

CLEANING VALIDATION AND RISK MANAGEMENT IN MANUFACTURING PHARMACEUTICALS FORMS WITH POTENT ACTIVITY

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Abstract

Cleaning validation in the manufacture of pharmaceutical forms is a critical step in implementing the rules of Good Manufacturing Practice (GMP). This process is very important because a drug may be contaminated with dangerous substances.

In this paper we validated the cleaning process of the production equipment in the technological process in which we obtained two semisolid topical anti-inflammatory drugs with different pharmacological activity, a topical non-steroidal anti-inflammatory drug (NSAID) (phenylbutazone) and an active potent steroid (clobetasol propionate) on the same production line, without compromising safety, quality and effectiveness, without the risk of cross contamination, ensuring quality production of both drugs.

Research can then be put into practice in the pharmaceutical industry for the manufacture of drugs with potent activity in the same facilities and can be extrapolated to other drugs belonging to the same class.

Rezumat

Validarea curățeniei în fabricarea de forme farmaceutice reprezintă unul dintre punctele critice în implementarea Regulilor de Bună Practică de Fabricație (RBPF). Acest proces este foarte important deoarece o formă farmaceutică poate fi contaminată cu substanțe periculoase.

În lucrare am urmărit să validăm procesul de curățare a echipamentelor de producție din cadrul unui proces tehnologic în care am obținut două medicamente antiinflamatoare semisolide topice cu activitate farmacologică diferită, un antiinflamator nesteroidian topic (fenilbutazonă) și un corticosteroid puternic activ (propionat de clobetasol) pe aceeași linie tehnologică, fără a pune în pericol siguranța, calitatea și eficacitatea acestora, fără a exista riscul contaminării încrucișate, asigurând calitatea fabricării celor două medicamente.

Rezultatele cercetărilor pot fi puse apoi în practică în industria farmaceutică pentru fabricarea de forme farmaceutice cu activitate puternică pe aceeași linie tehnologică și pot fi extrapolate și la fabricarea altor forme farmaceutice aparținând aceleiași clase farmaceutice.

Keywords: cleaning validation, pharmaceutical forms, technological process, activity potent, quality risk management.

Introduction

According to the rules of Good Manufacturing Practice (GMP) “The sites and equipment must be situated, designed, created, adapted and maintained in order to comply with the operations which must be performed. Their position and design must reduce to a minimum level the risks of errors and must allow an efficient cleaning and maintenance in order to avoid the cross contamination, the deposits of dust or dirt and, generally, any unwanted effect on the products’ quality.” [1].

The concept of quality applied to a product includes all the properties and characteristics through which it meets the needs of the users [22].

In order to reduce to a minimum the risk of severe medical accidents caused by the cross contamination, the manufacturing of certain drugs, such as: certain antibiotics, certain hormones, certain cytotoxic drugs, certain strongly active drugs or non-medicamentary products must not be made in the same facilities. For these products, in exceptional cases, the principle of the company activity in the same facilities may be accepted only if special cautions are taken and necessary validations are performed [1].

For economic reasons, it is not profitable for a manufacturer to dedicate a separate technological line only for the manufacturing of a strongly active drug. In such a case, the strongly active drug should be manufacture on the same technological line according to the company activity rules, but with special cautions and with all the necessary validations checked.

In this work, we present the results of the cleaning validation of the equipment within a manufacturing technological line where there were also manufactured two topic semisolid anti-inflammatory drugs with different pharmacological activity, containing a nonsteroidal anti-inflammatory drug and a potent corticosteroid, applying the risk management related to the facilities’ hygiene and cleaning the manufacturing equipment. According to the limits imposed by admissibility and to the results obtained following the performance of the cleaning process validation, the manufacturing of the two drugs within the same technological line will be considered acceptable or not.

In order for this issue to be accepted and authorised, we had to implement the quality management system in drugs’ manufacturing and to prove that within the same manufacturing section, within the same

technological line, it is possible to develop two drugs with different pharmacological activity, insuring the quality of the activities performed, of the manufacturing process and implicitly the quality of the end product. Therefore end the products obtained will comply with the purpose they were created for, they will be compliant with the exigencies provided in the marketing authorisation and will not expose the patients to any risk caused by deficiencies concerning their safety, quality and efficiency.

Materials and Methods

The cleaning validation of the manufacturing equipments was made on a technological manufacturing line of topic semisolid forms within a GMP certified drug company [1, 6, 7, 8].

