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Cleaning validation using thermogravimetry

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Abstract The health authorities require that equipment used in the pharmaceutical industry is clean prior to use. The main raison is to prevent any contamination of the drug products. This article demonstrates that thermogravimetry (TG) can be used for the determination of the residual impurities during the cleaning validation of the equipment of pharmaceutical production plants. The accuracy and the recovery rate of this method are comparable with those of the classical analysis method (determination of the distillation residue using a rotary evaporator). The fully automation of the testing equipment even allows its utilization around the clock by plants operators, leading to a significant reduction of the time necessary for the cleaning validation and to an increase of the plant capacity.

Keywords Thermogravimetry · Cleaning validation · Residual impurities · Pharmaceutical production

Introduction

The health authorities require that equipment used in the pharmaceutical industry is clean prior to use. The main raison is to prevent any contamination or adulteration of the drug products. In the past, a number of products have been recalled due to cross-contamination. Therefore, the FDA and other authorities expect firms to have written general procedures on how cleaning process will be validated. The aim of this study is to present the techniques usually used and demonstrate that thermogravimetry (TG) can be a good replacement for these methods.

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Sampling and current analysis techniques

As CFR sect. 211.67 states, "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements" [1]. Cleaning validation should be performed to confirm the effectiveness of a cleaning procedure [2, 3].

The FDAs guidelines require, "that the basis of any limits is scientifically justifiable" [4]. Nevertheless, FDA does not intend to set acceptance specifications or methods for determining whether a cleaning process is validated.

An often used acceptance criteria is typically set at 1/1000 reduction of the lowest therapeutic dose of the previous drug product (active ingredient in most cases) or the LD50 toxic dose of the cleaning solutions. If the calculated limit is larger than 10 ppm carryover of the residual contaminants, then the acceptance criteria is set at a more rigorous 10 ppm (depending on medical opinion and/or safety considerations).

Sampling methods

There are two general types of sampling. The first is the direct method of sampling the surface of the equipment. Another method is to use rinse solutions. The advantages and drawbacks of these methods are the following:

Direct surface sampling

The swab technique typically involves moistening a polyester swab with highly purified water (acidified with phosphoric acid, if necessary) to wipe a measured area in a systematic multi-pass way always going from clean to dirty areas to avoid recontamination (e.g., 10 side-by-side strokes vertically, 10 horizontally, and 10 each with the flip side of the swab in each diagonal direction).

Advantages

- Areas hardest to clean and which are reasonably accessible can be evaluated;
- Residues that are dried out or are insoluble can be sampled by physical removal.

Disadvantages

- The type of sampling material used (e.g., the adhesive used in swabs) can interfere with the analysis of the samples;
- In some cases, the plant needs to be dismounted to perform the sampling.

Rinse samples

The equipment is washed with water (to remove the inorganic salts) and with organic solvents (usually heated up to the boiling point to clean the head space of the equipment) and the final washing solution is analyzed.

Advantages

- A larger surface area may be sampled;
- Inaccessible systems or ones that cannot be routinely disassembled can be sampled and evaluated.

Disadvantage

- Residues or contaminant may not be soluble or may be physically occluded in the equipment.

The rinse sampling is a method commonly used in multipurpose pilot plants in the pharmaceutical industry: indeed this method is well adapted for very various and often unknown contaminants: traces of starting materials, side-products, salts, traces of product, potentially oil of stirrer motor, etc.).

Classical techniques for the cleaning validation

Due to the very large variety of possible contaminants, it is usually very difficult to perform a quantitative determination of each species; therefore, the global concentration of the residual contaminants must be determined. This concentration must be below the limit defined in the internal Standard Operation Procedure describing the cleaning method. Some classical methods for the cleaning verification are described hereunder:

Distillation residue

A sample of the rinse solution (250–500 mL) is placed in a glass flask and the solvent is evaporated using a rotary evaporator. The mass difference between the empty flask before the test and the same flask after the solvent evaporation (containing the residual contaminants) is used to calculate the total residues concentration. This technique is very convenient because all soluble substances are considered. Nevertheless, its fast implementation right round the clock is difficult in a pilot plant because the necessary laboratory personal often does not work over the night. Moreover, this test cannot easily be carried out by plant operators who often do not have the qualification to perform this delicate analytical work (use of a sensitive laboratory balance, manipulation of the dried flasks without external contamination, cooling of the flask in a desiccator).

