Aseptic filling of sterile powders: some elements of Statistical Process Control and Preventive Maintenance

1. INTRODUCTION

A precise and accurate dosing of sterile powders under aseptic conditions in vials still represents a challenge in the pharmaceutical field and this is even more true when it comes to small quantities of high-potency active substances.

To conduct this important operation of the pharmaceutical industry effectively and efficiently, microdosing machines are available that can fill up to over 20,000 vials per hour.

Among the various filling methods available, the one that uses a vacuum / pressure system is very popular.

In general, after washing and depyrogenation, the sterile glass vials, thanks to a conveyor belt, pass over weighing cells to determine the tare and then reach the dosing discs. These, usually two and operating in parallel, dispense the sterile powder using the vacuum/pressure dosing system. The vials then continue to other weighing cells for the determination of the net weight and then move towards the capping and crimping stations.

The heart of the process is therefore represented by the two dosing discs which are entrusted with the task of accurately and precisely dispensing the sterile powder into the glass vials. For this purpose, each disc contains calibrated chambers (or dosing ports) which are filled in succession with dry product by means of a vacuum action that compacts the powder inside them.

The sterile powder is fed from a loading hopper above the dosing discs.

After loading, the dosing disc rotates 180° and, replacing the vacuum with positive pressure air, delivers the dose into the vial below.

It is evident from this that the weight of each dose is a function of different variables such as, for example:

- the volume of the dosing chamber, which varies depending on the amount of powder to be dosed and which is adjusted by acting on the height of the chamber itself, as the diameter cannot be changed.

- the force of the vacuum applied which guarantees the permanence of the powder in the chamber during the 180° rotation of the dosing disc,
- the positive pressure value applied for the quantitative expulsion of the powder from the dosing chamber into the vial below.

These parameters are therefore those which affect the reproducibility of the dose delivered into the vial.

Microdosing machines are, in general, equipped with self-adjusting weight systems in order to compensate for any variations in the weights delivered that exceed pre-established operating limits.

Aim of this post is showing how, thanks to rather simple statistical tools, it is possible to obtain useful information about the performance of the filling process from operational data and monitor its variability so as to be able to intervene in a preventive manner.

2. EXPERIMENTAL SECTION

Table 1 shows an extract of the typical database that a microdosing machine generates during the filling process.
In the chosen case study, it consists of about 12600 data (almost 6300 for each dosing disc, A / B) relating to a possible *Target Fill Weight* (TFW) of 1000 mg and corresponding to as many vials filled with sterile powder.
Each row of Table 1 contains: the progressive number of the vial, the dosing disc that filled it (A or B), the number of the chamber in which the powder was contained (chambers are 12 for each disc), the height of the chamber itself and the amount of net powder transferred into the sterile vial.

Table 1

Sample	Dosing Wheel	Dosing Chamber	Chamber Height (mm)	Net Weight (mg)
1	A	5	22,90	938
2	B	5	22,90	943
3	A	6	22,90	944
4	B	6	22,90	954
5	A	7	23,30	961
6	B	7	23,30	970
7	A	8	23,30	971
8	B	8	23,30	977
9	A	9	23,50	974
10	B	9	23,50	986
…	…	…	…	…
…	…	…	…	…
12534	A	11	24,10	1000
12535	B	11	24,10	999
12536	A	12	24,10	1006
12537	B	12	24,10	1001
12538	A	1	24,10	998
12539	B	1	24,10	987

The dataset constitutes a *multiple entry table* as each row refers to a given vial while the columns are each related to a specific analytical parameter, or *variable*. This data table, in statistical jargon, is usually referred to as the *data matrix*.

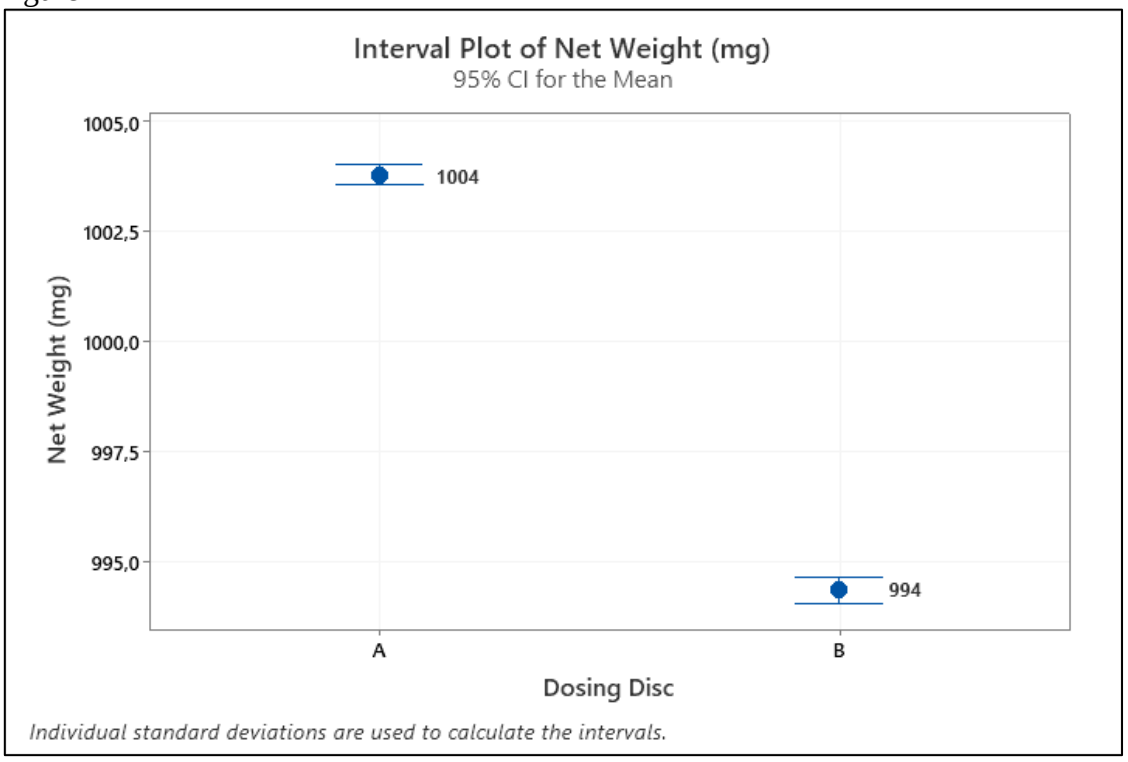
Data analysis and their visualization were conducted using Minitab 20 (GMSL S.r.l. - Via Giovanni XXIII, 21 - 20014 Nerviano (Milan), Italy).

3. RESULTS AND DISCUSSION

To begin, it is convenient to look at the process from a very general point of view by observing it as the result of two processes that occur concomitantly each on a given dosing disc.

Figure 1 shows an *interval plot* that compares average filling weights, and relative confidence intervals, for the two dosing discs A and B.

Figure 1



This graph immediately shows a difference of 10 mg, on average, between the quantity dosed by disc A compared to that dosed by disc B.

By itself this difference may not be that relevant, but a careful examination of Figure 1 also shows that the confidence interval calculated on the weights delivered by disc B appears wider than the corresponding relative to disc A. This difference is best visualized by the histograms in Figure 2 which represent the weight distributions delivered by the two dosing discs.

Figure 2

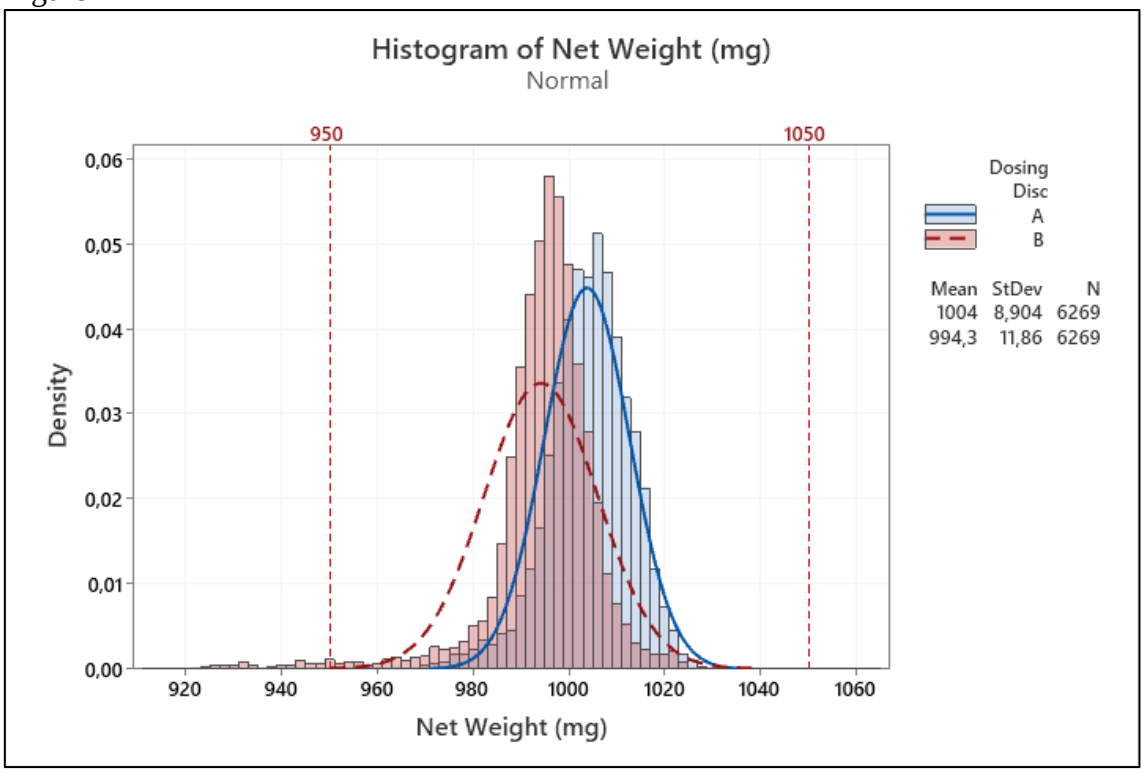


Figure 2 clearly shows that, although both weight distributions tend to be centered around the target value of 1000 mg, the one relating to disc A is less broad. It is in fact characterized by a lower standard deviation with respect to the distribution of the weights delivered by disc B (8.904 *vs*. 11.86) and is all within ± 5% of the target value (*i.e.*, 950 - 1050 mg).

At this point it is interesting to analyze the two weight distributions separately.

Figure 3 shows an *I-MR control chart* relating to the first three hundred weight values delivered by disc A. *Chart I* shows that, after an initial phase of centering the weight, this is kept practically constant and all values are within ± 3 standard deviations. Only one value is at the upper limit of +3 standard deviations from the mean. The *moving range* (MR) *control chart*, which computes subgroups of dimension 2 from sample observations, also shows all values strictly within limits. This behavior is also confirmed in the subsequent weight values.

Figure 4, for example, shows the following three hundred weight values delivered by disc A which are acceptable and practically all within the limit of ± 3 standard deviations.

