Review Article
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Basics of cleaning equipment for pharmaceutical ingredients manufacturing

Summary

Introduction: In the manufacturing of pharmaceutical ingredients, one of the most critical parts of the process is the cleaning procedure. Cleaning is essential to prevent cross-contamination in a multipurpose plant. Various guidelines establish the main criteria to consider when developing an effective cleaning process. Proper development and design of cleaning procedures are of paramount importance to ensure that equipment is free from previously used raw materials, intermediates, and reagents. Clear limits must be defined to guarantee that the next product manufactured will be free of residual compounds.

Intention: The basics of cleaning procedures sometimes are not completed explained, mainly because is part of the know-how companies, the intention of this paper is to show in a simple way the cleaning procedure including some important data mainly for the newcomers to this area.

Results: This review will compile the basics of cleaning process after the synthesis of pharmaceutical ingredients or intermediates. Some of these issues are solvent selection, cleaning design, guidelines to be follow, validation, execution and troubleshooting that generates deviations.

Keywords: cleaning, pharmaceutical ingredient, validation, limits, contamination, control

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Introduction

The manufacturing of pharmaceutical ingredients generally involves the use of reactors, centrifuges, dryers, mills, and auxiliary equipment such as filters, pumps, and containment systems. These pieces of equipment are used to carry out various unit operations, including loading, chemical reactions, cooling, heating, transfer, crystallization, precipitation, drying, and particle size reduction. The Figure 1 shows the manufacturing cycle of a pharmaceutical ingredient.[1]

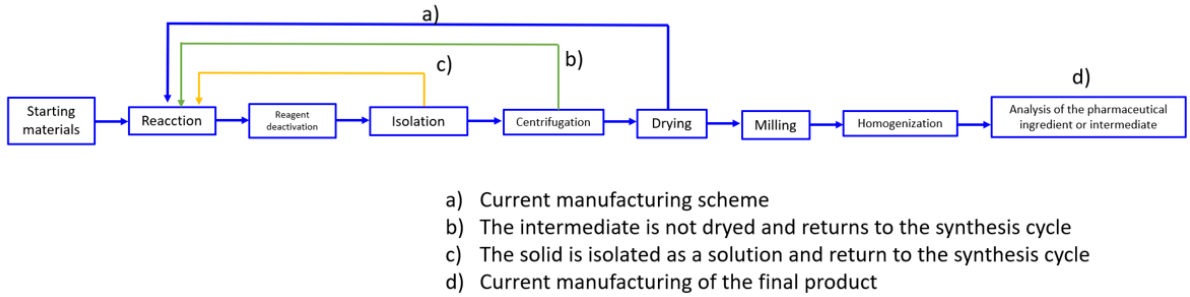

Figure 1 Manufacturing cycle of an active pharmaceutic ingredient.

In this context, the reaction takes place in the reactors, where reagents, solvents, and organic substances are loaded. In the next step of the process, centrifuges remove all the mother liquors and retain the isolated product. The residual molecules in this equipment are primarily the main product and traces of process-related impurities. The following step is the drying of the product. The function of the dryer is to eliminate the residual solvent of the product. Therefore, the dryer contains only the main product and solvent; any residue is the product itself. The same applies to mills and homogenizers, as they only encounter the isolated product. (Figure 2) schematically illustrates, the manufacturing equipment used for an intermediate or a pharmaceutical ingredient (API), along with their associated residues.

After the manufacturing of a pharmaceutical ingredient or intermediate, it is essential to clean the equipment thoroughly to eliminate all organic and inorganic substances and prevent cross-contamination.

Before entering the cleaning process itself, several key questions must be addressed:

a) How should the equipment be cleaned?

b) Is the cleaning sequence the same for a reactor as for a centrifuge or mill?

c) What solvent or reagent is required?

d) How will samples be taken, and what limits should be established?

e) How can we ensure that a new product will not be contaminated by the previous one?

f) Is it necessary to validate the cleaning method? What about the equipment cleaning procedure?

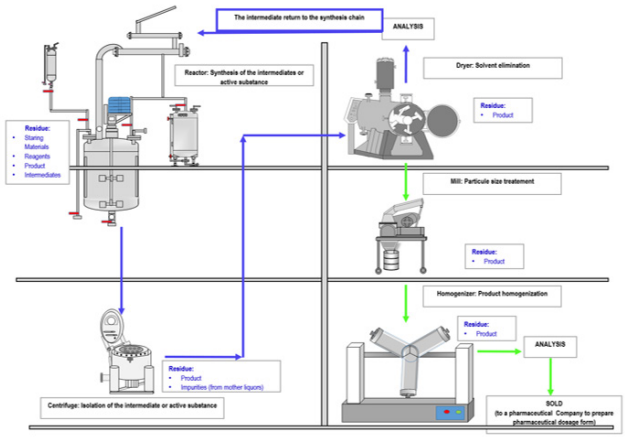

Figure 2 Equipment used for the manufacturing of active pharmaceutical ingredient and the potential residue.

To answer these questions, it is essential to understand some basic concepts related to cleaning in pharmaceutical manufacturing equipment. The aim of this paper is to present the fundamentals of the cleaning process following the synthesis of intermediates or active pharmaceutical ingredients.

Reasons for cleaning the equipment

The main reason for performing cleaning is to avoid cross-contamination, which can occur if a new product is synthesized in unclean equipment. A useful analogy is preparing soup in a dirty frying pan that was previously used to fry eggs, that is clearly unacceptable. Similarly, it is not feasible to synthesize a new product in a reactor containing organic or inorganic residues, especially because the biological activities of different compounds can vary significantly. For example, imagine combining an antipsychotic drug like **Risperidone** with a proton pump inhibitor such as **Omeprazole**. This unintended mixture could expose the patient to potentially catastrophic consequences.

To illustrate this situation, Figure 3 presents two scenarios:

Case A shows the undesired situation: after completing the synthesis of Product A, the reactor is used directly for the synthesis of Product B without any cleaning. In this hypothetical case, Product B would be contaminated with residues from Product A.

The desired situation occurs when, after completing the synthesis of Product **A**, a proper cleaning procedure is carried out. This procedure includes a defined cleaning sequence documented in a cleaning batch record (instructions), followed by sampling, analysis, and final approval.

