OMCL Network of the Council of Europe
QUALITY ASSURANCE DOCUMENT

PA/PH/OMCL (14) 89 R

Evaluation and Reporting of Results
Annex 2A

Full document title and reference	Evaluation and Reporting of Results – Annex 2A Examples of Re-test Programmes for Quantitative Tests PA/PH/OMCL (14) 89 R
Document type	Guideline
Legislative basis	/
Date of first adoption	August 2014
Date of original entry into force	October 2014
Date of entry into force of revised document	April 2015
Previous titles / other references / last valid version	Last adopted version : PA/PH/OMCL (14) 89
Custodian Organisation	The present document was elaborated by the OMCL Network / EDQM of the Council of Europe
Concerned Network	GEON

ANNEX II A OF THE OMCL NETWORK GUIDELINE

"EVALUATION AND REPORTING OF RESULTS"

EXAMPLES OF RE-TEST PROGRAMMES FOR QUANTITATIVE TESTS

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INTRODUCTION

This document is an Annex to the core document "Evaluation and reporting of results", *PA/PH/OMCL (13) 113* (in its current version), and it should be used in combination with it when planning, performing and documenting the evaluation process and reporting of results of quantitative tests with the expected Gaussian distribution.

The core document contains the Introduction, Scope and General requirements for the evaluation of results (in routine cases or otherwise) and the reporting of results.

The proposed approaches are only relevant if the decision is based on observed repeatability[1]. In all cases, the laboratory should make a decision based on documented and sound scientific judgement. This Annex should not be taken as a list of compulsory requirements. It is left to the professional judgement and background experience of each OMCL to decide on the most relevant procedures to be undertaken in order to give evidence that the evaluation and reporting of results is well managed. Although two or three initial determinations are described in most examples of this Annex, each OMCL is responsible for deciding what is the minimum number of replicates that should be taken into account for a certain test.

This Annex presents several examples of the evaluation of results for quantitative testing of medicinal products, which could be set up in combination with the general requirements given

[1] Observed repeatability should be interpreted as repeatability between independent sample determinations, either with regards to standard deviation (sd) or relative standard deviation (RSD).

in the core document. The examples are not intended to be all-inclusive, and other valid approaches may be adopted for evaluation of the acceptability of test results.

This document is based on publications in *Pharmeuropa* Vol. 9, No. 1, 148-156 (1997) and *Pharmeuropa* Vol. 11, No. 4, 571-577 (1999). The proposals for approach 1, 2 and 4 were tested against datasets obtained from proficiency tests and have been shown to be satisfactory for making a decision.

The following table gives an overview about the described possible approaches and the situations where they may be applied, see below:

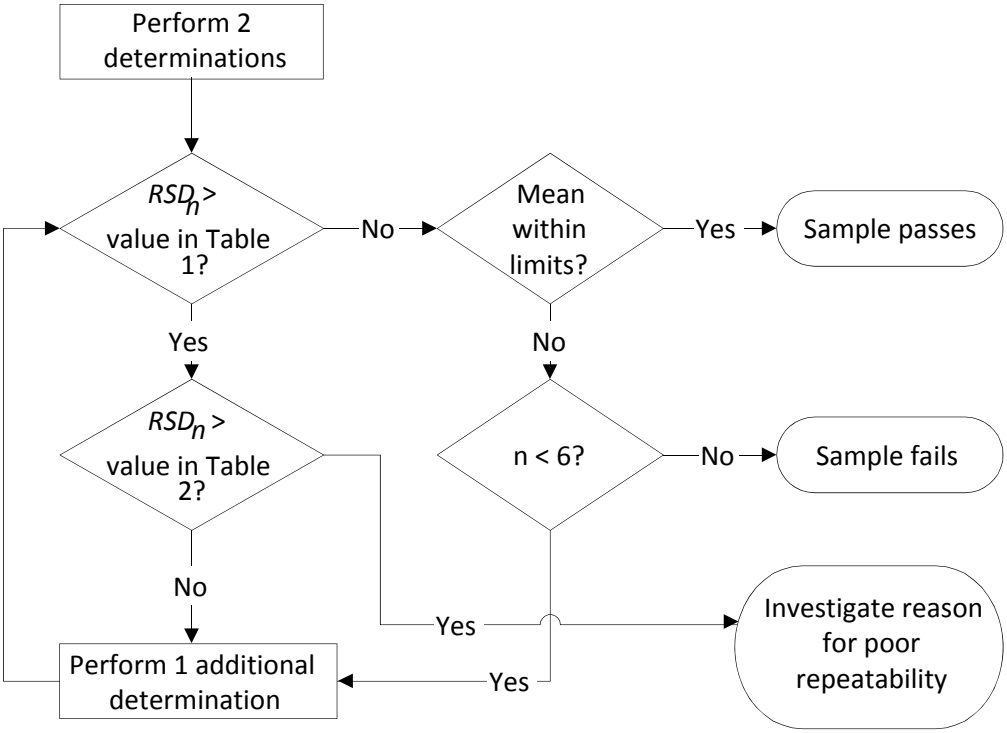
Approach	**Title**	**Situation**
1	Assay of active pharmaceutical ingredient, 2 initial determinations	This approach is applicable for chemical APIs only
2	Assay of active pharmaceutical ingredient, 3 initial determinations	This approach is applicable for chemical APIs only
3	Impurity tests (e.g.: Related substances by HPLC)	This approach is intended for trace level tests and may be used for quantitative impurity tests, where an analytical/instrumental response is obtained (peak area, for example)
4	Finished products	This approach is intended for finished products with fully validated methods regarding repeatability and intermediate precision described in the MA file. It is not intended for analyte concentrations at trace level.
5	Products with insufficient validation data	This approach is to be regarded as a tool to establish an acceptance criterion for precision of the replicates of a certain test, when the laboratory has no other mean to evaluate the dispersion of the results, as the available validation data are scarce or there are no validation data at all. It is not intended for analyte concentrations at trace level.
6	Retest programme based on statistical assay layouts (Ph. Eur. 5.3)	Results from bioassays in statistical layout according to Ph. Eur. 5.3. For combination of assays it is desirable that the individual results are obtained in identical or similar assay layouts.
7	Retest programme based on known intermediate precision	This approach is intended for assays with well-known in-house intermediate precision, e.g. in OCABR routine analysis
8	Approach for cases of unexplained lack of repeatability	For products where sample recovery problems are encountered or there is no plausible reason for lack of precision

