TECHNICAL SPECIFICATION

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Water quality — Sampling —

Part 25:
Guideline on the validation of the storage time of water samples

Qualité de l'eau — Échantillonnage —

Partie 25: Lignes directrices pour la validation de la durée de conservation des échantillons d'eau

Reference number
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Foreword

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This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 6, *Sampling (general methods).*

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document addresses the need for harmonized and reliable data on stability, which is essential for the expression of recommendations for both normative and regulatory purposes. It describes a methodological framework that enables laboratories to produce quality data that can be shared and even monetized[7].

It enables laboratories to study the stability of parameters when using the physico-chemical parameters measurement system: organic micropollutants, inorganic and organometallic micropollutants, nutrients and macropollutants in aqueous matrices (surface water, ground water, residual urban and industrial water and drinking water). It covers the sampling, transport and laboratory storage operations.

NOTE This document does not cover solid matrices from aquatic environments (suspended solids, sediments).

Water quality — Sampling —

Part 25:
Guideline on the validation of the storage time of water samples

1 Scope

The purpose of this document is to describe test plans and different operating methodologies of these test plans to define and verify the acceptable length of stability of a substance in a sample under specified conditions of preservation (temperature, matrix, light, addition of a stabilizer, where appropriate, type of preservation etc.) before starting analytical protocols (chemicals and physico-chemicals analysis). Biological and microbiological methods are excluded.

It is necessary to have an analytical method with performances that have already been characterized (repeatability, intermediate precision, trueness, accuracy and uncertainty) in order to perform the stability study and implement its test plans.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/TS 21231, *Water quality — Characterization of analytical methods — Guidelines for the selection of a representative matrix*

3 Terms, definitions and symbols

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
analytical process
detailed description of a measurement according to one or more measurement principles and to one given measurement method, and including any calculations intended to obtain a measurement result

3.2
batch
<production, material> definite amount of test material prepared by the laboratory at a given point in time under supposedly identical conditions

3.3
chronological stability study
study in which individual samples prepared at the same time (i.e., as a batch), under identical conditions, are measured as time elapses (e.g. one sample immediately, one after three months, the next one after six months, etc.)

[SOURCE: ISO Guide 35:2017, 8.3.2.1]

3.4
homogeneity
uniformity of a specified property value throughout a defined portion of a reference material (RM)

Note 1 to entry: Tests for homogeneity are described in ISO Guide 35[2].

Note 2 to entry: The “defined portion” may be, for example, an RM batch or a single unit within the batch.

Note 3 to entry: See also IUPAC Compendium on Analytical Nomenclature[6].

[SOURCE: ISO Guide 30:2015, 2.1.12]

3.5
influence parameter
intrinsic characteristic of the matrix, independent of the analyte concentration, a variation of which is liable to modify the analytical result

[SOURCE: ISO/TS 21231:2019, 3.3.1]

3.6
influence parameter of the conditions of preservation
characteristic related to the conditions of storage and preservation of the sample, independent of the analyte concentration

Note 1 to entry: E.g. container material, storage temperature, influence of light and/or relative humidity.

3.7
integrity
property that the parameter(s) of interest, information or content of the sample container has not been altered or lost in an unauthorized manner or subject to loss of representativeness

[SOURCE: ISO 5667-3:2018, 3.1]

3.8
intermediate precision condition of measurement
condition of measurement, out of a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over an extended period of time, but may include other conditions involving changes

Note 1 to entry: The changes can include new calibrations, calibrators, operators, and measuring systems.

Note 2 to entry: A specification for the conditions should contain the conditions changed and unchanged, to the extent practical.

Note 3 to entry: In chemistry, the term “inter-serial precision condition of measurement” is sometimes used to designate this concept[8].

3.9
isochronous stability study
experimental study of “reference” material stability in which units exposed to different storage conditions and times are measured in a short period of time

[SOURCE: ISO Guide 35:2017, 3.9]

3.10
matrix
all the constituents of the laboratory sample other than the analyte

Note 1 to entry: By extension, a matrix is defined by the analyst as waters characterized by a homogeneous behaviour with regard to the analytical method used.

[SOURCE: ISO/TS 21231:2019, 3.3.3]

3.11
maximum acceptable delay before analysis
MaxADs
maximum acceptable delay between the end of the sampling process and the start of the analysis operations, resulting from the stability study, that the laboratory uses to plan the analyses

3.12
maximum acceptable deviation for the stability study
MADs
maximum acceptable deviation relative to the assigned value of the sample at T_0, used to determine the maximum acceptable delay before analysis

3.13
measurement precision
closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 2 to entry: The "specified conditions" may be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-1:1994[1]).