Although this illustration may seem exaggerated, cross-contamination can and does occur in real-life scenarios. For example, in 2020, Fresenius Kabi issued a voluntary nationwide recall of a lot of Dexmedetomidine Hydrochloride (a sedative) injection due to cross-contamination with Lidocaine (an anesthetic). This contamination posed a serious risk, especially for patients with lidocaine allergies, as it could potentially lead to anaphylaxis, a life-threatening reaction.[2]

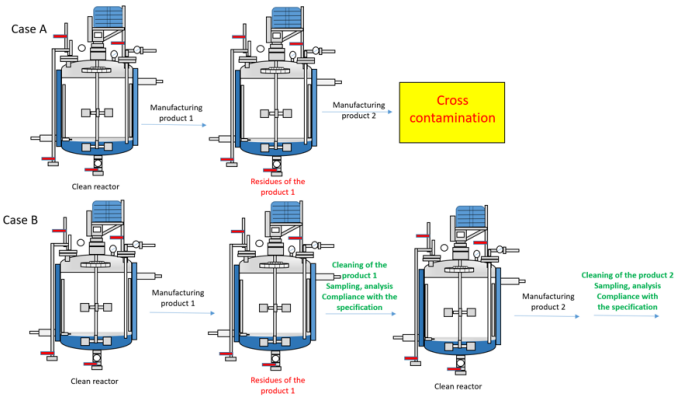

Figure 3 Example of cross contamination.

In summary, using unclean equipment can lead to serious problems and potentially harm the final consumer even resulting in death. These arguments highlight the critical importance of cleaning procedures. To regulate this area, several guidelines have been published and will be discussed later in this paper.

Continuing with the previous tessiture, proper equipment cleaning is essential to:

a) Guarantee the quality of intermediates and pharmaceutical ingredients

b) Prevent cross-contamination

c) Ensure compliance with current regulations

d) Inhibit microbial growth

e) Maintain the integrity and longevity of manufacturing equipment

Additional benefits of cleaning (and its validation) include:

a) Reduced quality-related costs

b) Reusability of equipment

c) Batch integrity

d) Microbial control

e) Product consistency

f) Informed business decision-making

Types of contaminants

After completing a chemical manufacturing process, it is necessary to eliminate all residues remaining in the equipment.

The ICHQ3A[3] guideline reported the type of materials that can be adhere to manufacturing equipment. In general, these are classified as:

i. **Raw materials:** These include all organic compounds introduced into the process, such as intermediates, building blocks, and starting materials.

ii. **By-products:** During synthesis, reactions often do not reach 100% conversion. As a result, undesired compounds commonly referred to as impurities may be generated and remain in the equipment.

iii. **Intermediates:** If chemical transformations are incomplete, a portion of intermediates or unreacted raw materials may adhere to the reactor walls or other equipment surfaces.

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iv. **Reagents, ligands, and catalysts:** These are essential for driving chemical reactions. Catalysts often require special removal steps after the process. Residual amounts of these substances may remain in the equipment if not properly cleaned.

v. **Solvents:** Solvents either organic or inorganic (e.g., water) are typically used to facilitate chemical reactions. Residual solvents can remain in the equipment, and water is of particular concern as it can promote microbial growth. A drying step after cleaning is often necessary to eliminate these residues.

vi. **Inorganic compounds:** These include substances such as filter aids, activated charcoal, inorganic reagents, and catalysts. All of these can leave residues that adhere to equipment surfaces.

vii. Once the potential contaminants of a reactor have been identified (Figure 4), it becomes essential to understand how to effectively remove all residual materials. However, before delving into specific cleaning methods, it is important to review the information provided in established cleaning guidelines.

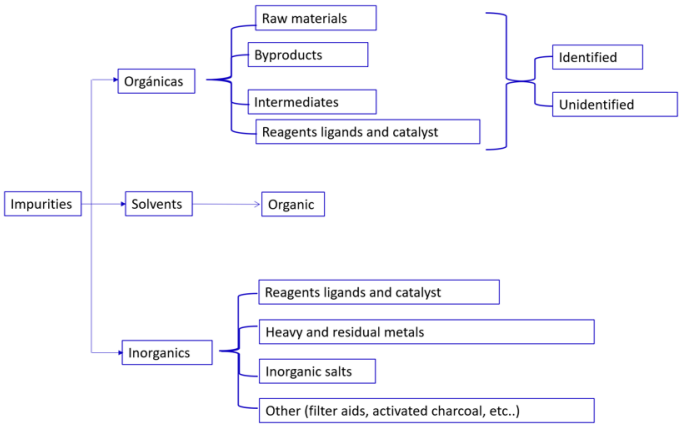

Figure 4 Residual material after a chemical process (ICHQ3A).

Guidelines

Cleaning guidelines typically summarize the key activities and expectations related to cleaning procedures. In this context, several regulatory authorities have published their concepts and instructions.

The following paragraphs will highlight the main points from some of the most essential guidelines:

Food Drug administration (FDA)[4]

This guideline, in the context of cleaning, focuses on controlling chemical residues. One of its key objectives is to prevent cross-contamination or adulteration. Written procedures must be in place for equipment cleaning, and the cleaning process must be validated, including the development of protocols and final reports.

The procedure should define cleaning levels and acceptance criteria. Residues must be identified, and for active ingredients (e.g., steroids), byproducts must also be characterized. Clean-in-place (CIP) systems are recommended for certain types of equipment. It is also necessary to determine the required number of cleaning cycles. Operators must be trained in the cleaning procedures, and equipment must be properly dried to minimize microbial proliferation.

Cleaning instructions must be included in the batch record or a similar controlled document. If the cleaning procedure is complex, it must be thoroughly documented. Analytical and recovery methods must be evaluated. Sampling methods include rinse sampling and direct surface sampling. Further details can be found in the guideline.

European medicines agency (EMA)

The European Medicines Agency (EMA) guideline introduces a risk-based approach to cleaning validation. It outlines the calculation of the Permitted Daily Exposure (PDE) to define acceptable limits for residual substances on manufacturing equipment.

Key concepts such as hazard identification and critical effects are addressed. These factors contribute to determining the No Observed Adverse Effect Level (NOAEL), which is the highest tested dose at which no adverse effects are observed. The route of administration is also considered in the risk assessment, as it influences the potential impact of residual substances.