Figure 3

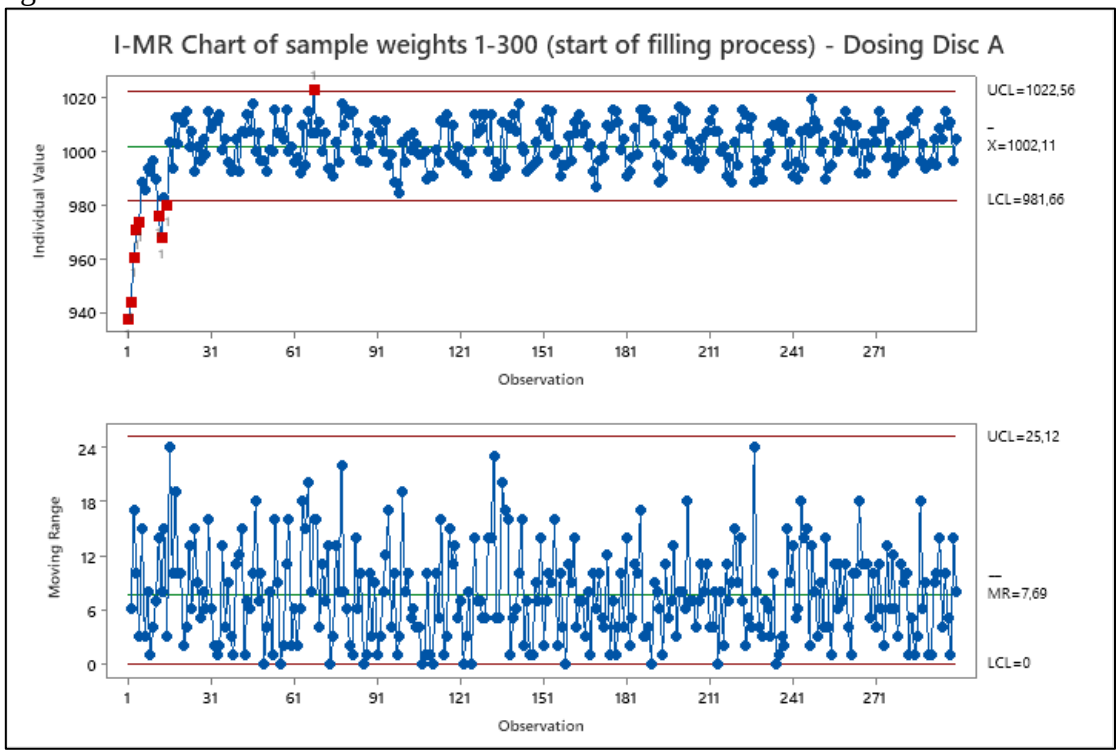


Figure 4

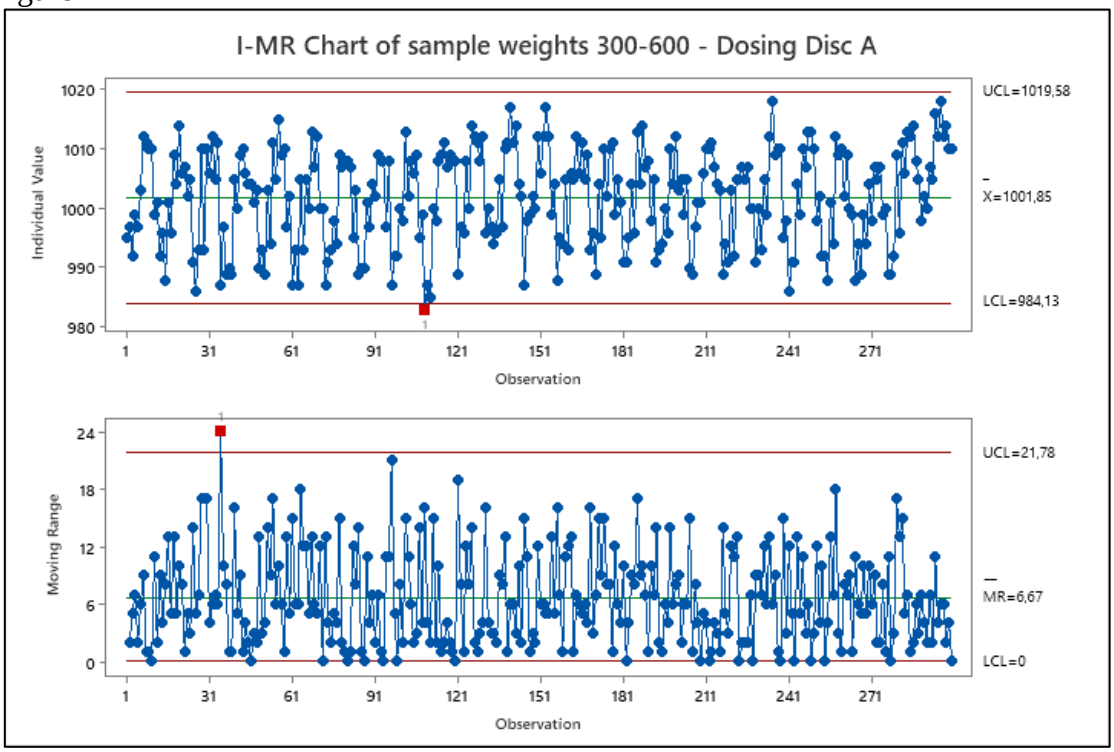
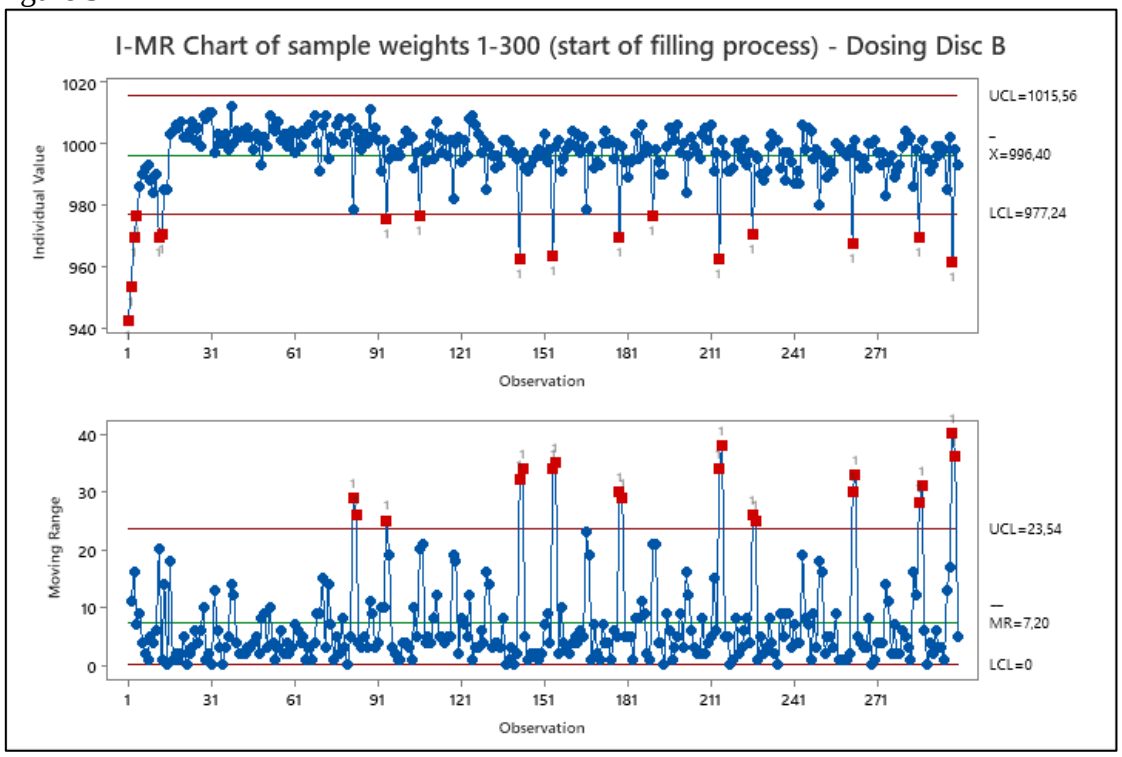


Figure 5, instead shows the *I-MR control chart* relating to the first three hundred weight values recorded for disc B.

Figure 5



The situation represented above, except for the initial centering phase, appears to be characterized by a greater variability than that observed for the dosing disc A (see Figure 3). Nevertheless, thanks to the self-compensation system, the weights realign themselves obtaining a situation that is practically ideal still in the middle of the filling process (Figure 6) and which remains practically unchanged until the end of the same (Figure 7).

Figure 6

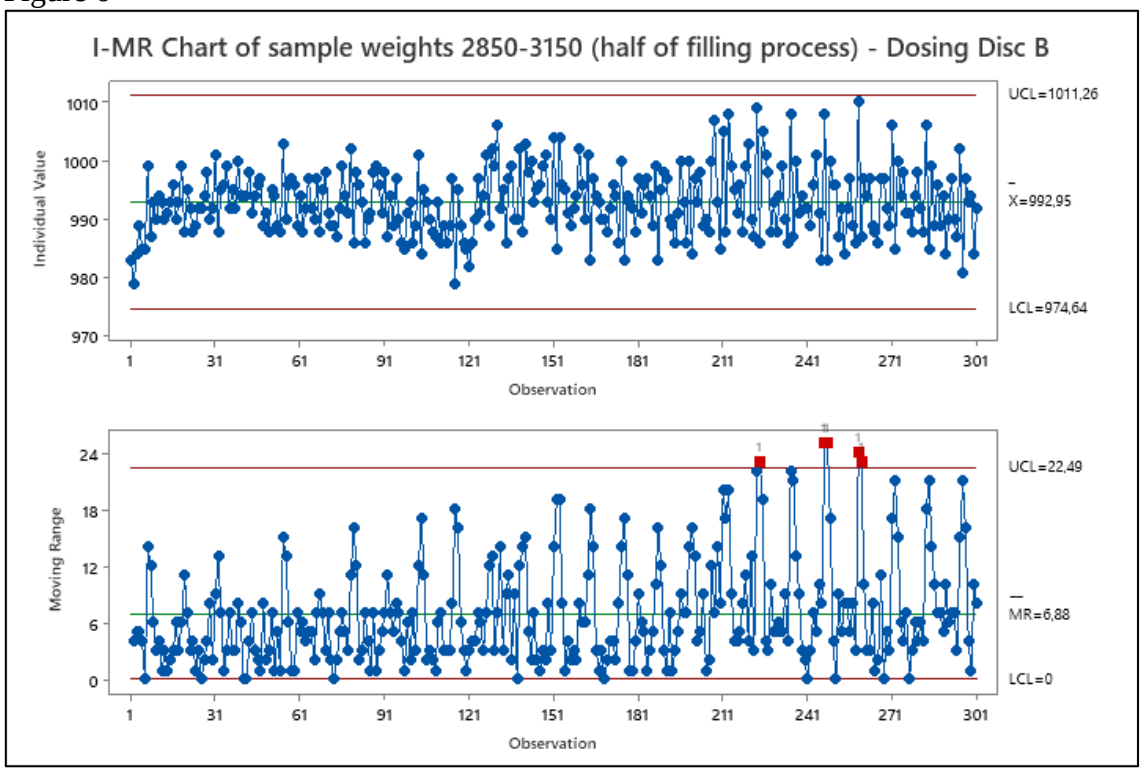
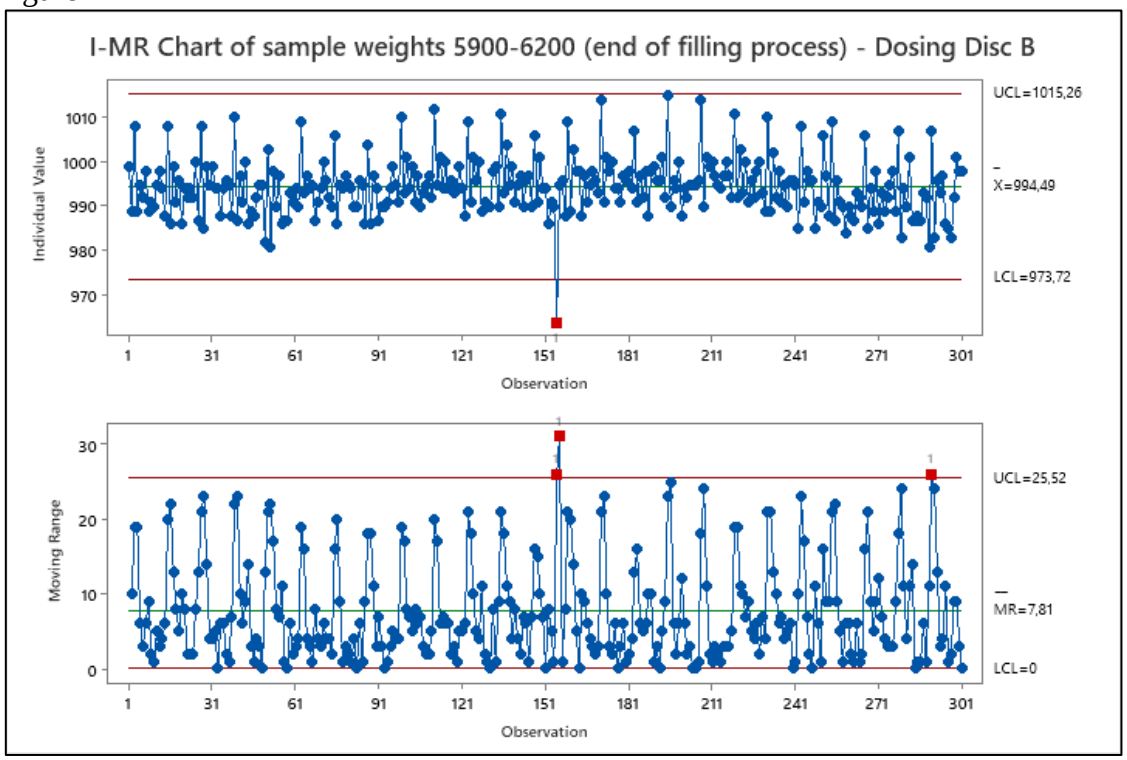