Note 3 to entry: Measurement precision is used to define measurement repeatability, intermediate measurement precision, and measurement reproducibility.

Note 4 to entry: Sometimes “measurement precision” is erroneously used to mean measurement accuracy[8].

3.14
measurement repeatability
measurement precision under a set of repeatability conditions of measurement

[SOURCE: JCGM 200:2012 (VIM), 2.21]

3.15
measurement reproducibility
measurement precision under reproducibility conditions of measurement

Note 1 to entry: I.e., condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects.

[SOURCE: JCGM 200:2012 (VIM), 2.25]

3.16
measurement trueness
closeness of agreement between the average of an infinite number of replicates measured quantity values and a reference quantity value

Note 1 to entry: Measurement trueness is not a quantity and thus cannot be expressed numerically, but measures for closeness of agreement are given in ISO 5725-1:1994[3].

Note 2 to entry: Measurement trueness is inversely related to systematic measurement error, but is not related to random measurement error.

Note 3 to entry: “Measurement accuracy” should not be used for ‘measurement trueness’.

[SOURCE: JCGM 200:2012 (VIM), 2.14]

3.17
minimum quantifiable deviation for the stability study (MQDs)
minimum deviation relative to the assigned value of the parameter in the sample at T_0, which can be unequivocally imputed to the instability

Note 1 to entry: This deviation takes account of the inhomogeneity of the test material and the intrinsic variability of all the measurement results over time used to determine the maximum acceptable deviations.

Note 2 to entry: The calculation of the minimum quantifiable deviation for the stability study depends on the type of study (chronological, pseudo-isochronous or isochronous) (6.1.1).

3.18
pseudo-isochronous stability study
stability study in which some of the steps, in particular the preparation, are performed under intermediate precision conditions, and in which the results of instrumental analyses are acquired under repeatability conditions

3.19
repeatability condition
condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time

Note 1 to entry: A condition of measurement is a repeatability condition only with respect to a specified set of repeatability conditions.

Note 2 to entry: In chemistry, the term “intra-serial precision condition of measurement” is sometimes used to designate this concept[8].

3.20
representative matrix
sample for which all the intrinsic characteristics are characteristics of a type of water or the source of a group of samples

[SOURCE: ISO/TS 21231:2019, 3.3.2]

3.21
reproducibility condition
condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects

Note 1 to entry: The different measuring systems may use different measurement procedures.

Note 2 to entry: A specification should contain the conditions changed and unchanged, to the extent practical[8].

3.22
sample preservation
any procedure used to stabilize a sample in such a way that the properties under examination are maintained stable from the collection step until preparation for analysis

[SOURCE: ISO 5667-3:2018, 3.2]

3.23
sample storage
process and the result of keeping a sample available under predefined conditions, usually for a specified time interval between collection and further treatment of a sample

Note 1 to entry: Specified time is the maximum time interval [see ISO 5667-3].

3.24
stability
characteristic of an analyte in an aqueous matrix, when stored under specified conditions, to maintain its property value within specified limits for a specified period from sampling to laboratory operations

3.25
stability interval
interval defined on the basis of the assigned value at T_0 and the maximum acceptable deviation for the stability study

3.26
storage time
period of time between filling of the sample container and further treatment of the sample in the laboratory, if stored under predefined conditions

Note 1 to entry: Sampling finishes as soon as the sample container has been filled with the sample. Storage time ends when the sample is taken by the analyst to start sample preparation prior to analysis.

Note 2 to entry: Further treatment is, for most analytes, a solvent extraction or acid destruction. The initial steps of sample preparation can be steps complementary to the storage conditions for the maintenance of analyte concentrations.

[SOURCE: ISO 5667-3:2018, 3.4]

4 Principle

The goal is to perform a series of tests, hereafter referred to as the “stability study”, to analyse the variations in the value of a given parameter, between an initial time and a maximum time, on samples representative of the scope of application of the measurement method of the parameter. The conclusions of these tests are used to determine the maximum acceptable delay before analysis (3.11) under the conditions of the study.