Total organic carbon (TOC)

This technique for the analysis of aqueous washing solutions is independent of the product and very sensitive [5]. The determination of TOC, which is automatically performed by the modern analyzers, is divided in three steps:

1. Acidification (removal and venting of inorganic carbon—IC—and purgeable organic carbon gases—POC).
2. Oxidation of the carbon in the remaining sample in the form of carbon dioxide (CO_2) and other gases. This can be done by high temperature combustion, high temperature catalytic oxidation, photo-oxidation, thermo-chemical oxidation, photo-chemical oxidation, or electrolytic oxidation.
3. Detection and quantification using conductivity and non-dispersive infrared (NDIR) to measure the CO_2 generated by the oxidation process.

Nevertheless, the final washing is often performed with an organic solvent in the pharmaceutical industry as many chemical reactions must be carried out in absence of water. Therefore, the method of the distillation residue is the more commonly used in pharmaceutical pilot plants.

Other special techniques are sometimes implemented, but they generally require the knowledge of the contaminants and a calibration is often required (e.g., MAESA

Multifunctional Active Excitation Spectral Analyzer [6]). Therefore, these methods are not described in detail in this article.

Thermogravimetry

This technique is based on the continuous recording of mass changes of a sample as a function of the temperature; the sample is put on a microbalance, which is placed in a furnace. The furnace temperature is controlled in a programmed temperature/time profile and the mass variations during this process are registered. Thermogravimetry is commonly used to investigate processes as thermal stability and decomposition, dehydration, oxidation, determination of volatile content, and corrosion studies at high temperatures.

Therefore, the variation of the sample mass during the evaporation of the solvent can be recorded; this application corresponds more or less to the classical distillation residue method (except that the evaporation is not performed under vacuum). Several equipment providers propose TG devices which are fully automated. Thus, the utilization is relatively easy and trained plant operators can perform the analyses. The TG analyzer used for this purpose must have a very sensitive balance which allows an accurate measurement of the residual mass at the end of the experiment which is performed with a quite small sample (a few grams).

Equipment

Due to the above-mentioned requirements, a *Netzsch STA 449 F1 Jupiter* thermal analyzer was tested for the determination of the cleaning validation in our laboratories. This analyzer offers highest TG resolution (0.025 µg) combined with a measurement range of 5 g. It is equipped with a silicon carbide SiC oven which allows measurements in a range from 25 to 1550 °C; the thermocouples are type S (Pt-10% Rh versus Pt).

The space containing the sample must be flushed with an inert gas during the experiment (see inlet "purge 1 or 2" on Fig. 1) to better eliminate the solvent vapors and avoid any condensation at the vapors outlet; moreover, the very sensitive balance system must also be protected with an inert gas (see inlet "protective"). This flushing minimizes the influence of external physical or chemical parameters. Our experiments were performed with helium because this gas is chemically inert and has no safety-related limitation.

The pressure variations due to the ventilation in the laboratory are compensated by a long fine gas outlet tube (about 3 m, diameter ca. 2 mm) which is connected to a buffer made of a glass tube filled with inert material such as glass wool.

Different sample containers can be used with this analyzer. Due to the low concentration of the impurities in the rinse samples, the containers should be as large as possible. Therefore, 5-mL beakers were implemented for the TG experiments (see Fig. 2). The standard material for these