Approach 1: Assay of active pharmaceutical ingredient, 2 initial determinations

Perform two determinations. If the RSD_2[2] is smaller than the RSD_{max} permitted for two determinations (see *Table 1*), and the mean falls within the content limits, the sample passes. If either of these two conditions is not met, one further determination is performed. If the RSD_3 of the three values meets the criterion and the mean of the three results falls within the content limits, the sample passes. This can be repeated up to a maximum of six determinations. The sample can only be rejected if the mean is outside the content limits and the criterion for the RSD_n is met. If at any stage the RSD_n is greater than the value listed in *Table 2*, further determinations are useless because it can be predicted that the *RSD* will not meet the criterion. Instead, the reason for the poor repeatability should be investigated. As a consequence, the sample can neither be accepted nor rejected.

This approach is illustrated in Figure 1.

Figure 1 - Decision tree for Approach 1



[2] Note that using RSD_2 is equivalent to using the relative range if the requirement for $n = 2$ is multiplied by $\sqrt{2}$.

Table 1- If the RSDn is greater than the values listed, additional assays are required up to a maximum total of 6 determinations

B	**Number of determinations (*n*)**				
	2	**3**	**4**	**5**	**6**
1.0	0.11	0.29	0.42	0.52	0.60
1.5	0.17	0.44	0.63	0.78	0.90
2.0	0.22	0.59	0.84	1.04	1.20
2.5	0.28	0.73	1.05	1.30	1.50
3.0	0.33	0.88	1.26	1.55	1.80

B = Upper specification limit – 100.

Table 2 - If the RSDn is greater than the values listed, additional determinations are useless

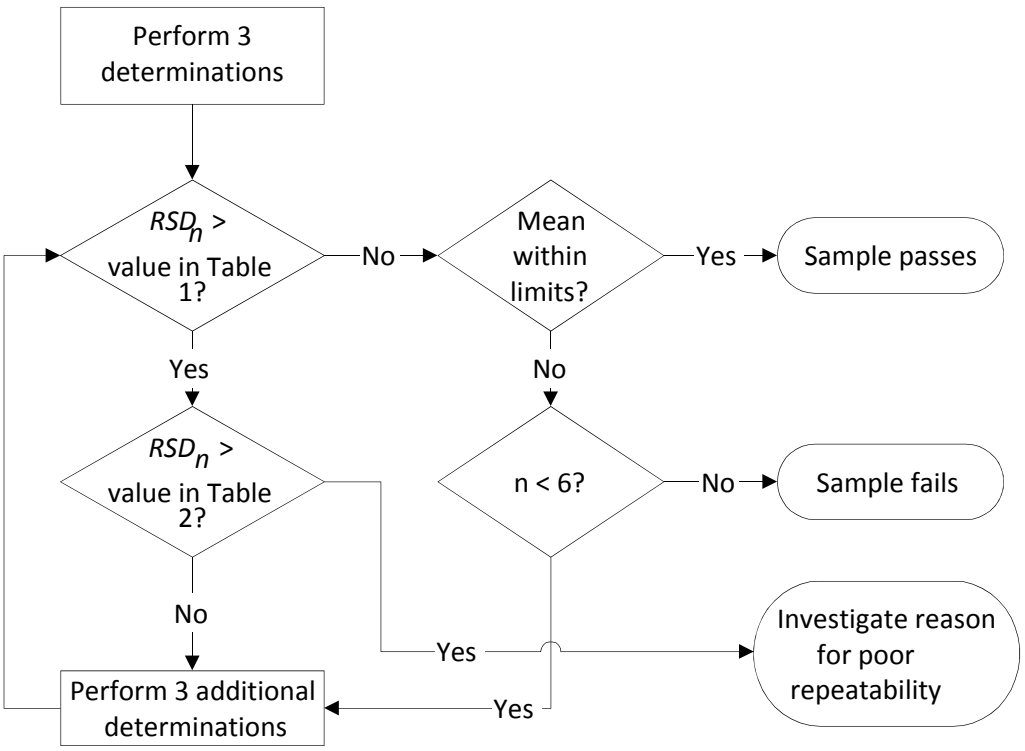
B	**Number of determinations (*n*)**				
	2	**3**	**4**	**5**	**6**
1.0	1.34	0.95	0.77	0.67	0.60
1.5	2.01	1.42	1.16	1.01	0.90
2.0	2.68	1.90	1.55	1.34	1.20
2.5	3.35	2.37	1.94	1.68	1.50
3.0	4.02	2.85	2.32	2.01	1.80