Figure 7



These control charts provide valuable detailed information, but do not account for the difference between the amount of powder dosed on average by the two discs and initially illustrated in Figure 1. To understand this aspect, it is necessary to analyze the behavior of the individual dosing chambers. Figures 8 and 9 show the interval plots of the individual chambers for each dosing disc.

Figure 8

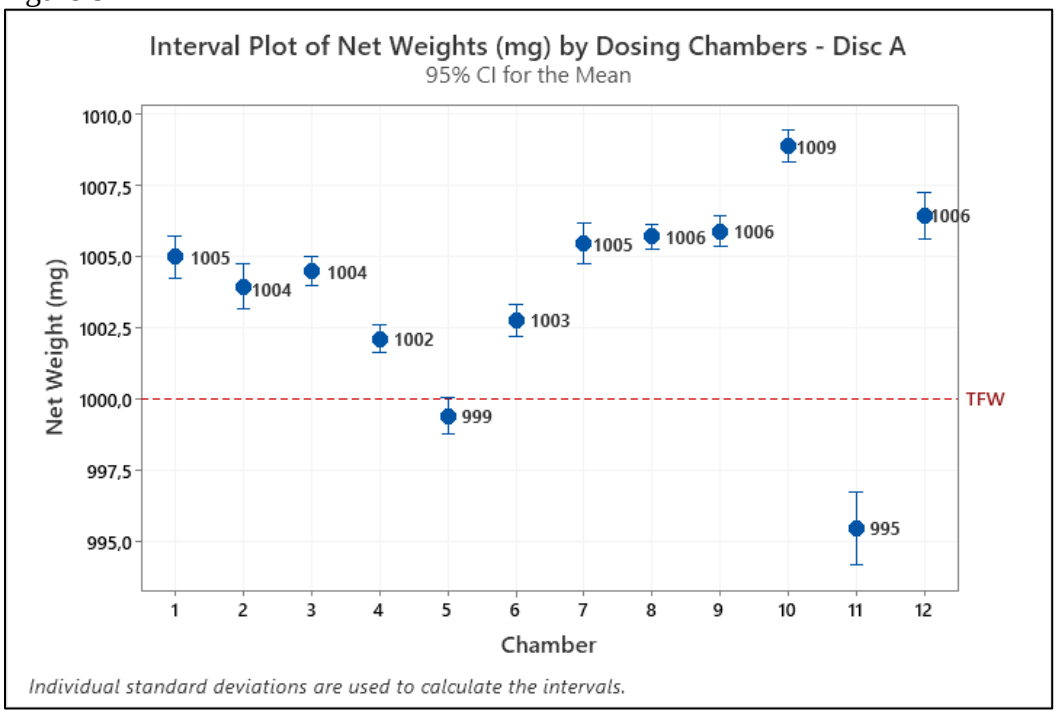
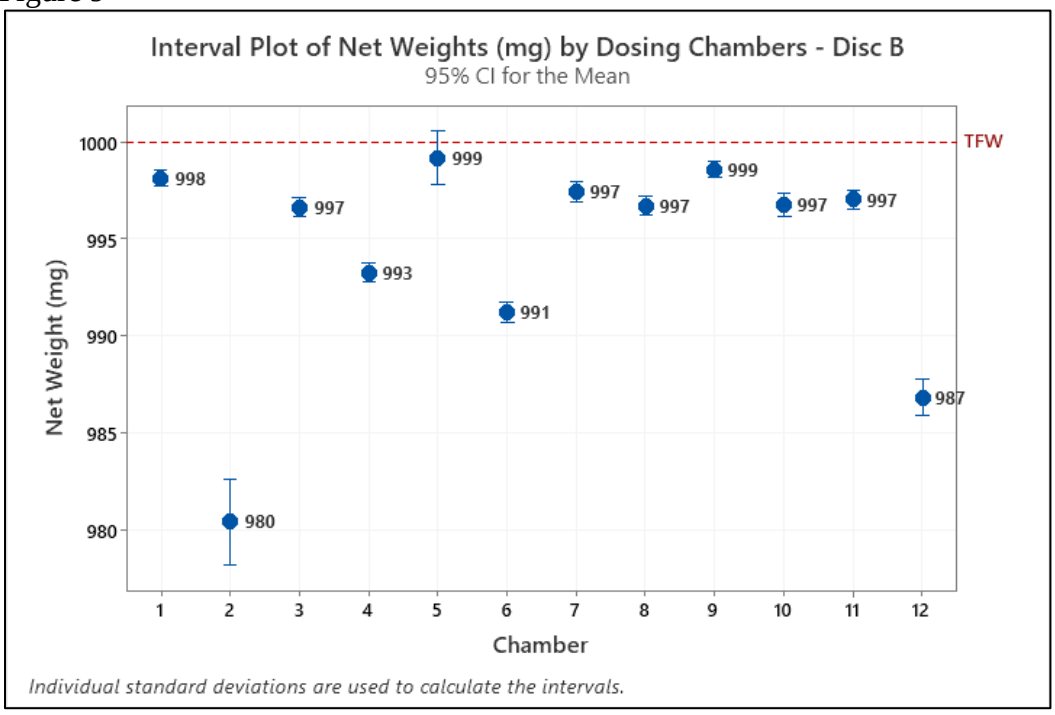
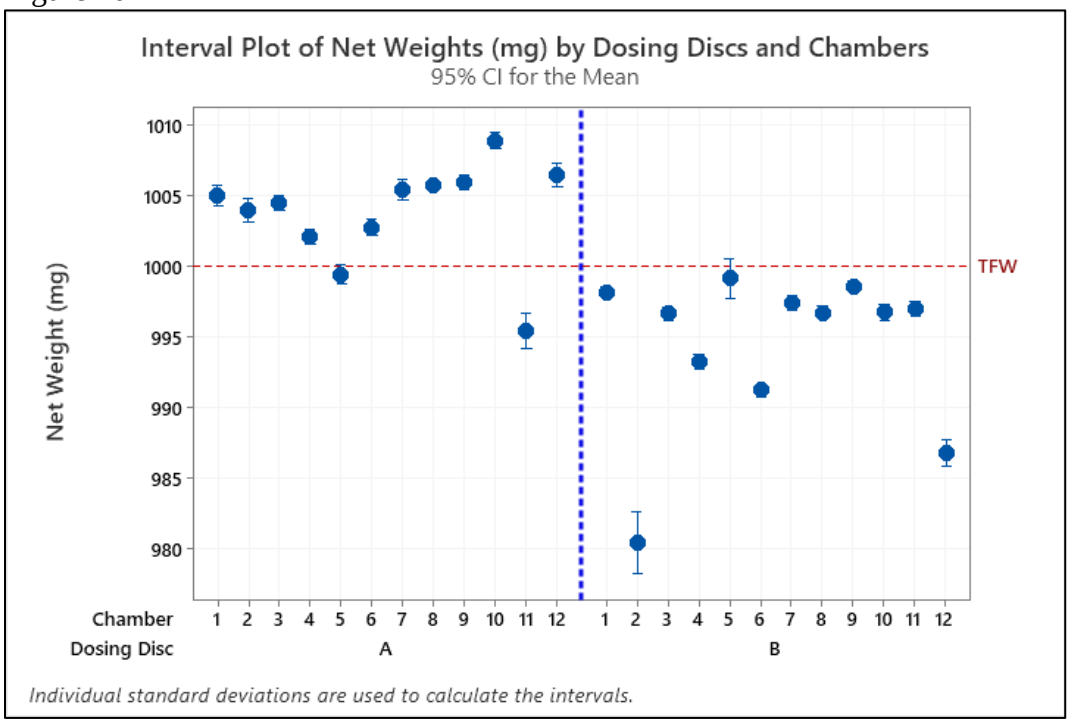


Figure 9



For easier viewing of the whole system, Figure 10 shows the interval plots related to the dosing chambers of the two discs.

Figure 10

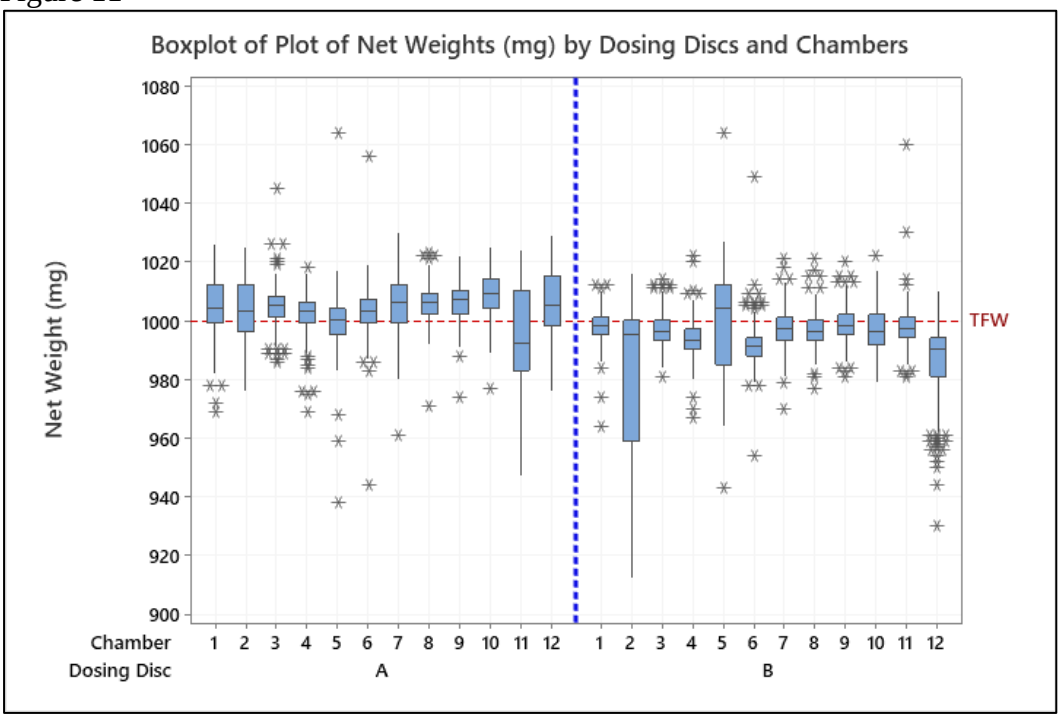


From the examination of Figures 8-10 it emerges that:

- chambers of the dosing disc B appear to deliver weight values smaller than the TFW of 1000 mg while those of disc A, apart from two (chambers 5 and 11), appear to deliver values greater than the TFW,
- some chambers, 11 for disc A and 2 and 5 for disc B, have a much higher variability than that exhibited by the others,
- chamber 12 of disc B shows an average weight value (987 mg - Figure 9) lower than the others except for that associated with chamber 2 (980 mg - Figure 9).