The stability study has six stages:

— Definition of the requirements (analytes, matrices, levels of concentration, storage conditions, length of storage, maximum acceptable deviation for the stability study (3.12)), see Clause 5;

— Definition of the experiments plan (type of study, number of time intervals and total length of the study), see Clause 6;

— Performance of the tests, see Clause 7;

— Validation of the data, see Clause 9;

— Using the results (based on a maximum acceptable deviation, 3.12), see Clause 10;

— Expression of the stability in the form of a maximum acceptable delay before analysis (3.11) and the duration of stability corresponding to the conditions and the criteria (maximum acceptable deviation for the stability study (3.12)) of the study, see Clause 10.

Since the stability study covers different stages of the data acquisition process according to the organization of the measurement system, examples of the organization of the measurement system are given in Annex A for reference.

The laboratory shall take the following factors into consideration:

— The method of determination used: for example, limit of quantification, repeatability, intermediate precision, accuracy, influence parameters of the matrix (3.10) on the performance of the method;

— The “sample” material of the stability study: for example, homogeneity, physico-chemical properties;

— Suitability of the sample material for use as test material in accordance with storage time (3.26).

— Definition of the influence parameters of the preservation conditions (3.6) assessed as part of the stability study: for example, time, temperature, addition of stabilizers;

— If studied, command of the storage and transportation conditions;

— Clearly defined acceptability criteria (maximum acceptable deviation, 3.12), with which the results of the study will be compared.

5 Definition of the scope of the stability study

5.1 Aim of the stability study

Based on the scope of application of the method, the experimental plan shall clearly define the aim of the stability study by specifying the measured analytes, the matrices (3.10) and the target concentration levels.

5.2 Selection of the maximum acceptable delay before analysis and the acceptance criteria

Respecting the maximum acceptable delay before analysis (3.11) may determine the quality of the results of the analysis more than certain performance data of the measurement methods (bias). This is the reason why the maximum acceptable delay before analysis shall be established and known before the routine application of a laboratory analysis method.

The assessment of the results of a stability study with the intension of drawing a conclusion on the stability, expressed as a maximum acceptable delay before analysis, of a given analyte in a representative matrix of the scope of application shall be based on the interpretation of the results in perspective of the requirements of the stability study. A maximum acceptable deviation (3.12) shall be set in order to come to a conclusion. This maximum acceptable deviation shall be chosen before the start of the study, because it determines the conditions of performance of the method and the admissibility of the data, in particular with regard to the measurement method.

There are five ways to determine the maximum acceptable deviation. They are, in order of relevance:

a) The application of a regulatory requirement, if one exists;

b) Twice the repeatability standard deviation for isochronous or pseudo-isochronous type 1 studies (Annex B), or twice the intermediate precision standard deviation for type 2 pseudo-isochronous studies (Annex B) or chronological studies; the values of the standard deviation of repeatability and intermediate precision being the values defined during the characterization of the method.

c) Use of the data from the stability study: dispersion at T_0 of the stability study. See Formula (1):

$$\text{Maximum acceptable deviation} = \text{Minimum quantifiable deviation} \quad (1)$$

d) The choice of an arbitrary value, determined according to the technical operational implementation constraints (e.g., the best available method offering a precision of 15 %) or a value from a ring trial (ISO 5667-3). In this case, the maximum acceptable deviation shall be greater than this value, e.g., 25 %.

e) A value derived from the temporal operational implementation constraints (e.g., the impossibility of performing an analysis before a given time due to the minimum transportation time). In this case, the maximum acceptable delay before analysis is fixed and the maximum acceptable deviation is based on the observations at the pre-determined time.

EXAMPLE If the temporal constraint is three days, (MaxADs =3 days) the maximum acceptable deviation is estimated according to the observations (dispersion, for example) at T_3.

If the minimum quantifiable deviation is greater than the maximum acceptable deviation, it is impossible to draw any conclusions about the stability. In this case, the laboratory should identify the causes (e.g., lack of homogeneity of the materials, lack of performances of the method, need to stabilize the materials) and repeat the study (e.g., with a different maximum acceptable deviation, another method, etc.).

5.3 Influence parameters of the maximum acceptable delay before analysis

This protocol applies to conditions clearly defined by the laboratory, which defines the maximum acceptable delay before analysis, the corresponding assessment criterion (see 5.2) and the factors likely to impact it.

