THE APPLICATION OF CLEANING VALIDATION PRINCIPLES ON DIETARY SUPPLEMENTS PRODUCTION EQUIPMENT

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Cleaning validation for pharmaceutical production equipment is a documented proof of the efficient cleaning, and one of prerequisites of good manufacturing practice in medicine production. Successful validation confirms the efficiency of the procedures of cleaning, washing, and disinfecting of the manufacturing equipment, and records results of the chemical and microbiological analyses, which are a prerequisite for a safe final dietary product.

The main goal of this study was to improve the cleaning process of the production equipment by using cleaning validation procedures on the solid form production line (capsules) in the Abela Pharm d.o.o. The validation principles that are used in manufacturing of medicines can be applied to determine more efficient cleaning methods that will ensure longer periods of the status clean in the production of dietary supplements. The outcome is a practical analysis of the production equipment in view of regulatory demands, confirming that the cleaning validation measures ensure prevention of unwanted microbial growth or removal of contamination from the production equipment in order to preserve the activity, efficacy, and safety of the final dietary product.

KEY WORDS: microbiological criteria, cleaning validation, dietary supplements

INTRODUCTION

Modern day pharmaceutical manufacture meets strict regulatory and practical demands regarding employee hygiene standards, work environment and cleanliness of production equipment (1, 2, 3). Expert teams that organize and manage pharmaceutical production implement hygiene principles, and afterwards monitor chemical and microbiological parameters that document efficacy of cleaning and disinfection processes in practice. All hygienic precautionary measures are taken in order to achieve the production of safe pharmaceutical product of standard quality that contains active components together with excipients without any impurities or microorganisms that might disturb its activity or affect badly on consumer health (4).

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In pharmaceutical practice, hygienic standards are implemented by the use of Good Manufacturing Practice, and are realized with the appliance of cleaning validation principles for the production of medicines. Cleaning validation in pharmaceutical manufacturing is a documented proof that the cleaning procedures repeatedly remove residues of previous products or cleaning agents up to maximum allowable scientifically and regulatory confirmed limits (1, 5).

Cleaning validation principles are obligatory in the manufacture of medicines, and they are also used in the production conditions that possess HVAC system (heating, ventilation and air-purification systems). In this research, validation principles are used as an additional data in regard to relevant literature, and are applied for non-HVAC production line system for the production of dietary supplements (5).

All preliminary activities and practical procedures of validation are used to define adequate routine activities that will in practice guarantee hygienic sustainment on the used equipment. Therefore, they represent prerequisites of continual work that conforms to the relevant regulatory demands, and have safe and efficient final pharmaceutical product as the main result. In order to obtain reliable data by analyzing chemical and microbiological parameters of equipment cleaning process, often needs the implementation of certified cleaning and disinfecting agents. If the process of harmonizing with the regulatory demands leads to the larger savings of the production resources, (bigger and more efficient use of equipment, space and employees), then multiple goals of safe and efficient production are achieved together with the preservation of natural resources. These multiple goals, as defined, serve as a starting point for the introduction of cleaning validation on multifunctional capsule production line in pharmaceutical company Abela Pharm d.o.o. Research conducted during August, 2015. was supposed to show the effects of implementation of cleaning protocols with the use of certified cleaning and disinfecting agents, in order to create a more efficient cleaning method and a longer lasting duration of status “clean” on the production equipment.

