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Research Article

Microbiological Skip Test: Fundamentals and Evaluation of Water Activity in Pharmaceutical Ingredients

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Abstract

Quality control is essential for Good Manufacturing Practices in the pharmaceutical industry. The time and costs associated with microbiological quality control are well known. It is proposed the reduction of traditional microbiological methods of counting the total number of microorganisms and research of specific pathogens using the water activity (Aw) method, a practice known as the skip test. The raw material selected as a prototype for this study met the criteria for the feasibility of reductions in analyses having synthetic origins, solid physical state, low or high pH in aqueous solution, and Aw results below 0.75 at a temperature of 25°C, and absence of nonconformity records. The risk management process was implemented using risk analysis with the mode, effect, criticality, failure analysis tool, and opening change control with the replacement of microbiological standard by Aw in 20 sequential batches after complete analysis of the first batch received in the year. It was possible to certify the microbiological quality of the selected pharmaceutical ingredients using the AW results, thereby confirming the feasibility of the skip test.

Keywords: Manufacturing; Quality; Control; Pharmaceutical; System

Introduction

Quality is defined by the set of programming, coordination, and execution operations, the purpose of which is to evaluate and ensure that medicines comply with the quality standards required by regulatory agencies. To obtain quality pharmaceutical products using pharmacopeial standards, the entire production chain must be monitored, that is, raw materials, qualified equipment, facilities, and professional staff [1-3].

To reach the objectives, the regulatory agencies in human health advocate norms to be strictly complied with by manufacturers, as in Brazil, the Agência Nacional de Vigilância Sanitária, Resolução de Diretoria Colegiada (RDC) no. 658, March 30, 2022, and the General Guidelines for Good Manufacturing Practices for Medicines in the pharmaceutical industry [4].

Among the quality control analyses, microbiological investigations are mandatory. In non-sterile pharmaceutical products, microbial levels are limited to guarantee harmlessness to human health and inertia of the medicine [5,12,13].

The standard method used in microbiological quality control laboratories for the analysis of raw materials and non-sterile drugs includes counting the total number of mesophilic microorganisms to determine the total number of mesophilic bacteria and fungi. The investigation of pathogenic microorganisms in selective culture media was also carried out according to official analysis compendiums [6-9].

Microbiological analyses of raw materials, specific laboratory conditions, material resources, and specialized labor are necessary to guarantee consistent results with the microbiological quality of the sample. In addition, the time dedicated to the complete series of analyses is highlighted, which is often incompatible with industrial market demand and financial investments. For instance, according to Pharmacopeia Brasileira 6 ed., it is necessary to research the specific pathogens *Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*, which means the use of additional selective methodologies and a dedicated time of approximately three days [6].

Given the importance of microbiological research in the context of the pharmaceutical industry and in compliance with laboratory logistics, it was proposed to reduce the methods of microbiological analysis by counting the total number of mesophilic microorganisms and researching pathogens, a practice already known as the skip test. Water activity (Aw) in the raw materials was determined according to the criteria established by the United States Pharmacopeia (USP). In addition to raw materials, products such as tablets obtained by direct compression, capsules, ointments, rectal suppositories, and non-aqueous liquids are suitable for performing the skip test by Aw [7,8,18]

This practice is based on the evidence of microbiological growth at minimum levels of water in the materials; that is, for

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Table 1: Minimum values of water activity (AW) required for the growth of representative microorganisms.