In particular, these factors may include:

— The container: ISO 5667-3 or the analysis standards applicable to the targeted parameters specify the recommended containers for the transportation of samples based on current knowledge. However, for certain classes of molecules, for which no normative guidelines exist, the compatibility of these general recommendations on the container material (plastic, glass, etc.) that may cause losses by adsorption, diffusion, the transfer of additives used in production, etc., should be verified;

— The storage temperature (ambient, refrigeration, freezing, etc.), which can impact the partition of the substances between the dissolved and particulate phases, or the enantiomer form, or their biological deterioration. ISO 5667-3 recommends that samples be preserved at 5 ± 3 °C during transportation. Therefore, this temperature range is the reference temperature of the study, in the absence of any other requirements. Other temperatures may be chosen for the study, for example when demonstrating the stability of analytical extracts in the laboratory. The temperature conditions of the study shall be clearly indicated, and they shall be monitored and documented throughout the stability study;

— The influence parameters of the matrix: pH, suspended solids, etc;

— Light, which can cause the photodegradation of certain organic molecules, e.g.: Benzo[a]pyrene, BDE209;

— The influence of sample pre-treatment on site, e.g. filtration[15];

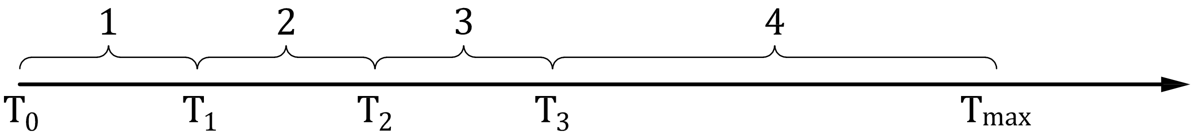
— The addition to the sample of stabilizing chemical agents (e.g., acid for metals, sodium hydroxide for cyanides, solvents, etc.).

5.4 Duration of the study

The duration of the study shall cover the initially planned maximum acceptable delay before analysis. The extrapolation of the MaxADs beyond T_{max} is not permitted. It is thus recommended to collect data beyond the planned MaxADs,

Interpolation between the two ends of a time interval is not permitted in order to define the maximum acceptable delay before analysis. Therefore, it is recommended to acquire additional information at different intermediate intervals of time.

The laboratory shall adapt the number of the intervals of time (Figure 1) of the study according to its initial knowledge of the stability of the analyte of concerned in order to minimize the risk of inconclusive studies.



Key

1 time interval 1

2 time interval 2

3 time interval 3

4 time interval 4

Figure 1 — Example of a diagrammatic illustration of the notion of time laps

5.5 Concentration levels

The levels of concentration to be tested (p) are chosen according to the requirements of the corresponding sector of activity, any regulatory requirements that may exist, the occurrence of data in the environment, for example, and the performances of the analytical method and its scope of application.

The experimental plan selects one of the two following approaches, depending on the complexity of the matrices and their knowledge of the method:

— At least two levels of concentration ($p \geq 2$) by representative sample ($n \geq 2$) of the matrix of the scope of application of the method shall be considered:

  — One low concentration level, different from the LOQ, ≤ 25 % of the scope of the method for one-decade methods or ≤ 10 % for more-decades methods,

  — One high concentration level, in the second half of the scope of application of the method.

— A minimum concentration level of p =1, ≤ 25 % of scope of the method for one-decade methods, resp. ≤ 10 % for more-decades methods, with several representative samples of the matrix (p x n ≥ 4). In this case two time laps should be taken.

The laboratory shall substantiate its choice of n=2 by demonstrating that its scope of application is restricted and, therefore, sufficiently described by two representative samples, in accordance with ISO/TS 21231.

NOTE 1 Performing stability studies at the limit of quantification (LOQ) is not generally relevant. The uncertainty of the methods at the LQ does not allow for the unequivocal interpretation of the data.

NOTE 2 When representative samples free of any background contamination of analyte cannot be found, the environmental background noise should be considered to determine the lowest level of contamination tested in the stability study.

5.6 Definition of the matrices and selection of the representative samples

The experimental plan defines the matrices or group of matrices for the study by referring to ISO/TS 21231. Consequently, the analyst selects representative samples of these matrices or group of matrices. The laboratory shall substantiate its choice.

The representativeness of the samples for the stability study is critically important. Two strategies can be considered:

— the use of natural samples, or

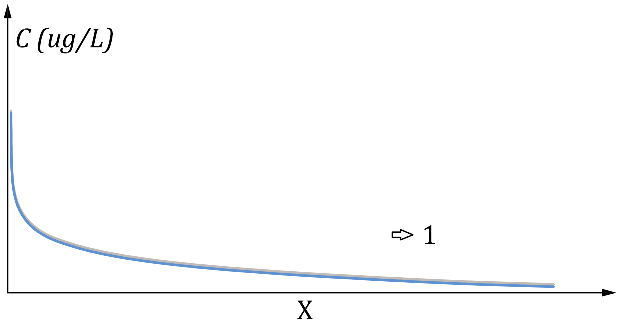
— the use of synthetic samples, which are natural samples whose physico-chemical characteristics are varied using the recipes in ISO/TS 21231; or the addition of influence parameters.

