B	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	B/AS 2%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	B/AS 5%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
Disinfectant and Antiseptic	AA 3%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	CS 2%	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	I 5%	IA	IA	IA	IA	IA

Legend: IA - Intrinsic Activity

The neutralizing solution used both in validation tests and in the microbiological quality testing, was regularly subjected to determination of efficacy trials and toxicity against the tested microorganisms to ensure the validity of its application. This determination was carried out through the recovery of microorganisms in different analysis groups: Formulation Test, Control Group with Diluent and Control Group with Neutralizer (26). The comparison between the test formulation and Diluent Control demonstrate the efficacy of the

neutralizing whereas the comparison between the control and Diluent, and control with Neutralizing solution shows the absence of intrinsic toxicity towards the test organisms.

Regarding the group of Intermediate Preparations for Oral Administration, most formulations were validated for the 1:10 dilution However, Parabens Concentrate formulation has been validated in the second dilution to *P. aeruginosa ATCC 9027*, *C. albicans ATCC* 10231 and *A. brasiliensis ATCC 16406*. This result is consistent with the chemical constitution of the formulation and to the use of tis preparation as a preservative of CM.

Also the Citric Acid 25% (w / v) Aqueous Solution formulation was validated on the second dilution for *P. aeruginosa* and *B. subtilis* microorganisms. The inhibition of growth of the referred microorganisms is probably related to the active substance inherent to Citric Acid 25% formulation.

Concerning preparations for Oral Use Nystatin Oral Suspension formulation was validated in the second dilution to the fungus *A. brasiliensis*. The inhibition of growth of the referred microorganism can be related to the antifungal activity of Nystatin.

In the group of disinfectants / antiseptics, Iodine 5% (w / v) Aqueous Solution formulation it was not possible to recover the microorganisms according to the established method. Iodine, active principle of the formulation is probably related to the inhibition of growth of the referred microorganisms. Therefore the inhibition of growth of these microorganisms, either the first or the second dilution, is justified by those antiseptic / disinfectant properties. This formulations exhibits intrinsic IA against the tested microorganisms.

3.2 Microbiological Quality

From January to December 2014, 421 microbiological quality tests were performed, corresponding to 27 different formulations, according to the route of administration: 8 intermediate preparations for oral use, 11 solutions/suspensions for oral use, 5 topical products and 3 disinfectant/antiseptic preparations.

From January to September 2014, the formulations were tested for microbiological quality after preparation (t0) and at the end of BU. From October to December 2014 additional tests were performed upon expired samples returned to the ambulatory section of PD-CHCB. These tests allowed for the assessment of microbiological quality of these preparations, after the BU-AP, in “real life” conditions, as they had been used by the patient and health professionals.

3.2.1 Intermediate Preparations for Oral Administration

Regarding intermediate preparations used for the preparation of CM for oral administration 8 different types of formulations were analyzed, from January to September (**Table 6**), which achieved 100% compliance in both t0 and immediatly after the BU date, according to the specifications of Ph. Eur. 8.0 (**Figure 1**).

Table 6 Tested intermediate preparations for Oral Administration and number of batches tested for each formulation.

Formulations	No. of Batches t0	No. of Batches BU
Simple Syrup	13	13
Simple Syrup with Parabens	1	1
Sodium Bicarbonate 1,4% (w/v) aqueous solution	31	31
Parabens concentrated solution	1	1
Methylcellulose gel 1% (w/v)	1	1
Citric Acid 25% (w/v) aqueous solution	1	1
Vehicle for Oral Solutions and Suspensions	7	7
Banana flavoring 10% (w/v) aqueous solution	2	2

The results of the microbiological quality analyzes are presented in **Figure 1**.