Approach 2: Assay of active pharmaceutical ingredient, 3 initial determinations

Perform three determinations. If the RSD_3 is smaller than the RSD_{max} permitted for three determinations (see Table 1), and the mean falls within the content limits, the sample can be accepted. If either of these two conditions is not met, three further determinations are performed (unless the *RSD* exceeds the value in Table 2, in which case further assays are useless). If the RSD_6 of the six values is smaller than the RSD_{max} permitted for six determinations, and the mean of the six values falls within the content limits, the sample can be accepted. The sample can only be rejected if the mean is outside the content limits and the criterion for the *RSD* is met. If the *RSD* is too large, the reason for the poor repeatability should be investigated and, in such circumstances, the sample can neither be accepted nor rejected.

This approach is illustrated in *Figure 2*

Figure 2 - Decision tree for Approach 2



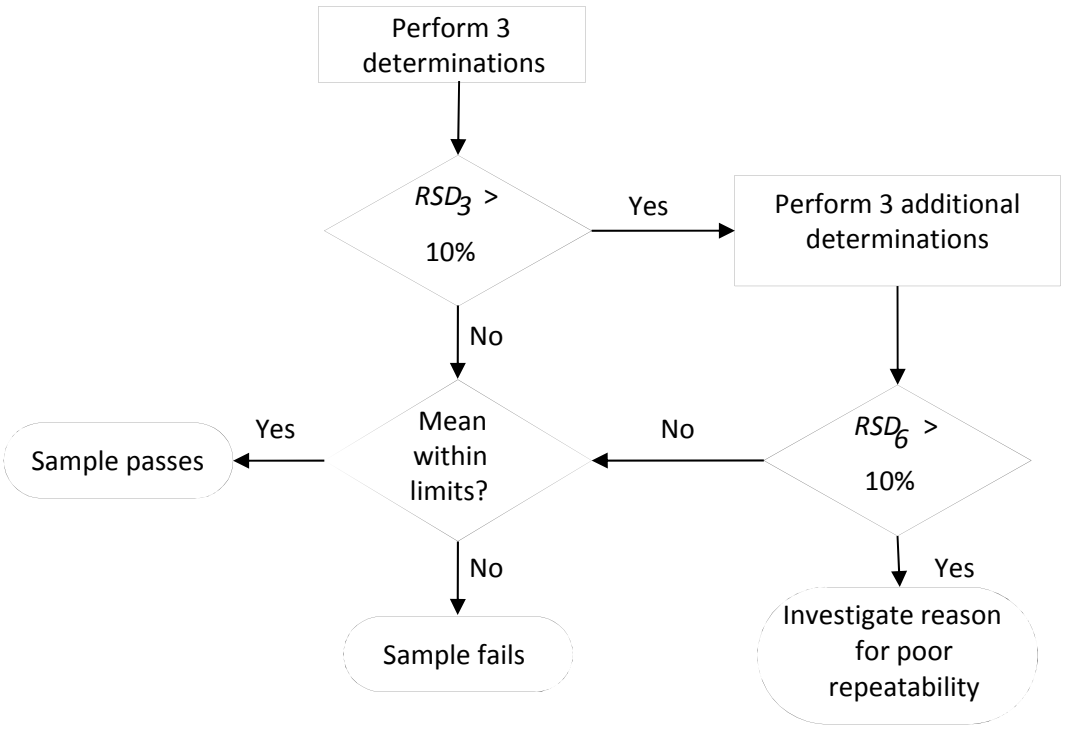
Approach 3: Impurity tests (e.g. Related substances by HPLC)

Approach 2 may be adapted to quantitative impurity tests, where an analytical/instrumental response is obtained (e.g. peak area). In that case, RSD_{max} is 10% when results are found near the Limit of Quantitation (LOQ)[3] or Reporting Threshold[4].

Perform three determinations. Responses below LOQ or with a signal-to-noise ratio of less than 10 should be disregarded. If the RSD_3 is smaller than or equal to 10%, and the mean complies with the specifications, the sample can be accepted. If this requirement is not met, three further determinations are performed. If the RSD_6 of the six values is smaller than 10% and the mean of the six values complies with the specifications, the sample can be accepted. The sample can only be rejected if the mean does not comply with the specifications and the criterion for the *RSD* is met. If the RSD_6 is still too large, the reason for the poor repeatability should be investigated and, in such circumstances, the sample can neither be accepted nor rejected.