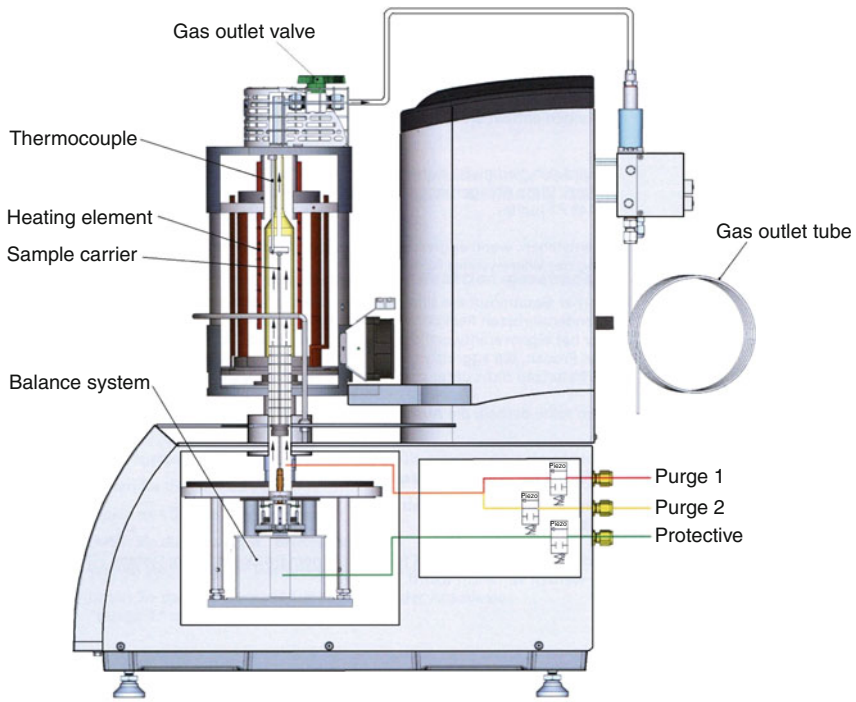

Fig. 1 *Netzsch STA 449 F1 Jupiter* thermal analyzer

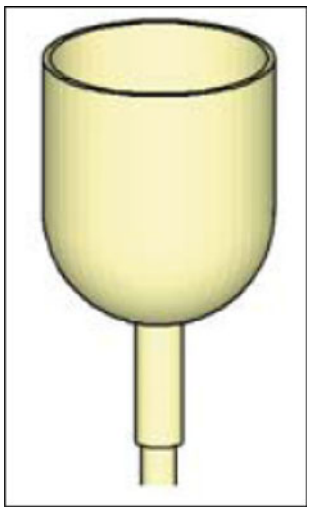

Fig. 2 Testing beaker

beakers is aluminum oxide, which is thermally resistant and can be used up to the maximum temperature of the furnace. Nevertheless, this material is slightly porous and some solvents, mainly with higher boiling points, can remain confined in this substance. The solvents absorbed in the aluminum oxide structure lead to imprecise results during the determination of the residual residues which are systematically higher than in the reality (mainly for low concentrations). Therefore, new beakers made in aluminum or glass were developed. Moreover, they are somewhat more lightweight that the original aluminum oxide beakers, which allows to perform the experiments with more rinse solution without exceeding the balance capacity.

Experimental part

Tests were performed using samples with known impurities concentrations. The samples were prepared by dissolving well-defined amounts of active substances or other impurities as salts in a pure solvent (e.g., acetone, ethanol, and water). Series of experiments were carried out with the same sample to check the repeatability of the method.

Rinse samples containing acetone

The applied temperature program for the furnace was the following:

- Isothermal phase at 30 °C, 10 min.
- Dynamic phase, heating up to 55 °C with 1 °C/min.
- Isothermal phase at 55 °C, 30 min.
- Dynamic phase, heating up to 105 °C with 2 °C/min.
- Isothermal phase at 105 °C, 5 min.
- Dynamic phase, cooling down to 30 °C with 2 °C/min.
- Isothermal phase at 30 °C, 30 min.