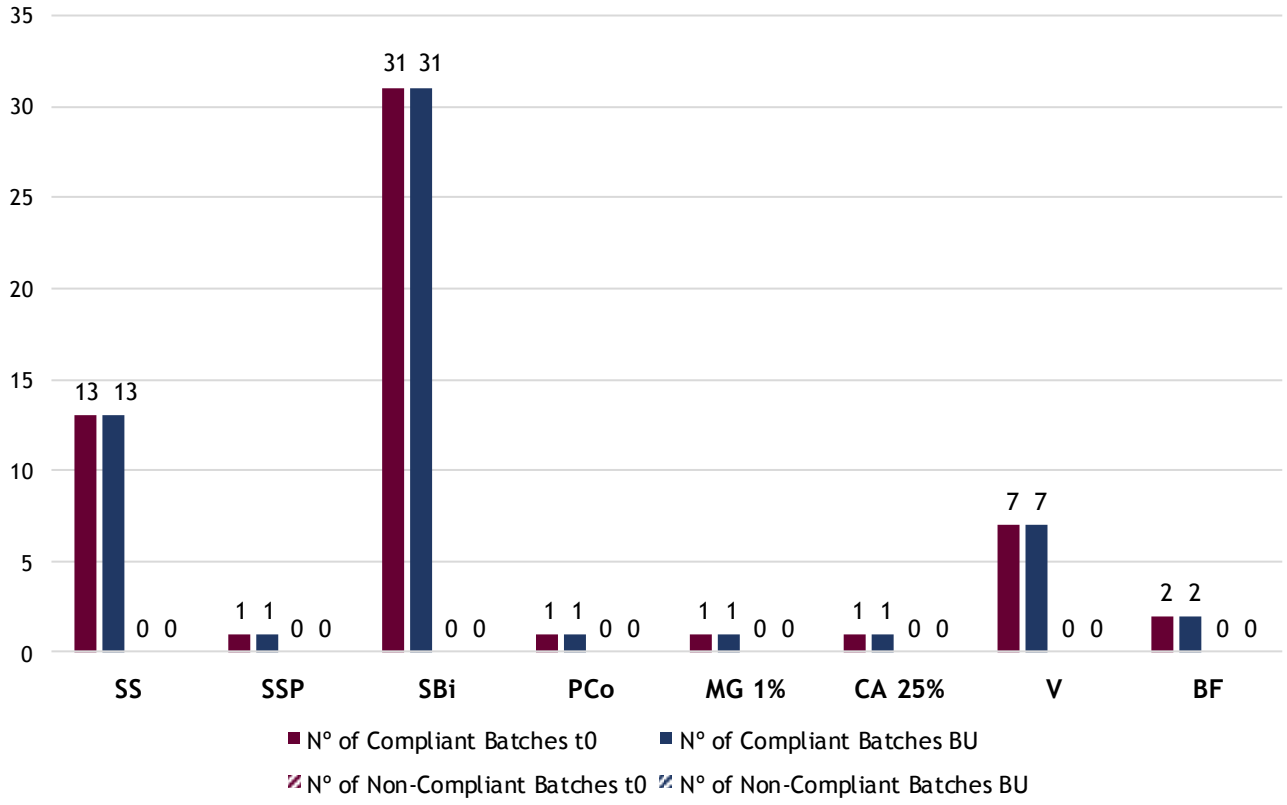

Figure 1 Microbiological quality results of intermediate preparations used for the preparation of compounded medicines for oral administration. Bars represent the number of batches classified as "compliant" or "non-compliant" according to the specifications of Ph. Eur. 8.0 for aqueous oral preparations.

Based on these results intermediate preparations were not further tested (from October to December) since the overall quality analyzes of the final preparations would also allow for indirect conclusions on the quality of intermediate ones.

Within the 8 formulations under study in this category, the Sodium Bicarbonate 1.4% (w/v) aqueous solution tested at t0 accounted for 2 batches with fungi count limit nearby the pharmacopeial limit Ph. Eur. 8.0 (Fungi 19 CFU < $2x10^1$ CFU TYMC), although still compliant with these specifications. Samples from the same batches did not show microbial counts at BU (Fungi 0 CFU <$2x10^1$ TYMC), sustaining the microbiological quality of the formulation. The remaining formulations presented scores within the established limits highlighting the microbiological quality of these formulations both after preparation and at the end of BU date.

3.2.2 Preparations for Oral Use

Regarding CM belonging to the class of solutions and suspensions for oral use, 11 formulations were tested at t0 and BU date. Also, Trimethoprim and Nistatine oral suspensions expired formulations returned from patients/health professionals were analyzed. **Table 7** described the formulations studied, as well as the number of batches analyzed at t0, BU and BU-AP

Table 7 Tested preparations for oral use and number of batches tested for each formulation.

Formulations	No. of Batches t0	No. of Batches BU	No. of Batches BU-AP
Trimethoprim 10 mg/mL oral suspension	12	12	2
Prednisolone 5 mg/mL oral suspension	16	16	0
Prednisolone 5mg/mL oral suspension preserved with Parabens	5	5	0
Nistatine oral suspension	39	39	1
Chloral Hydrate 10% (w/v) syrup	23	23	0
Amiodarone 0.5% (w/v) oral suspension	2	2	0
Propranolol HCl 0.1% (w/v) oral suspension	1	1	0
Nitrofurantoine 5 mg/mL oral suspension	3	3	0
Ursodeoxycholic Acid 4% (w/v) oral suspension	1	1	0
Ursodeoxycholic Acid 5% (w/v) oral suspension	2	2	0
Omeprazole 0.4% (w/v) oral suspension	4	4	0

The results of the microbiological quality analysis obtained for these formulations are presented in **Figure 2**.