Additional relevant concepts and methodologies are detailed within the guideline, supporting a scientifically justified and health-based approach to cleaning validation.

International council of harmonization (ICHQ7)

The International Council of Harmonization in the guideline good manufacturing practice guide for active pharmaceutical ingredients (ICHQ7)[5] also addresses cleaning validation as a critical step to prevent cross-contamination. A key consideration is that multiple intermediates or active pharmaceutical ingredients (APIs) may be synthesized using the same equipment, which is cleaned using a common procedure. In such cases, a representative intermediate or API can be selected for cleaning validation based on its solubility and cleaning difficulty.

Residue limits must be established based on the potency, toxicity, and stability of the intermediates and APIs. The guideline emphasizes the importance of having a cleaning validation protocol and final reports, including details on sampling methods, analytical methods, and acceptance limits. Visual inspection is also recognized as a useful tool for detecting macroscopic residues. An important aspect highlighted in the guideline is the need for continued monitoring after validation to ensure the cleaning process remains in a validated state.

Norma 164 (Cofepris, México)

The Norma 164 is the official guideline for México (NOM-164-SSA)[6] this document highlights the importance of the cleaning validation process. The cleaning procedures must be appropriate to the nature of the intermediates or active substances involved. It is essential to evaluate the interaction between different cleaning agents. A worst-case scenario approach may be applied, considering factors such as concentration, toxicity, and stability. Validation should be performed over three consecutive cleaning cycles, and all results must meet the acceptance criteria to be considered successful. Analytical methods used in the validation must be validated themselves and must have sufficient sensitivity to detect impurities in relation to the established limits. Additionally, the concept of cleaning hold time (the maximum time equipment can remain clean before reuse) must be taken into account.

Active pharmaceutical ingredients committee (APIC)

The guideline "Guidance on aspect of cleaning validation in active pharmaceutical ingredient plants" from the Active pharmaceutical ingredients committee (APIC)[7] outlines key concepts related to cleaning validation. One of the main principles is the integration of cleaning validation into an effective quality system, along with the assessment and mitigation of risks to patients.

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The document addresses six main topics:

a) Acceptance criteria: including Maximum Allowable Carryover (MACO) and health-based exposure limits (mg/day).

b) Levels of cleaning: illustrated with diagrams and types of cleaning.

c) Control of the cleaning process: covering visual inspection, analytical verification, standard operating procedures, and detailed process descriptions.

d) Bracketing and worst-Case rating: with examples and classifications based on residue solubility.

e) Determination of residue amounts including examples, classifications, and calculation methods.

f) Cleaning Validation Protocol: with examples of sampling methods, calculations, dirty hold time, and clean hold time.

g) The guideline includes several diagrams that help clarify key concepts. It also features a section of frequently asked questions answered by experts, and a glossary of cleaning-related terms. Figure 5 presents the main international guidelines associated with cleaning in pharmaceutical manufacturing.

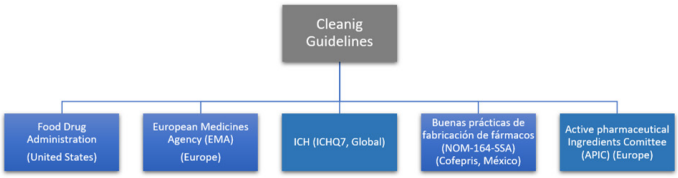

Figure 5 Main guidelines associated to the cleaning procedures.

Cleaning procedure

The cleaning procedure must include all the necessary steps to perform an effective cleaning. This procedure should describe all unit operations involved. Readers may be more familiar with a master batch record for manufacturing; similarly, a detailed document must be established for the cleaning procedure.

It is essential that all cleaning procedures comply with Good Manufacturing Practices (GMP) and are integrated into the quality system.

The cleaning batch record or procedure should include the following data:

a) Main page: This should contain information related to the batch, product, codes, dates, and solvent sequence.

b) Subsequent pages: Include equipment information, descriptions, auxiliary systems, etc.

c) Safety considerations: All reagents and solvents have inherent toxicity and specific handling requirements. Therefore, all safety measures must be clearly indicated in the instructions. Since various solvents are used in cleaning procedures, safety practices such as blanketing (removing oxygen from inside equipment) and grounding (to prevent electrostatic discharge) are crucial.

d) Unit operations: Solvents and reagents must clean all parts of the equipment. This may involve operations such as reflux (distilling the solvent within the reactor) or recirculation using pneumatic pumps. The main goal is to "wash" the equipment walls to remove all residual materials. Hoses must also be included in these operations (Figure 6).

e) Waste management: The cleaning procedure involves the use of a certain amount of solvent to rinse or wash the equipment. These solvents may contain residual compounds. Therefore, all effluents must be handled and disposed of in a safe and appropriate manner, in compliance with environmental and safety regulations.

f) Visual inspection: A verification step must be carried out by a second person (the first being the operator who performs the cleaning). For equipment used in the manufacture of pharmaceutical ingredients, the visual inspection must be adapted to the specific equipment type. This means that the inspection of a reactor differs from that of a fluid bed dryer or a mill. Nevertheless, the core objective remains the same: to ensure cleanliness and absence of residues. Some parts that must be visually inspected include:

- **Reactors**: internal walls, agitator, baffles, sight glass, reflux line, manway, solvent inlet line, discharge line, etc.
- **Centrifuges**: sight glass, basket, loading line, discharge line, cover, etc.
- **Dryers**: paddles, body, and discharge line.

Figure 7 illustrates some of these equipment parts (non-exhaustive list).

g) **Sampling:** There are two main methods, the rinse and the swab. The rinse is using a determined solvent quantity in which the main compound of the previous product is soluble. After finishing the cleaning procedure, the specific solvent quantity "rinse" the equipment. A sample of these rinse is analyzed in Quality Control to determine the residual quantity of the main compound. The result must comply with the specification. Is the residual quantity being below the specification the cleaning is approved, on the contrary it will be necessary open a deviation and propose a new cleaning procedure.