The picture provided by Figure 10 is perhaps even more immediate if, instead of the *interval plots*, the *box plots* are used as in Figure 11 where, already at a glance, the variability that exists between chamber and chamber is evident while remaining at inside of the same dosing disc.

Figure 11



The greater variability associated with chamber 11 of disc A and with chambers 2 and 5 of the disc B is here even more graphically evident (Figure 11).

To have a greater level of detail it is worth considering the two dosing discs separately (Figures 12 and 13).

Figure 12

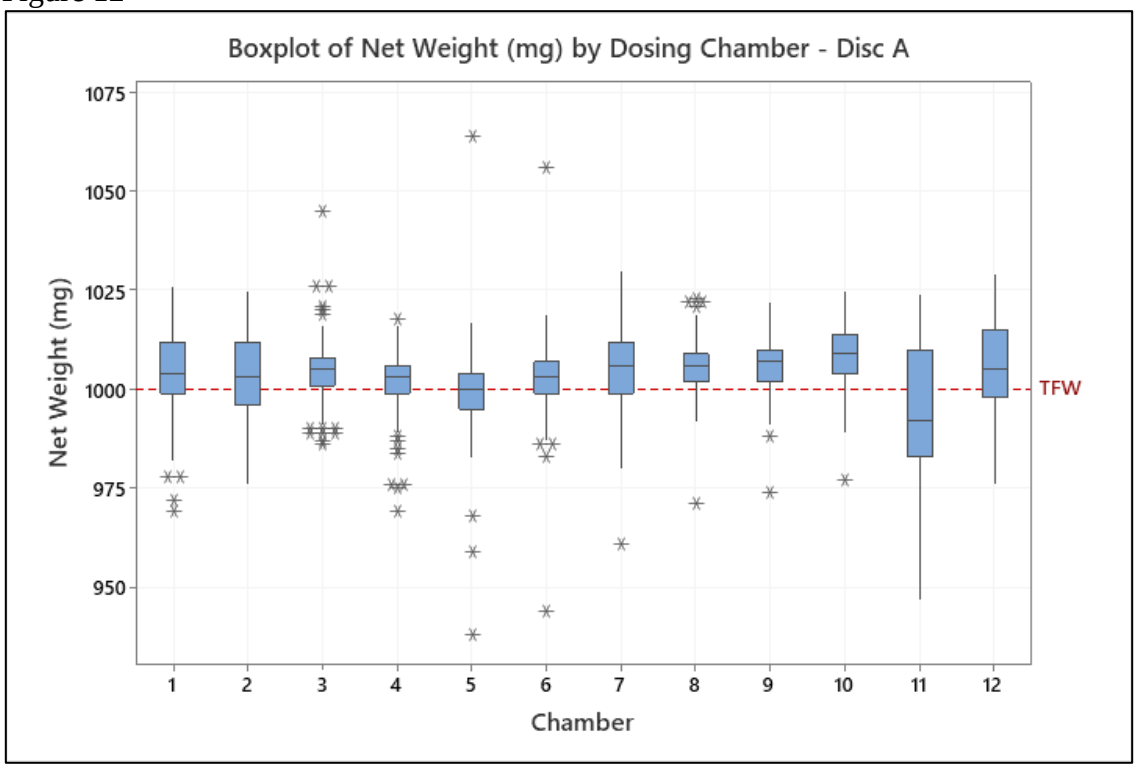
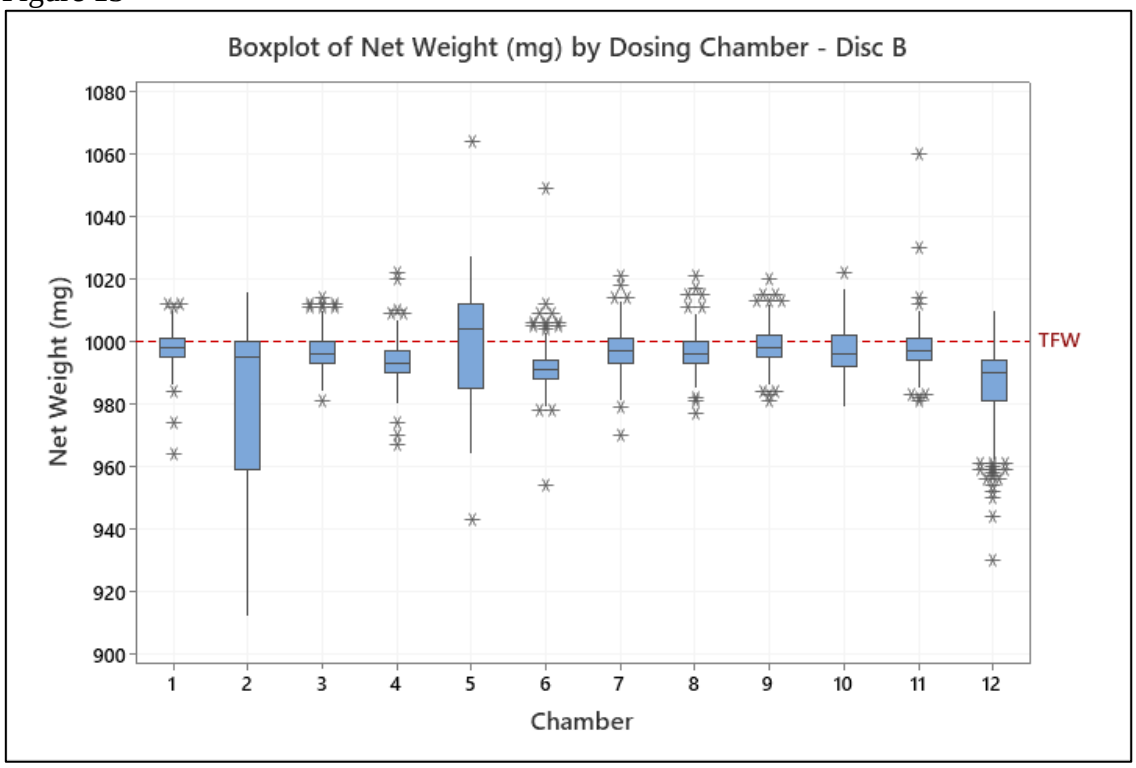


Figure 13

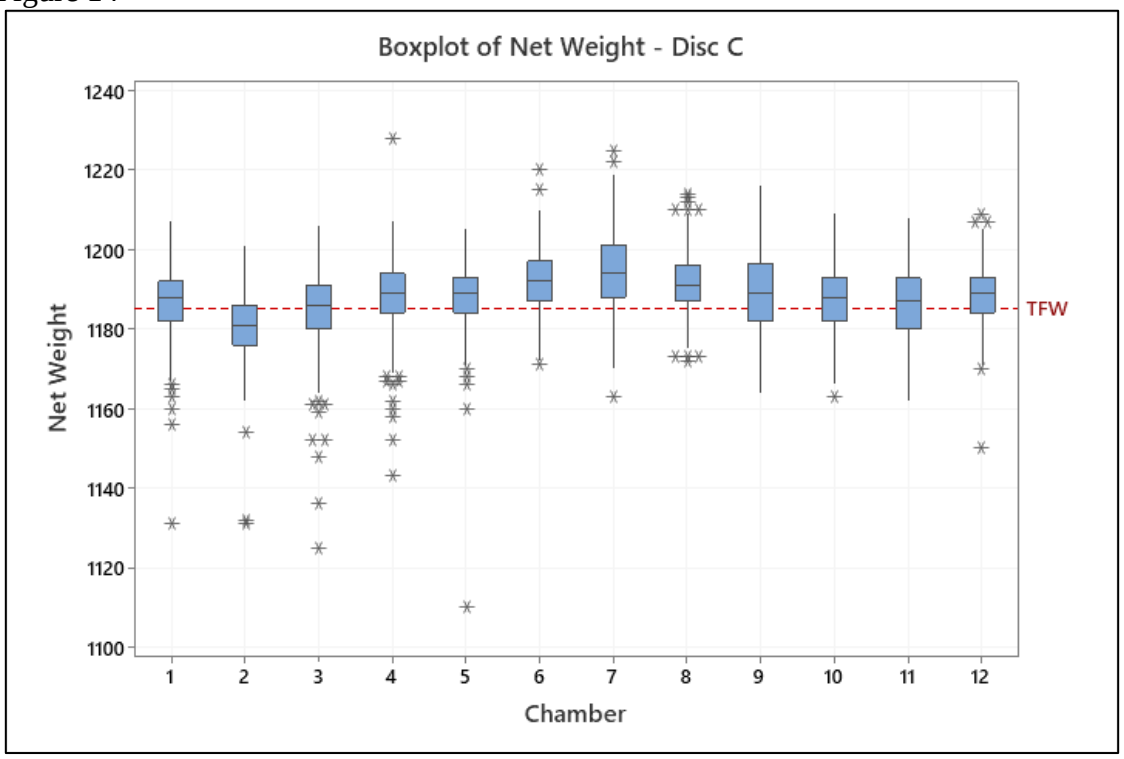


In Figure 12 are displayed some different types of boxplots such as, for example, those related to:

- fairly symmetric distributions with no outlier (*e.g.*, chambers 2 and 12)
- symmetric distributions, much narrower than the previous ones, but with outliers (*e.g.*, chamber 3)
- clearly asymmetrical distributions (*e.g.*, chamber 11).

To get an idea of the appearance that a similar boxplot has in the case of a dosing disc characterized by greater uniformity between the different chambers, refer to Figure 14 which shows the trend of a further dosing disc which will be indicated as C for distinguish it from the previous ones. The visual comparison of the box plots in Figure 14 with those shown in Figures 12 and 13 already shows the difference.

Figure 14



For a more precise comparison between the behaviors of the various chambers in Figure 12, the four box plots relating to the weights delivered by the chambers are shown (Figure 15), while the corresponding histograms are displayed in Figure 16.