Bactérias	Atividade de Água (Aw)	Bolores e Leveduras	Atividade de Água (Aw)
Pseudomonas aeruginosa	0.97	*Rhyzopus nigricans*	0.93
Bacillus cereus	0.95	*Mucor plumbeus*	0.92
Clostridium botulinum, Tipo A	0.95	*Rhodotorula mucilaginosa*	0.92
Escherichia coli	0.95	*Saccharomyces cerevisiae*	0.90
Clostridium perfringens	0.95	*Paecilomyces variotti*	0.84
Lactobacillus greeniscens	0.95	*Penicillium chrysogenum*	0.83
Salmonela spp	0.95	*Aspergillus fumigatus*	0.82
Enterobacter aerogenes	0.94	*Penicillium liso*	0.81
Bacillus subtilis	0.90	*Aspergillus flavus*	0.70
Micrococcus lysodekticus	0.93	*Aspergillus niger*	0.77
Staphylococcus aureus	0.86	*Zygosachharomyces rouxii (levedura osmófila)*	0.62
Halobacterium halobium (bactéria halofílica)	0.75	*Xeromyces bisporus (fungos xerofílicos)*	0.61

Fonte: USP, 2023.

microbiological growth to occur, limiting levels of water activity are required, as described in Table 1 [7,18].

Aw is the measure of the free water in a given sample, which is determined by the ratio between the vapor pressure of water in the sample and the vapor pressure of pure water at a constant temperature [8]. In the microbiological skip test process by Aw, the determinations were made by the activity analyzer equipment to quantify the free water in raw materials or pharmaceutical products subject to the implementation of the reduction, and that meet the following criteria: be of synthetic origin, present lower Aw to 0.75 at a temperature of 25°C, have a low or high pH value measured in aqueous solution, and do not contain surfactants, nutrients, and chemical agents that inhibit the growth of microorganisms [6].

To reduce or replace microbiological tests with water activity, risk analyses must be carried out using the Q9 guide of the International Conference on Harmonization (ICH), which addresses quality risk management with technical justifications or change control for due modification, according to the impact that the replacement of the method will cause in the context of quality control [10].

The implementation of skip tests is applicable in several laboratory areas to enable the reduction of analyses, reduce analytical errors, implement new technologies, reduce the costs of materials used, and reduce re-analyses in the laboratory.

Therefore, a reduction in microbiological pharmacopeial tests in active pharmaceutical ingredients and excipients was proposed based on the results of Aw in the pharmaceutical industry in Brazil.

Materials and Methods

To conduct the microbiological skip test, nine pharmaceutical ingredients of synthetic origin were selected, as recommended. For each raw material, a survey of the results of the quality control of the last 10 batches was conducted, observing the microbiological reports in particular, and the existence of Nonconformity Reports (NCR). The pH data of the sample in aqueous solution were also observed.

The evaluation of Aw in detriment of the reduction in the frequency of microbiological tests was carried out in two batches of each raw material, the first and the last being valid and available for future reference (sufficient samples of raw materials and products kept in storage to allow the future analysis of the product) [4].

The pilot samples, their respective manufacturing and expiration dates for each batch, and the manufacturer information are listed in (Table 2).

Table 2: Pharmaceutical ingredients selected to reduce the frequency of microbiological tests.

Raw material	Fabrication	Validity	Manufacturer
Ascorbic acid	Sep-20	Sep-23	Northeast Pharmaceutical Group
	Nov-21	Nov-24	
Aspartame	Jan-22	Jan-25	Sinosweet co., ltd
	Mar-22	Mar-25	
Twilight Yellow Dye	Jun-23	Jun-27	Sencient colors s.a
	Nov-21	Nov-27	
Crospovidone	Jun-20	Jun-23	sp Chemical llc
	Apr-22	Apr-25	
Cumarine	Nov-21	Nov-23	Iffect Chemphar co., ltd.
	Dec-21	Dec-24	
Omeprazole Pellets 8,5%	Jan-21	Oct-23	Cornileus Pharmaceuticals
	May-23	Feb-25	
Micronized polyethyleneglycol 6000	Jun-21	Jun-23	Polioles s.a. de c.v.
	Jun-22	Jun-24	
Paracetamol 90%	Oct-20	Oct-23	Anqiu lu'an Pharmaceutical co, ltda
	Jul-22	Jul-25	
Povidone K-30	Oct-20	Oct-23	Isp Chemicals LLC
	Oct-21	Oct-24	