Each type of matrix in the scope of application of the method shall be studied. The influence parameters of each sample shall be measured and recorded at least at T_0.

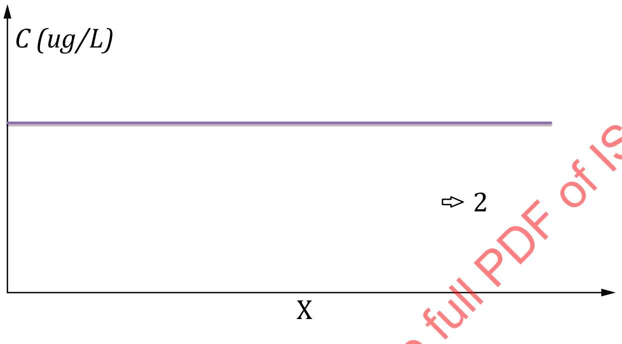
To guarantee the representativeness of the study and cover the entire range of the influence parameters of the matrix for the parameter(s) of interest, these samples shall have different intrinsic characteristics by type of matrix (surface water, ground water, for example), and include the extreme values of the influencing parameters. For example, the content of suspended solids and the pH for the methods used to analyse organic analytes, or the content of suspended solids and the conductivity for nutrients (see ISO/TS 21231).

5.7 Guidelines to the stability studies of transformation products

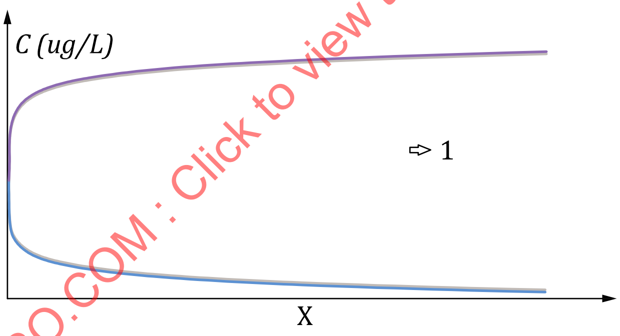
Whenever a stability study is made of a parameter that is known to be a transformation product (metabolite, by-products of oxidation or hydrolysis, for example) of a parent compound (analyte) that may be present in the sample, it is imperative to include the measurement of the parent compound (analyte) in the stability assessment of the transformation product. Transformation products usually have longer half-lives than the parent compound. Consequently, the stability study may erroneously conclude to the stability (Figure 2, b) of the transformation product. In real sample, where the parent compound is present, stability study will lead to instability conclusion of the transformation product together with the parent compound (Figure 2, c).



2a) Stability study – parent compound



2b) Stability study – transformation product (alone)



2c) Stability study – parent compound/transformation product mixtures

Key

X time

1 instability

2 stability

Figure 2 — Stability study of parent compound/transformation product mixtures

The maximum acceptable delay before analysis set for the transformation product cannot be longer than the maximum acceptable delay before analysis of the parent compound, irrespective of the results achieved in the stability study of the transformation product. If this condition is not met, there is a risk of bias in the estimate of the concentration of the transformation product in the sample. The maximum acceptable delay before analysis will be set at the shortest period of time. If this period of time is incompatible with the imperatives of the laboratory, the laboratory shall use a means of stabilizing the parent compound.

6 Definition of the experimental plan

Stability studies shall be associated with a homogeneity study of the material.

There are three possible approaches to stability studies: chronological, isochronous and an intermediate approach, known as pseudo-isochronous (Annex B). Table 1 compares these approaches.

The study shall be documented (Annex C). In particular, every laboratory should substantiate its choices in the corresponding records of this study.

Table 1 — Comparative description of the different approaches to stability studies

*	Chronological stability study	Isochronous stability study	Pseudo-isochronous stability study
Advantages	Ease of implementation	Performance of measurements under repeatability conditions	A flexible approach that is compatible with the requirements of the study and the operational constraints of the laboratory and the scope of application
	Acquistion of the results in the course of the study	Very discriminatory in order to highlight inhomogeneity and instability	
	Production of the test material in one batch	Production of the test material in one batch	Production of the test material in one batch or in several batches
Drawbacks	Need for a measurement method with a very good intermediate precision	Need to temporarily store the samples under conditions of stability	Availability of the results at the end of the study
	Less discriminatory in order to highlight inhomogeneity and instability	Availability of the results at the end of the study	Need for a highly reproducible method to prepare the test materials in order to achieve good intermediate precision

The admissibility of the stability study requires reliable results. Consequently, internal quality checks are necessary throughout the study: blanks, yield, limit of quantification check, for example.