Figure 15

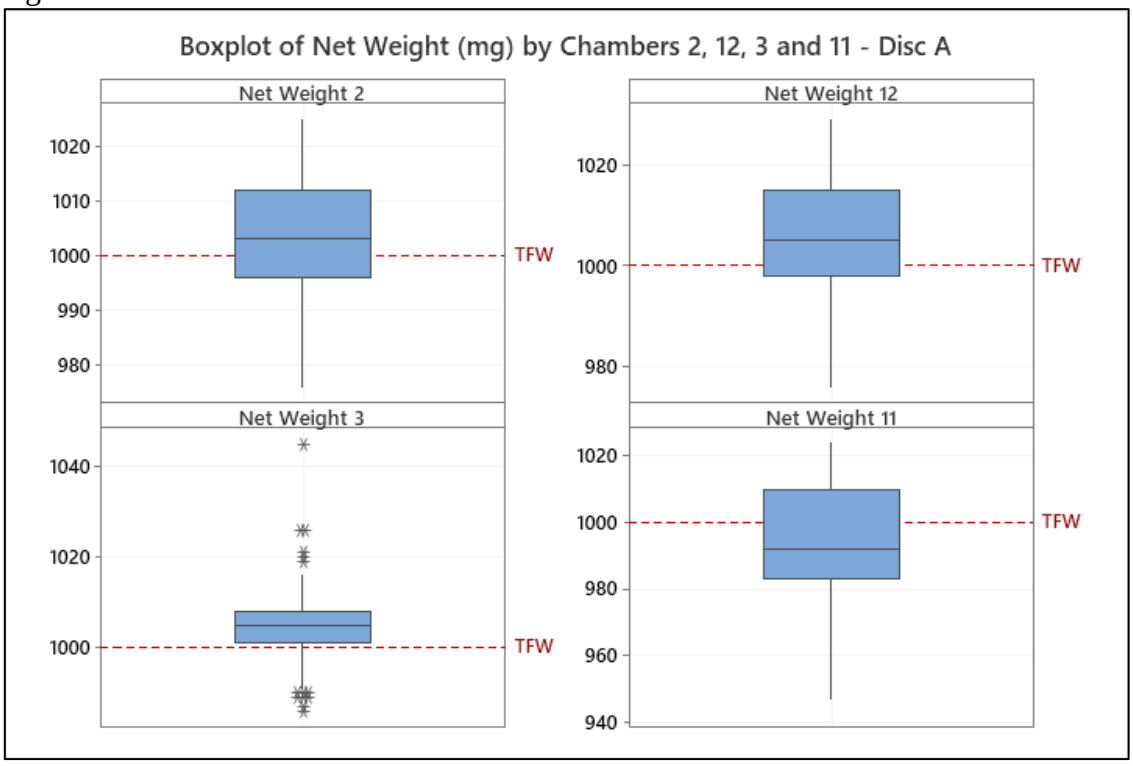
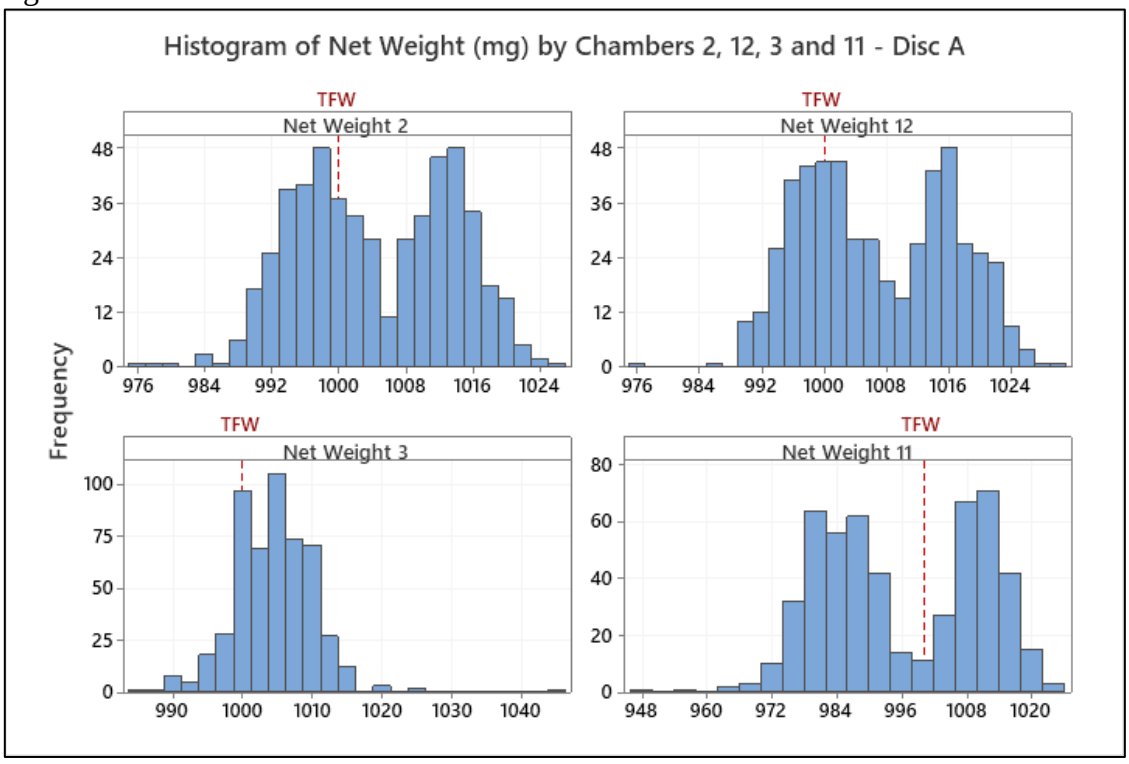


Figure 16



From the comparison between Figures 15 and 16 it emerges that the increase in the variability of the weights leads to distributions that are increasingly bimodal with a maximum for the distribution relative to the weights supplied by chamber 11. In the latter case, it is true that the average weight is 995 mg, as can be seen from Figure 8, but this value is the average of two distributions respectively centered around 980 and 1010 mg. In practice, there are two subpopulations that differ from each other by 30 mg.

The only case in which there is a mono-modal distribution is that of chamber 3 which is then the one with the narrowest weight distribution.

A similar behavior is also found for disc B (Figure 13). As can be seen in fact from Figures 17 and 18 shown below:

- apparently narrow distributions (*e.g.*, chambers 3 and 6 in Figure 17) show a mono-modal trend characterized, however, by numerous outliers which ensure that the mean and median are significantly different from each other
- broader distributions (*e.g.*, chamber 5) have bimodal distributions which, with a further increase in the data dispersion, return to mono-modal, but heavily tailed. This is particularly evident in the case of chamber 2 where the weights dispensed extend over a range of 90 mg (from about 915 mg to over 1005 mg).

Figure 17

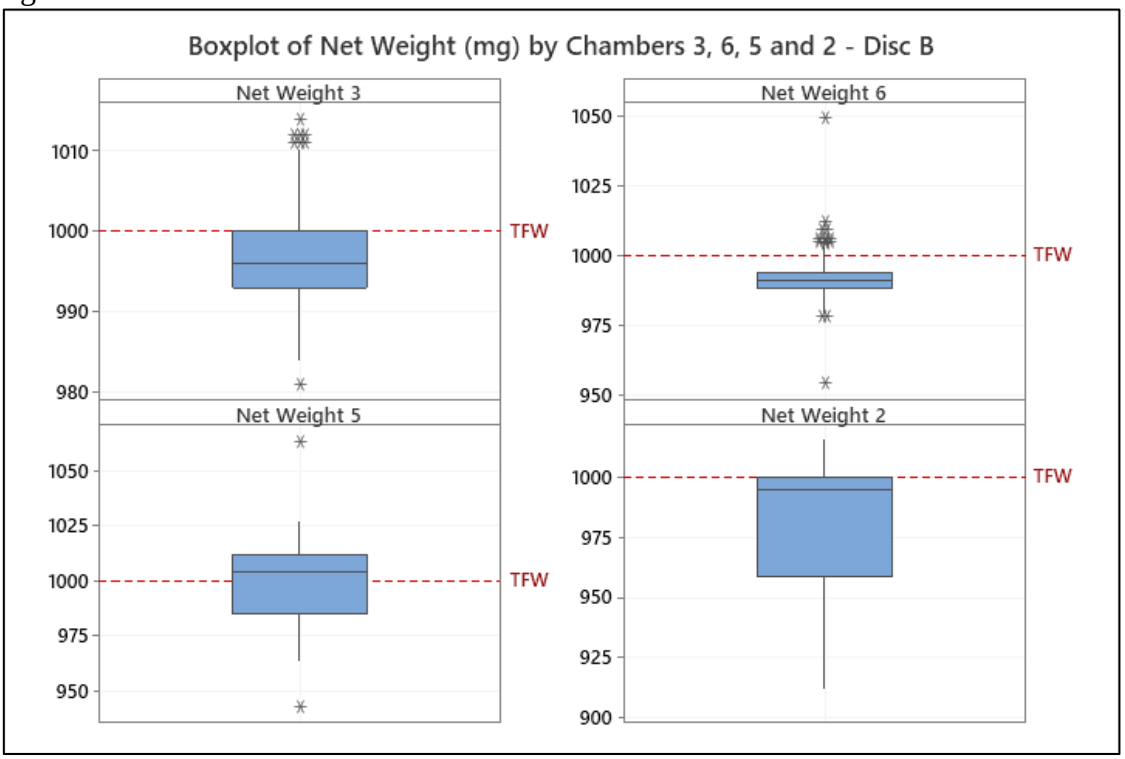
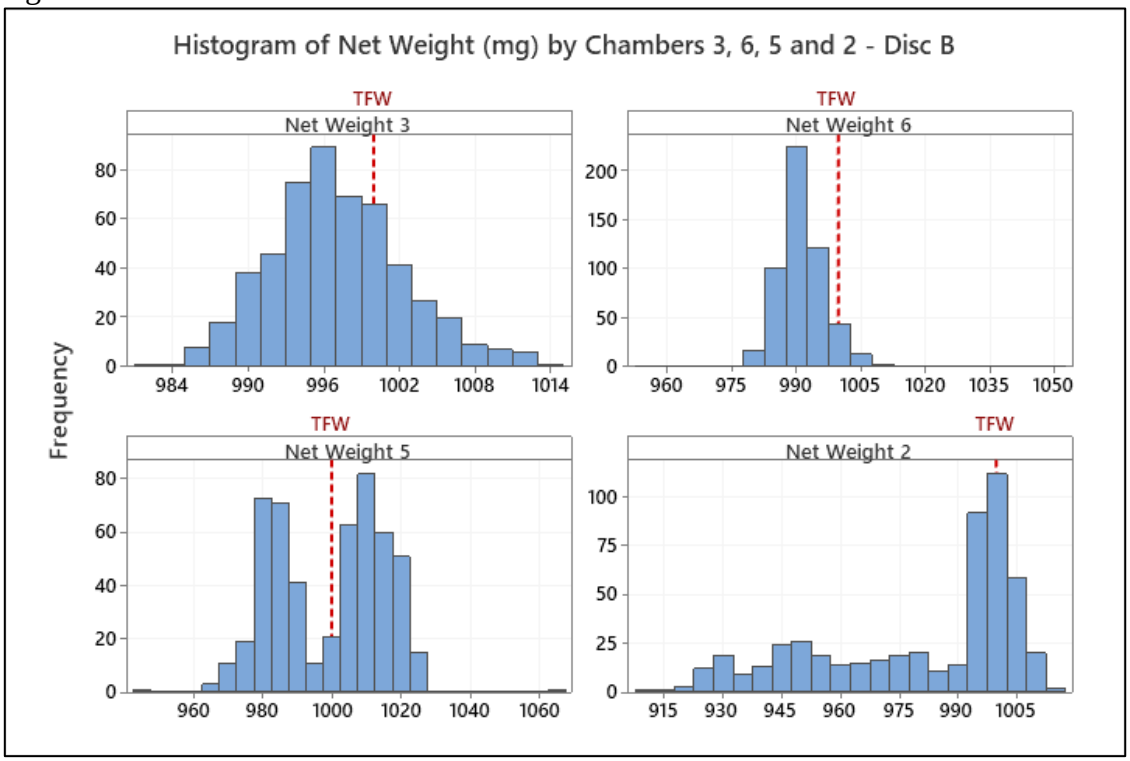


Figure 18



From this simple preliminary analysis, it emerges, in general, different performances between the dosing chambers inside each disc and an overall more deteriorated situation for disc B than disc A.

To deepen the relationship between the weights dispensed and the heights of the dosing chamber, one of the best cases available was chosen and precisely chamber 3 of disc A for which a *graphical summary report* is provided in Figure 19.