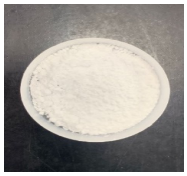
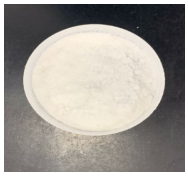
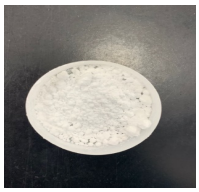
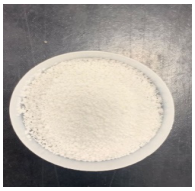
Aw was determined using a water activity analyzer Aqualab model, manufactured by Pawkit. The equipment was calibrated with a standard solution of lithium chloride (0.250 Aw, 13.41 mol/kg) and sodium chloride (0.760 Aw, 6.0 mol/kg). The analyses were performed in triplicate, and the results were expressed as the mean of the results with the respective standard deviation (s). The tests were performed using approximately 1g of sample. Each analysis lasted for 5 min. According to FB 6 ed, the Aw specification is 0.75 at 25°C; therefore, in raw materials whose value is above the specification, the analysis reduction cannot be carried out and the traditional microbiological analysis will remain.

Results and Discussion

The chosen pharmaceutical ingredients were solid and did not present potential conditions for spore germination or microbiological growth. Descriptions of the chosen samples and their respective illustrative photos are presented in Table 3.

In the survey of information on the last 10 batches of each raw material, there was no RNC opening record. The microbiological quality control obtained by the analyses certificate of the last 10 batches of raw materials did not show results close

Table 3: Physical state, aspect, and illustrative photo of the raw materials.

Raw material	Physical State - Appearance	Illustrative photo
Ascorbic acid	Solid - Fine, white, or slightly yellowish crystalline powder	
Aspartame	Solid - White or almost white crystalline powder	
Twilight Yellow Dye	Solid - Fine powder, reddish-orange	
Crospovidone	Solid–white to creamy white powder	
Cumarine	Solid-crystal, white	
Omeprazole Pellets 8,5%	Solid – Spherical, white, or off-white balls	
Micronized Polietilenoglicol 6000	Solid – Odorless white solid, creamy consistency, in the form of powder or flakes	
Micronized polyethyleneglycol 6000	Solid – White granules	
Povidone K-30	Solid - White to slightly cream powder	

to the limits of microbiological contamination, according to the specifications established by FB 6 ed [6] (Table 4).

According to the certificate of analysis provided by the manufacturer, all raw materials met the pH determination criterion in the aqueous solution, that is, low or high, not close to the neutrality value.

Analyses were performed on the first future reference sample (valid batch) and the last future reference sample (valid batch) for each selected raw material. The results are presented in (Table 5). Note that all samples have less than the limit specification of 0.75 Aw at 25°C, one of the criteria for applying the microbiological skip test [6].

According to the criteria predefined by the current regulation and the results obtained in the Aw evaluation, the selected raw materials for the implementation of the skip test in the industry were subject to the risk management process. Risk assessments are necessary to carry out change control related to analysis reductions, as required by regulations [3].

For the implementation of the skip test, an assessment of the criticality of change was performed for each raw analyzed material, based on prior knowledge of the routine of the production processes, observing the situation of the complete microbiological analysis of all batches received, and proposals for changes to replace batch-to-batch microbiological analyses.

It is up to the regulatory affairs sector to document the process of the microbiological skip test in the industry, before the regulatory Agency in Brazil, Anvisa.

According to RDC 73/2022, the implementation of the analysis reduction for pharmaceutical ingredients can be immediate, and does not require an individual protocol, Product Change History (HMP), or a document available in the company in which information regarding the annual history of the product and document is sent to Anvisa [5].

