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ANALYTICAL CHALLENGE

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Solution to Quality Assurance Challenge 3

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Solution

The subject of the quality assurance challenge 3 was controlling limit values in production batches. In the example presented here the residual monomer concentration of styrene in polysterene used for pharmaceutical purposes was not to exceed a specified limit.

The task was to prove experimentally that the specified precision has been reached. Furthermore, the upper limit for batch release must be specified. Four standard samples were analyzed, and four replicate determinations were carried out for each sample. The results obtained are once again listed in Table 1.

We assumed 0.480 m% for the upper limit of styrene monomer. Styrene concentration in polystyrene was determined by headspace gas chromatography. The specified standard deviation of the method determined at s=0.010 m% was obtained by a round robin analysis performed among eight laboratories, with each laboratory conducting five replicate measurements.

Before the precision can be calculated, the data sets must first be checked for outliers. Possible outliers are then to be eliminated.

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Test for outlier

In the quality assurance challenge 2 (published in ABC issue 383/1) we used the test by Grubbs et al., but here Dixon's test [1] will be applied for the testing. Although both tests are appropriate, Dixon's test is one of the most popular tests for the detection of an outlier because it is easy to calculate [2]. It is based on a comparison of the difference between the suspect value x_1 and its direct neighbour, with the overall range for n=3–7 – as in the present case – or a modified range for n>7. The test value, $\widehat{M}$ is given by

$$\widehat{M} = \left|\frac{x_1 - x_2}{x_1 - x_k}\right| \quad (1)$$

The calculated $\widehat{M}$ -value is compared in the usual way with the critical value for testing outliers by Dixon at a chosen significance level. An outlier is detected if the calculated $\widehat{M}$ exceeds the critical M (= 0.765) for n=4 at p=95%.

The results are listed in Table 2.

Answer to question (a): the value 0.398 for the second standard is an outlier at the 95% level of significance; therefore, it is removed from the data set.

Note: The same results are obtained by the Grubb's test.

Homogeneity of the variances

For the calculation of the overall standard deviation of the data set of Table 1, it is necessary to determine whether the group variances are homogeneous. Tests appropriate for more than two variances are those of Bartlett [2] or Cochran [3]. The simple Cochran test is based on comparing s_{max}^2 with the sum of all the variances, but it requires equal numbers of data in each data set. As this is not the case after having removed an outlier in the second data set, Bartlett's test is used here.

Table 1 Data presented on the residual monomer concentration of styrene in polysterene in four replicate determinations for each of four standard samples

Standard	m% Monomer			
1	0.469	0.419	0.445	0.436
2	0.333	0.342	0.348	0.398
3	0.436	0.400	0.415	0.421
4	0.308	0.321	0.301	0.315

The test value $\widehat{\chi}^2$ is calculated by Eq. (2).

$$\widehat{\chi}^2 = 2.303 \cdot \left(df \cdot lgs^2 - \sum df_j \cdot \lg s_j^2 \right) \quad (2)$$

The overall standard deviation 's' is calculated by Eq. (3):

$$s = \sqrt{\frac{\sum SS_j}{df}}$$

SS_j is the sum of squares in the set j:

$$SS_j = \sum \left(\bar{x}_j - x_{ij} \right) \quad (3)$$

The degree of freedom df is given by Eq. (4).

$$df = n - m \quad (4)$$

with the overall number of the value n and the number of the samples *m*.

s_j and df_j are the standard deviation and the degree of freedom in each sample j, respectively.

The $\widehat{\chi}^2$ -value calculated by Eq. (2) is compared to the critical of the chi-square distribution. If the $\widehat{\chi}^2$ -value is smaller than the critical chi-square value we assume homogeneity of variances or – in other words – homoscedasticity.

The results of Barlett's test using the outlier-free data sets are listed in Table 3.

Answer to question (b): The calculated chi-square value is smaller than the critical value at the significance level p=95%. The null hypothesis that the variances are homogeneous is accepted.

Test of the precision of the procedure

Once we have ensured the homogeneity of the group variances, based on the outlier-eliminated data sets we can show that the specified precision of s=0.010 m% is reached. From the variance in Table 3, a standard deviation of s=0.0145 m% is obtained for the analytical method.

Comparison of two variances is performed by means of an *F*-test. The test value $\widehat{F}$ is given by Eq. (5)

$$\widehat{F} = \frac{s_1^2}{s_2^2} \quad (5)$$

in which s_1^2 is the variance of the laboratory and s_2^2 is the specified value. The following test value is calculated to $\widehat{F} = 0.298$. This value is compared with the critical *F*-value at the chosen significance level p.