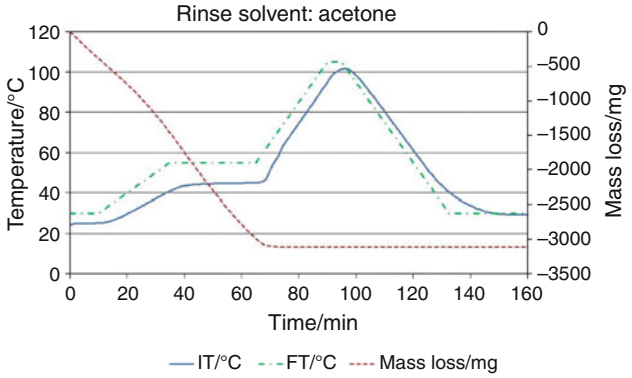

Fig. 3 Temperature measured in the beaker (IT), programmed furnace temperature (FT), and mass loss during an experience with a rinse sample containing acetone

Table 1 Residual contaminants concentrations (in ppm) determined with three series of rinse samples containing acetone

	Series 1	Series 2	Series 3
Measured conc./ppm	31.7	23.2	12.4
True concentrations/ppm	33.2	22.8	12.4
Recovery rate/%	95.50	101.75	100

After the evaporation of the main part of acetone at 55 °C, the content of the beaker was heated up to 105 °C for a short period to eliminate all residual solvent and water traces (the solvents usually used in a production plant are not completely water-free). The mass loss is registered during the progress of the experiment (see Fig. 3). The temperature measured in the beaker (IT) is below the programmed furnace temperature (FT) due to the cooling effect of the solvent evaporation (endothermic process). During the cooling phase, IT is higher than FT due to the thermal inertia of the furnace construction materials. The residue concentration is calculated according to Eq. 1:

$$\mathrm{RC} = \frac{M_R}{\Delta M} \times 10^6 \quad (1)$$

where RC is the residue concentration (ppm), M_R is the residual mass after the experiment completion (mg), and ΔM is the mass loss (mg).

The residual mass M_R is automatically determined by comparison with the initial mass of the empty beaker before starting the experience (tare of the balance system). The temperature of the gas outlet system of the analyzer must be set above the boiling temperature of the solvent (considering the worst case, water) to avoid any condensation in the outlet tube.

Many tests were performed with three rinse samples to determine the recovery rate of the method. The results are summarized in Table 1 and plotted in Fig. 4. The accuracy

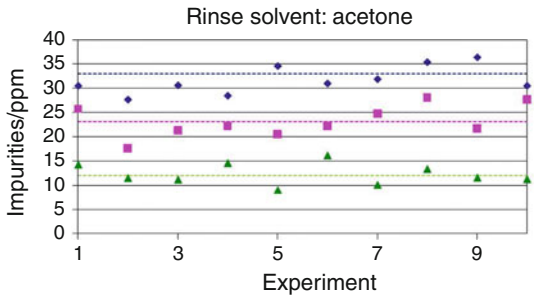

Fig. 4 Residual contaminants concentrations (in ppm) determined with three series of rinse samples containing acetone

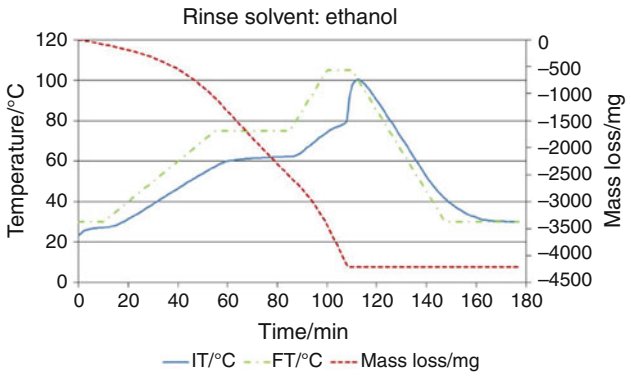

Fig. 5 Temperature measured in the beaker (IT), programmed furnace temperature (FT), and mass loss during an experience with a rinse sample containing ethanol

of the method is sufficient for the assessment of the cleaning effectiveness and the recovery rate is comparable to the traditional distillation residue method.

Rinse samples containing ethanol

The temperature sequence of the furnace was slightly modified due to the higher boiling point of ethanol:

- Isothermal phase at 30 °C, 10 min.
- Dynamic phase, heating up to 75 °C with 1 °C/min.
- Isothermal phase at 75 °C, 30 min.
- Dynamic phase, heating up to 105 °C with 2 °C/min.
- Isothermal phase at 105 °C, 10 min.
- Dynamic phase, cooling down to 30 °C with 2 °C/min.
- Isothermal phase at 30 °C, 30 min.