This approach is illustrated in *Figure 3*.

Figure 3 - Decision tree for Approach 3



[3] Quantitation limit, as defined in "Validation of Analytical Procedures: Text and Methodology Q2(R1)", ICH, 1994.

[4] Reporting threshold, as defined in the European Pharmacopoeia 7.4, Chapter 5.10. Control of impurities in substances for pharmaceutical use".

Approach 4: Finished products

For recently-registered products with fully validated analytical methods, information regarding repeatability and/or intermediate precision of the test method is supplied in the application file. The repeatability might be reported with different degrees of freedom depending on the experimental design. The minimum degrees of freedom is 5, as given in the ICH guideline.

During the assessment process, the performance characteristics of the quality control procedures are evaluated against the specification limits proposed by the manufacturer. When approved, the results of the tests performed must fall within the specification limits.

When a product is to be tested at an OMCL, the MAH file is consulted in order to find suitable conditions for the test method and also to get information on its performance characteristics. The repeatability of the results obtained during testing can therefore be used as a quality indicator and can be checked against the values given in the dossier. Nevertheless, the OMCL might find that internal quality control criteria for evaluating the repeatability of the results of the test are suitable for the intended purpose.

The observed standard deviation varies between testing events, following a skewed distribution. To test whether standard deviations (or, rather, variances) are not significantly different, the quotient of two variances is calculated and compared to the critical F-value at a specified probability for the relevant degrees of freedom. In Tables 3 and 4, the critical F-values at the 5% level have been used to calculate the maximum allowable standard deviations under the assumption that the observed repeatability is not significantly worse than that reported in the dossier. If the OMCL chooses to use its own in-house repeatability criteria, the use of the data described in Tables 3 and 4 is not applicable and the comparison should be done with the predefined RSD criteria. This should be taken into account in points 3, 4, 5 and 6 below.

1. Find the RSD and the degrees of freedom for the repeatability in the dossier.
2. Perform three determinations and obtain the results in % of the label claim. Calculate the mean and the relative standard deviation.
3. Check in the relevant Table 3 or Table 4 if the RSD obtained for the repeatability is larger than the critical value given in the table corresponding to the reported value in the dossier.

4 If the RSD is not larger than the value in the table and the mean is within the acceptance range, the sample passes.

5 If the RSD is not larger than the value in the table, but the mean is outside the acceptance range, perform three more determinations and calculate the mean and the standard deviation of the six determinations.

6 If the RSD is still not larger than the value in the table and the new mean is within the acceptance range, the sample passes.

7 If the RSD is not larger than the value in the table, but the mean is outside the acceptance range, calculate the confidence interval using the standard deviation from

the independent determinations performed by the laboratory and/or from the MA dossier, the corresponding t-value and square root of 6 according to the following formula:

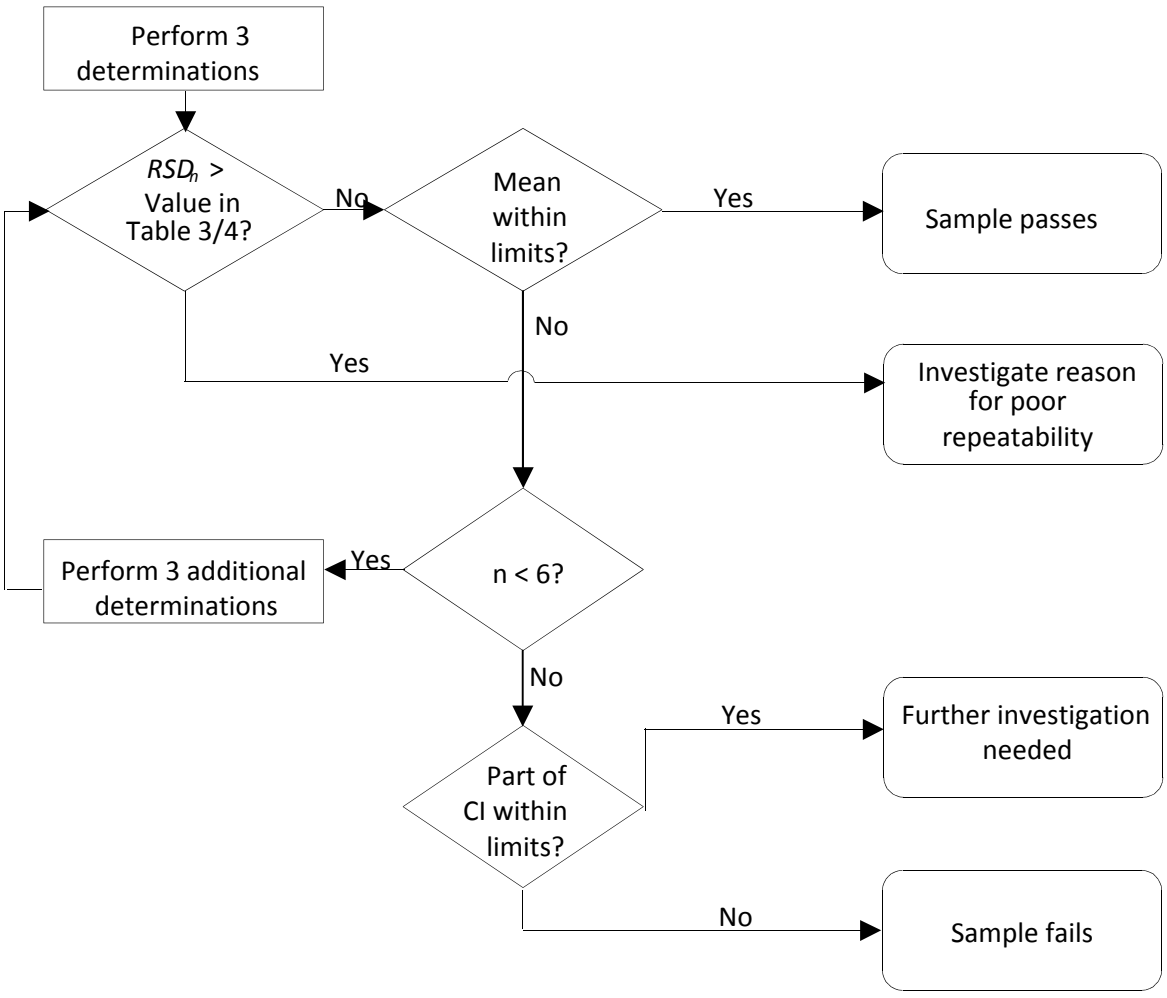
$$\overline{x}_6 \pm \frac{t_{(95\%,\nu)} \times \hat{\sigma}_{(\nu)}}{\sqrt{6}}$$