Figure 19

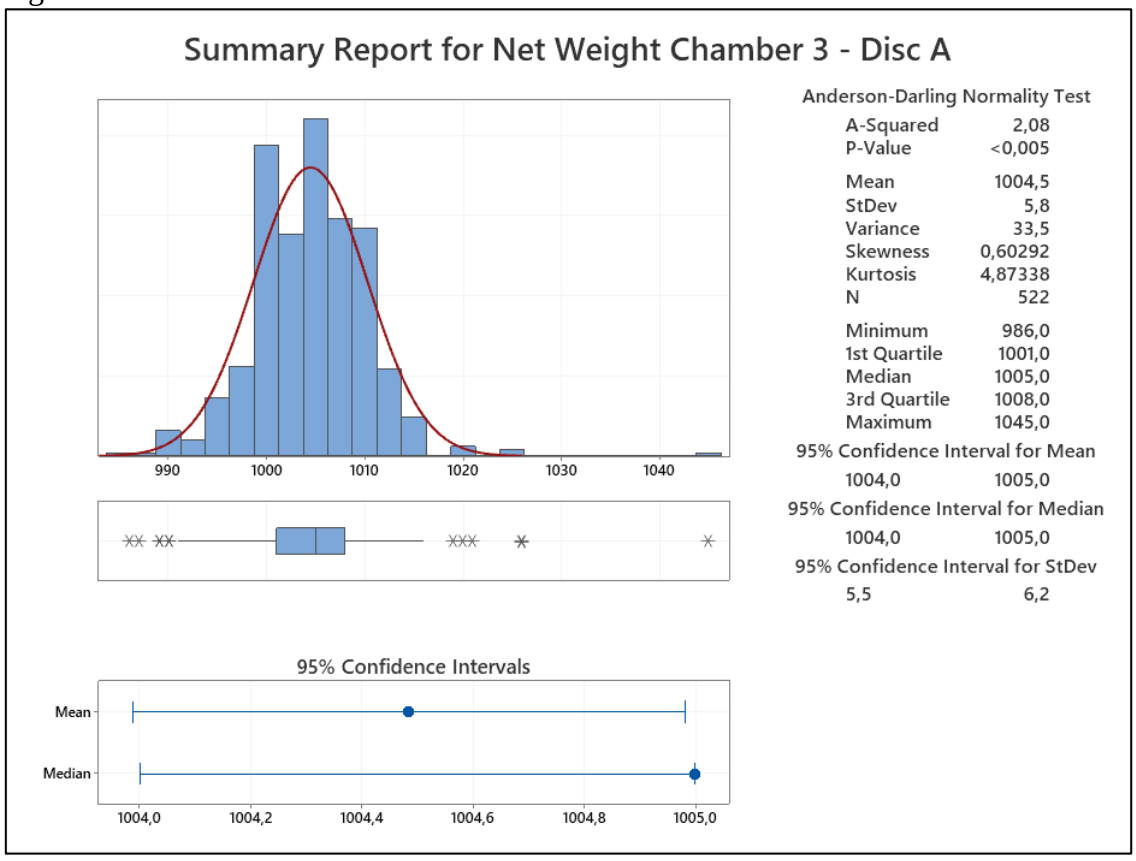


Figure 19 shows that the weight distribution, apart from some outliers, is on the whole quite contained as it is practically ranging from 990 to 1020 mg.

Figure 20, on the other hand, shows the trend of the weights as a function of the different heights that the dosing chamber has taken during the powder distribution process. Although the graph is burdened by the number of datapoints (522) it is evident, already at a glance, the existence of a *curvilinear relationship* between the two variables.

The "curvilinear" nature of this relationship is also confirmed by interpolating the data with a linear and a quadratic model (Figures 21 and 22). The transition from the linear model (Figure 21) to the quadratic one (Figure 22) raises R-Sq from 27% to 34.6%.

Figure 20

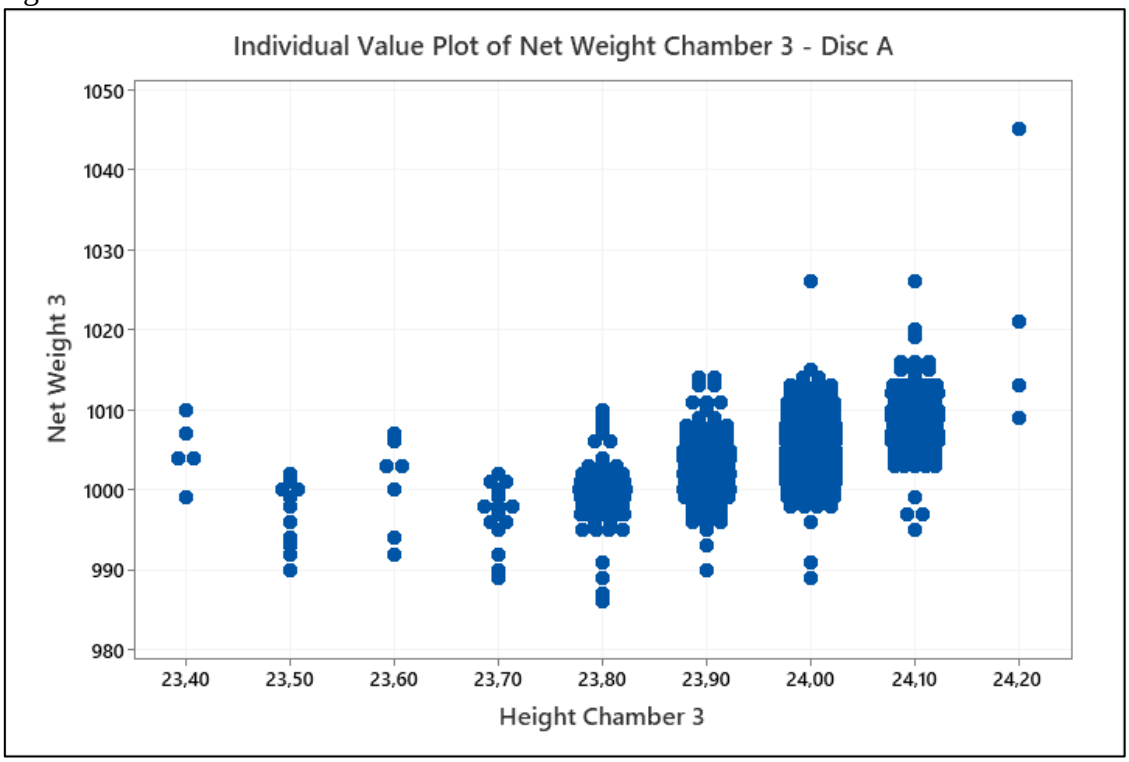


Figure 21

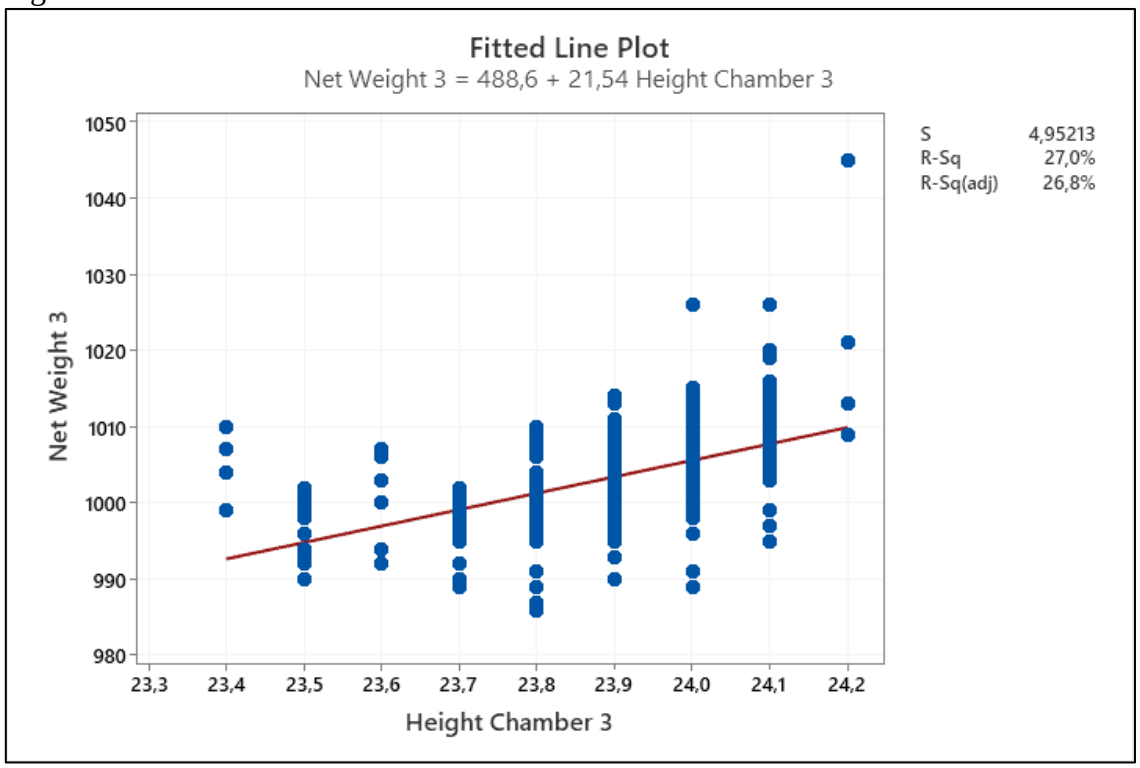
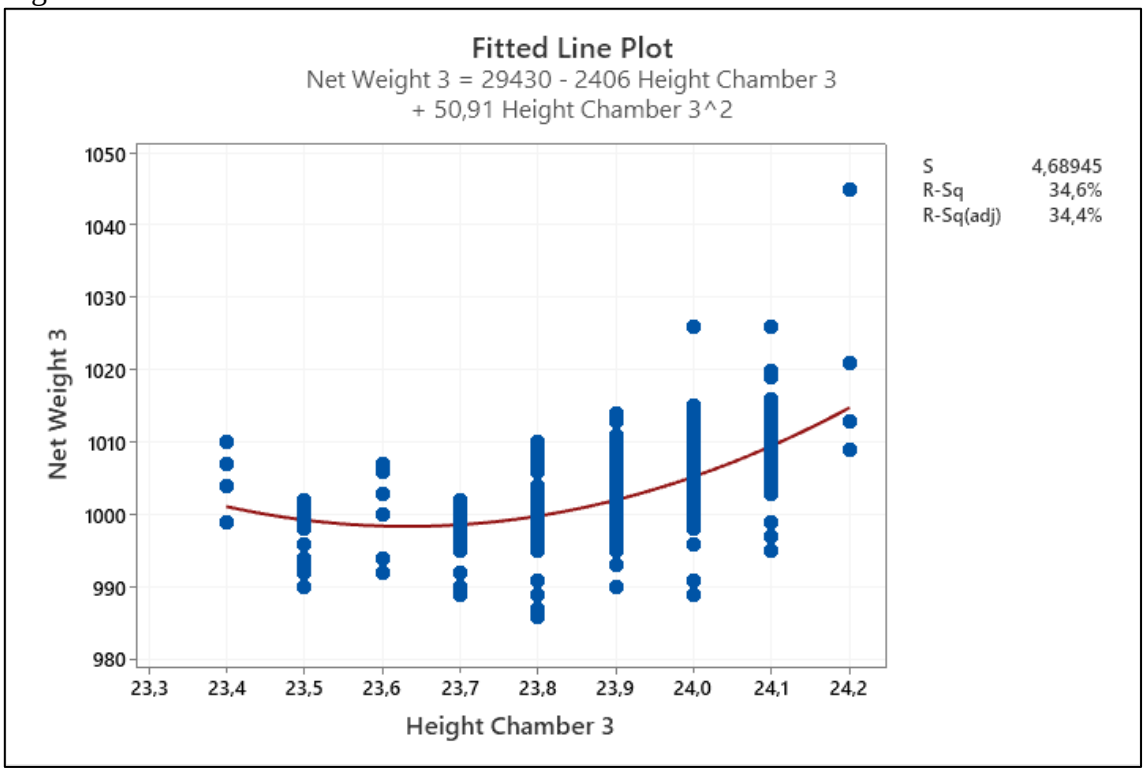


Figure 22



The Analysis of Variance associated with the model, and summarized here below, confirms the statistical significance of the quadratic term as well as the linear one.