The score for the criticality assessment ranges from 01 to 05 for the classification of items considered critical (impact on safety, product quality, effectiveness, employee safety, legal requirements, impact on facilities, qualification/validation, impact on processes/systems, and documentation). The sum of the points determines the criticality classification, considering a weighting system for the items considered most critical [15].

Risk analyses were performed using the quality tool Analysis of Mode, Effect, Criticality, and Failure (AMECF), considering the possible risks of implementing the skip test [14].

For this study, the criticality analysis of the raw materials in the skip test implementation process obtained a final score with a medium impact, making it feasible to carry out the risk analysis.

After this process, the industry requests Change Control (CC) according to Good Manufacturing Practices for Medicines (GMP) [4,5]. The open CC for the implementation of the microbiological skip test of the selected raw materials indicates the

Table 4: Results of the microbiological quality control of the selected raw materials referring to the last 10 batches.

Total count of mesophilic microorganisms and pathogen research	
Limits	**Total count of mesophilic microorganisms and pathogen research**
≤2000 UFC/g for bacteria	accordingly
≤200 UFC/g for fungus	
Absence de *Escherichia coli, Pseudomonas aeruginosa* e *Staphylococcus aureus* (1g)	

Fonte: Brazil (2019)

Table 5: Result of water activity (AW) of the pilot samples, first and last sample of future reference, expressed as the mean of the three measurements and respective standard deviation (s).

Sample	Aw (s)	Standard Deviation (s)
Ascorbic acid	0.34	±0.006
	0.33	±0.006
Aspartame	0.38	±0.006
	0.25	±0.01
Twilight Yellow Dye	0.03	±0.01
	0.03	±0.006
Crospovidone	0.47	0
	0.09	±0.006
Cumarine	0.46	±0.006
	0.49	±0.038
Omeprazole pellets 8,5%	0.44	±0.063
	0.42	±0.078
Micronized polyethyleneglycol 6000	0.52	±0.01
	0.51	±0.01
Paracetamol 90%	0.64	±0.01
	0.62	±0.006
Povidone K-30	0.18	0
	0.19	±0.006

methodology that will be changed, the proposed change, the sectors involved, and the impact assessment of the change request in each sector.

The CC is in progress, individually for each raw material; however, crospovidone and ascorbic acid are already approved with CM, awaiting the disposal of skip test implementation actions by the sectors involved, which confirms the viability of this practice in the pharmaceutical industry.

Conclusions

The raw materials selected for this pilot study met the criteria for reduction and/or replacement of microbiological pharmacopoeial analyses, which can be applied to several pharmaceutical ingredients that can be used by the industry.

The Aw results indicate the precision and accuracy of the method used, which was confirmed to be an analytical alternative to expensive traditional methods.

It was possible to prove the feasibility of reducing microbiological analysis and skipping the test by evaluating Aw in pharmaceutical ingredients according to GMP guidelines, attesting to the microbiological quality of the batches for entry into the production flow.

The AMECF tool for risk analysis proved to be adequate in the context of the pharmaceutical quality system, an action foreseen by GMP. It emphasizes the importance of individual development after the completion of the protocol and issuance of the report, proving that the raw materials are suitable for the reduction of analyses.

The reduction of microbiological analyses by the determination of Aw is a reliable laboratory strategy that is compatible with the industrial demand required by the pharmaceutical market, without compromising the safety of its products.

This pilot study confirmed the potential of microbiological skip testing, which can be gradually applied with due risk management protocols.

Author Statements

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Author Contributions

Natália Silvério de Freitas: Investigation, Formal analysis, Roles/Writing - Original draft, Investigation, Methodology, Validation.

Adriana Nascimento Sousa: Writing - review & editing

Hussain Ullah: Writing - review & editing

Wagner da Nova Mussel: Data curation, Roles/Writing: Original Draft

Maria Betânia de Freitas-Marques: Conceptualization; Data curation, formal analysis, Investigation; Methodology; Validation; Visualization; Roles/Writing; original draft and project administration.

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