**EXPERIMENTAL**

Risk analysis for Bulardi ® Probiotik capsules was performed together with sampling plan, and alongside with a validation cleaning protocol for the multifunctional capsulizing equipment, prior to experimental work. Risk analysis for all products manufactured on a multifunctional solid dosage form equipment for the manufacture of capsules included comparing results for: solubility as a risk factor, pharmacology as a risk factor and formulation as a risk factor (2, 6, 7). Moreover, different sampling places were chosen for direct sampling (microbiological smear) and indirect sampling (chemical washings) on the production equipment in order to determine status “clean/dirty”. Considering above mentioned risk factors for products manufactured on the production equipment for solid dosage form, a worst case was discovered to be Bulardi® Probiotik, capsules as it contains *Saccharomyces boulardii*, a probiotic yeast insoluble in water, with many residues which could lead to microbiological contamination of equipment and therefore contamination of the next product manufactured on the same production line.
Because of this reason, new work procedures were devised for cleaning and disinfecting of equipment. They included reports and recommendations from certified manufacturers of cleaning and disinfecting agents used in pharmaceutical industry, as well as the results of the try-out experiments of cleaning and disinfection of samples manufactured on the production line for solid dosage forms- capsules (8, 9).

This study compares results of cleaning and disinfection of multipractical capsulizing equipment using ethanol, 70% solution (Ethanol assessment) and using certified cleaning and disinfecting agents (Validation assessment) while performing defined cleaning validation principles (8,9). In order to properly carry out the comparison, both examinations included tests for worst case scenario previously discovered by risk analysis on the same capsulizing machine.

Three consecutive batches of the worst case product of the same size were analyzed in both experiments. The same sampling places were used on the equipment for microbiological smears and chemical washings (Table 1). Samplings were defined to be representative for cleaning process of all parts of the equipment (10). Parts of equipment especially exposed to product or hard to reach were chosen for microbiological smears, whereas chemical sampling was performed from hardly accessible points on the equipment.

Table 1. Microbiological and chemical sampling spots on production equipment for solid dosage forms

<table>
<thead>
<tr>
<th>Equipment name</th>
<th>Number of sample points</th>
<th>Type of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixing machine</td>
<td>3</td>
<td>MB1, MB2</td>
</tr>
<tr>
<td>Blistering machine</td>
<td>3</td>
<td>MB3, MB4, MB5</td>
</tr>
<tr>
<td>Capsulating machine</td>
<td>10</td>
<td>MB6, MB7, MB8, MB9, MB10, MB11, MB12</td>
</tr>
<tr>
<td>Tools</td>
<td>2</td>
<td>MB13</td>
</tr>
<tr>
<td>Total:</td>
<td>18</td>
<td>13</td>
</tr>
</tbody>
</table>

First phase of testing included the analysis of the sampling places after cleaning/disinfection of equipment with ethanol, 70% solution, performed immediately before the production (up to 1h) of three consecutive batches of Bulardi® Probiotik capsules (Ethanol assessment).

Validation plan for the second phase of testing was devised with certified cleaning agents- alkaline detergent (potassium hydroxide with nonionic surfactants), alkaline disinfectant without aldehydes (dodecyl dimethyl ammonium chloride and n-(3-amino-propyl)-n-dodecylpropane-1,3-diamine) and acidic disinfectant based on hydrogen peroxide and peracetic acid (8). Parameters of analysis were determined during the method of cleaning validation, as well as the acceptable limits and status time of “clean” and “dirty”. A competitive cleaning validation (during the routine production of product in-
tended for market placement) (11) was performed on three consecutive batches of Bulardi® Probiotik, capsules, with the same batch size and with careful documentation of work phases.

During the second phase of testing, samples with the status “dirty”, that lasted for 8h±2h, were collected, as well as samples with status “clean”, which lasted 12h±4h after the application of determined validation principles of cleaning (Validation assessment).

This research provided comparison of results with status “clean” during both phases of testing.

MATERIAL AND METHODS

Material and methods of visual inspection

Before the sampling process of equipment parts began, dry and well-lit surface was observed from multiple angles to assess work surface (12).