The mass loss, the sample, and furnace temperatures are registered during the progress of the experiment (see Fig. 5). The results are summarized in Table 2 and plotted in Fig. 6. They confirm the results obtained with acetone.

Table 2 Residual contaminants concentrations (in ppm) determined with three series of rinse samples containing ethanol

	Series 1	Series 2	Series 3
Measured conc./ppm	37.0	25.6	14.2
True concentrations/ppm	35.8	24.5	13.3
Recovery rate/%	103.35	104.49	106.77

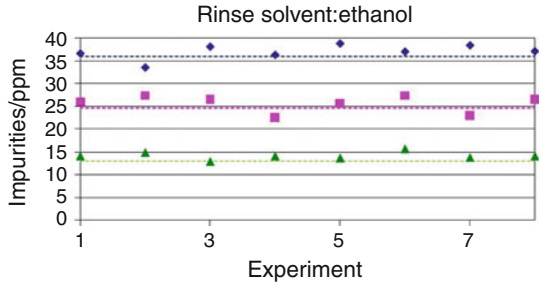

Fig. 6 Residual contaminants concentrations (in ppm) determined with three series of rinse samples containing ethanol

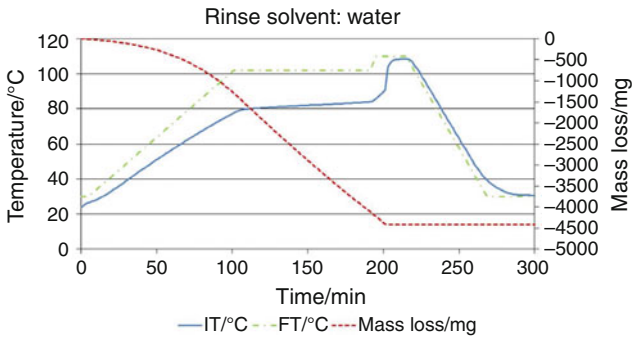

Fig. 7 Temperature measured in the beaker (IT), programmed furnace temperature (FT), and mass loss during an experience with a rinse sample containing water

Rinse samples containing water

Here too, the temperature program for the furnace was somewhat modified due to the higher boiling point of water:

- Isothermal phase at 30 °C, 5 min.
- Dynamic phase, heating up to 102 °C with 0.75 °C/min.
- Isothermal phase at 102 °C, 90 min.
- Dynamic phase, heating up to 110 °C with 2 °C/min.
- Isothermal phase at 110 °C, 20 min.
- Dynamic phase, cooling down to 30 °C with 1.5 °C/min.
- Isothermal phase at 30 °C, 30 min.

The mass loss, the sample, and furnace temperatures are registered during the progress of the experiment (see Fig. 7). The duration of the different heating and cooling

Table 3 Residual contaminants concentrations (in ppm) determined with four series of rinse samples containing water

	Series 1	Series 2	Series 3	Series 4
Measured conc./ppm	31.7	21.9	13.5	8.2
True concentrations/ppm	32.1	22.1	12.9	7.0
Recovery rate/%	98.71	99.44	112.34	117.76

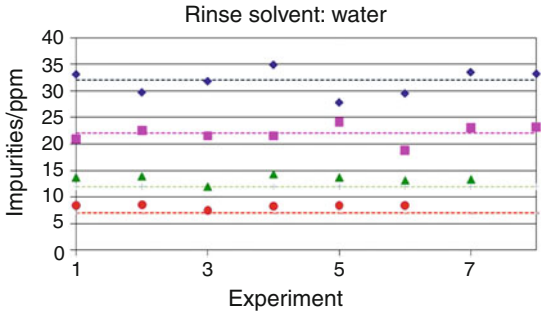

Fig. 8 Residual contaminants concentrations (in ppm) determined with four series of rinse samples containing water