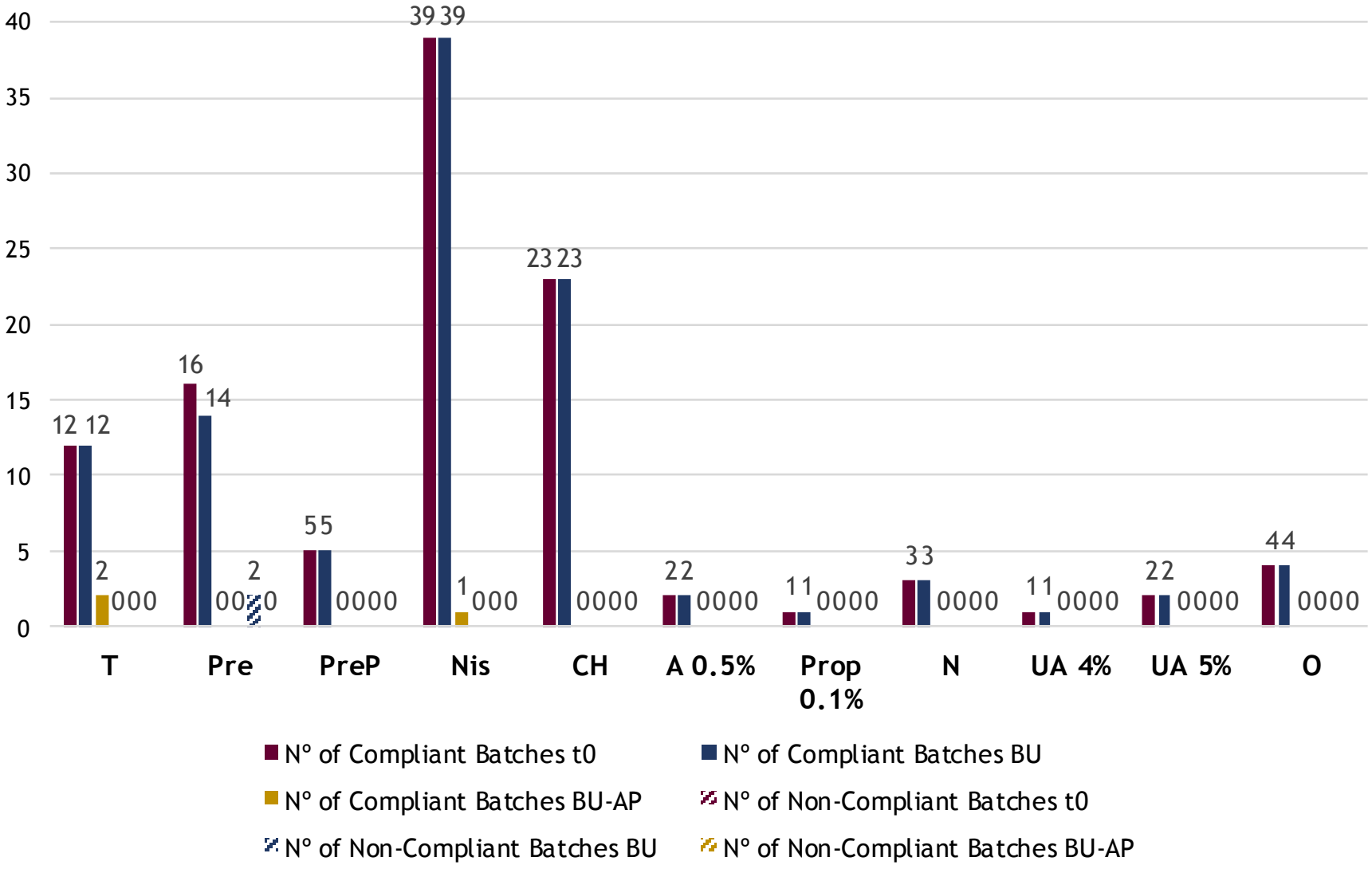

Figure 2 Microbiological quality results of solutions and suspensions for oral use. Bars represent the number of batches classified as "compliant" or "non-compliant" according to the specifications of Ph. Eur. 8.0 for aqueous oral preparations

As can be seen in **Figure 2**, 100% of batches analyzed at t0 and BU-AP were in compliance with the requirements of Ph. Eur. 8.0. However, at the end of BU Prednisolone 5 mg/ml oral suspension formulation presents two batches with scores above the limits established by Ph. Eur. 8.0 as well as one batch with scores in the limit (Fungi 20 CFU = $2x10^1$ CFU TYMC).

Prednisolone is the active ingredient of Prednisolone 5 mg/ml oral suspension formulation consisting of a glucocorticoid hydrocortisone derivative with anti-inflammatory and immunosuppressive properties that are used in the treatment of numerous pathological conditions. Because it is an aqueous formulation this preparation is particularly susceptible to microbiological contamination and in the absence of added preservatives, these formulations are even more vulnerable to microbial contamination due to successive openings of multidose containers used for packaging. Although this formulation is based on simple syrup, which contains a high concentration of sacarose excepted to ihibit microorganisms proliferation, this result highlights the importance of conducting microbiological quality tests in order to confirm the need of including, preservatives to guarantee the required microbiological safety (25). In consequence of these results the PD-CHCB replaced the original formulation of prednisolone by a formulation using a vehicle with parabens, (Prednisolone 5 mg/ml oral suspension preserved with Parabens), in order to avoid the risk of contamination during storage. As can be seen in **Figure 2**, this new formulation at both t0 and BU tests showed microbial counts within the limits of Eur. Ph. 8.0 demonstrating the suitability of the Parabens preservative power, on the stability of the formulation.

3.2.3 Topical Application Preparations

In this category 5 different preparations were analyzed during the 12 month study. Similarly o the procedure taken in the solutions and suspensions for oral use, analysis were performed for t0 and BU, as well as PD-CHCB samples.

The formulations studied, as well as the number of batches analyzed at t0, BU and BU-PA are presented in **Table 8**.

Table 8 Tested preparations for Topical Application formulations and number of batches tested for each formulation.

Formulations	No. of Batches t0	No. of Batches BU	No. of Batches BU-AP
Nitroglycerin 0.25% (w/v) + Cinchocaine 0.5% (w/v) ointment	**22**	**22**	2
Potassium Permanganate 0.01% (w/v) aqueous solution	**2**	**2**	0
Fusidic acid 2% (w/v) + Betamethasone 0.1% (w/v) ointment	**2**	**2**	0
Betamethasone 0.1% (w/v) + Salicilated Vaseline 2% (w/v) ointment	**4**	**4**	0
Betamethasone 0.1% (w/v) + Salicilated Vaseline 5% (w/v) ointment	**1**	**1**	0

The results of the microbiological quality analysis obtained for these formulations are presented in **Figure 3**.