Material and methods of ATP monitoring

Identification of biological and/or organic material on equipment was preliminary tested using the fast ATP bioluminescence method, via ATP smears (ATP apparatus Lumitester PD-20, LuciPac Pen, Kikkoman, Japan). ATP smears (bioluminescence test for adenosine triphosphate) determine total microbiological burden or in other words, the presence of bacteria, yeasts, molds, biofilm formations or organic residues. Cleanliness status by bioluminescence was assessed based on results of ATP measurements and the result was given in relative light units (RLU). The results of ATP smears confirm that the presence of total microbiological burden: bacteria, yeasts (such as Saccharomyces boulardii), and other organic material after cleaning and disinfection process are within acceptable limits (13, 14). This non-selective preliminary analysis quantifies any microbiological burden on the production equipment. Therefore, it is used as a primary method for the assessment of the efficacy of cleaning and disinfection processes.

Material and methods of the assessment of purified water chemical washings (HI)

A total of 100 ml of purified water was sampled from final washings of inaccessible parts of the equipment, and was tested for (10):
1. pH value- determined by potentiometric technique (15)
2. electroconductivity- determined (µS/cm at 20°C) by conductometric method (16)
3. total and composite alkaline properties- determined by titrimetric method (17)

Material and methods of microbiological testing (MB)

1. Sterile cotton wool or synthetic material- smears were used for microbiological sampling (alginate or rajone) (18) after the addition of sterile saline solution. After the sampling, a smear was placed inside a test tube containing sterile sali-
ne solution. With each set of samples, a negative control was also delivered to the laboratory (*blank sample*). Surface area used for sampling was 100 cm$^2$.

A total count of aerobic mesophile bacteria was done at 30 °C, using horizontal method for determination of microorganism count via surface inoculation technique (19, 9, 14).

2. Determination of the count of *Enterobacteriaceae* was done by horizontal method for determination and identification of *Enterobacteriaceae* via colony-count approach (20).

**RESULTS AND DISCUSSION**

**Ethanol assessment**

During the first assessment, visually inspected spots were sampled on the production line treated with ethanol, 70% solution. Microbiological burden was determined afterwards by ATP smears, and chemical washings and microbiological smears were tested immediately before the production process began (status “clean” after 1h- Table 1). Visual inspection previously determined that the surface equipment was clean, whereas the results of ATP assessment were \( \leq 200 \) RLU. The results of chemical sampling and average values obtained after the production of three consecutive batches of Bulardi® Probiotik, capsules are graphically presented in Figures 1 and 2.

![Figure 1](image_url)

**Figure 1.** Graphical view of pH values of chemical washings HI1-HI5 during *Ethanol assessment* (average values of three consecutive results)
Immediately before production began (up to 1h) microbiological smears were sampled and aerobic mesophile bacteria count was assessed. Obtained results are shown as a graphical image in Figure 3. Results for the samples of microbiological smears for *Enterobacteriaceae* are not graphically presented, and they all equaled less than 1 CFU/cm².

**Figure 2.** Graphical view of conductivity results of chemical washings HI1-HI5 during *Ethanol assessment* (average values of three consecutive results)

**Figure 3.** Graphical view of Aerobic mesoph. bacteria count in microbiological smears MB1-MB13 during *Ethanol assessment* (average values of three consecutive results)
Validation assessment

Second phase of analysis included the effects of cleaning after the production of three consecutive batches of Bulard® Probiotik, capsules. Same sampling places from the first testing were used (Table 2). However, certified cleaning agents were used this time (8) after the status “clean” that lasted 12h± 4h.

Visual inspection of the equipment was found to be clean. The results of chemical washings are shown in Figures 4 and 5, whereas the results of microbiological smears—aerobic mesophile bacteria count are shown in Figure 6. Results for the samples of microbiological smears for Enterobacteriaceae are not graphically presented, and they all equaled less than 1 CFU/cm² (2, 14).