Polynomial Regression Analysis: Net Weight 3 versus Height Chamber 3

The regression equation is

Net Weight 3 = 29430 - 2406 Height Chamber 3 + 50,91 Height Chamber 3^2

Model Summary

S	R-sq	R-sq(adj)
4,68945	34,63%	34,37%

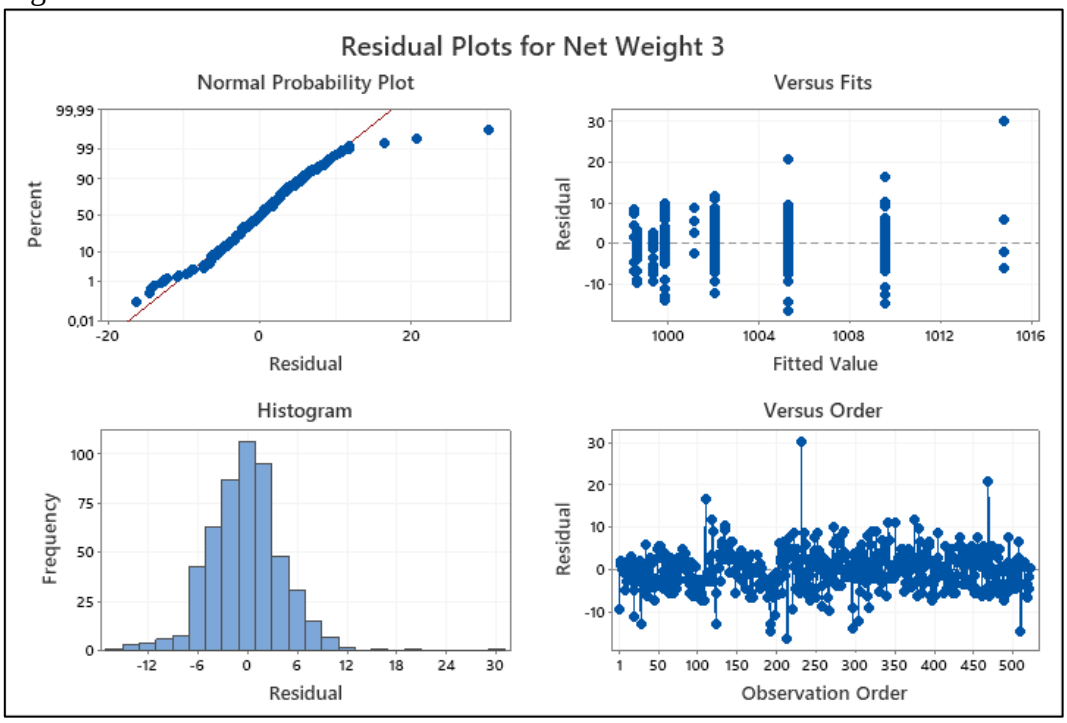
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	6045,1	3022,55	137,45	0,000
Error	519	11413,3	21,99		
Total	521	17458,4			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	4706,11	191,90	**0,000**
Quadratic	1	1338,98	60,89	**0,000**

Figure 23



The quadratic model can explain only 34.6% of the total variability expressed by the data as R-sq, which is defined by the formula:

$$R - sq = \frac{Variance\ explained\ by\ the\ model}{Total\ variance} \times 100$$

evaluates the dispersion of the experimental data around the regression curve and, in this case, this dispersion is significant (Figure 22). Indeed, considering the values of heights assumed by the chamber during the process and replacing the experimental data with their average, R-sq raises from 34.6% to 92.2% as shown here below.

Polynomial Regression Analysis: Average Net Weight 3 versus Bin Height Chamber 3

The regression equation is

Average Net Weight 3 = 46930 - 3880 Bin Height Chamber 3 + 81,93 Bin Height Chamber 3^2

Model Summary

S	R-sq	R-sq(adj)
2,51851	92,16%	89,55%

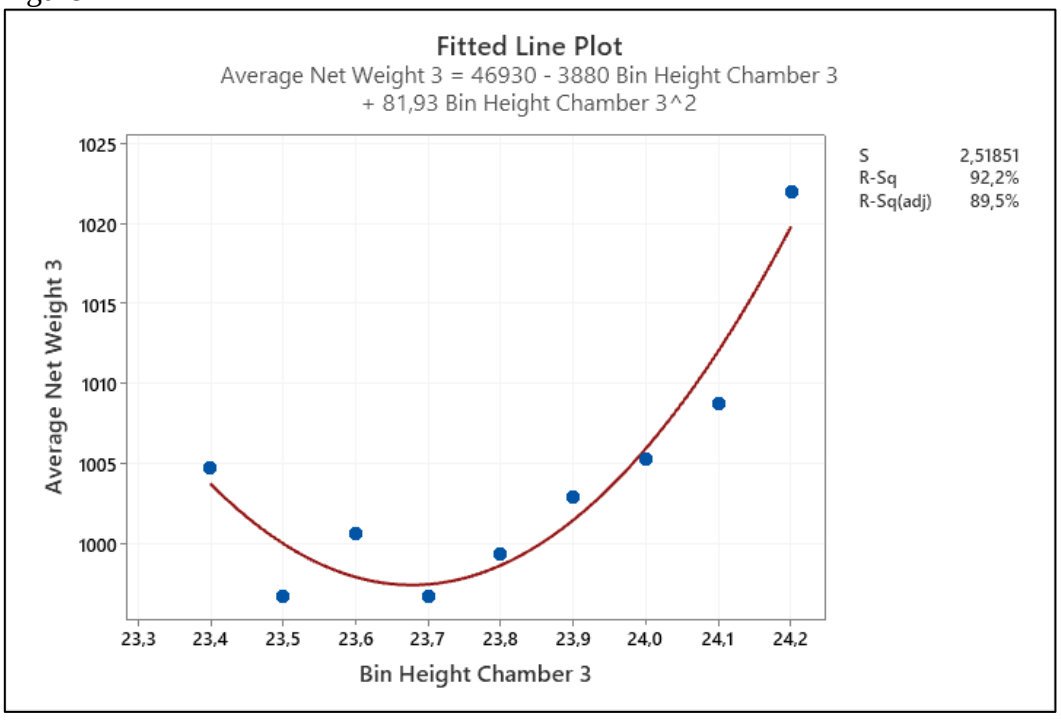
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	447,421	223,710	35,27	0,000
Error	6	38,057	6,343		
Total	8	485,478			

Sequential Analysis of Variance

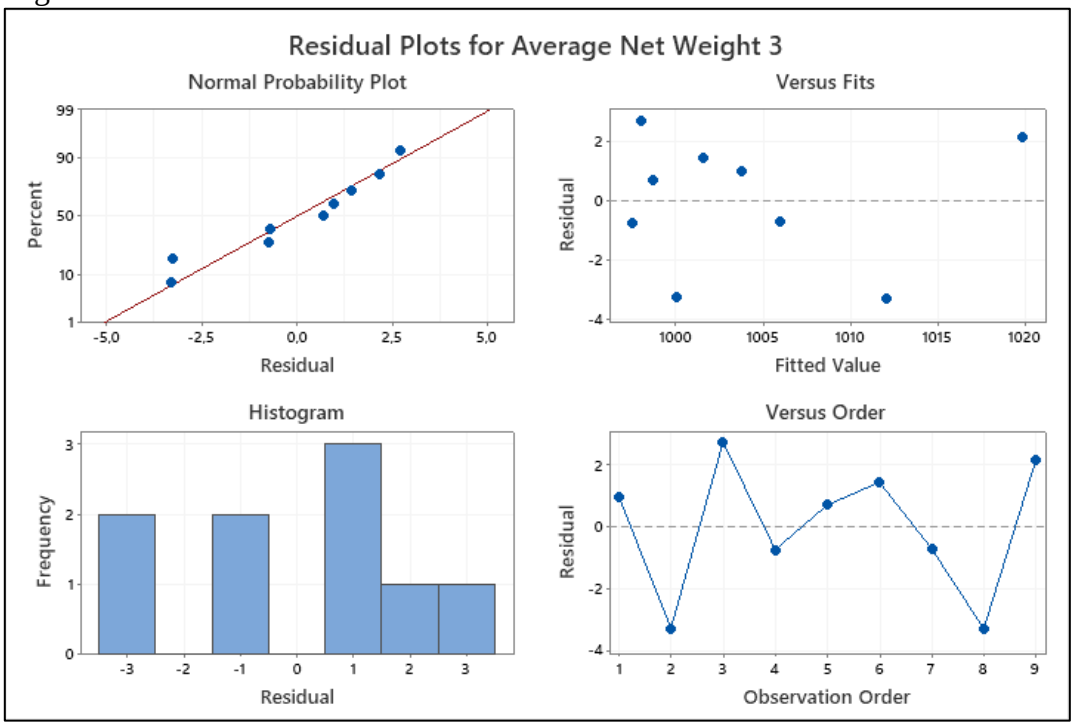
Source	DF	SS	F	P
Linear	1	240,679	6,88	0,034
Quadratic	1	206,742	32,59	0,001

Figure 24



In this last case, the analysis of variance associated with the model shown above indicates the quadratic term as the most relevant.

Figure 25



This result shows that, in practice, the amount of dosed powder is essentially a function of the chamber volume which is fixed from time to time by adjusting its height.
Following this quadratic dependence, the direct proportionality between the chamber volume and the quantity of dosed powder occurs only starting from a certain height of the chamber itself, *i.e.*, starting from values greater than 23.7 mm.

In the case of the dosing disc C of Figure 14, which shows a more uniform variability between chambers compared to the two discs considered so far, the examination of the weight distribution of a typical chamber (chamber 12) instead yields a completely different picture compared to what we have just seen.
First of all (Figure 26), the weights distribution is quite symmetrical even if with some outliers, but, above all, there is a substantial absence of linear correlation between the weight distribution and the chamber height during the filling process. (Figure 27). The value of *Bravais-Pearson's linear correlation coefficient*, ρ, is in fact equal to -0.031.