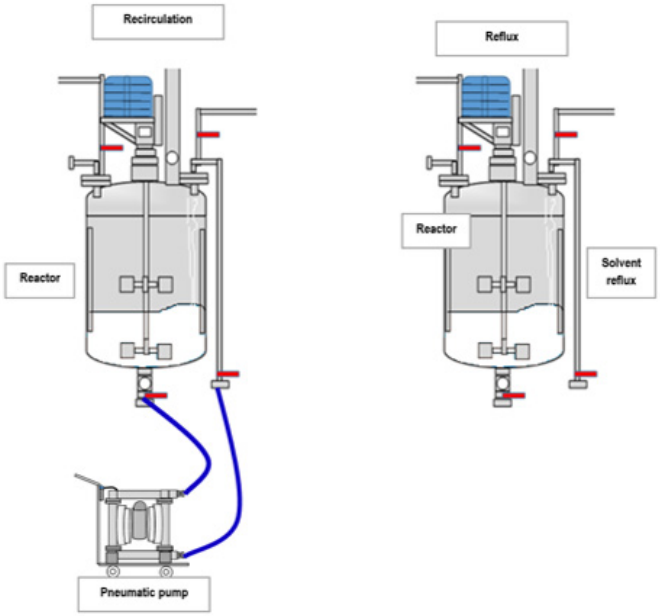

Figure 6 Example of unit operations used in the cleaning of equipment.

The second method is the swab. A swab is a fibrous material that is used to wipe a surface to remove residues from the surface. The sample is taken with a defined wiping motion, the surface is known and

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must be the area of most difficult access. Then the potential residue is extracted with a specific solvent. Swabs used should be compatible with the residues (intermediates or active substances) and stable against the recovery compounds.[8] The recovery is quantified with an analytical method. And the result is compared against a specification. If the content is below than the specification the result is approved if no, the cleaning is rejected. Both methodologies are described in the Figure 8.

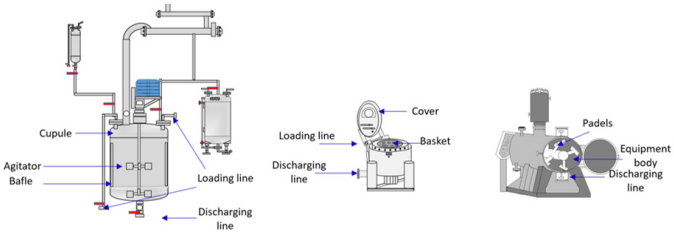

Figure 7 Example of parts to be include in the visual inspection.

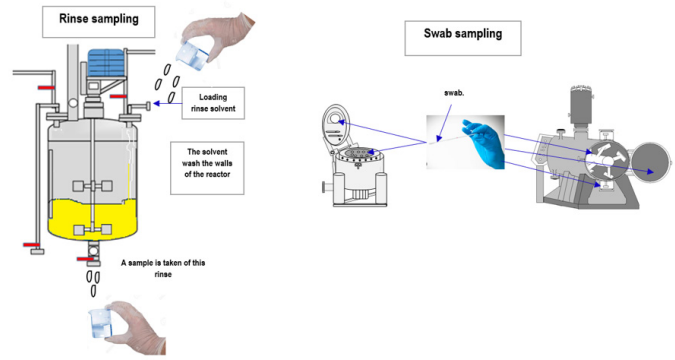

Figure 8 Types of sampling procedures.

Sampling. There are two main methods for sampling: **rinse** and **swab**.

1) Rinse sampling: This method uses a defined quantity of solvent in which the main compound of the previous product is soluble. After completing the cleaning procedure, the equipment is rinsed with this solvent. A sample of the rinse is then analyzed by Quality Control to determine the residual amount of the target compound. The result must comply with the established specification. If the residual level is below the specification, the cleaning is approved. Otherwise, a deviation must be opened, and a new cleaning procedure proposed.

2) Swab sampling: A swab is a fibrous material used to wipe a surface and collect any remaining residues. The sample is taken using a defined wiping pattern over a known surface area, typically one that is difficult to clean. The swab is then extracted with a specific solvent. Swabs must be compatible with the residues (intermediates or active substances) and stable in the presence of recovery solvents. The extracted sample is analyzed using an appropriate analytical method, and the result is compared to the specification. If the content is below the limit, the cleaning is approved; if not, it is rejected. Both methodologies are illustrated in Figure 8.

h) **Analysis.** The analysis part will be discussed in further paragraphs.

i) **Closing the cleaning procedure.** Once the visual inspection, sampling, and analytical approval have been completed, the cleaning procedure can be formally closed. Proper execution of all these steps is essential to prevent cross-contamination.

Solvent selection

Solvent Selection for Rinse: To select an appropriate rinse solvent, solubility trials must be conducted in the laboratory. For example, if compounds A and B are charged into a reactor and react under specific conditions to form compound C, and compound C is isolated in that same equipment, then compound C will be the primary residual substance. Therefore, its solubility must be evaluated. Compound C should be tested in various solvents commonly used options include methanol, ethanol, toluene, acetone, diluted hydrochloric acid, and diluted sodium hydroxide. Solubility is assessed based on the solubility ratio, and companies may refer to the United States Pharmacopeia (USP) solubility classification table or establish their own internal criteria.

In a theoretical case, if methanol proves to be the most effective solvent for dissolving compound **C**, then methanol will be selected for the cleaning process (e.g., loading, reflux, distillation). These activities are summarized in Figure 9.

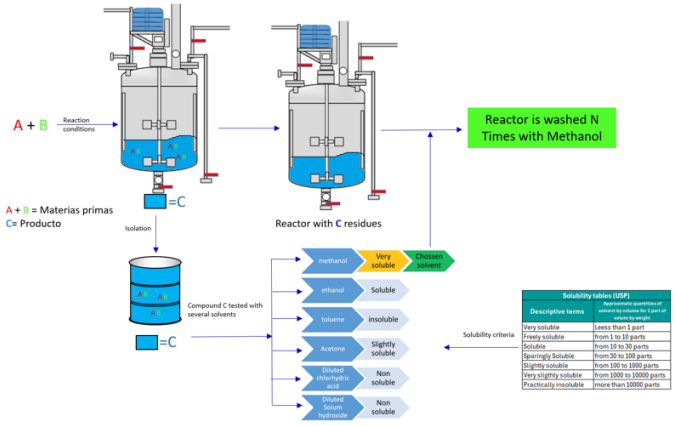

Figure 9 Solvent selection for a cleaning procedure.