8.1 If no part of the confidence interval is inside the acceptance range, the sample fails

8.2 If part of the confidence interval is inside the acceptance range, further investigation is needed.

This approach is illustrated in *Figure 4*.

Figure 4 - Decision tree for Approach 4



If the OMCL decides to use internal quality control criteria to evaluate the repeatability of the results of the test, Tables 3 and 4 are not applicable. The steps 1 to 8, as well as the decision tree (Figure 4), can be applied as long as the comparison of the obtained RSD is performed with the internal quality control criteria (step 3).

Table 3 - Maximum acceptable observed RSD for validation based on 5 degrees of freedom (95% degree of confidence)

Repeatability from validation (%) **ν=5 (ICH)**	df=2	df=3	df=4	df=5
0.40	0.96	0.93	0.91	0.90
0.50	1.20	1.16	1.14	1.12
0.60	1.44	1.40	1.37	1.35
0.70	1.68	1.63	1.59	1.57
0.80	1.92	1.86	1.82	1.80
0.90	2.17	2.09	2.05	2.02
1.00	2.41	2.33	2.28	2.25
1.10	2.65	2.56	2.51	2.47
1.20	2.89	2.79	2.73	2.70
1.30	3.13	3.02	2.96	2.92
1.40	3.37	3.26	3.19	3.15
1.50	3.61	3.49	3.42	3.37
1.60	3.85	3.72	3.65	3.60
1.70	4.09	3.95	3.87	3.82
1.80	4.33	4.19	4.10	4.04
1.90	4.57	4.42	4.33	4.27
2.00	4.81	4.65	4.56	4.49

df: degree of freedom

Table 4 - Maximum acceptable observed RSD for validation based on 6 degrees of freedom (95% degree of confidence)

Repeatability from validation (%) **ν=6** (ICH)	df=2	df=3	df=4	df=5
0.40	0.91	0.87	0.85	0.84
0.50	1.13	1.09	1.06	1.05
0.60	1.36	1.31	1.28	1.26
0.70	1.59	1.53	1.49	1.47
0.80	1.81	1.75	1.70	1.68
0.90	2.04	1.96	1.92	1.89
1.00	2.27	2.18	2.13	2.10
1.10	2.49	2.40	2.34	2.30
1.20	2.72	2.62	2.55	2.51
1.30	2.95	2.84	2.77	2.72
1.40	3.17	3.05	2.98	2.93
1.50	3.40	3.27	3.19	3.14
1.60	3.63	3.49	3.41	3.35
1.70	3.85	3.71	3.62	3.56
1.80	4.08	3.93	3.83	3.77
1.90	4.31	4.15	4.04	3.98
2.00	4.53	4.36	4.26	4.19

df: degree of freedom

Approach 5: Products with insufficient validation data

For products where the validation data is limited or there is no validation data at all, the criteria for precision should be determined in order to decide upon the acceptance of a result. This approach is to be regarded as a tool to establish an acceptance criterion for precision of the replicates of a given test, when the laboratory has no other means to evaluate the distribution of the results.