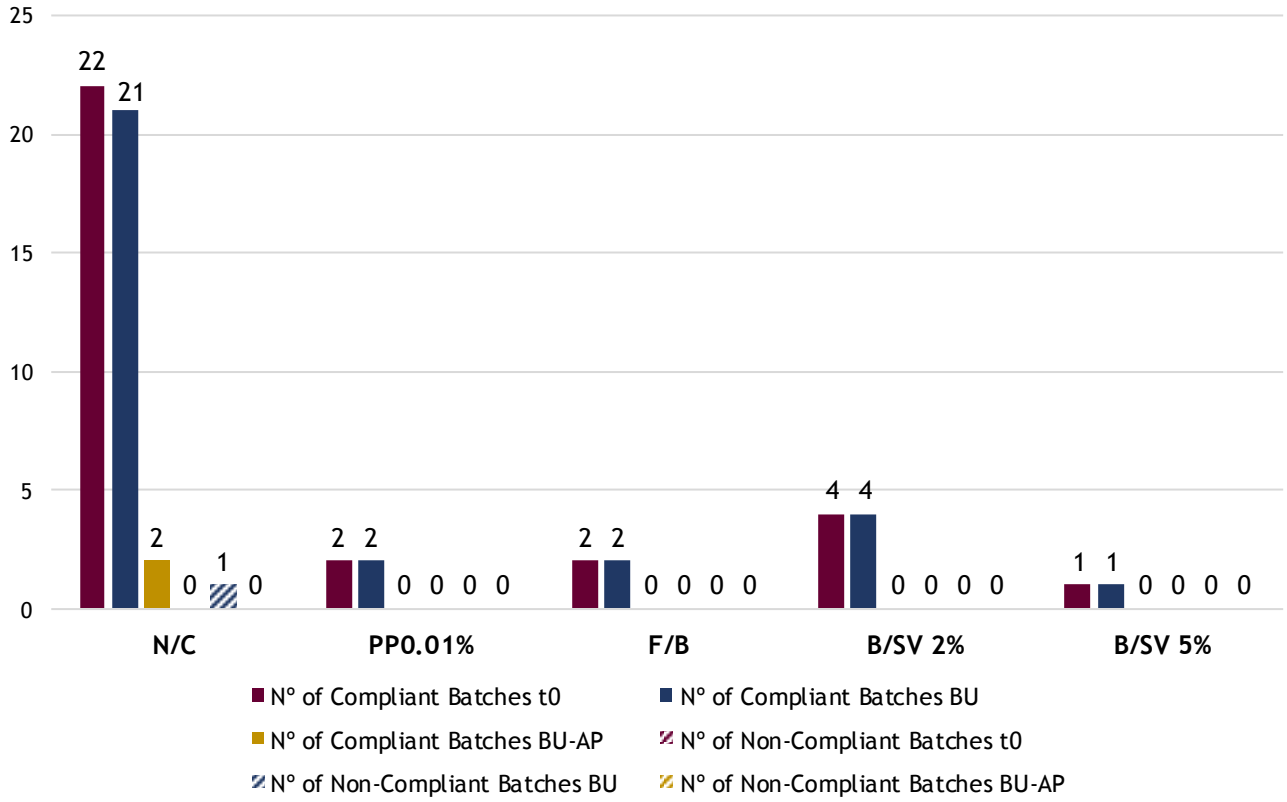

Figure 3 Results of microbiological quality control of formulations for Topical Application. Bars represent the number of batches classified as "compliant" or "non-compliant" according to the specifications of Ph. Eur. 8.0 for topical preparations.

Figure 3 shows 100% compliance for batches analyzed at t0, BU and BU-AP. These data sustain thet microbiological quality both after the BU and after the use by patients or at the hospital wards. However, for one batch of Nitroglycerin 0.25% (w/v) + Cinchocaine 0.5% (w/v) ointment formulation, CFU counts were above the limits established by the Ph. Eur. 8.0 the BU. Also, one batch BU presented counts veruy closer to the limits (Fungi 15 CFU < $2x10^1$ CFU). These results point to the need for greater vigilance regarding the CM microbiological quality of this formulation in order to assess the real need to improve the formulation. Despite the non-compliance has been verified in only 1 batch at the BU, it suggests the need to eventually redefine the validity period for this formulation or otherwise, the addition to the formulations of excipients that can improve its stability, like preservatives, in case these counts persist in further analyzes.

As this formulations l is prepared to be applied in damaged skinit is particularly relevant to avoid that microbial contamination may eventually have serious repercussions on the health of the patient which is already naturally weakened.

3.2.4 Desinfectant and Antiseptic Preparations

In the category of Disinfectant and Antiseptic 3 different formulations were tested during this study. Samples analyzed at both t0 and BU showes 100% compliance, according to the specifications of the Ph. Eur. 8.0. The formulations studied, as well as the number of batches analyzed at t0 and BU are described in **Table 9**.

Table 9 Tested Desinfectant and Antiseptic preparations and number of batches tested for each formulation.