The specified standard deviation was determined by a round robin analysis performed among eight laboratories, each conducting five replicate measurements. Therefore, the degree of freedom df_2 is 32 (df_2=40–5). With df_2=32 and df_1=11 (the degree of freedom of the laboratory) the critical *F*-value is 2.103 at the significance level p=95% (at simplest received from Excel with the function f_x=FINV (5%; 11; 32)).

Answer to question (c): The $\widehat{F}$ -value calculated is smaller than the critical *F*-value at the significance level p=95%. The precision of the analytical procedure of the laboratory does not differ from the specified value. The precision is O.K.

Note: Obviously, when introducing an analytical method in a laboratory, both precision and *accuracy* have to be documented. This is the subject for a further analytical challenge...

Table 2 Data for the outlier (OL) test by Dixon

Standard	$x_1=x_{min}$	x_2	$\widehat{M}_l$	Result	$x_1=x_{max}$	x_2	$\widehat{M}_u$	Result
1	0.419	0.436	0.340	No OL	0.469	0.445	0.480	No OL
2.	0.333	0.342	0.138	No OL	0.398	0.348	0.769	**Outlier**
3	0.400	0.415	0.417	No OL	0.436	0.421	0.417	No OL
4	0.301	0.308	0.350	No OL	0.321	0.315	0.300	No OL

Table 3 Results of Barlett's test

Standard	SS_j	s_j^2	$\log s_j^2$	df_j	$df_j \cdot \log(s_j^2)$
1	0.001303	0.000434	−3.36226	3	−10.08678
2	0.000114	0.000057	−4.24413	2	−8.48825
3	0.000666	0.000222	−3.65364	3	−10.96094
4	0.000225	0.000075	−4.12542	3	−12.37626
Sum	0.002308			11	−41.91224
m=3	s^2=0.0002098	log s^2=−3.678251		df· log s^2=−40.46076	
chi-square calculated: $\hat{\chi}^2$= 3.343					
Value from the chi-square distribution: $\chi^2(p$=95%; df=4−1) = 7.815					

Maximum allowable mean of the analytical results for two replicate determinations

The maximum allowable mean of the analytical results is given by the difference between the specified critical value T_0 and the one-sided confidence interval ($CI_{one\text{-}sided}$) because for controlling of the limit value only the upper value is of interest [3]. This difference is calculated by Eq. (6).

$$T_0 - CI_{one-sided}(x) = T_0 - \frac{s \cdot t(\overline{P}_{one-sided}; df)}{\sqrt{n}} \quad (6)$$

In our example, T_0=0.480 m%, s is the standard deviation of the laboratory (s=0.0145 m% with df=11), and n=2 (two replicates in the analysis). The statistical factor for the one-sided test is again received from Excel with the function f_x=TINV(0.10; 11) to 1.796 because $t(\overline{P}_{one-sided} = 95\%; df = 11) \approx t(P_{two-sided} = 90\%; df = 11)$.

Answer to question (d): With $CI_{one\text{-}sided}$ (x)=0.01839 m%, the maximum allowable mean of the analytical results is 0.4616 m% styrene monomer. This value must not be exceeded if the production batch is to pass the quality check.

Analysis of two production batches

In routine analysis, the determination of the monomer content of the batches should be carried out based on double determinations. Therefore, the allowed difference between a pair of individual values must be established. Pearson's criterion can be used for this [4].

According to Pearson, the maximum difference (x_{max}−x_{min}) of the two replicate determinations is given by Eq. (7):

$$(x_{max} - x_{min}) = D(P; n_j) \cdot s \quad (7)$$

The Pearson factor D(P; n_j) for two replicate determinations (n_j=2) is 2.77 at the significance level p=95% [4].

With s=0.0145 m%, the critical difference is calculated to be 0.0401 m%.

The differences between the replicates for the two production batches are: batch 1: 0.01 m%, batch 2: 0.007 m%. In each batch, the difference is smaller than 0.0401 m%; averaging is allowed. The mean value of batch 1 $(x_1 = 0.461m\%)$ is smaller than the maximum allowable mean value 0.4616 m%, but $(x_2 = 0.462m\%)$ exceeds the critical value.

Answer to question (e): Batch 1 may be released for selling, but the release of batch 2 is not allowed.

Note: With the false two-sided statistic factor P ($p_{two\text{-}sided}$ = 95%; df = 2.201) the CI(x) calculated is 0.02254 m%, and the maximum allowable mean value is 0.457 m% monomer.

With this critical value also, the release of Batch 1 is not allowed. This result is false!

References

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