The specification for a given test can be regarded as an interval, where:

- the lower limit is [Central value - Maximum Error],
- the upper limit is [Central value + Maximum Error],

with no further tolerances to be applied.

If the maximum error is considered as expanded uncertainty (U), the interval for the specification can be regarded as [Central value +/- U], or [Central value +/- 2*RSD][5], taking into account that the main component of the global uncertainty of a test is most frequently its precision.

As an example, for the specification of an assay test of [95.0 – 105.0%]:

- the central value is 100.0%
- the maximum error is 5.0%
- the lower limit is 95.0% (= 100.0-5.0%)
- the upper limit is 105.0% (= 100.0+5.0%)
- the expanded uncertainty U (= 2*μ) is 5.0%
- the relative uncertainty μ is the maximum RSD (μ ≈ RSD), taking into account that the main uncertainty component is precision
- the maximum expected RSD for the test can be assumed to be 2.5%.

The criteria for precision that could be applied to these cases are described in Table 5.

Table 5 - Approach 5: Criteria for precision

Sample	Specification (Assay)	Maximum error	Global Estimated Precision (RSD)
API	99.0 – 101.0 %	1 %	0.5 %
Finished Product	95.0 – 105.0 %	5 %	2.5 %
General approach	(100-x) – (100+x)%	x %	x/2 %

Steps 2 to 8 of Approach 4 can be applied directly.

[5]This proposal for precision criteria should be regarded as an approach to solving the problem of a lack of precision criteria for a given test. It should not be confused with the concept of specifications established for European Pharmacopoeia monographs.

Approach 6: Re-test programme based on statistical assay layouts (Ph. Eur. 5.3)

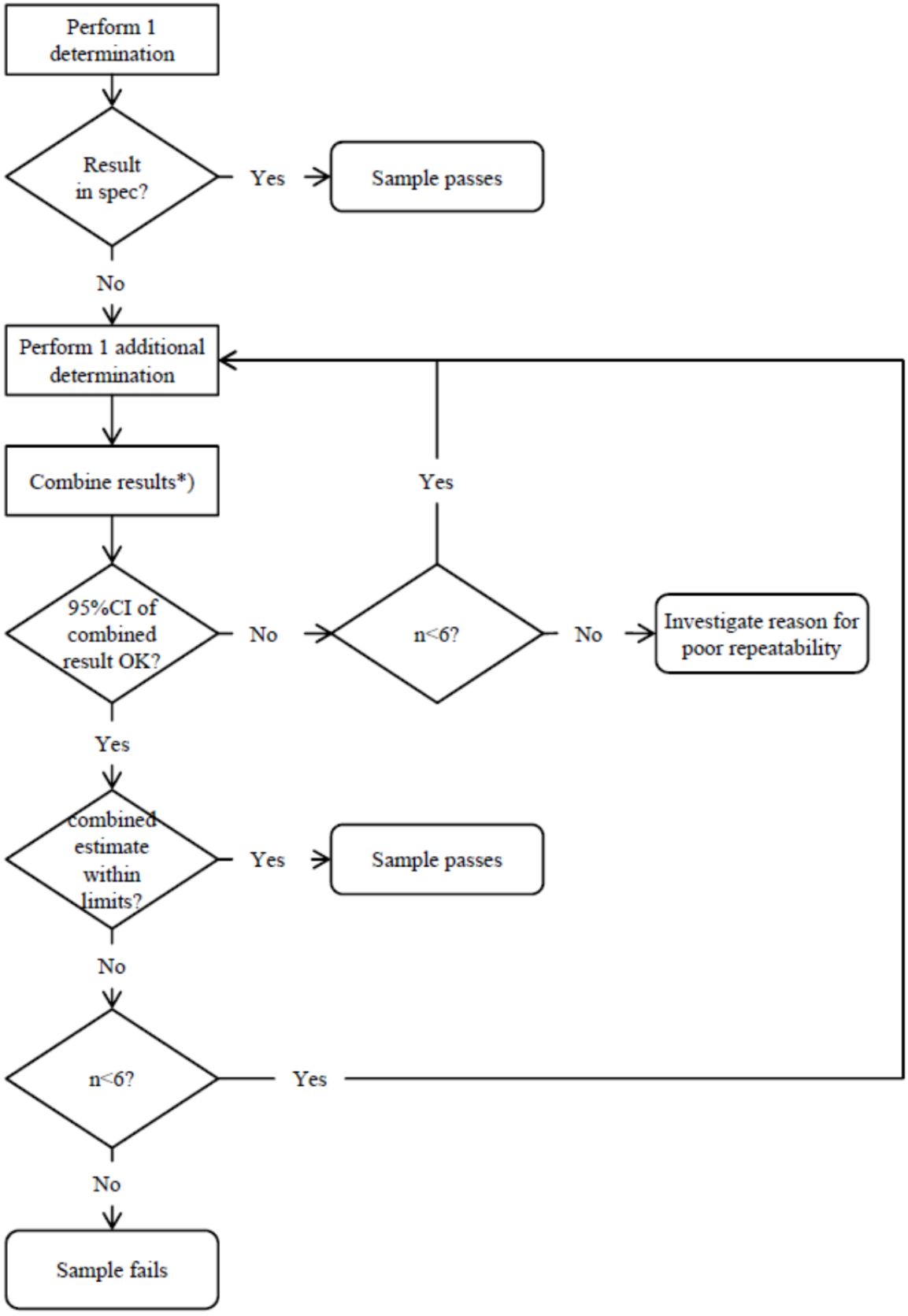
Results from bioassays in a statistical layout according to Ph. Eur. 5.3 (e.g. parallel lines, slope ratio, probit) may be combined according to Ph. Eur. 5.3 (6) or the relevant CombiStats functions. To combine assays, it is desirable that the individual results are obtained according to identical or similar assay layouts.