Figure 26

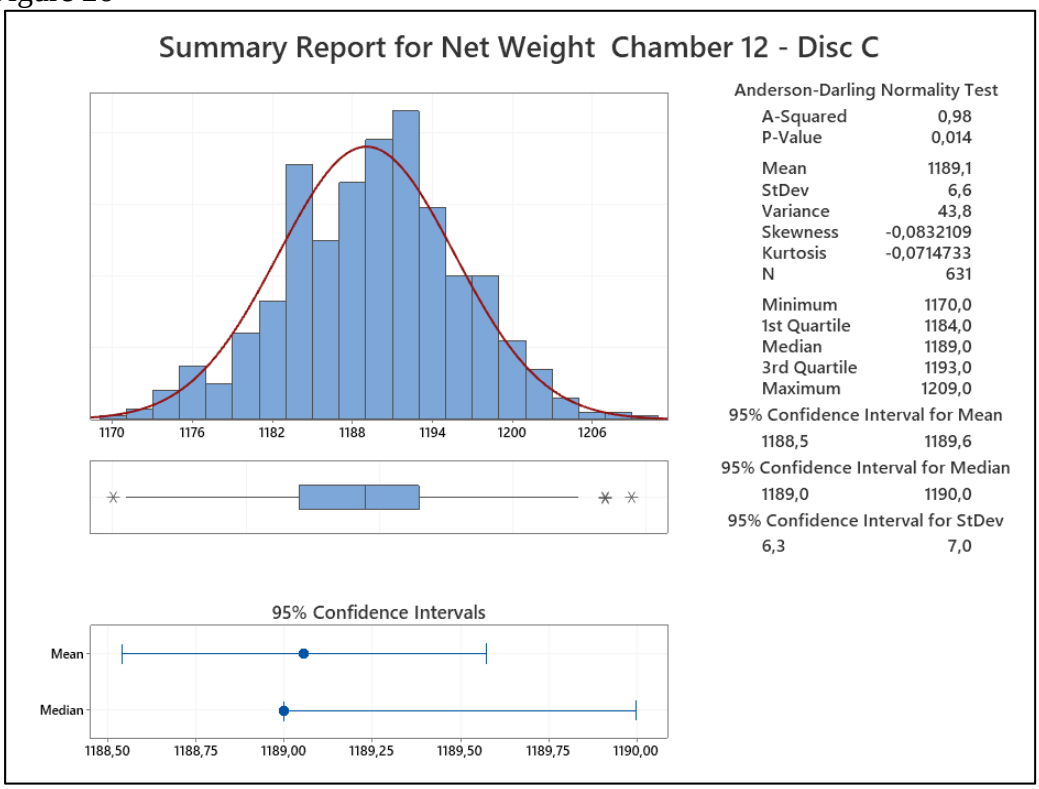
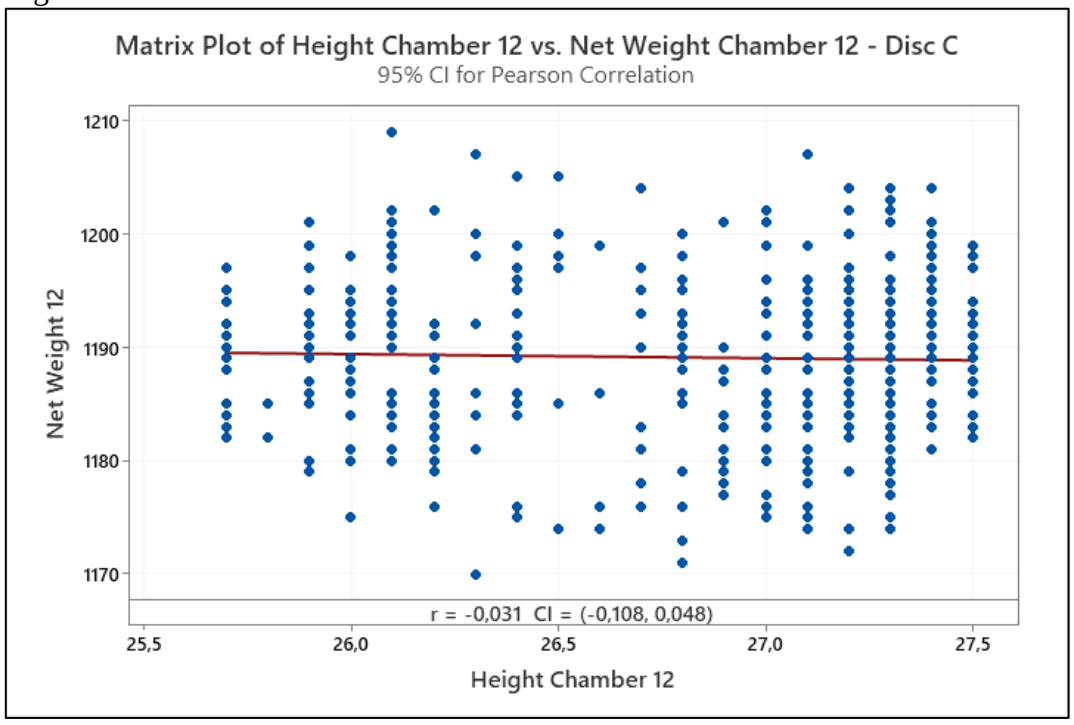


Figure 27



The analysis conducted so far has shown the details of the operation of the individual chambers and dosing discs.

To summarize the operation of the whole microdosing machine and quickly identify any abnormal behavior, a *summary index* is required.

In this regard, Descriptive Statistics offers a simple but powerful tool: the *coefficient of variation*. It is a *relative variability index*, *i.e.*, a pure number that does not depend on the unit of measurement of the variables, but which is not normalized (*i.e.*, not between 0 and 1). It is defined as the ratio between the standard deviation and the arithmetic mean of the data distribution, that is:

$$CV = \frac{\sigma}{\mu}$$

Table 2 here below summarizes the coefficient of variation values relating to each individual chamber and each dosing disc among those considered so far.

Table 2

Chamber	Dosing Disc		
	A	B	C
1	0,85	0,51	0,60
2	0,93	2,61	0,64
3	0,58	0,54	0,74
4	0,59	0,57	0,66
5	0,76	1,61	0,60
6	0,67	0,61	0,62
7	0,85	0,65	0,80
8	0,49	0,55	0,58
9	0,61	0,51	0,81
10	0,65	0,67	0,66
11	1,48	0,61	0,70
12	0,95	1,13	0,57

In Figure 28 the trends of the variation coefficient relating to the chambers of dosing discs A and B are superimposed. What has been said since the beginning of this post, namely that disc B exhibits a behavior characterized by greater variability, is clearly evident here.

Figure 28

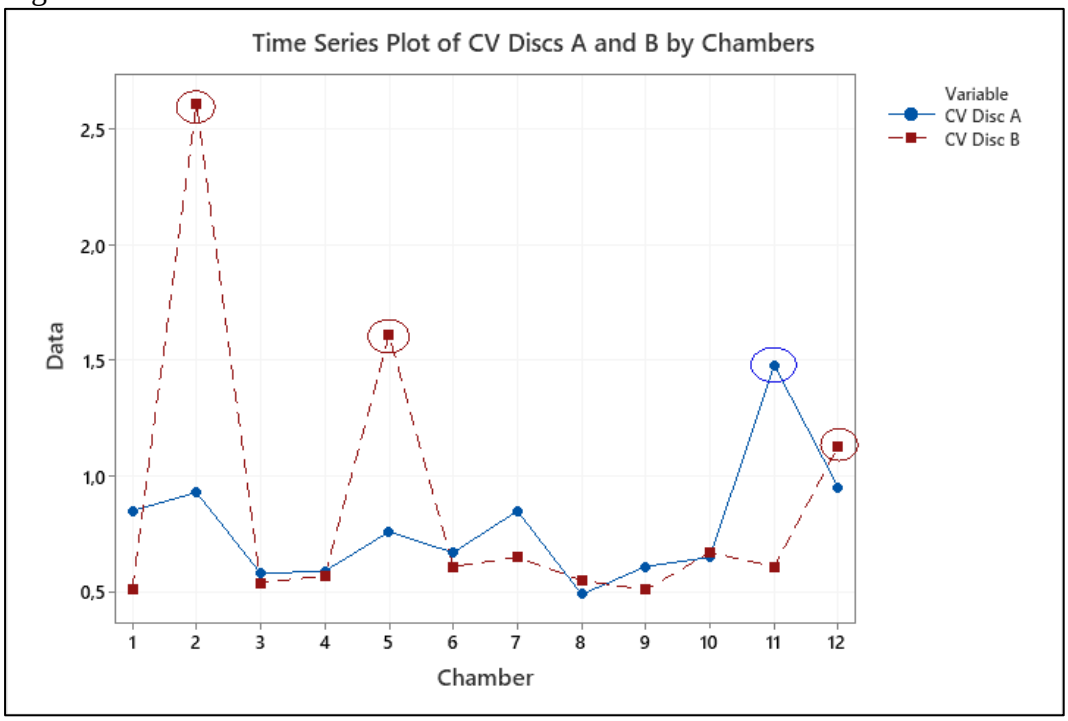
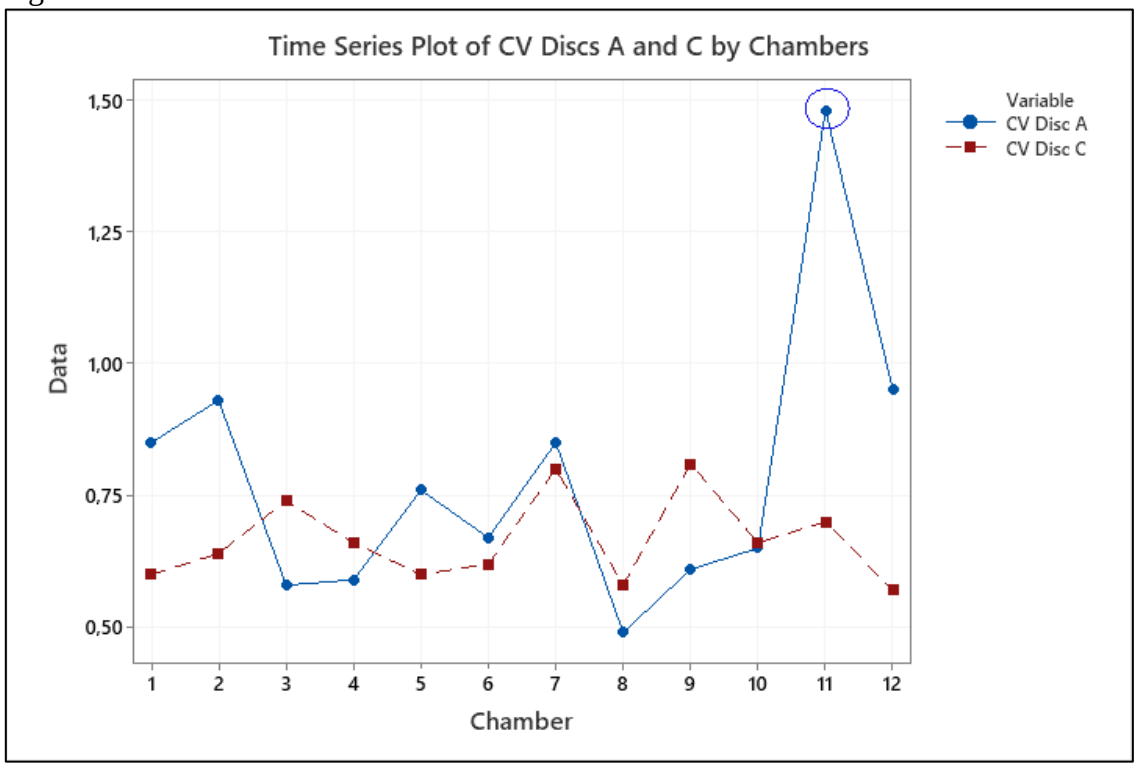


Figure 28 immediately shows the chambers affected by anomalous variability, namely 11 for dosing disc A and 2, 5, 12 for disc B.
For illustrative purposes, Figure 29 compares the trends of the coefficient of variation relative to dosing discs A and C.

The *coefficient of variation* is therefore a simple summary index that allows to monitor, on the basis of the weights delivered by the dosing discs, the behavior of individual chambers. By building a case history it is possible to study the behavior of the chambers of a given disc with respect to the different products and dosages for which it is used. On this basis it is then possible to define limits of acceptability and intercept in time natural phenomena of deterioration.

Figure 29



4. CONCLUSIONS

All processes are affected by variability which, while being an enemy of Quality, is also its best ally because it continuously sends signals which, if collected and analyzed, allow to limit its influence on the process itself.

A typical example of this is precisely the process of dispensing sterile powders under aseptic conditions considered in this post.

The discs of the microdosing machine, and the chambers contained therein, are subjected to a continuous operational stress which leads to an inevitable deterioration of their performance.

To what extent can this deterioration be accepted?

When should preventive actions be taken to limit it?

These questions are answered by the Descriptive Statistics which, thanks to a simple summary index, the *coefficient of variation*, allows to compare the variability of each chamber over time, build a case history, set limits of acceptability and then indicate when it is time to intervene in a preventive way.

Furthermore, the statistical methods allow us to go into even more detail of the filling process, modeling it and verifying its consistency between the different dosing chambers and over time.

It is worth noting that the approach and methods presented here are applicable to similar processes, at least in some respects, such as compression to produce tablets, *etc*.

Once again, and as pointed out in previous posts, statistical methods show how it is possible to "simplify complexity" and extract practical and ready-to-use knowledge from complex data sets by capturing their information content.

5. BIBLIOGRAPHY

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