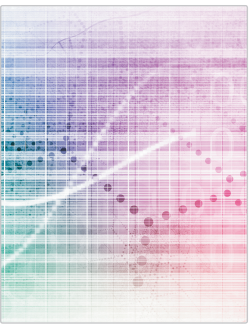

Methods for Identifying Out-of-Trend Data in Analysis of Stability Measurements—Part II: By-Time-Point and Multivariate Control Chart

Máté Mihalovits and Sándor Kemény

In Part I of this article series, the authors discussed the regression control chart method for identifying out-of-trend data in pharmaceutical stability studies. In Part II, the by-time-point method and the multivariate control chart method are investigated, and improved approaches are suggested. The method is illustrated using real data sets.

This article is a continuation of Part I, in which the authors discussed the identification of out-of-trend (OOT) data in the stability studies of single batches using a regression control chart method (1).

If several batches are considered, there are two different time scales. The first one is the life within batches, which has been covered in Part I (1). The regression control chart uses this within-batch context. The second time scale is the order of batches. Two approaches are followed in this Part II article. The by-time-point method uses the between-batches context only, while the multivariate approach uses both between-batches and within-batches contexts.

Two statistical concepts have been suggested in the literature (2). The first concept was based on known variance and expected value of distribution of measurements. This method was referred to as the Shewhart method in Part I. In the current situation, however, where only a small sample size is available, it is not justified to assume known variance and expected value. The second method suggested was to use the tolerance interval, but this is not a proper approach either. The tolerance interval gives the limits within which a certain proportion of population falls and is not relevant for a single data point. The proper concept that one should use when a single observation is in question is the prediction interval, using *t*-distribution (1).

Detecting OOT data using the by-time-point method

In this method, the result for the new batch is compared with points of earlier batches belonging to the same time of batch life.

Use of by-time-point method, original proposal

The Pharmaceutical Research and Manufacturers of America (PhRMA) Statistics and Stability Expert Teams (2)

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suggest calculating the tolerance interval with $\pm ks$ limits around the mean of data of earlier batches at the same time point. From tables or calculating with approximations, k can be found. If the new data fall out of the interval that belongs to the respective time point, they are OOT. Along with the tolerance interval, Torbovska and Trajkovic-Jolevska (3) calculate the earlier described Shewhart control limits (assumed known expected value and variance), based on a z (standard normal) statistic.

Suggested use of by-time-point-method

In the by-time-point situation, neither the Shewhart interval nor the tolerance interval are the proper intervals to use. As explained previously, the prediction interval is to be used with Student's t-distribution.

Equation 1 gives the calculation for prediction interval:

$$\bar{y}_i - t_{\alpha/2} s_{y(i)} \sqrt{1+\frac{1}{n}} < y_i^* < \bar{y}_i + t_{\alpha/2} s_{y(i)} \sqrt{1+\frac{1}{n}} \quad \text{[Eq. 1]}$$

where, $\bar{y}_i$ is the mean of the reference data that belongs to the *ith* time point, y_i^* is the new measured data at *ith* time point, $s_{y(i)}$ is the sample standard deviation of reference data that belongs to the *ith* time point, and n is the number of reference data at the *ith* time point (number of historical batches). $t_{(\alpha/2)}$ is the critical value of Student's t-distribution at one sided $\alpha/2$ level with $(n-1)$ degrees of freedom. If the inequality is satisfied, that is the y_i^* is within the interval, the data are accepted, otherwise they are OOT. In this method, a 5% significance level was used instead of the 0.27% level, which is accepted in quality engineering applications as discussed under Shewhart method in Part I (1). The reason for this choice is to keep the level of error of the second kind sufficiently low. A type II error (second kind) occurs when the null hypothesis is false, but erroneously fails to be rejected.

The calculation can be improved using pooled standard deviation. For this purpose, it should be proven that the error variances at different time points are equal, which can be tested by Bartlett and Levene tests, for example. For the authors' data, the hypothesis of homogeneity of variances is accepted (details of tests are not shown here). The pooled sample variance (s_p) is calculated in **Equation 2** as:

$$s_p = \sqrt{\frac{\sum s_{y(i)}^2}{p}} \quad \text{[Eq. 2]}$$

where p is the number of time points.