Cleaning agents

Chemical reactions are generally expected to produce only the main product**.** However, in practice, most processes generate several side reactions, resulting in byproducts such as dimers, trimers, overreaction products, and related compounds. These byproducts often have different solubility profiles, which may require the use of alternative reagents for effective cleaning.

For example, diluted acids or bases most commonly hydrochloric acid and sodium hydroxide are frequently used. These reagents are advantageous because they are widely available, cost-effective, and relatively easy to handle. Additionally, they can form organic salts, which is a well-known method for altering the solubility of organic substances in water, primarily due to the formation of ionic bonds.[9]

Other acids such as phosphoric acid and acetic acid may also be used, as well as bases like sodium bicarbonate, depending on the nature of the residues and the cleaning requirements.

Material of construction of the equipment

Some chemical processes are not highly efficient. A good example, based on the author's experience, is the Biginelli reaction. This process typically yields a low output approximately 30%. Its advantage lies in the ability to generate complex molecular structures from simple building blocks. However, recovering only 30% of the

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desired product means that around 70% of the starting materials result in various byproducts.

Consequently, a significant amount of residue remains in the reactor, requiring the use of stronger cleaning agents. After manufacturing the next step is the cleaning but not all cleaning agents are compatible with the equipment's construction materials. In pharmaceutical ingredient synthesis, there are mainly three types of construction materials used in reactors:

i. **Stainless steel:** The most used material for active substance synthesis. It is suitable for basic and neutral processes but incompatible with strong acids. However, it can tolerate weak acids such as phosphoric and acetic acid.

ii. **Hastelloy:** A highly resistant alloy capable of withstanding the entire pH range. It is used in processes involving drastic pH changes. Strong acids like hydrochloric acid and strong bases like sodium hydroxide can be used for cleaning without restrictions. The main drawback is its high cost.

iii. **Enamel coated reactors**: These reactors are lined with a glass--like enamel and are suitable for acidic media but cannot withstand basic conditions. They are typically used for gentle agitation processes and can be cleaned with strong acids.

In summary, the construction material of the equipment must be carefully considered when designing a cleaning procedure.

Acceptance criteria

The aim of cleaning is to eliminate residues from equipment to an acceptable limit in order to prevent cross-contamination**.** These limits must be established based on a sound scientific rationale. The APIC guideline[10] refers that the acceptance criteria should be based on the Acceptable Daily exposure (ADE) or Permitted Daily Exposure (PDE). The ADE / PDE define limits the expositions of the patients per day with acceptable risk.

In other cases where availability of pharmacological or toxicological data is limited, for example for chemicals, raw materials, starting materials, active pharmaceutical ingredients cleaning limits are based on the threshold of toxicological concern (TTC), LD 50 and general cleaning limits may be calculated. In these cases, carcinogenic, genotoxic and potency effect of these structures should be evaluated by toxicologist.

Taking in account the previous arguments there are some ways to set the cleaning acceptance criteria.

- Using health-based data, ADE (acceptance daily exposure)
- Basic on the products therapeutic daily dose
- Based on the lethal dose value (LD50)
- General limit based on maximum concentration and batch size.

Using health-based data, ADE (acceptance daily exposure)

The Maximum Allowable Carryover (MACO) should be based upon the Health-Based Exposure Limits (HBEL), which can be an Acceptable Daily Exposure (ADE) or Permitted Daily Exposure (PDE), calculated when sufficient data is available. The principle of MACO calculation is that you calculate your acceptable carry-over of your previous product, based upon the HBEL, into your next material:

$$MACO = \frac{Hbel\ previous \times MBS\ next \times PF}{TDDnext \times SF}$$

a) MACO Maximum Allowable Carryover: acceptable transferred amount from the previous product into your next material (mg)

b) HBEL Health-Based Exposure Limit (mg/day) of the previous compound

c) MBSnext Minimum batch size for the next material(s) (where MACO can end up) (mg)

d) TDDnext Maximum Therapeutic Daily Dose for the next material (mg/day)

e) PF Purging Factor reflects the ability of a process to reduce the level of the previous product in the downstream synthetic route of the next material (in case the next material is not yet the final API). The default value is "1" unless R&D can provide case-specific purging ability evidence (e.g. in case of control LOD limitation.)

f) SF Safety factor reflects the effects from the interaction between previous products.

Based on the products therapeutic daily dose

The HBEL should be calculated as an Acceptable Daily Exposure (ADE) or Permitted Daily Exposure (PDE). They are effectively comparable with each other and represents an estimate of a daily exposure that is unlikely to cause an adverse effect if an individual is exposed, by any route, at or below this dose every day for a lifetime. They are determined to protect patients and are calculated by following formulas in mg/day:

$$ADE = \frac{POD \times BW}{UFc \times MF \times PK}$$

$$\frac{POD \times BW}{F1 \times F2 \times F3 \times F4 \times F5}$$

i. ADE Acceptable Daily Exposure (mg/day)

ii. PDE Permitted Daily Exposure (mg/day)

iii. POD Point Of Departure

iv. BW Is the weight of an average adult (e.g. 50 kg cfr EMA guideline)

v. UFc Composite Uncertainty Factor: combination of factors which reflects the inter-individual variability, interspecies differences, sub-chronic-to-chronic extrapolation, LOEL-to-NOEL extrapolation, database completeness.

vi. NOAEL No Observed Adverse Effect Level (mg/kg/day)

vii. NOEL No Observed Effect Level (mg/day)

viii. MF Modifying Factor: a factor to address uncertainties not covered by the other factors

ix. PK Pharmacokinetic Adjustment

x. F1-F5 Adjustment factors to account for uncertainties. Refer to EMA Guidance 2 for further explanation.

Based on the lethal dose value (LD50)

In cases where no other data is available and only LD50 data is available the HBEL can be based upon LD50 data. Calculate NOEL according to the following equation and use the result for the establishment of HBEL. Note the 2000 is an empirical constant.

$$NOEL = \frac{LD50 \times BW}{2000}$$

General limit based on maximum concentration and batch size.

Companies may choose to have a MACO upper limit as an internal policy, if MACO calculations result are less stringent, or toxicological data for intermediates are not known, the approach of a general limit may be suitable. The general limit is often set as an upper limit for the maximum concentration (MAXCONC) of a contaminating substance in a subsequent batch.

