THE DRINKING WATER QUALITY DIVISION

GUIDANCE ON

SAMPLE AND SAMPLE EXTRACT STABILITY TRIALS

DWQD GUIDANCE ON SAMPLE AND SAMPLE EXTRACT STABILITY TRIALS

Purpose

The purpose of these trials is to demonstrate that the maximum permitted delay between sampling and analysis does not result in "a material alteration in the concentration or value for the measurement or observation of which the sample is intended" (regulation 16(2)(c)).

In terms of a statistical trial, the hypothesis to be tested is that the change in the mean of repeated measurements before and after storage is not greater than a target figure.

A successful stability trial must be conducted prior to adopting sample preservation conditions or sample extract preservation conditions which differ from, or storage times which are longer than, those documented in ISO 5667 Part 3:2003, or other authoritative source ie a standard method. Each sample matrix of interest must be fully tested. In practice, only the worst case or worst cases need be tested. For example testing nitrite stability in waters with low colony counts will not yield useful information.

Results of trials carried out in other laboratories may be used, provided the laboratory can show that the preservation and storage conditions are identical (not just similar) and that the sample matrices of interest to the laboratory were included in the trial and the results of the trial were fully satisfactory and robust (ie reproduced in three or more independent laboratories).

Specification of requirement

For regulatory analysis the appropriate target value is one half of the maximum permitted trueness error. For most parameters this is 5% of the value at the PCV. For many organic parameters it is 12.5%. Significance is at the two-sided 95% confidence level, and the power is set at 90%. Each sample matrix type of interest must be tested separately.

The specification, design, calculation and interpretation given in this document are all derived from NS30 pages 113 to 120, 137 to 139 and 148.

Design of Trial

The trial should consist of spiking of a pre-determined number of samples to the PCV. All samples must be collected by filling a series of bottles from the same source (eg a single tap). The true concentrations of the parameter must show negligible variation from one sample to another. If filling a series of bottles directly from the tap may not yield such samples, a bulk sample should be taken which is then mixed and sub-divided into a series of bottles. Precision of spiking is of paramount importance and more important than the absolute value spiked. If precision of spiking is likely to cause problems, consideration should be given to spiking a bulk sample, which can then be sub-divided into a series of bottles.

One set of samples is analysed on day 0, with a further set analysed at each selected time interval with all sample preservation and storage conditions applied exactly as it is intended to apply them to regulatory samples. It would be prudent to also include times less than the full period desired for routine storage of samples in the trial.
The estimated minimum number of samples (n) required to be analysed on each day of testing to show whether the change is significant is given below

Standard deviation (%PCV)	Number of samples to detect 12.5% change	Number of samples to detect 10% change	Number of samples to detect 5% change
1	2	2	2
2	2	2	5
3	2	3	10
4	3	7	17
5	5	**7** (mercury)	**26**
6	6	10	38
7	9	13	51
8	11	17	67
9	14	22	85
10	17	**26 (**tetrachloromethane)	104
11	21	32	126
12	24	38	150
12.5	**26**	41	163
38	38	59	234
20	67	104	416

These figures are minimum values of n for which the equation $(t_\alpha + t_\beta)\, s\sqrt{(2/n)} \leq \delta$ is true, where δ is the target change, subject to a minimum of 2 for a statistical comparison to be made. This indicates that the test will probably be sufficiently powerful to identify the target change as being a statistically significant difference. Figures in bold relate to the maximum permitted precision relevant to the maximum permitted change. These numbers are only estimates of the actual numbers required because the actual distribution of data will not be known until after the test is completed, and either more or fewer replicates may be needed in practice. Reasons for large deviations from the expected standard deviation should be investigated to determine if there is any reason for the unexpected change in performance, which may invalidate the trial. Large within batch variations can also lead to wrong conclusions being drawn. Prior to undertaking trials steps should be taken to ensure that between batch errors are not significant. The most common cause of significant between batch errors is variation in the true value of calibration standards. If it is not possible to reduce such errors to a magnitude which will not adversely affect the trial, means should be adopted to measure and compensate for such errors, such as those described in NS30 or DD ISO ENV 13530:1998

The same design can also be used to test alternative preservation and pre-treatment methods. In these cases, storage times should be the same and between batch errors can be eliminated by analysing both sets of samples in the same analytical batch.

Calculation

The significance of any observed difference is determined using a t-test. The following is an example calculation, with expected standard deviation of 2% and target change 5%

	Day zero	Day x
	101.0	94.0
	100.3	93.2
	98.8	92.9
	101.2	96.5
	99.9	92.8
Mean	100.225	94.05
Standard deviation	1.11	1.72

Pooled standard deviation	1.45
Mean difference	6.175
Standard Error (of differneces)	1.024
t statistic (calc)	**6.032**
Degrees of freedom	6
Critical value (.05) (from tables)	**2.447**

Conclusion: there is a real difference between the means.

Interpretation

$t_{0.05}$ for 6 degrees of freedom = 2.447 (from tables). The observed value is greater than the tabulated value and therefore there is a real difference between the two means. The numerical value of the change is also greater than the target value and therefore there is a significant change.

If the observed value of t is greater than the tabulated value and the change was less than or equal to the target change, the change is less than (or equal to) the target and samples may be stored for up to the tested period under the conditions tested.

If the observed value of t is less than the tabulated value and the change was equal to or greater than the target change, the trial was not sufficiently powerful to show a significant change and must be repeated with more replicates.

If the change is less than the target change and the observed value of t was less than the tabulated value then, provided the trial was sufficiently powerful and would have identified any difference in excess of the target change as being significant, there has been no significant change and samples may be stored for up to the tested period under the conditions tested. The test is sufficiently powerful if the target change is substituted for mean difference in the formula for the t test and the value of t then calculated is greater than the tabulated value. If it is not greater then the trial was not sufficiently powerful to show a significant change and must be repeated with more replicates.