Formulations	No. of Batches t0	No. of Batches BU
Acetic Acid 3% (w/v) aqueous solution	5	5
Colloidal Silver 2% (w/v) aqueous solution	3	3
Iodine 5% (w/v) aqueous solution	4	4

The results of the microbiological quality analysis obtained for these formulations are presented in **Figure 4**.

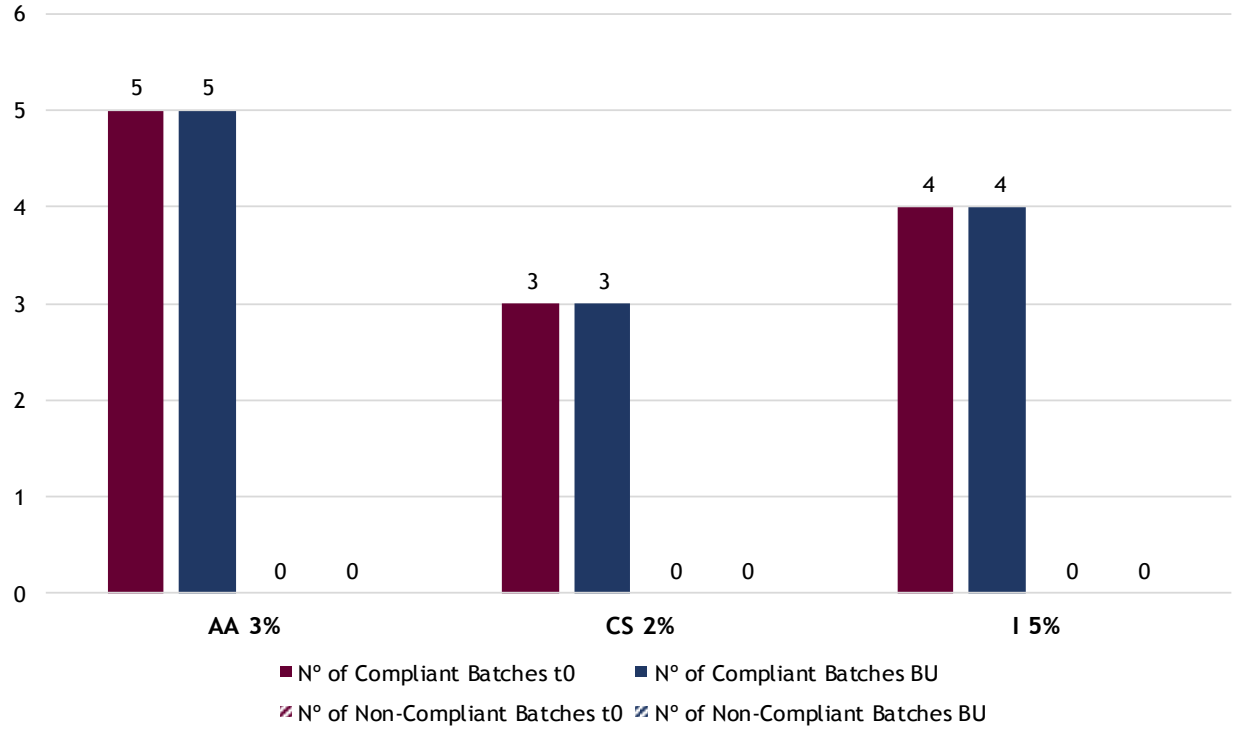

Figure 4 Results of microbiological quality control of the Desinfectant and Antiseptic preparations. Bars represent the number of batches classified as "compliant" or "non-compliant" according to the specifications of Ph. Eur. 8.0 for topical preparations.

The analyzed formulations of the disinfectant / antiseptic group always presented scores within the limits established by the Ph. Eur. 8.0. which demonstrates the microbiological quality of these products either after preparation and at the end of BU date.

As mentioned above, due to the nature of these formulations is plausible that the active substance inherent to these preparations has an intrinsic antibiotic effect that potentiates the inhibition of microbiological contamination. In fact, the antimicrobial effects associated to Acetic Acid 3% (w / v) aqueous solution (39), Colloidal Silver 2% (w / v) aqueous solution

(40) and Iodine 5% (w / v) aqueous solution (41) are described in the literature explaining the obtained results. Thus it can be concluded that the antimicrobial effect inherent to the active ingredient of these formulations guarantees the microbiological quality of the formulations ensuring their therapeutic effectiveness.

The overall results presented for the analyzes of microbiological quality control of CM highlight the adequacy of the procedures implemented in PD-CHCB concerning the quality and safety of these medicines. This department, which is certified according to the standard ISO 9001:2008, since 2011, has developed and implemented written procedures concerning, for example, control of equipments, restricted access to compounding laboratory, cleaning and management of material used for compounding, procedures of compounding, control of raw material and final preparations.

Chapter 4

Conclusions and Future Perspectives

The CM emerge as individualized therapeutic alternatives, as they constitute a mean to personalized therapeutics, complementing the therapeutic arsenal available by the pharmaceutical industry that fails to meet all the patient needs.

The preparation of CM in hospital and community pharmacies is an important factor in public health being urgent to ensure the quality and safety of these products. This requirement has been increasingly demonstrated due to various public health problems associated with the preparation of CM, specially those that require sterility.