It was studied the manufacturing of two pharmaceutical products with substances which belong to two different therapeutic classes: a topic nonsteroidal anti-inflammatory drug (AINS) under the pharmaceutical form of *cream* and a strong corticosteroid (SC) under the pharmaceutical form of *unguent*.

The cleaning procedures were established considering the physical and chemical properties of active substances and of the pharmaceutical form which is manufactured with these manufacturing equipment [16].

The total removal of the traces of active substances and of cleaning agent was argued by the physical, chemical and microbiological analyses imposed.

Selecting the product after having performed the cleaning validation

The selection method of the two drugs after having performed the cleaning process validation was made according to the principle “the worst case”, using the “bracketing” method.

The objective of the “bracketing” method was to demonstrate that there is a scientific justification for the “worst case” of evaluation of the drugs in the cleaning validation programme of the manufacturing equipment. The first thing that was made was to develop groups and subgroups – that we called “bracketing”, out of which we subsequently selected “the worst cases” according to the evaluation results. In the case of the drugs evaluated in order to select the “worst case”, the “bracketing” method was applied for the following data: the activity of the active substance; the amount of the active substances, the size of the product manufacturing series; the solubility of active substances in water and in a solution of NaOH 0.1 mol/L; the method of dispersion of the active substance in the cream / unguent base.

We took as reference the solubility of active substances in water and in a solution of NaOH 0.1 mol/L, because the cleaning of the manufacturing equipment was performed mainly with alkaline detergents and water [5].

Applying the “bracketing” method, we selected a potent corticosteroid – *clobetasol propionate* and a topic nonsteroidal anti-inflammatory drug – *phenylbutazone*, representing the “worst case” of the therapeutic class they belong to, from the list of drugs prepared within the same manufacturing section at the date when we initiated this study.

We have chosen to make the cleaning validation after the manufacturing of the two drugs selected from therapeutic classes and with different pharmacological activities in order to demonstrate the fact that the consecutive manufacturing on the same technological line of the two drugs is possible and accepted, it does not influence and does not endanger the quality of products and does not present any risk.

The cleaning agents

The liquid detergent used to degrease the tools contains: alkaline ammonium salt (organic acid), sodium hydroxide, phosphates (sodium salt), glycol ether polyethylene alkyl amine (C₁₂₋₁₈), alkyl ether (C₁₂₋₁₈) polyethylene glycol (< 8 ethylene glycol (EO) molecules) – polypropylene glycol, purified water.

The disinfectant used contains: hydrogen peroxide, acetic acid, peracetic acid, 1- hydroxy ethane diphosphonic acid and purified water and is efficient against a wide range of microorganisms: bacteria, yeasts, moulds, spores, viruses.

Selecting the sampling points and the sampling technique

The sampling points were chosen taking into account the surfaces of the equipment coming into contact with the product which is being manufactured. The sampling was made *after every rinsing cycle* (step I, II and III) with purified water for the physical and chemical analyses and for the microbiological analyses.

The techniques used for sampling were *the tampon method* and the *rinsing method*. The tampon method was used for plane surfaces, and the rinsing method was used for the areas difficult to access [15, 20].

Acceptation criteria [18, 19]

Limits for microorganisms:

- for the total number of germs/cm² = 1 CFU (colony forming unit)/1 cm²
- for pathogenic microorganisms = ABSENT

Limits for traces of active substance = ABSENT

Limits for traces of cleaning agents:

- determining the traces of disinfectant by determining the conductivity = less than 4 ppm
- determining the traces of disinfectant by “analytic strip test for peroxide” = less than 1 ppm
- determining the traces of disinfectant (titrimetric) = ABSENT

Methods of analysis for the quantitative determination of active substance traces

Method of analysis of the clobetasol propionate – HPLC method

The quantitative determination of the traces of clobetasol propionate was made by an HPLC method, with UV detection, at the wavelength $\lambda = 240$ nm [9, 10, 11, 17].

After sampling, the sterile hydrophobic cotton tampon was brought in a measuring bottle of 10 mL. The tampon was washed with 2 mL of methanol, and then was brought to sign with the mobile phase.

The reference solution 0.02 % was obtained by dissolving 20 mg of *SC active substance* in mobile phase and was diluted at 100 mL with mobile phase.

Chromatographic conditions: it was used an HPLC device Agilent 1100, a liquid chromatograph under pressure provided with UV lamp, recorder, automatic system of integration of the peak areas. It was used a chromatographic column of $l=150\text{cm} \times \text{Ø}=4.6\text{mm}$, of stainless steel, with stationary phase of octadecylsilyl silica gel for chromatography (R), C18 Zorbax Eclipse XDB with the diameter of the internal particles of 5 μm .