Establish MACO, based on a general limit, using the following equations.

MACO = MAXCONC x MBS

MACO Maximum Allowable Carryover: acceptable transferred amount from the investigated product ("previous"). Calculated from general ppm limit.

MAXCONC General limit for maximum allowed concentration (mg/kg or ppm) of "previous" substance in the next batch.

MBS Minimum batch size for the next product(s) (where MACO can end up)

E.g. for a general limit of 100 ppm: MACO = 0.01% of the minimum batch size (MBS), and for a general limit of 10 ppm: MACO = 0.001% of the minimum batch size (MBS).

A general upper limit for the maximum concentration of a contaminating substance in a subsequent batch (MAXCONC) is often set to 5-500 ppm (100 ppm in APIs is very frequent) of the previous product into the next product depending on the nature of products produced from the individual company (e.g. toxicity, pharmacological activity,…). The general limit should be supported by a scientific/ documented rationale.

Particularly, this method is very useful in industrial process development.

Swab limit

As it was commented previously it is necessary to set limit, in case of the swab the limit is associated to the sampling surface. So, the limits must follow the next equation:

$$Target\ value\left[\mu\frac{g}{dm2}\right]\frac{MACO\ [\mu g]}{Total\ surface[dm2]}$$

The total quantity of the surfaces must be below the MACO.

Rinse limit

For the case of the rinse the limit must follow the next equation

$$Target\ value\left(\frac{mg}{L}\right)=\frac{MACO(mg)}{Volume\ of\ rinse\ or\ boil\ (L)}$$

For quantitation a solvent sample (e.g. 1 l) is taken, the residue in the sample is determined by a suitable analytical method and the residue in the whole equipment is calculated according to the following equation:

CO (mg)= V x (C- Cb)

i. CO Carry Over, true (measured) total quantity of substance (possible carry over) on the cleaned surface in contact with the product, calculated from results of rinse tests.

ii. V Volume of the last rinse or wash solvent portion in L

iii. C Concentration of impurities in the sample in mg/L

iv. Cb Blank of the cleaning or rinsing solvent in mg/L. If several samples are taken during one run, one and the same blank can be used for all samples provided the same solvent lot was used for the whole run.

v. Requirement: CO < Target value.

All limits are established for every company and follow the quality systems, the data presented here are a summary of the APIC guideline content, for more details it is suggested consult the original reference. In the other hand, some examples can be consulted.[11]

Validation method

In a multipurpose plant, validating the cleaning procedure is mandatory to prevent cross-contamination and to ensure documented evidence of control. In this context, process validation [12] is defined as the collection and evaluation of data from the process design stage through commercial production that establishes scientific evidence that the process consistently delivers a quality product. Validation encompasses a series of activities throughout the product and process lifecycle, including cleaning procedures.

Process validation consists of three main stages:

i. **Stage 1: Process design:** The commercial manufacturing process is defined during this stage, based on knowledge gained through development and scale-up activities. For cleaning procedures, solubility trials should be conducted during scale-up to verify that the selected solvent effectively removes all residues.

ii. **Stage 2: Process qualification:** This stage evaluates whether the process design can consistently support reproducible commercial manufacturing. After each production run, the equipment must be cleaned, and the cleaning process must be executed and evaluated.

iii. **Stage 3: Continued process verification:** Ongoing assurance is obtained during routine production to confirm that the process remains under control. After each manufacturing run, cleaning effectiveness must be assessed. To maintain the validated state, cleaning must comply with established criteria. If validation fails, an investigation must be initiated, and corrective or preventive actions (CAPA) must be implemented.

As with chemical processes, cleaning validation must consider the following elements:

a) **Understanding the Source of Variation:** Identify the types of molecules to be cleaned, including intermediates and byproducts.

b) **Detecting the presence and degree of variation:** Evaluate residues from each unit operation. Variability may occur between batches or operators. A minimum of three consecutive successful cleaning runs is typically required for validation.

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c) **Understanding the impact of variation on the process and product attributes:** Final product attributes must be considered. For example, polymorphism can affect solubility and compromise cleaning effectiveness.

d) **Controlling Variation According to Risk:** Variation should be controlled through well-defined cleaning batch records, specifying unit operations, solvents, and reagents.

These principles apply to the cleaning process. The next step involves validating the analytical method used to assess cleaning effectiveness.

Validation of the analytical method

When a new process is validated in the plant, it often requires the creation and validation of new analytical methods**.** In this context, it is essential to determine whether the analysis can be performed using a newly developed method or an official pharmacopeial method. The primary objective is to generate reliable data regarding cleaning efficiency.

Analytical method validation. It is necessary to ensure the method is suitable for its intended purpose. Key parameters to consider include:

a) **Accuracy**: This refers to the closeness of agreement between the measured value and a known reference value. Accuracy can be assessed by comparing test results with established standards.

b) **Precision**: Precision reflects the consistency of results when the method is applied repeatedly under similar conditions. It includes three levels:

c) **Repeatability:** Also known as intra-assay precision, it measures consistency under the same operating conditions over a short time interval. Typically, six replicate samples are prepared and analyzed at 100% concentration.

d) **Intermediate precision**: This assesses variability within the same laboratory, across different days, analysts, and instruments. For example, two analysts each prepare six sample solutions using the same method.

e) **Reproducibility**: This evaluates precision across different laboratories. Each lab prepares and analyzes six sample solutions using the same validated method.