Figure 4. Graphical view of pH values of chemical washings HI1-HI5 during Validation assessment (average values of three consecutive results)

Figure 5. Graphical view of conductivity results of chemical washings HI1-HI5 during Validation assessment (average values of three consecutive results)
Figure 6. Graphical view of Aerobic mesoph. bacteria count in microbiological smears MB1-MBI3 during Validation assessment (average values of three consecutive results)

Testing of final products

Samples of final products for three consecutive batches of Bulardi® Probiotik, capsules during validation assessment, as well as samples of the following final product (Herbiko PropoMucil, capsules) manufactured on the same production line, were tested for microbiology (bacteria count, yeast and mold content) and heavy metal content, according to the regulations for dietary supplements in the Republic of Serbia (5).

Cleaning validation principles were applied in this research during the manufacturing conditions without HVAC system.

All of the obtained results, as stated in literature, were analyzed and assessed according to the regulatory requirements for acceptable contamination levels (2, 5, 10, 12,).

Table 2 describes the acceptance criteria for visual and analytic method used during the first and second phase of testing.
Table 2. Acceptance criteria for visual and analytic method

<table>
<thead>
<tr>
<th>Acceptance criteria</th>
<th>Acceptance criteria limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual acceptance criteria</td>
<td>• cleaned, dry and well illuminated area observed from different sides</td>
</tr>
<tr>
<td>ATP monitrong criteria</td>
<td>• fast determination of microbiological burden with ATP smears: ≤200 RLU (21).</td>
</tr>
<tr>
<td>Chemical acceptance criteria</td>
<td>• acidic and alkaline properties: no change in color after the addition of indicators: methyl orange or bromthymol blue (or pH value between 5.0-7.0) (22)</td>
</tr>
<tr>
<td>Microbiological acceptance criteria</td>
<td>• aerobic mesophile bacteria: ≤ 10 CFU/cm² and Enterobacteriaceae from 0 to 1 CFU/cm² (2, 3, 14).</td>
</tr>
</tbody>
</table>

Comparing the results obtained during Ethanol assessment and Validation assessment of chemical and microbiological parameters the following was concluded:
- the results obtained in Ethanol assessment and Validation assessment show large differences regarding the duration period of status “clean”. With Ethanol assessment status clean equaled 1h, while in Validation assessment it equaled 12h ± 4h.
- chemical burden samples (acceptance criteria for pH value equaled from 5.0 to 7.0 and acceptance criteria for conductivity equaled no more than 4.3 µS/cm, measured on 20°C) present slightly higher results of conductivity for samples obtained during Validation assessment that points out the need for a more detailed rinsing of equipment due to the difficult removal of certified cleaning agent residues in contrast to the easy removal of residues of ethanol.
- microbiological burden of smears for aerobic mesophile bacteria (acceptance criteria of less than 10 CFU/cm²) and for Enterobacteriaceae (acceptance criteria of less than 1 CFU/cm²) were within specified limits on all samples, after performed cleaning process and as well as after defined time of status “clean”. These results comply with the relevant literature (14). MB1 sampling place during Ethanol assessment presented a relatively higher microbiological burden (bioburden) probably due to insufficient action of used disinfecting agent (10).
- the results of chemical washi ng and microbiological smears obtained from all three batches were inside the allowable limits. The absence of any residues of active components of Saccharomyces boulardii was also confirmed, as well as the absence of residues of cleaning agents, lubricants, and other potential contaminants on the manufacturing equipment. Cleaning procedure with certified cleaning agents was deemed appropriate for the worst-case final product, and for all other products manufactured on the same production line for solid dosage forms without HVAC system. It was also confirmed that
cross-contamination could not be achieved after performing the defined manufacturing procedures. - microbiological results obtained during Ethanol assessment presented higher values of standard deviation, while the results obtained during Validation assessment show a slightly smaller values of standard deviation (first phase of testing showed 6 results to be above 1 CFU/cm², whereas the second phase of testing showed only three results above 1 CFU/cm²). These results led to a conclusion that higher microbiological efficacy of cleaning was presented after the appliance of specified validation protocol, and also - better uniformity of the process itself. (10).