Also, a new $t_{\alpha/2}$ value is to be used in calculations, as the degrees of freedom of standard deviation is changed from $(n-1)$ to $p(n-1)$.

For illustrational purposes along with prediction limits, Shewhart limits (α=0.05), confidence limits (α=0.05), and tolerance limits (P=0.99, γ=0.95) are calculated as well. None of these limits are appropriate to use in the current situations, except the prediction limits. These limits are discussed in detail below.

Shewhart limits can be calculated by **Equation 3**:

$$SL = \bar{y} \pm z_{\alpha/2}\sigma_y \quad \text{[Eq. 3]}$$

where σ_y is the assumed variance, equal to s_y. As the known variance is assumed, there is no room for pooling. One may, however, use the pooled standard deviation as a substitute of σ_y. The latter is falsely assumed to be known because of the small sample size. When the pooled estimated variance is substituted, σ_y is taken to be equal to s_p.

Confidence limits can be calculated by **Equation 4**:

$$CL = \bar{y} \pm t_{\alpha/2}\, s_y/\sqrt{n} \quad \text{[Eq. 4]}$$

For the calculations with pooled standard deviation, s_p is substituted with s_y in **Equation 4** and a new $t_{\alpha/2}$ is obtained with degrees of freedom of s_p^2.

Tolerance limits can be calculated by **Equation 5**:

$$TL = \bar{y} \pm k_1 s_y \quad \text{[Eq. 5]}$$

To calculate the tolerance factor (k_1), different approximations could be used. Howe (4) suggests the following formula (**Equation 6**):

$$k_2 = \sqrt{\frac{\nu\left(1+\frac{1}{n}\right) z_{\frac{1-P}{2}}^2}{\chi_{1-\gamma,\nu}^2}\left(1+\frac{\nu-2-\chi_{1-\gamma,\nu}^2}{2(n+1)^2}\right)} \quad \text{[Eq. 6]}$$

This approximation is recommended if

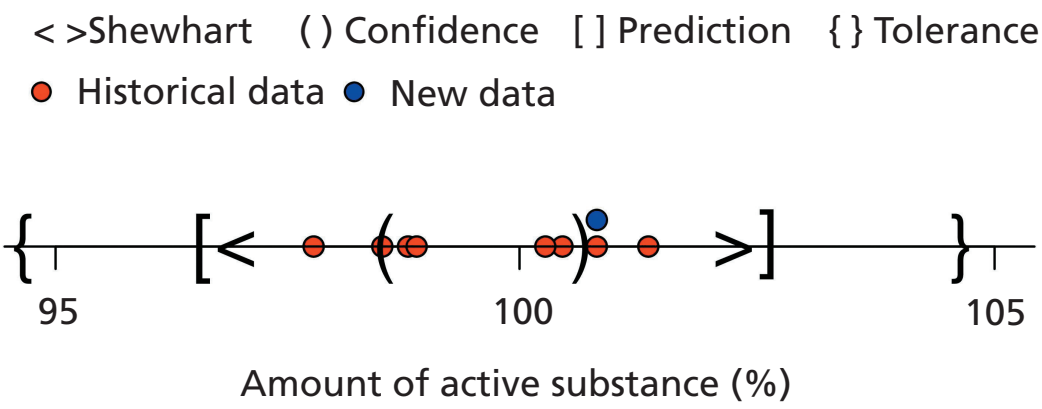
$$\nu \le \left(1+\frac{1}{z_{1-\gamma}^2}\right) n^2 \quad \text{[Eq. 7]}$$

conforms to the current situation. In **Equation 6**, n is the number of historical points, ν is the degrees of freedom of s_y^2, z_{1-P} is the critical value of standard normal distribution at one-sided $(1-P)$ level, and $\chi_{1-\gamma,\nu}^2$ is the critical value of chi-square distribution at one-sided $(1-\gamma)$ level with ν degrees of freedom. For the calculations with pooled standard deviation, s_r is substituted with s_y in **Equation 5** and the degrees of freedom of s_p^2 (ν) is used in **Equation 6** ($\chi_{1-\gamma,\nu}^2$ also changes) and in **Equation 7**.