In summary:

Mean difference greater than target?	Observed t greater than tabulated value?	Would difference equal to target change have observed t greater than tabulated value?	Proposed new storage arrangements satisfactory?
Yes	Yes	N/A	No
Yes	No	N/A	No*
No	Yes	N/A	Yes
No	No	No	No*
No	No	Yes	Yes

* Trial not sufficiently powerful to test the original hypothesis. Repeat trial using more replicates.

THE DRINKING WATER QUALITY DIVISION

SAMPLE PRESERVATION AND PREPARATION FOR METALS ANALYSIS OF DRINKING WATER

DWQD GUIDANCE ON SAMPLE PRESERVATION AND PREPARATION FOR METALS ANALYSIS OF DRINKING WATER

Purpose

Regulation 16(2)(c) requires Scottish Water to ensure that samples are kept at such temperature and in such conditions as will secure that there is no material alteration in the concentration or value for the measurement or observation of which the sample is intended. This is relevant to a number of parameters.

In order to fulfil the requirements of the above regulation in respect of metal determinands, the sample needs to be preserved at the time of sampling or as soon as it enters the laboratory. Further pre-treatment may then be necessary to ensure that all the metal of interest present in the sample is in the form and state required for analysis. In practice this usually means that any of the metal of interest present in particulate form, or adsorbed onto particles in the sample or the surface of the sample container, is taken into solution. The exception to this is antimony where the oxidation state may also be of prime importance.

In practice, sample preservation and pre-treatment for metal determinands are interlinked and must be considered together. Some laboratories assume that the preservation stage is adequate to dissolve any particulate material present, as long as there is a suitable time lapse between sampling and analysis. Other laboratories have adopted a practice of only subjecting samples to a rigorous pre-treatment if particulate material can be seen in the sample or the sample has an elevated turbidity. These practices have been shown to be unreliable and should not be used for regulatory analysis.

Best Practice for sample preservation

Best practice for sample preservation is documented in ISO 5667 Part 3:2003.

The recommendations on best practice for pre-cleaning sample containers is given in paragraph 3.2.2.4. Acid-washed plastic or borosilicate glass containers may be used, except for mercury, for which acid-washed borosilicate glass containers must be used.

For most metals the recommended preservation is acidification to pH1 to 2 with concentrated nitric acid. The exceptions are mercury which additionally requires the addition of potassium dichromate, and antimony, for which hydrochloric acid should be used if analysis is to be by hydride generation.

Best practice for sample pre-treatment

Best practice for sample pre-treatment for metal determinands is documented in ISO 15587 Parts 1 and 2. These recommend digestion by boiling with aqua regia or nitric acid. The nitric acid method is not suitable for antimony when it is determined by hydride generation. Procedures are described using both open and closed systems, with digestion using standard heating devices and microwave digestion.

Certain analytical procedures may specify alternative preservation and pre-treatment regimes. It is acceptable to use these methods, provided they are properly documented and published by an authoritative source of analytical methods. They also need to be validated as part of the overall method. Where the method is dependent on specific preservation and pre-treatment regimes these should always be used (this advice is contained within the ISO Standards) (eg some methods for mercury analysis).

Analytical blanks

Analytical blanks should always be subject to preservation and pre-treatment regimes identical to regimes applied to samples. They should be processed with the batch of samples to which they relate using the same bottle/batch of each reagent. If blank correction is applied, it should be these blanks which are used to correct all samples in the batch.

When carrying out trace analysis, contamination of reagents and standards is a significant source of error and should be minimised by only using the grade of reagent or standard appropriate to the method being used. Standards and reagents suitable for AAS have been found to be unsuitable for ICP-MS work.

It may be necessary to also run reagent blanks, prepared in the same way as standards, for blank correction of standards.

Regulatory Requirement

The DWQR expects Scottish Water's laboratories to follow best practice in order to meet the requirements of regulations 16(2)(c) and 16(5)(b), unless an alternative method can be shown to be equally satisfactory. Real samples and, if necessary, spiked real samples containing concentrations of each metal of interest at concentrations close to the PCV including natural particulate material should be used for testing. For lead analysis this includes particles of metallic lead, and naturally occurring particles of oxides, hydroxides or salts of all metals. The principles of testing and calculation of results given in the accompanying guidance on stability trials should be followed.

Alternative Preservation and Pre-treatment Regimes

Samples for mercury should always be preserved at the time of sampling because of the highly volatile and highly reactive nature of the determinand.

The ISO method recommends preservation of the sample at the time of sampling. If this is adopted, then a representative aliquot may be taken from the sample for pre-treatment prior to analysis.

The DWQR accepts that Scottish Water may prefer not to use bottles with acid preservative for samples taken at consumers' taps, with the exception of mercury. This is on health and safety grounds. In such circumstances the samples should be preserved immediately on arrival at the laboratory. However the whole sample container should then be subject to an additional pre-treatment step before any sample is withdrawn. This is to take account of any metals that have adsorbed onto the surface of the container and should be applied for both plastic and glass sample containers.

The additional pre-treatment step may be to heat the acidified sample container in an oven at 80ºC for 12 hours (in practice over night) before cooling the contents and sub-sampling for analysis. At least one laboratory has shown this to be satisfactory and comparable with preservation at the time of sampling. This procedure can also be used with samples preserved at the time of sampling. In either case a further digestion step may be required prior to analysis, especially for lead analysis.

Laboratories may wish to establish which combination of procedures is most appropriate for their sample matrices.