The mobile phase A was obtained by mixing 10 volumes of methanol (R), 42.5 volumes of a solution 7.85 g/L solution of *monosodium phosphate monohydrate* (R) which was adjusted to pH – 5.5 with a solution of 100 g/L of sodium hydroxide (R) and 47.5 volumes of *acetonitrile* (R).

The flow was of 1.0 mL/min, with the UV detection at the wavelength $\lambda=240$ nm, at room temperature. The volumes of samples and reference solutions for injection were of 10 μL . The time of analysis was the time of retention multiplied by 3 for the active substance.

The analysis method of phenylbutazone – HPLC method

The quantitative determination of the phenylbutazone traces was made by an HPLC method, with UV detection, at a wavelength of $\lambda = 254$ nm [12, 13, 14].

After sampling, the sterile hydrophobic cotton tampon was brought into a volumetric flask of 100 mL. The tampon was washed 4 times, each time with 20 mL of methanol, then was brought to sign with methanol. 2.5 mL of this solution was transferred in a volumetric flask of 25 mL and was filled to sign with the mobile phase.

Standard solution: 50 mg of SC active substance were dissolved into 50 mL of mobile phase. The solution was brought to volume, at 100 mL, in a volumetric flask with mobile phase and the *A solution* was obtained. 2.5 mL of A solution were brought to volume, at 25 mL, in a volumetric flask with mobile phase.

Chromatographic conditions: For the HPLC method it was used a liquid chromatograph Agilent 1100 under pressure provided with a UV lamp, a recorder, an automatic system of integration of the peak areas. It was used a chromatographic column of 150 x 4.6 mm, of stainless steel, with Zorbax Eclipse XDB C8 octyl silane solid phase (C8), the diameter of the internal particles of 5 μm and with the mobile phase consisting of a mixture of HPLC acetonitrile and bidistilled water in a ratio of 75:25 (v/v), and at 100 mL of mobile phase it was added 0.1 mL of orthophosphoric acid (R). The flow was of 1 mL/min, with UV detection at the wavelength $\lambda = 254\text{nm}$, at a temperature of 25°C. The volumes of the samples for injection were of 20 μL . The retention time was of 2.75 minutes.

The microbiological evaluation

The evaluation of the microbial contamination was made according to the European Pharmacopoeia: the assay of the total number of microorganisms (aerobic bacteria) and the determination of the total number of yeasts and filamentous fungi. Microbiological examination of non-sterile products: microbial enumeration tests; *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Microbiological examination of non-sterile products: test for specified microorganisms [3].

Each pharmaceutical product should be submitted to predictive microbiology studies on the behavior of microorganisms under different physical, physicochemical or chemical conditions such as temperature, water activity, pH, or antimicrobial compounds [21].

The analysis methods for the quantitative determination of the traces of disinfectant agent

Three methods were applied in order to detect the traces of disinfectant, namely: the determination of conductivity, the titrimetric method and the determination of the peracetic acid – “analytic strip test for peroxide”.

Determination of the disinfectant traces by determining the conductivity

After having measured the sample conductivity by means of the conductivity meter InoLab730, the result was introduced in the equation: $y = 0.159 \cdot x + 0.3705$, where y is the conductivity in $\mu\text{S/cm}$ and x is the concentration of P3-cosa in ppm.

Determination of the disinfectant traces by using the titrimetric method

The sample was titrated with a solution 0.1N sodium thiosulphate until the disappearance of blue colour. The results were expressed according to the following equation: the volume of sodium thiosulphate 0.1N used for titration $(n/10) \times 0.064 = \% \text{ disinfectant}$.

Determination of the traces of disinfectant by determining the peracetic acid "analytic strip test for peroxide"

The strip test was deeply introduced in the sample solution for 1 second and then was drawn out of the solution the excess of liquid was removed and the reaction zone was compared visually with the colour scale after 15 seconds.

Results and Discussion

There were performed 6 types of determinations in order to verify the cleaning of the manufacturing equipment of topic semisolid forms, namely:

Quantitative determination of the traces of active substance (SC and NSAID) - by HPLC method.

For ***clobetasol propionate*** – on the chromatogram, at the time of retention of the active substance (14.3 minutes), detection signal appeared (under the limit of detection), but other signals appeared under 13 minutes, maybe because of the traces of disinfectant detected, according to the results obtained and recorded; the detection of the active substance traces during the *steps I, II and III* of rinsing was within the imposed limits.