Table I: Prediction-, Shewhart-, confidence-, tolerance limits, by-time-point method using s_p. LPL is lower prediction limit and UPL is upper prediction limit. LSL is lower Shewhart limit and USL is upper Shewhart limit. LCL is lower confidence limit and UCL is upper confidence limit. LTL is lower tolerance limit and UTL is upper tolerance limit. The observed data in the row of the grey pair of boxes are OOT considering the limits specified in the columns.

Time (month)	y	s_p	y^*_i	LPL	UPL	LSL	USL	LCL	UCL	LTL	UTL
0	99.6	1.481	100.9	96.4	102.7	96.7	102.5	98.5	100.6	94.6	104.6
3	98.1	1.481	97.3	95.0	101.3	95.2	101.0	97.1	99.2	93.1	103.1
6	97.6	1.481	97.7	94.4	100.7	94.7	100.5	96.5	98.6	92.6	102.6
9	97.4	1.481	98.4	94.3	100.6	94.5	100.3	96.4	98.5	92.4	102.4
12	96.5	1.481	96.5	93.3	99.6	93.6	99.4	95.4	97.5	91.5	101.5
18	95.5	1.481	99.5	92.3	98.6	92.6	98.4	94.4	96.5	90.5	100.5
24	95.5	1.481	96	92.4	98.7	92.6	98.4	94.5	96.6	90.5	100.5
36	92.2	1.481	93.7	89.1	95.3	89.3	95.1	91.2	93.2	87.2	97.2

Figure 1: Statistical intervals for the by-time-point method at 0-month time point.



It is important to note that the method does not use the time dependence function. This approach is an advantage, because an assumed function may falsify the conclusion. At the same time, it is a drawback, since the functional time dependence, which is an essential feature of stability data, is not used at all.

Example I: By-time-point method

Using the data set found in Part I (1), the statistical intervals based on calculations with the pooled standard deviation (**Equation 2**) are given in **Table I**. The grey coloring indicates that the observed data in the row of the grey pair of boxes are OOT considering the limits specified in the columns. Considering the correct interval (prediction limits), the data point at 18 months is OOT, while the other data are accepted points just as they were at regression control chart in the first article of the series (1). The mistakenly used confidence interval would not contain y* at the 0-, 18-, 36-month time points; therefore, these would be OOT, while if the tolerance intervals were used, all the points would be accepted. The Shewhart limit approach would find the 18-month time point as OOT.

The calculated intervals at 0-month time point are illustrated in **Figure 1**. The confidence interval is narrow and even some historical points are out of that range.

Detecting OOT batch by multivariate method

In this section, the observed batch is compared to earlier batches. As compared to the previous section, whole sets of data within the batch are compared (time function itself as with regression control charts) to those of historical batches (as with by-time-point method).

The time function, if linearity is assumed, is characterized by two parameters: slope and intercept. If the time function is unchanged, both parameters are unchanged.

The originally proposed slope control chart method

In the seminal paper by the Statistics and Stability Expert Teams (2), only the slopes are considered in the stability study. It is checked if the slope of the new batch belongs to the same distribution as that of the earlier batches. More precisely, it is assumed that estimated slopes of batches follow a normal distribution with the same expected value

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and variance. This assumption needs to be checked. The authors suggest constructing regression lines separately for each batch using data up to the time point in question. If the slope of the new batch is out of the tolerance interval calculated for those of the historical batches, the data in question are OOT.

Remarks to the originally proposed slope control chart method

As explained previously, the prediction interval is to be used given that the interval for one future observation (slope of a new batch) is in question. This is not only sound but advantageous as well, because the calculation of the prediction range is simpler than that of the tolerance range. The prediction range is narrower than the tolerance range, thus the test criterion based on the prediction range is stricter. This method is an advantage for reducing the risk of false conclusion, enabling a manufacturing company to detect disorder earlier.