Additional details and guidance can be found in the work of Sharma and colleagues. [13]

6.2 Analytical methodologies

The choice of analytical methodologies depends on the type of sample to be analyzed, and various techniques may be employed.[14]

a) **Ultraviolet (UV) spectroscopy**: This method is suitable for molecules containing chromophores. UV analysis is widely used due to its simplicity, ease of development, and affordability of reagents.

b) **High performance liquid chromatography (HPLC)**: One of the most commonly used techniques, HPLC is employed to identify and quantify specific analytes. The sample is injected into a chromatographic column to separate the target compound from other species. Detection is typically performed using UV spectroscopy, conductivity, or evaporative light-scattering detectors. HPLC is standard in pharmaceutical manufacturing.

c) **Gas chromatography (GC)**: Like HPLC, GC separates analytes using a chromatographic column. However, it uses a carrier gas instead of a liquid solvent. GC is particularly useful for analyzing low molecular weight compounds.

d) **Total organic carbon (TOC)**: TOC analysis involves oxidizing the sample and measuring the resulting carbon content using infrared spectroscopy or conductivity. It is a non-specific method used to assess overall organic contamination.

e) **Atomic absorption spectroscopy (AAS)**: This technique is specific for detecting metal ions. It can be used to quantify cations or anions in active substances and is also useful for detecting catalysts such as palladium.

f) **Titrations**: Acid-base or complexometric titrations can be used to determine the upper limits of certain cleaning agents. Although not commonly used in pharmaceutical manufacturing, they remain a valid option for specific applications.

g) **Conductivity**: Conductivity measures the ionic content of a solution and provides a non-specific estimate of residual cleaning agents, such as acids or bases. Dilute solutions typically exhibit linear behavior, making this method useful for monitoring general cleanliness.

Cleaning procedures design considerations

Cleaning procedures are typically designed based on the solubility of the main compound in a specific solvent. However, other factors must also be considered to effectively address cleaning challenges. Sometimes, cleaning methodologies fail because the process generates additional intermediates, or simply because the chosen procedure is not appropriate.

When designing a cleaning procedure, some basic considerations should be taken into account:

Are there multiple intermediates in the process?

- Is any salt used or generated as an intermediate, reagent, or byproduct?
- Is there an excessive amount of interphases?
- Is the process yield low?
- Are the residual compounds stable?
- Are polymorphs present?
- Are there traces of reagents with strong odors?

a) **Process intermediates:** Although the cleaning process is designed to remove the main compound, all intermediates must be considered. These may have different solubility properties, which can lead to cleaning failures. This issue may not be evident during visual inspection.

b) **Salts:** If a salt is used or generated, a water rinse should be considered due to the high solubility of ionic compounds in water. Relying solely on organic solvents may leave salt residues in the equipment, resulting in ineffective cleaning.

c) **Interphases:** If a significant amount of interphases is present during the settling phase, there is a risk of these compounds adhering to the equipment walls. In such cases, an alternative solvent may be required to remove the residue.

d) Low yield: A low process yield often indicates the presence of byproducts or resins. This information should be factored into the cleaning procedure design.

e) Residue stability: Ideally, equipment should be cleaned immediately after a campaign. In practice, cleaning depends on plant priorities. Therefore, a time frame must be established to ensure residue stability, known as the dirty hold time the period during which residues remain stable in the equipment. This time is defined by each company and is typically 7 days.

f) Conversely, the cleaning hold time refers to the period during which cleaned equipment can remain unused before being revalidated for production. This period also varies by company but is commonly around 30 days depending on the rationale used.

g) Polymorphs: Based on the authors' experience, if a molecule exhibits polymorphism, its solubility properties may vary, potentially causing cleaning failures. Although rare, this can be the root cause of a deviation.

h) Reagents with Strong Odors: Certain reagents with strong odors can pose challenges, as even trace amounts may be detectable by humans. A common example is a thiol derivative. In such cases, decontamination with sodium hypochlorite is recommended to eliminate odors. This step is optional, as thiol residues may be difficult to detect through analysis or visual inspection.

i) Heat Tints: Reactors are exposed to high temperatures during reactions and drying, often without nitrogen purging. Sometimes, the construction material reacts with heat and oxygen (biradical), producing rainbow-like discolorations inside the equipment. This effect can be removed through acid passivation (e.g., with phosphoric or hydrochloric acid).

Staff training

All operators and supervisors must be trained in both the cleaning procedure and the sampling procedure. It is mandatory to explain the unit operations involved in cleaning and to maintain records of all actions performed, with special emphasis on visual inspections, as proper execution is critical to preventing cross-contamination. Additionally, operators must be trained in swab sampling techniques and qualified by the Quality department.

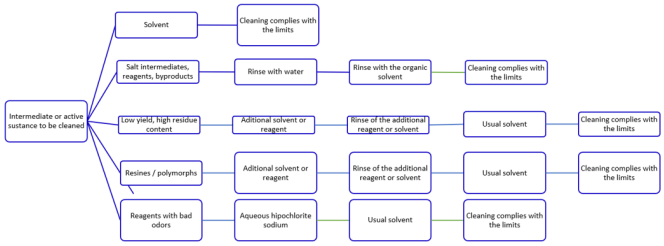

Figure 10 Alternatives to eliminate all the residual compound of an equipment.

Deviation

Cleaning validation must be conducted following a formal protocol. All activities must be documented and integrated into the quality system. Several factors can lead to cleaning failures and deviations, including insoluble intermediates, salts, resins, or interphases that adhere to equipment surfaces.

Investigations should consider all these potential causes, and additionally include the possibility of human error meaning that some operations may not have been executed correctly.

To support effective investigations, the Food and Drug Administration (FDA) has recently published a manual outlining recommended methodologies.[15] The main investigative tools include:

Brainstorming

Process Mapping

Cause-and-Effect Analysis

The 5 Whys Technique

Bracketing and the worst case

Once cleaning procedures are designed, certain sequences can be standardized for groups of molecules or product types. For example, if a reactor is loaded with compounds such as sodium chloride, sodium bicarbonate, sodium hydroxide, hydrochloric acid, etc., the cleaning procedure can include water washes due to the high solubility of these salts in water.

Similarly, if a family of compounds is soluble in methanol, a general cleaning procedure using methanol can be developed for that group. Other structurally similar molecules may also be cleaned effectively using the same solvent.

In general, solvent sequences can be implemented, allowing target residues to be removed using standard methods. However, there will inevitably be exceptional cases where solubility is very low. In such situations, a combination of cleaning agents such as hydrochloric acid, sodium hydroxide, and organic solvents may be required.

Conversely, if no general procedure is applicable, a new cleaning process must be developed for each specific residue. This often involves the use of specialized and sometimes expensive solvents. An additional drawback is the need to manage and treat these solvents as chemical waste.

Prevention of microbiological growth in equipment

It is mandatory to prevent microbial growth in order to avoid contamination of manufacturing products. The main cause of microbial growth is the presence of residual water in equipment.

Certain equipment, such as reactors and dryers, can be dried using heating fluids circulated through the equipment jacket. Additionally, simple heating under vacuum conditions can effectively remove water.