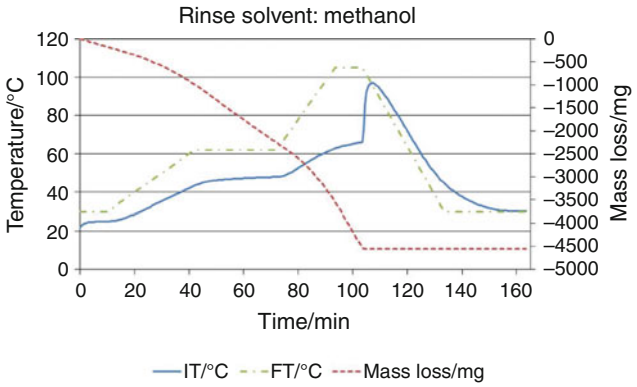

Fig. 9 Temperature measured in the beaker (IT), programmed furnace temperature (FT), and mass loss during an experience with a rinse sample containing methanol

phases can be shortened without negative influence on the result. The results are summarized in Table 3 and represented in Fig. 8. They confirm the previous results obtained with acetone and ethanol. The recovery rate is similar to the conventional distillation residue method.

Similar methods were developed for the other solvents commonly used for the cleaning operations in a pharmaceutical manufacture. Figures 9 and 10 show the mass loss, the sample, and furnace temperatures registered during the progress of experiments with rinse samples containing methanol or dimethylformamide.

After the first phase for the evaluation of thermogravimetry and for the development of the testing methods, series of experiments were performed in parallel with the classical distillation residue method using real samples for

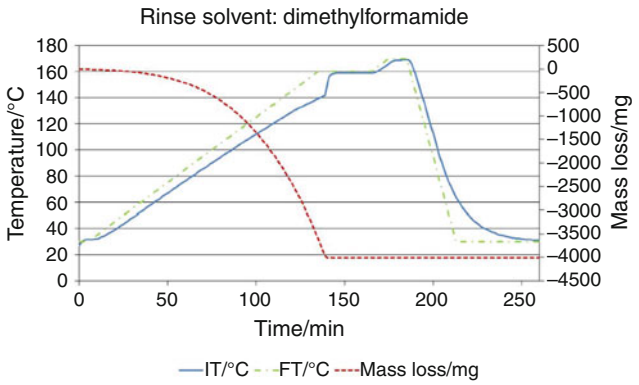

Fig. 10 Temperature measured in the beaker (IT), programmed furnace temperature (FT), and mass loss during an experience with a rinse sample containing dimethylformamide

Table 4 Comparison between the determination of the residues concentrations (in ppm) using the classical distillation residue method and thermogravimetry

Sample	Solvent	Residues concentration/ppm	
		Distillation residue	Thermogravimetry
1	Acetone	6	5.9 ± 0.1
2	Ethanol	27	29.0 ± 0.2
3	Ethanol	13	13.8 ± 1.6
4	Ethanol	5	4.7 ± 0.3
5	Acetone	17	18.5 ± 1.9
6	Acetone	58	53.1 ± 1.4
7	Acetone	0.6	0.8 ± 0.3
8	Acetone	<3	1.2 ± 0.2
9	Acetone	<3	2.1 ± 0.8
10	Ethanol	3.4	2.7 ± 0.3
11	Acetone	11	12.3 ± 0.3
12	Acetone	109	111 ± 1
13	Methanol	14	14.5 ± 1.0

the pilot plant. Some results are summarized in Table 4. Only one determination was carried out with the traditional method during the official cleaning validation of chemical reactors. On the other hand, several tests were performed using thermogravimetry to check the repeatability of the method.

Conclusions

The tests demonstrate that thermogravimetry can be used for the determination of the residual impurities during the cleaning validation of the equipment of pharmaceutical production plants. The accuracy and the recovery rate of this method are comparable with those of the classical analysis method (determination of the distillation residue

using a rotary evaporator). The fully automation of the testing equipment even allows its utilization around the clock by plants operators, leading to a significant reduction of the time necessary for the cleaning validation and to an increase of the plant capacity.

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