A more profound improvement considers the trend line as a whole, thus both parameters (i.e., slope and intercept) are considered. The authors do not see any reason for allowing different slopes but not different intercepts. The difficulty is that as the estimate of the intercept and slope are statistically not independent, the prediction intervals may not be calculated separately. For separate intervals of two mutually dependent variables, Bonferroni inequality could be used for example; whereas for a joint interval, Hotelling T-square distribution could be used. Using Bonferroni inequality, a rectangle prediction region is obtained, which is easier to calculate, but is less accurate. If using Hotelling T-square distribution, an ellipse region is calculated, which might be harder to handle but more accurate. This method is, in fact, profile monitoring, which is established in reference 5 and dealt with in many papers (6, 7).

Another point of possible improvement is that for the historical batches, all points may be used for the regression, not just the ones until the time point in question (i.e., the most recent point of the test batch). This would lead to more sensitive test criterion because of the larger amount of information used. This approach gives additional difficulty as the variance-covariance matrix of parameters estimated from previous batches and from the actual batch is different, thus the homoscedasticity assumption is not justified. Therefore, this improvement is not utilized for the time being; the same number of points is considered for all batches.

The calculations in the following can be used every time a new data point is obtained, and a new regression line is fitted with the new data included. The data of historical batches are taken into account only up to the time point of the new data of the observed batch, and separate regression lines are fitted to those data.

The prediction region for the new pair of parameters is calculated in **Equation 8**:

$$\frac{n}{n+1}(\boldsymbol{X}-\boldsymbol{x}^*)^T\boldsymbol{S}^-(\boldsymbol{X}-\boldsymbol{x}^*) \le \frac{p(n-1)}{n-p}F_{p,n-p,\alpha}$$

[Eq. 8]

where n is the number of historical parameter pairs (historical batches), p is the number of parameters (here 2), $F_{p,n-p,\alpha}$ is the upper critical value of F-test with (p,n-p) degrees of freedom at one sided α level. The (X-x*) is the matrix of the difference of mean vector of the historical parameters (X) and vector of the new parameters (x*):

$$\boldsymbol{X}-\boldsymbol{x}^* = \begin{bmatrix}\bar{b}_0 - b_0^* \\ \bar{b}_1 - b_1^*\end{bmatrix}$$

[Eq. 9]

The $(X-x^*)^T$ is the transpose of the matrix:

$$(\boldsymbol{X}-\boldsymbol{x}^*)^T = [\bar{b}_0 - b_0^* \quad \bar{b}_1 - b_1^*]$$

[Eq. 10]

S^- is the inverse of the variance-covariance matrix:

$$\boldsymbol{S}^- = \begin{bmatrix} s_{b_0}^2 & cov(b_0;b_1) \\ cov(b_0;b_1) & s_{b_1}^2\end{bmatrix}^- = \frac{1}{\det(S)}\begin{bmatrix} s_{b_1}^2 & -cov(b_0;b_1) \\ -cov(b_0;b_1) & s_{b_0}^2\end{bmatrix}$$

[Eq. 11]

where det(S) is the determinant of S:

$$\det(S) = s_{b_0}^2 s_{b_1}^2 - cov(b_0;b_1)^2$$

[Eq. 12]

Performing the matrix multiplications, **Equation 13** is obtained:

$$\left(\frac{n}{n+1}\right)\left(\frac{1}{s_{b_0}^2 s_{b_1}^2 - cov(b_0;b_1)^2}\right)\left[(\bar{b}_0 - b_0^*)^2 s_{b_1}^2 + (\bar{b}_1 - b_1^*)^2 s_{b_0}^2 - 2(\bar{b}_0 - b_0^*)(\bar{b}_1 - b_1^*)cov(b_0;b_1)\right] \le \frac{2(n-1)}{n-2}F_{p,n-p,\alpha}$$

[Eq. 13]

where n is the number of reference batches, s_{b0} and s_{b1} are the sample standard deviations of historical intercepts and slopes, respectively, $cov(b_0;b_1)$ is the estimated covariance between the parameters, and $\bar{b}_0$ and $\bar{b}_1$ are the means of the parameters of historical batches.