For other types of equipment such as centrifuges, mills, and homogenizers a rinse with alcohol (e.g., methanol, ethanol, or isopropanol) followed by a nitrogen blow is effective. In this process, the alcohol "drags" the residual water, and the nitrogen "drags" the residual alcohol. Since alcohol is more volatile than water, this method ensures that no residual solvent remains in the equipment.

Clean in place (CIP) systems

A very useful tool for executing cleaning procedures is the Clean-in-Place (CIP) system. CIP refers to the automated cleaning of plant components without the need to dismantle or open the equipment, and with minimal or no manual intervention from operators.[16]

Advantages of using CIP systems include:

a) No need to dismantle equipment

b) Reduced solvent consumption for rinsing

c) Minimal manual handling

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d) Cost-effective

e) Reduced personnel exposure to cleaning solvents

f) Improved cleaning results compared to conventional solvent loading methods because of the radial distribution (Figure 11).

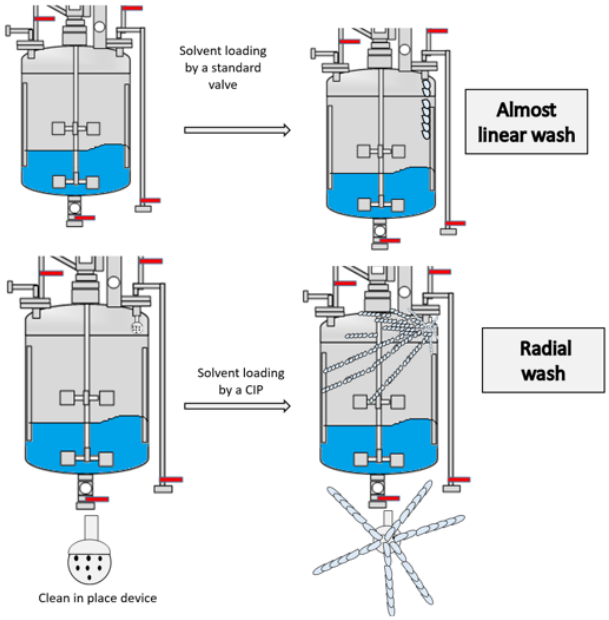

Figure 11 Effect of clean in place system in the rinse of a reactor.

Change management and quality system

During the development and validation of cleaning processes, several changes may occur. All activities related to these changes must be supported and documented within the quality system. These changes may include:

a) Updates to the cleaning validation plan

b) Revisions to standard cleaning procedures

c) Solvent studies for residue removal

d) Cleaning protocols

e) Validation reports

f) Analytical method validation

g) Reference standard registration

h) Documentation of deviations

In addition, before, during, and after the validation process, personnel from Quality Assurance, Quality Control, and Production must coordinate all activities, requirements, questions, and potential issues that may arise. This cross-functional collaboration is essential to ensure a successful cleaning validation.

Literature example of cleaning validation

Cleaning validation is part of the industry's know-how, which makes it difficult to find detailed and comprehensive reports in the literature. Nonetheless, Balmos et al. (2014) published a cleaning validation study that serves as a valuable example of the steps required to carry out a successful validation. [17] Although the study focuses on pharmaceutical dosage form production, it provides a useful case study.

The work involved the cleaning validation of two semisolid topical anti-inflammatory pharmaceutical ingredients with different pharmacological activities: phenylbutazone (a non-steroidal anti-inflammatory drug) and clobetasol propionate (a potent corticosteroid). Both were manufactured on the same production line. The objective was to ensure that phenylbutazone was completely removed during cleaning to prevent contamination of clobetasol propionate.

The procedure followed these steps:

i. **Solubility trials:** The selected solvent needed to effectively solubilize both active ingredients, be readily available, and ideally have minimal environmental impact.

ii. **Worst case selection:** Using the bracketing method and production experience, phenylbutazone was identified as the worst-case compound.

iii. **Disinfectant selection:** A test was conducted to choose the most effective disinfectant agent. Note: this step is not typically performed in synthesis reactors.

iv. **Sampling point identification:** Critical sampling points were defined, such as the manway, loading line, and recirculation line. A risk analysis was conducted to identify the most difficult areas to clean.

v. **Acceptance criteria definition:** Acceptance limits were established.

vi. **Analytical method development:** A validated analytical method was implemented to assess cleaning effectiveness.

The most notable part of the study was the analytical evaluation. Six parameters were monitored over a 12-month period, and all results complied with the established limits. As a result, the cleaning process was considered successfully validated.

The relevance of this example lies in its methodology, which is often missing in other publications on this topic. A flow diagram summarizing the study was also included (Figure 12).

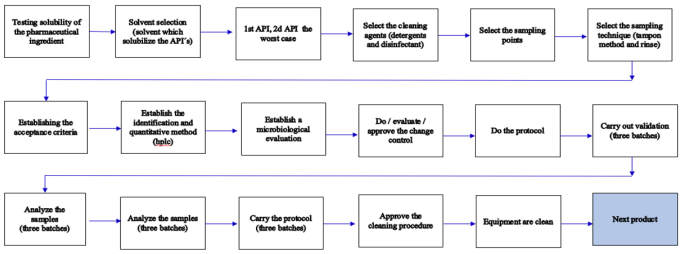

Figure 12 Methodology of cleaning validation by Balmos et al.[17]

Conclusion

In the manufacturing of active pharmaceutical ingredients (APIs), it is crucial to properly clean equipment after synthesis to prevent cross-contamination. Several regulatory guidelines establish the minimum requirements for cleaning procedures.

Cleaning procedures must be tailored to the types of residues generated by the chemical process, which are closely related to their solubility properties. Acceptance criteria must be clearly defined, and the analytical method used to verify cleaning effectiveness must be validated, covering all steps of the current validation process.

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Visual inspection plays a critical role in detecting residues, and personnel must be properly trained to perform it effectively. For each cleaning procedure, a protocol and corresponding report must be generated. A cleaning batch record must be implemented, and any deviations from the protocol must be documented, investigated, and addressed through corrective and preventive actions (CAPA).

Overall, the cleaning procedure is one of the most important aspects of API manufacturing, as it ensures product integrity, prevents cross-contamination, and protects patient safety.

Acknowledgments

None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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