The results from this study highlight the microbiological quality of non-sterile CM at the time of preparation demonstrating the adequacy of the procedures implemented in the PD-CHCB. Moreover, the evaluation of the microbiological quality of the preparations at the end of BU, made possible to identify needs to redefine the beyond-use datefor (Nitroglycerin 0.25% (w/v) + Cinchocaine 0.5% (w/v) ointment) or improve formulations increasing their quality and microbiological stability.

As the microbiological quality of the Intermediate Preparations for Oral Use formulations is reflected in the microbiological quality of the final preparations and in consequence of the results obtained after 9 months of analyzes, we decided to replace the intermediate formulations with samples received in the ambulatory service after being used by the patients or CHCB health professionals (in the wards). This strategy was certainly an added value to the study as we were able to measure the microbiological quality and effectiveness of these medicines throughout their route of use, from the time of preparation until the end of their use in normal conditions. Altgough the number os batches analyzed was limited the results pointed to adequate microbiological stability of these preparations. It would have been interesting to extend this study tomore batches in these conditions.

The analysis of certain disinfectants / antiseptic formulations may not be relevant when compared with other type of preparations, due to their intrinsic antimicrobial activity relevance towards another category of formulations.

In conclusion, within the 421 analysis of microbiological quality only 3 showed non accordance results with the requirements of Ph. Eur. 8.0. These results highlight the suitability of the GCP implemented in PD-CHCB and the microbiological safety of these medicines. The implementation of these procedures in other hospitals/community pharmacies may help other pharmacists to increase and assure the microbiological quality of compounded medicines.

The microbiological quality assessment of non-sterile CM products is a preventive strategy concerning public health that should be implemented in order to contribute with an added value to patient safety policies.

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The references were listed following the Vancouver style.

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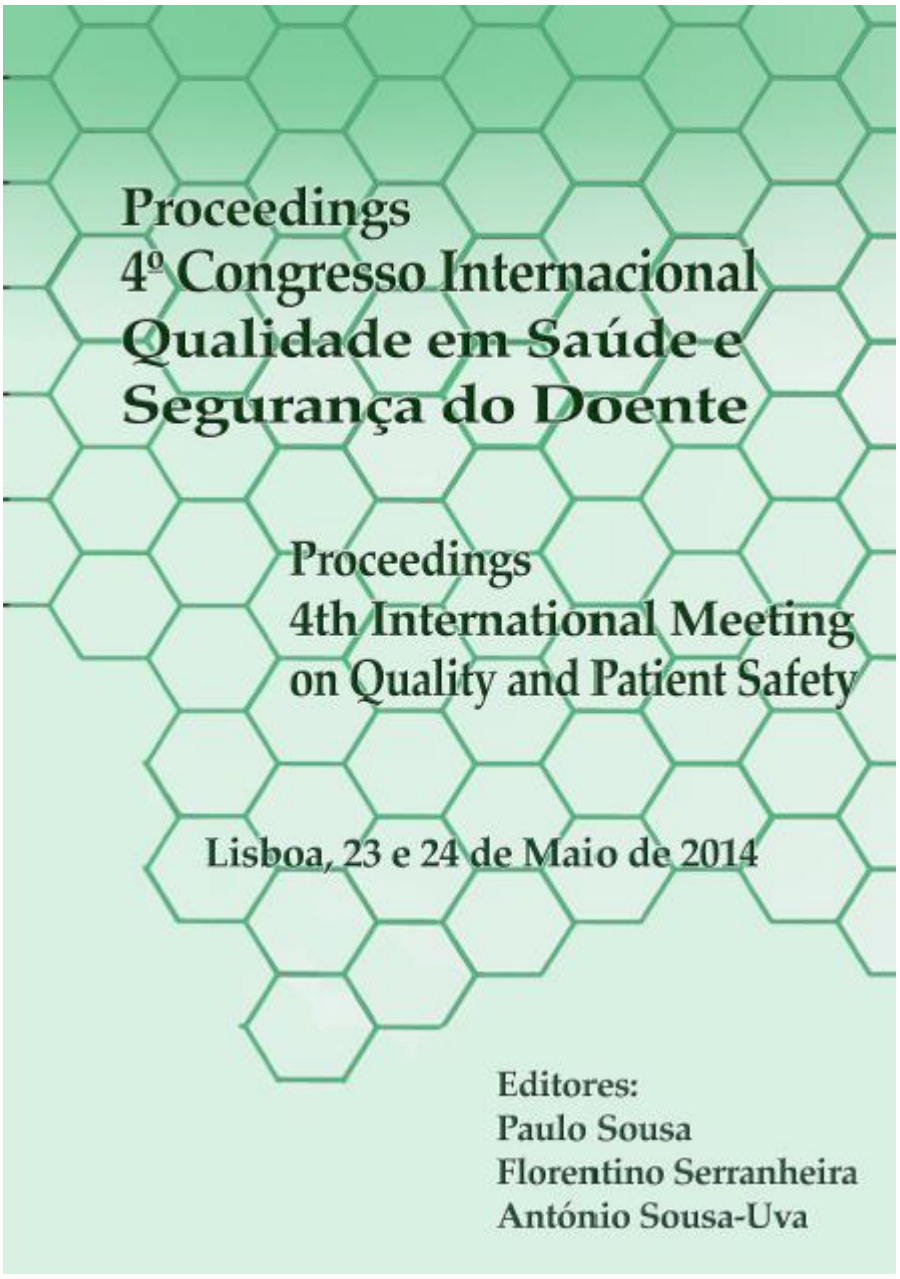
Attachments

Annex 1

Palmeira-de-Oliveira, Rita; Palmeira-de-Oliveira, Ana; Luís, Catarina; Bogas, Elisabete; Morgado, Manuel; Guardado, Mónica; Fonseca, Olímpia. "Controlo de qualidade microbiológica de manipulados não estéreis produzidos no Centro Hospitalar Cova da Beira, EPE". 4º Congresso Internacional de Qualidade em Saúde e Segurança do Doente. Lisboa. Maio 2014. **ISBN**: 978-989-20-4745-4.