Covariance is estimated as follows:

$$cov(b_0; b_1) = \frac{\sum_{i=1}^{n}(b_{0_i} - \bar{b}_0)(b_{1_i} - \bar{b}_1)}{n-1} \quad \text{[Eq. 14]}$$

If the inequality (**Equation 13**) is satisfied, the (b_0^*, b_1^*) is within the region and the batch is accepted, otherwise it is OOT. The acceptance region is a spatial shape. By calculating and plotting b_0^* and b_1^* pairs to the critical value of F, the region can be obtained, as illustrated in the following.

For illustrational purposes, regions calculated by the Shewhart method (Shewhart region) (α=0.05), confidence region (α=0.05), and tolerance region (P=0.99, γ=0.95) are calculated as well. These calculations, however, are not appropriate to use in the current situations.

The Shewhart region is calculated by (8):

$$n(\boldsymbol{X} - \boldsymbol{x}^*)^T \boldsymbol{S}^- (\boldsymbol{X} - \boldsymbol{x}^*) \leq \chi^2_{(1-\alpha,p)} \quad \text{[Eq. 15]}$$

The confidence region can be calculated as follows (8):

$$n(\boldsymbol{X} - \boldsymbol{x}^*)^T \boldsymbol{S}^- (\boldsymbol{X} - \boldsymbol{x}^*) \leq \frac{p(n-1)}{n-p} F_{p,n-p,\alpha} \quad \text{[Eq. 16]}$$

while the tolerance region is calculated by (8):

$$(\boldsymbol{X} - \boldsymbol{x}^*)^T \boldsymbol{S}^- (\boldsymbol{X} - \boldsymbol{x}^*) \leq k_2 \quad \text{[Eq. 17]}$$

where k_2 is the tolerance factor taken from reference 9 for P=0.99 and γ=0.95. Its value is 48.77.

This method is not sensitive enough when a single point is in question. The reason is that a single OOT data will not have an impact big enough on the stability profile to make the observer detect the batch as an OOT batch. However, the method is more sensitive and, therefore, should be used when the question is whether the observed batch is OOT (in other words, all the points are OOT in the batch). Parameters of stability profile with less data are less certain, therefore, the acceptance region is wider. As more data become available, the uncertainty of parameters gets smaller, and detection of OOT batch becomes more certain. One should start using this approach after the third data point is obtained in the observed batch, and keep using the method at every time point when new data are obtained, until the end of the stability study. In the example calculation, all data of the observed batch (and, therefore, all data of historical batches) are considered, including the last point.

Example II: Hotelling T-square joint prediction region

In the example calculation, all data of the observed batch (and, therefore, all data of historical batches) are taken into account. Intercepts and slopes of Batch I –VIII from **Table II** were used as reference data and the method was used to observe data of Batch IX.

Figure 2: Joint statistical regions for slope and intercept.

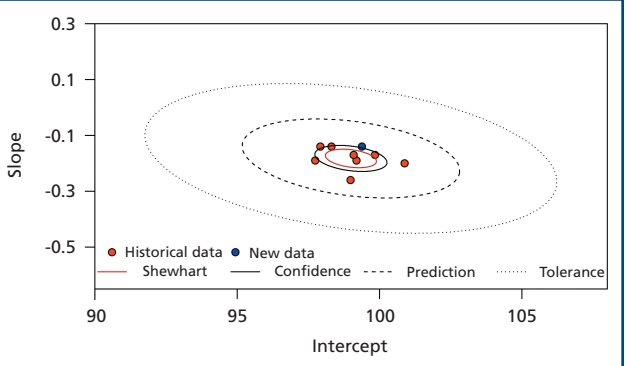

Table II: Parameters of regression lines of batches.

	Intercept	Slope
Batch I.	97.92	-0.14
Batch II.	98.31	-0.14
Batch III.	99.19	-0.19
Batch IV.	97.74	-0.19
Batch V.	99.09	-0.17
Batch VI.	98.98	-0.26
Batch VII.	99.84	-0.17
Batch VIII.	100.88	-0.20
Batch IX.	99.38	-0.14

Table III: Terms to calculations in Equation 13.