Assay combination may also be applied to individual assays for which the 95% CI exceeds the validity criteria (e.g. 95-105% - *Note*: this is an exception to all other approaches, which only consider valid test results). In all cases, the 95% CI of the combined result should meet the validity criteria defined by the method.

CombiStats calculates three types of combinations. Which combination you should use depends on whether the intervals are homogeneous. There are no strict rules as to which of the three should be used, but the following 'rule of thumb' can be of use:

- If the p-value for homogeneity is more than 0.100, the confidence intervals are sufficiently homogeneous to use the weighted combination.
- If the p-value is less than 0.100, the confidence intervals tend to be heterogeneous and it would be better to use the semi-weighted combination.
- The unweighted combination should only be used if there are enough assays, e.g. at least six.

Figure 5 - Decision tree for Approach 6:



*) choose mode of combination considering guidance in text

Approach 7: Re-test programme based on known intermediate precision

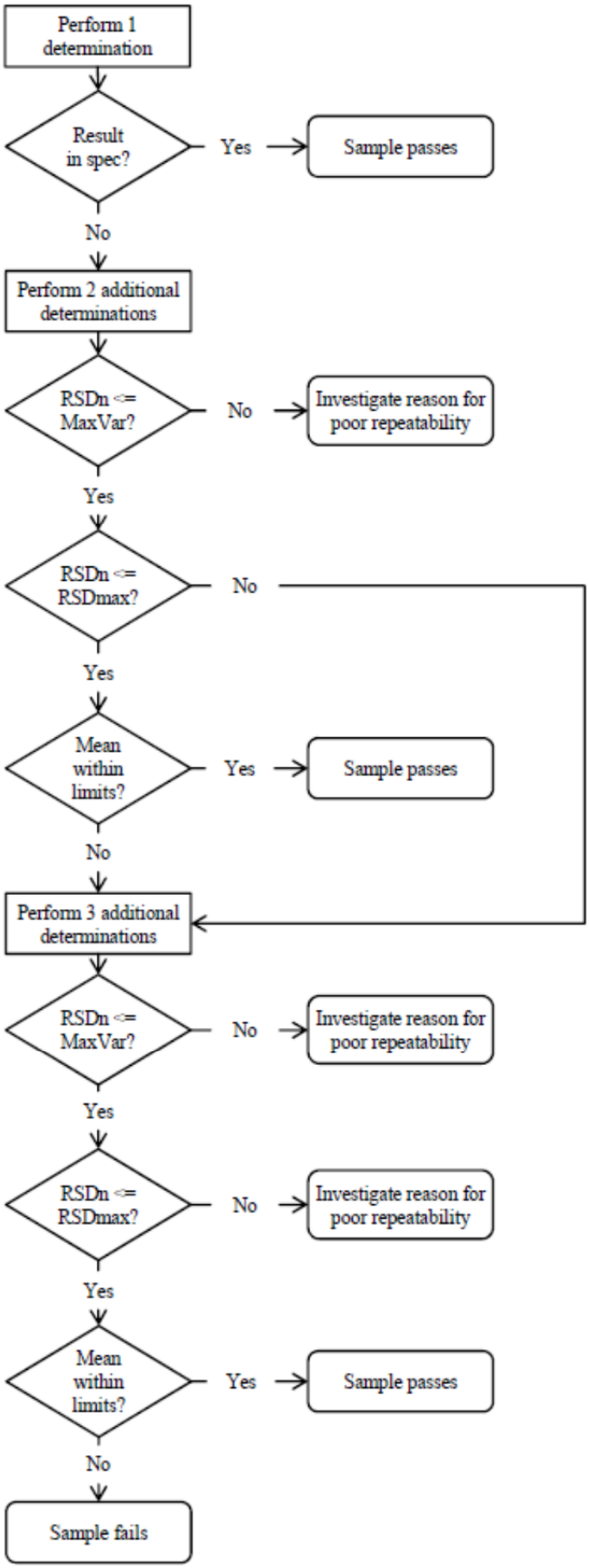
This approach is intended for assays with well-known in-house intermediate precision, e.g. in OCABR routine analysis. The benefit of the approach is that the re-test programme can be abandoned if it is evident after the first three tests that the repeatability of the method is insufficient to reach a conclusion.
If the assay follows a statistical layout, the combination of assay results (Approach 6) should be considered as an alternative.