Controlo de qualidade microbiológica de manipulados não estéreis produzidos no Centro Hospitalar Cova da Beira, EPE

Microbiological quality control of non-sterile products compounded in Centro Hospitalar Cova da Beira, EPE

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1. RESUMO

Os medicamentos manipulados dão resposta às necessidades terapêuticas de populações especiais e situações clínicas para as quais a indústria farmacêutica não disponibiliza produtos adequados. Estes medicamentos são preparados em farmácias comunitárias ou hospitalares estando sujeitos a testes de controlo de qualidade facilmente mensuráveis que não incluem a qualidade microbiológica das preparações. Este trabalho teve como objetivo avaliar a qualidade microbiológica das preparações não estéreis manipuladas no Centro Hospitalar Cova da Beira, EPE (CHCB), no momento da preparação e no término do prazo de validade atribuído, de acordo com as especificações da Farmacopeia Europeia 8.0. Entre janeiro e março de 2014, foram analisadas 71 preparações, correspondendo a 17 formulações diferentes (6 preparações intermédias, 7 soluções/suspensões para uso oral e 4 produtos para aplicação tópica). Todas as preparações apresentaram conformidade com as especificações da farmacopeia no momento da preparação. Contudo, as formulações «solução oral de prednisolona 5mg/mL» e «pomada de nitroglicerina 0,25% e cinchocaína 0,5%» apresentaram contagens de microrganismos superiores aos limites aprovados pela farmacopeia, no término da validade. Os resultados obtidos põem em evidência a adequação dos procedimentos implementados, nos Serviços Farmacêuticos do CHCB para garantir a qualidade microbiológica dos medicamentos manipulados. Adicionalmente verifica-se que a avaliação da qualidade microbiológica destas preparações representa uma ferramenta importante na redefinição de prazos de validade, no sentido de garantir a segurança da sua utilização.

ABSTRACT

Pharmaceutical compounding often represents the solution for therapeutic personalization whenever commercial alternatives are not available. These products are compounded in community and hospital pharmacies and undergo general quality control tests that do not include the microbiological quality evaluation. This work aimed to evaluate the quality of non-sterile formulations compounded at Centro Hospitalar Cova da Beira, EPE (CHCB)

immediately after preparation and up to the defined expiration date, in accordance to the European Pharmacopoeia (Eur.Ph.). From January to March 2014, 71 preparations were analysed corresponding to 17 different formulations (6 intermediate preparations, 7 oral solutions/suspensions and 4 topical preparations). All preparations were in accordance with the pharmacopoeial specifications immediately after preparation. However, for the formulations «Prednisolone oral solution (5mg/mL)» and «Nitroglycerine and cinchocaine ointment (0.25%/0.5%)» the results of microbial counts exceeded the defined limits after storage up to the expiration date. These results show that the compounding practices adopted by the PD-CHCB are able to assure the microbiological quality of compounded products. Also it has become clear that the microbiological quality control tests may be used to redefine the expiration date of formulations that have been shown not to be stable throughout the storage period, improving their safety of use.

Annex 2

Palmeira-de-Oliveira, R., Palmeira-de-Oliveira, A., Luís, C., Morgado, M., Guardado, M., Abrantes, A., Fernandes, A., Gonçalves, A., Ribeiro, M., Bogas, E., Nascimento, V., Augusto, R., Lages, M., Freire, I., Fonseca, O. "*Metodologia implementada no controlo de qualidade microbiológica de manipulados não estéreis no Centro Hospitalar Cova da Beira*". III Workshop de Qualidade em Saúde. Universidade da Beira Interior. Covilhã. 6 de Junho de 2014.

V Encontro de Investigadores da Qualidade

III Workshop da Qualidade em Saúde

Comissão Organizadora
Álvaro Rosa, ISCTE // António Ramos Pires, Instituto Politécnico de Setúbal // Henrique Lopes, Instituto Superior de Educação e Ciências // Luís Lourenço, Universidade da Beira Interior // Margarida Saraiva, Universidade de Évora // Patrícia Moura e Sá, Universidade de Coimbra // Paulo Sampaio, Universidade do Minho

SESSÕES DE COMUNICAÇÕES

MESA CS09 - KEYNOTE SPEAKERS (11:30 - 13:00)

Economia da Saúde e Qualidade: uma história de 35 anos de SNS virada para o futuro
Professor Doutor Henrique Lopes

Gestão do Conhecimento em Semântica de labelling de materiais de saúde
Professor Doutor Mário Macedo

Paradoxos da Regulação Social dos Processos da Qualidade em Saúde
Professor Doutor Carlos Silva