$s^2_{b_1}$	$s^2_{b_0}$	$cov(b_0; b_1)$	$\bar{b}_1 - b_1^*$	$\bar{b}_0 - b_0^*$	η
1.073	0.0015	-0.0124	-0.39	-0.04	8

Using the terms from **Table III**, **Equation 18** is obtained from **Equation 13**:

$$0.6822 \leq F_{2,6,0.05} \quad \text{[Eq. 18]}$$

Where F with degrees of freedom 2 and 6, at one-sided 0.05 level is 5.14. As the **Equation 18** is satisfied, the batch can be accepted as non-OOT. The prediction region can be illustrated by calculating max values of (b_0^*, b_1^*) to $F_{2,6,0.05}$ that satisfy **Equation 13**. Every (b_0^*, b_1^*) within the region is accepted as non-OOT. **Figure 2** illustrates the calculated regions. One should keep in mind that the prediction region is the proper approach in the current problem.

As the new data (slope and intercept) are found within the prediction region, they are non-OOT. By mistakenly using the confidence region, some of earlier batches and the new batch are found to be OOT. If the tolerance region approach is used, all batches would be accepted as non-OOT.

contin. on page 45

Advances in transdermal patch drug delivery and measurement

Medherant, a bioadhesives company formed as a spin-out from the University of Warwick (UK) in 2015, is developing its TEPI Patch technology, a higher-dosage drug-delivery patch with a constant rate of drug release. The patch is formulated by mixing the drug with an adhesive that enables high drug loading. The company's first patch, containing ibuprofen, is being manufactured for clinical trials.

Dr. Gabit Nurumbetov, principal scientist at Medherant, has developed an improved instrument to test the release of drugs from a transdermal patch. The device, patented by Medherant, is an improved, miniaturized, and multiplexed version of a Franz or diffusion cell, which is a device commonly used to measure the amount of drug that permeates across human skin.

"In essence, the cell is a vial with a modified top part where you can place human skin and your formulation (gel, cream, or patch). The volume below the skin is filled with a biological fluid, which is taken out for analysis through a sampling port," explains Nurumbetov. "The amount of drug permeated across the skin is then measured by means of chromatographic and/or spectroscopic methods allowing estimation of parameters such as API flux, patch area efficacy, and others."

The improved diffusion cell, which is patented by Medherant, can test more than 100 formulations per day, compared to approximately 12 in the same period with traditional Franz cells, reports the company.

Drug release in a transdermal formulation is complex. "It is affected by physico-chemical properties of the adhesive and drug and the presence or absence of additional excipients in a formulation," says Nurumbetov. "Some adhesives can chemically 'hold' drug molecules leading to a lesser amount of drug delivered. Also, if the molar mass of a drug is higher than 500 Daltons, it is likely to be not suitable for transdermal applications. Additional chemicals (permeation enhancers) in the formulation can also affect the drug-delivery performance." The new instrument will allow high-throughput testing for faster development.

—Jennifer Markarian

homogenous mix. It also can include measuring and quantifying adhesion characteristics throughout the manufacturing process.

Finished-product testing analyzes the final product's physical properties to help ensure the patch is produced with the desired physical characteristics and performs as expected. Adhesion and tack testing, for example, can help determine if the product will adhere to a user's skin for a given period of time. Manufacturers must first define what levels of adhesion level and tack are required in a finished product and then implement the methods or tools to measure for those levels. **PT**

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Conclusion

The by-time-point method and multivariate control chart were discussed in this article as methods that could be used to identify OOT data and OOT batches in pharmaceutical stability studies. The earlier suggested methods are improved here. The most important part of the improvement is the use of the prediction region concept instead of the tolerance interval or Shewhart method concepts. Also, for the earlier suggested method, the slope control chart is improved so that not just the slope of the observed batch is considered in the stability study but also the intercept. The multivariate control chart using Hotelling T-square distribution satisfies the requirements mentioned. The by-time-point method is a way to identify OOT data, while the multivariate approach is less sensitive to detect this kind of phenomena and should be used to detect an OOT batch instead. Also, the multivariate approach uses the time dependence function within the batch, which means more information from the data set is used, hence, giving sound conclusions. The three methods discussed in Part I and Part II are possible ways to identify OOT data or batches in stability studies. The sensitivity of the methods to detect OOT results are not studied yet, therefore, one should decide if the data can be accepted by using all methods and drawing the conclusion from the results.

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