For ***phenylbutazone*** – on the chromatogram, at the time of retention of the active substance (2.75 minutes), one detection signal appeared during the *step I*, and for the *steps II and III*, there was no other detection signal (under the limit of detection), only the specific signal for the injection peak at 1.5 minutes according to the results obtained and recorded.

Determination of the total number of germs/cm² or /mL and of the pathogenic microorganisms:

- the total number of germs/cm² and /mL was within the limits admitted for the *steps I, II, III* and no pathogenic microorganisms developed during the *steps I, II, III*;
- for the samples used by the *rinsing method*, it was made an analysis of the purified water before introducing the parts of the device. As we may notice, the results' values are almost identical, so the purified water did not change the microbiological parameters – *the total number of germs/mL and pathogenic microorganisms* – after rinsing.

Determination of disinfectant traces – titrimetric method

- for *step I*, when adding sodium thiosulphate 0.1N, the aqueous solution bleached, which indicated the fact that the solution presented traces of disinfectant, and for the *steps II and III*, the aqueous solution did not change the blue colour, which indicated the fact that the solution did not present traces of disinfectant.

Determination of disinfectant traces – by determining the conductivity

- for *step I*, the conductivity was above the limit admitted (more than 4 ppm), and for the *steps II and III*, the conductivity diminished, which led to results within the limit admitted (less than 4 ppm).

Determination of disinfectant traces – analytic strip test for peroxide – by the semi-quantitative determination of the traces of peracetic acid

- it was noticed a slight bluish coloration of the strip test for the *step I*, corresponding to the standard strip 1.3 ppm – above the limit admitted
- it was noticed that for the *steps II and III* the strip test did not change the colour, even after 20 minutes (less than 0.8 ppm).

(Tables I, II, III, IV).

Table I

The results of the microbiological, physical and chemical determinations in case of the pharmaceutical form with phenylbutazone by the *method of rinsing*

Analysis	Admitted limits	Results			
		Sample no. 5 (dosing systems)			
		Purified water	Step I	Step II	Step III
Total no. of germs/ ml	>100 CFU/1 mL	3	5	4	5
Pathogenic germs <i>Escherichia Coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas sp.</i>	Absent Absent Absent	Absent Absent Absent	Absent Absent Absent	Absent Absent Absent	Absent Absent Absent
Determination of traces of active substance – phenylbutazone HPLC method)	Under 10 ppm	14.1		Under the limits of detection	Under the limits of detection
Determination of traces of disinfectant (conductivity)	Under 4 ppm	4.59		1.44	0
Determination of traces of cleaning agent – “analytic strip test for Peroxide”	Under 1 ppm	> 1.3		< 0.8	< 0.8
Determination of traces of disinfectant (titrimetric method)	Absent	2.56 ppm		Absent	Absent

Table II

The results of the microbiological, physical and chemical determinations in case of the pharmaceutical form with clobetasol by the *method of rinsing*

Analysis	Admitted limits	Results			
		Sample no. 5 (dosing system)			
		Purified water	Step I	Step II	Step III
Total no. of germs / mL	>100 CFU/1 mL	3	4	5	5
Pathogenic germs <i>Escherichia Coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas sp.</i>	Absent Absent Absent	Absent Absent Absent	Absent Absent Absent	Absent Absent Absent	Absent Absent Absent
Determination of traces of active substance – clobetasol propionate – HPLC method	Absent	Under the limits of detection		Under the limits of detection	Under the limits of detection
Determination of traces of disinfectant (conductivity)	Under 4 ppm	3.33		0.81	0
Determination of traces of cleaning agent – “analytic strip test for peroxide”	Under 1 ppm	> 1.3		< 0.8	< 0.8
Determination of traces of disinfectant (titrimetric method)	Absent	1.28 ppm		Absent	Absent

Table III

The results of the microbiological, physical and chemical determinations in case of the pharmaceutical form with phenylbutazone by the *tampon method*