- microbiological burden, or aerobic mesophilic bacteria count in Validation assessment, presented the highest values in the following sampling places: MB4, MB6, and MB10, most probably due to a slightly lesser bactericidal and bacteriostatic actions of the disinfecting agents on less accessible places od sampling.(10)

- using validated process of cleaning and disinfection with certified cleaning agents has confirmed that the status period of “clean”, lasted longer than previously defined time period. Microbiological burden of sampling places was slightly improved.

- one-time wash and disinfection achieved its goal of production of safe products, together with a more rational resource management in production process. (10)

- the obtained results confirmed that the addition of certified detergents and disinfectants contributes to a more efficient cleaning process, avoids the cleaning and disinfection of the equipment immediately before the production and extends the status time of “clean”. Status time of “clean” was extended from 1 hour to 12 hours ± 4 hours.

- Samples of final products of three consecutive batches of the product Bulardi® Probiotik, capsules during Validation examination, as well as samples of the next final product (Herbiko PropoMucil, capsules) manufactured on the same production line presented microbiological results (bacteria contents, yeast and mold count) and heavy metal content to be within limits of defined regulatory aspects in the Republic of Serbia; the absence of any residues of active components of Saccharomyces boulardii, as well as the absence of any residues of the cleaning agent, lubricants, and other potential contami-nants on the manufacturing equipment.

CONCLUSION

Cleaning validation was implemented by a multidisciplinary team of Abela Pharm d.o.o that contributed to a better understanding of the process in details, thus enhancing the production process for capsulated dietary supplements. The efficacy and status time of “clean” after the cleaning and disinfection process of the equipment with 70% ethanol was compared with cleaning and disinfection process with certified pharmaceutical industry agents.

The primary objective of the cleaning validation process has been achieved. Cleaning procedure and status time of “clean” and “dirty” were validated after three consecutive batches for the selected worst case product during which every documented potential contaminants were reduced to predetermined levels of acceptance (18). The obtained
results confirmed that the addition of certified detergents and disinfecting agents contribute to a more efficient cleaning process and status time “clean” was extended which has proved to be a contributing factor to a more rational management of resources in the production without HVAC system and equipment, space and employee engagement were better utilized.

Quality and safety of all dietary supplements manufactured on the production line for solid dosage forms was confirmed in this research. Routine use of this validated process will be continued in order to produce pharmaceutically active, efficient and safe final products. Abela Pharm d.o.o. professional team will continue to implement cleaning validation and revalidation procedures on the production line for the manufacture of capsulated dietary supplements.

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**ПРИМЕНА ПРИНЦИПА ВАЛИДАЦИЈЕ ЧИШЋЕЊА НА ОПРЕМИ ЗА ПРОИЗВОДЊУ ДИЈЕТЕТСКИХ ПРОИЗВОДА**

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Валидација чишћења опреме у фармацевутској производњи је документовани доказ успешног чишћења и представља један од захтева добре производњачке праксе у производњи лекова. Успешна валидација потврђује поступке прања, чишћења и дезинфекције опреме и документује резултате хемијских и микробиолошких анализа који су предуслов чисте опреме и безбедног финалног дијететског производа. Циљ овог рада је унапређење чишћења процесне опреме применом поступка валидације чишћења на производној линији за производњу дијететских производа тј.
чврстих форми- капсула у Abela Pharm д.о.о. Принципи валидације чишћења се пре свега примењују у производњи лекова. Резултати валидације добијени у овом истраживању представљају практичну анализу процеса чишћења производне опreme које је усклађено са регулаторним захтевима. Финална потврда успешно обављене валидације чишћења су резултати који потврђују превенцију нежељеног микробног раста и уклањање потенцијалне контаминације са производне опреме у циљу очувања активности, ефикасности и безбедности дијететског производа.

Кључне речи: микробиолошки критеријуми, валидација чишћења, дијететски производи

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