Based on the distance from the targeted specification limit to the nominal value, a maximal variation based on the specification of MaxVar is calculated that is considered acceptable for reaching a conclusion. Additionally, twice the imprecision of the in-house method validation RSDval is defined as RSDmax. The RSD from the re-tests is compared to both the MaxVar and the RSDmax.

B	distance from targeted specification limit to nominal value (in percent of nominal value)	absolute ([100*specification limit/stated potency]-100)
RSDval	inter-assay precision known from in-house validation or control chart	
RSDmax	maximum expected RSD	RSDmax=2*RSDval
MaxVar	maximum acceptable variation based on specification	MaxVar=B*1.265*SQRT(1/n-1)

Re-tests have to be performed on independent samples and dilutions, but for pragmatic considerations, they may be performed together in up to two re-test series (the first series may be performed with two repeats in one test run, while the second series can be performed with three repeats). Variability caused by the dilutions of the reference should be monitored by a control chart.

Figure 6 - Decision tree for Approach 7:



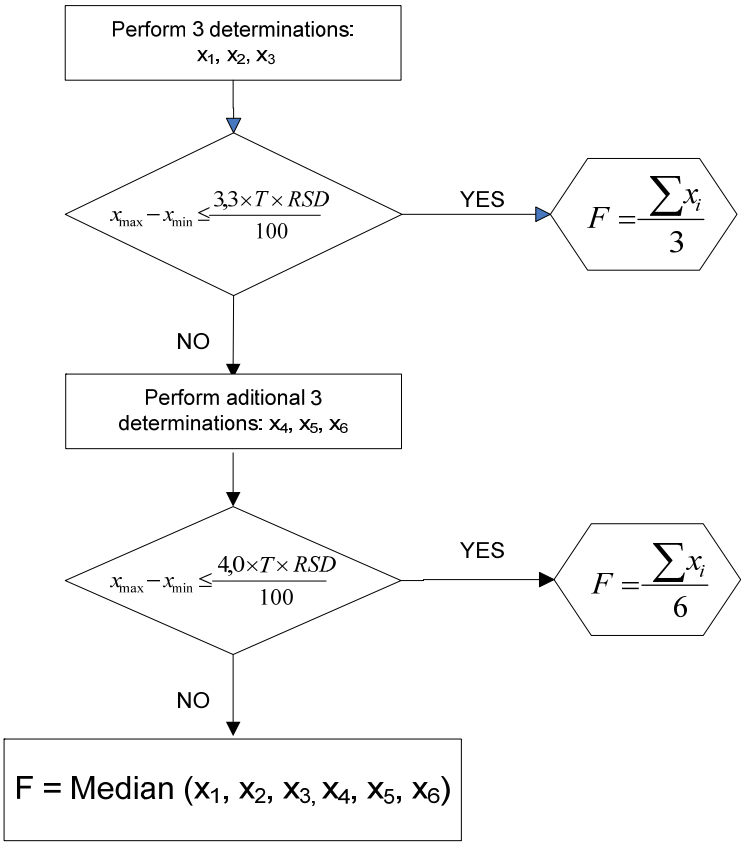
Approach 8: Approach for cases of unexplained lack of repeatability

For products where sample recovery problems are encountered or there is no plausible reason for lack of precision, ISO standard 5725-6:1994 "Accuracy (trueness and precision) of measurement methods and results -- Part 6: Use in practice of accuracy values" can be applied.

This ISO standard assumes that all replicates are generated under repeatability conditions, regardless of the test being performed on the sample in duplicate or in triplicate. It enables the use of all the determinations performed, based on the statistical difference between the lower and the highest value, without having to exclude results which, apparently, are outliers.

The decision for acceptance of replicates of a sample, regardless of the number, depends on the difference between the maximum value and the minimum value obtained, defining an amplitude. This value is compared with $\left(\frac{C \times T \times RSD}{100}\right)$. T is the average of all the sample results. C is a constant that depends on the number of replicates analysed, and is described in ISO 5725-6:1994 ($C_{n=2} = 2.8$; $C_{n=3} = 3.3$; $C_{n=4} = 3.6$; $C_{n=6} = 4.0$).

Figure 7 - Decision tree for Approach 8, for triplicates.



As an outcome, several scenarios can be predicted:

- The first set of replicates is accepted: the final result is their arithmetic mean;
- The first set of replicates is not accepted: the test must be repeated;
- After repeating the analysis, the two sets of replicates are accepted: the final result is their arithmetic mean;
- After repeating the analysis, the two sets of replicates do not pass the acceptance criteria: the final result is the median.

This approach is an alternative to the cases where the precision criteria for six replicates are not met and there is no apparent explanation for the observed lack of repeatability.

Although OMCLs can apply this approach, it is strongly recommended that the individual results are critically analysed. The OMCL must decide the most adequate way to report when a final result is the median.

If applicable, this approach may be used when the investigation of the reasons for poor repeatability of a test, performed using approaches 1, 2, 3 or 4, indicates that there is no plausible reason for the lack of precision.