Analysis	Admitted limits	Results			Results			Results			Results			Results		
		Sample no. 1 (preparation vessel I)			Sample no. 2 (transfer pump I)			Sample no. 3 (preparation vessel II)			Sample no. 4 (transfer pump + dosing cone)			Sample no. 6 (tampon vessel)		
		Step I	Step II	Step III	Step I	Step II	Step III	Step I	Step II	Step III	Step I	Step II	Step III	Step I	Step II	Step III
Total no. of germs / cm ²	<1CFU/cm ²	0.18	0.13	0.08	0.22	0.27	0.25	0.16	0.21	0.13	0.2	0.13	0.2	0.22	0.27	0.17
Pathogenic germs <i>Escherichia Coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas sp.</i>	Absent Absent Absent	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.
Determination of traces of active substance – phenylbutazone HPLC method)	Less than 10 ppm	14.7	Under the limit of detection	Under the limit of detection	16.3	Under the limit of detection	Under the limit of detection	20.1	Under the limit of detection	Under the limit of detection	17.9	Under the limit of detection	Under the limit of detection	14.7	Under the limit of detection	Under the limit of detection
Determination of traces of disinfectant (conductivity)	Less than 4 ppm	3.96	0.81	0	4.59	1.44	0	3.33	0.18	0	3.96	1.44	0	4.59	0.81	0
Determination of traces of cleaning agent – “analytic strip test for peroxide”	Less than 1 ppm	>1.3	<0.8	<0.8	>1.3	<0.8	<0.8	>1.3	<0.8	<0.8	>1.3	<0.8	<0.8	>1.3	<0.8	<0.8
Determination of traces of disinfectant (titrimetric method)	Absent	2.56 ppm	Absent	Absent	1.28 ppm	Absent	Absent	0.64 ppm	Absent	Absent	1.92 ppm	Absent	Absent	1.28 ppm	Absent	Absent

Table IV
The results of the micro-biological, physical and chemical determinations in case of the pharmaceutical form with clobetasol by the *tampon method*

Analysis made for the Strongly active corticosteroid	Admitted limits	Results			Results			Results			Results			Results		
		Sample no. 1 (preparation vessel I)			Sample no. 2 (transfer pump I)			Sample no. 3 (preparation vessel II)			Sample no. 4 (transfer pump + dosing cone)			Sample no. 6 (tampon vessel)		
		Step I	Step II	Step III	Step I	Step II	Step III	Step I	Step II	Step III	Step I	Step II	Step III	Step I	Step II	Step III
Total no. of germs / cm ²	<1CFU /cm ²	0.25	0.18	0.13	0.4	0.35	0.17	0.21	0.28	0.1	0,25	0,18	0,21	0,25	0,3	0,15
Pathogenic germs <i>Escherichia Coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas</i>	Absent Absent Absent	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.	Abs. Abs. Abs.
Determination of traces of active substance – clobetasol propionate (HPLC method)	Absent	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection	Under the limit of detection
Determination of traces of disinfectant (conductivity)	Less than 4 ppm	3.96	1.44	0	4.59	2.07	0	3.96	1.44	0	4.59	1.44	0	3.96	0.81	0
Determination of traces of cleaning agent – „analytic strip test for Peroxide”	Less than 1 ppm	>1.3	<0.8	<0.8	>1.3	<0.8	<0.8	>1.3	<0.8	<0.8	>1.3	<0.8	<0.8	>1.3	<0.8	<0.8
Determination of traces of disinfectant (titrimetric method)	Absent	2,56 ppm	Absent	Absent	1,92 ppm	Absent	Absent	2,56 ppm	Absent	Absent	1,92 ppm	Absent	Absent	1,92 ppm	Absent	Absent

The cleaning method was accepted because the results of the analyses were within the limits imposed by the validation protocol.

After having applied the cleaning process validated during 12 months, it was made an evaluation of this process after a quarterly monitoring. The results obtained following the evaluation of the cleaning process of the manufacturing equipment were within the limits admitted during 12 months. For this reason, the cleaning process of the manufacturing equipment validated is considered efficient for the removal of residues, of the degradation compounds and of the cleaning agents in order not to exist any risk related to the cross contamination of active substances.

Conclusions

The results obtained following the determinations for validating the cleaning process of the manufacturing equipment in the case of the two the two drugs considered were within the limits admitted during the validation period for the *steps II and III*. For this reason, the cleaning process of the manufacturing equipment is considered efficient for the removal of residues, the degradation products and the cleaning agents, in order not to exist any risk relating to the cross contamination of active substances.

The monitoring may be reduced as much as possible during the routine phases, and at the end of 12 months. These results may be integrated in an annual evaluation report of the cleaning process.

By the cleaning validation and by the application of the quality risk management, it was demonstrated the fact that the manufacturing on the same technological line of the potent active pharmaceutical drugs together with other pharmaceutical substances from different therapeutic classes (in case of all the non-sterile topic semisolid forms) is possible and does not influence the quality of the products manufactured on these manufacturing equipment and at the same time it is accepted and there is no risk of cross contamination of active substances.

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Manuscript received: 12th September 2012