MESA I - TRABALHOS A CONCURSO (14:30 - 16:00)

Gestão da prevenção das Infeções associadas aos cuidados de saúde (IACS): contributos dos modelos ISO 9001 e a abordagem por Processos
Pilar Baylina // Paulo Moreira

Maturidade de um serviço de RX – CHLC
Cristina Almeida

Gestão do Movimento Hepático com Tomografia Computorizada a Quatro Dimensões: Revisão da Literatura
Flávia Neves Horácio // Margarida Eiras // Nuno Pimentel

Concordância na informação registada em notificação de queda e no processo clínico eletrónico
Elsa Guimarães // Joel Teixeira // Ana Azevedo

MESA II - TRABALHOS A CONCURSO (16:00 - 17:30)

A Qualidade em imuno-hemoterapia
Margarete Figueiredo

Política Qualidade no CHCB
Anabela Almeida

Incidentes e eventos adversos
Cristina Padez // Marisa Silva // Sandra Almeida

Metodologia implementada no controlo de qualidade de manipulados não estéreis no CHCB
Vários

Annex 3

Luís, Catarina; Palmeira-de-Oliveira, Rita; Palmeira-de-Oliveira, Ana; Gaspar, Carlos; Bogas, Elisabete; Morgado, Manuel; Guardado, Mónica; Fonseca, Olímpia. "Controlo de qualidade microbiológica de manipulados não estéreis: a experiência do centro hospitalar cova da beira". 7th Week APFH – 17th National Symposium. Associação Portuguesa de Farmacêuticos Hospitalares. Centro de Congressos do Estoril. Lisboa. Novembro 2014.

Controlo de qualidade microbiológica de manipulados não estéreis: a experiência do centro hospitalar cova da beira

Luís, Catarina [b]; Palmeira-de-Oliveira, Rita [a,b]; Palmeira-de-Oliveira, Ana [b]; Gaspar, C.[b]; Bogas, Elisabete [a]; Morgado, Manuel [a]; Guardado, Mónica [a]; Fonseca, Olímpia [b]

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INTRODUÇÃO
Os medicamentos manipulados permitem a individualização terapêutica por adequação da dosagem e/ou forma farmacêutica de um medicamento. Estes medicamentos são preparados em farmácias, de acordo com os requisitos legais das Boas Práticas a Observar na Preparação de Medicamentos Manipulados que determinam a obrigatoriedade de testes de controlo de qualidade facilmente mensuráveis neste nível de produção. Estes não contemplam a avaliação da qualidade microbiológica visto que não se encontra, geralmente, acessível à escala oficinal.

OBJETIVO
Avaliar a qualidade microbiológica das preparações não estéreis manipuladas nos Serviços Farmacêuticos (SF) do Centro Hospitalar Cova da Beira (CHCB) de janeiro a junho de 2014.

MÉTODOS
Foi estabelecido um protocolo entre o CHCB e o Labfit (spin-off da Universidade da Beira Interior dedicada à investigação, desenvolvimento e controlo de qualidade de produtos de saúde e cosméticos) para avaliação da qualidade microbiológica das preparações não estéreis segundo as especificações da Farmacopeia Europeia 8.0 (ensaio de «Qualidade Microbiológica de Preparações Não Estéreis»). As amostras foram recolhidas em material estéril e processadas no prazo de 72 h após a preparação tendo sido armazenadas nas condições preconizadas para cada formulação (temperatura ambiente ou 2-8ºC). Para cada lote foram também recolhidas amostras para análise no término de validade. Foram realizadas contagens de aeróbios totais e fungos/leveduras e confrontadas com as especificações da farmacopeia

para as preparações orais aquosas e preparações de uso tópico (≤ $2x10^2$ para aeróbios totais e ≤$2x10^1$ para fungos).

RESULTADOS

De janeiro a junho de 2014, foram realizadas 223 análises, correspondendo a 19 formulações diferentes (7 preparações intermédias, 8 soluções/suspensões orais e 4 produtos de aplicação tópica). Todas as preparações apresentaram conformidade com a farmacopeia no momento da preparação. Contudo, um lote de «solução oral de prednisolona 5mg/mL» e de «pomada de nitroglicerina 0,25% e cinchocaína 0,5%» apresentou contagens de microrganismos superiores aos limites, no término da validade.

DISCUSSÃO/CONCLUSÃO

Os resultados põem em evidência a adequação dos procedimentos dos SF do CHCB para garantir a qualidade microbiológica dos medicamentos manipulados. Para as formulações para as quais se verificaram não conformidades no prazo de validade será necessário avaliar os resultados de mais lotes para concluir sobre a necessidade de redefinição do seu prazo de validade. A avaliação da qualidade microbiológica de manipulados constitui uma ferramenta importante na garantia da qualidade e redefinição de prazos de validade, no sentido de garantir a segurança da sua utilização.

Oral communication distinguished by **APFH award 2014**.

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"*Controlo de qualidade microbiológica de manipulados não estéreis e a experiencia do Centro Hospitalar da Cova da Beira*" apresentada no

17º SIMPÓSIO NACIONAL DA APFH,

foi contemplada com o **Prémio APFH 2014** para

MELHOR COMUNICAÇÃO ORAL.

MABatista

(Dra. Aida Batista – Presidente APFH)

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