Moreover, all the equipment shall imperatively be calibrated and checked before use.

6.1 Determination of the value of the quantity of the material at T_0 and at other times during the study

Table 2 exemplifies the number of measurements of a study.

Table 2 — Examples of number of tests to be performed

	Number of tests														
Number of time intervals	1	1	2	3	3	4	4	2	1	1	7	7	4	2	2

Key

m total number of measurements to be made under repeatability or precision conditions for the stability study

n number of representative samples

p number of concentration levels

q number of measurements to be made under repeatability conditions for each representative sample at each time interval T_i

r number of measurements to be made under repeatability conditions for each representative sample at the initial point in time T_0

Table 2 *(continued)*

	Number of tests														
Number of representative samples (materials): n	2	4	4	2	4	2	4	5	6	3	4	2	5	6	3
Number of concentrations: p	2	1	1	2	1	2	1	1	1	2	1	2	1	1	2
Characterization at T_0: Number of measurements to be performed under repeatability conditions at T_0: r	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Number of measurements to be performed under repeatability conditions at each day T: q	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2
Total numbers of measurements to be performed: m	24	24	36	36	36	44	44	35	30	30	68	68	55	42	42

Key

m total number of measurements to be made under repeatability or precision conditions for the stability study

n number of representative samples

p number of concentration levels

q number of measurements to be made under repeatability conditions for each representative sample at each time interval T_i

r number of measurements to be made under repeatability conditions for each representative sample at the initial point in time T_0

The data at T_0 influences the acceptance and the interpretation of the stability study, so this data shall be reliable. The measurements at T_0 are used to determine the so-called reference value of the concentration levels chosen in the study materials and their homogeneity. To this end, at least three replicates r shall be made per material at the initial point in time T_0, i.e., r=12 at T_0.

The laboratory shall check the homogeneity of the material prior to the characterization at T_0.

For the other time intervals, the minimum number of measurements q to be made under repeatability conditions will depend on the test plan chosen by the laboratory (Table 2):

If there are at least three-time intervals and $n \times p = 4$, at least two measurements under repeatability conditions ($q = 2$) are taken per condition (material, time interval), therefore:

— at T_0, the number of measurements performed is $n \times p \times r$ with r=3, i.e. $n \times p \times r = 12$;

— for each time interval after T_0, $n \times p \times q = 8$ measurements;

— for the 3-time intervals, $n \times p \times q \times 3 = 24$ measurements.

The total number of measurements is $m = 12 + 24 = 36$, and $m = r + q = 36$ at least.

6.2 Test materials

6.2.1 Preparation

This step shall be properly controlled to avoid:

i. insufficient homogeneity of the test material that prevents the results from being interpreted and requires the test to be repeated;

ii. an under-estimation or an over-estimation of the stability.

and, therefore, a wrong decision resulting in poor data quality.

Usually, the test materials for these studies are natural samples, synthetic materials or natural samples that are spiked.

The notion of the representativeness of a sample includes the delay before the preparation of the stability test materials at T_0.

The materials used in the stability study shall be prepared as soon as possible after the samples are taken in order to optimally preserve their representativeness[15]. The means of preservation between taking the sample and its preparation shall not alter the representativeness of the sample, e.g., if the study is dedicated to whole water samples, on site filtration is not allowed.

The experimental plans required to conduct the stability studies may require large volumes of samples that, in a large majority of cases, shall be adjusted to meet the need for the representativeness of the matrix. In particular, this may involve making additions (spiking) of the studied parameter. This step involves significant technical particularities, such as the need for a large container that is compatible with the requirements of the analysis of micropollutants in the form of traces (nature of the container, cleaning and/or packaging).

When the method requires only small sample intake (a few millilitres), new sample intake cannot be taken in a container that has already been used for a previous time interval. The conditions of preservation may have been modified (water/air exchanges, return to the ambient temperature, etc.).

This preparation stage is determined by a consideration of the above factors, irrespective of the approach adopted for the stability study (chronological, isochronous, pseudo-isochronous).

Spiking shall take place on a matrix at ambient temperature.

The gravimetric or volumetric means used to make additions shall be calibrated and checked beforehand.

Two preparation methods can be used: spiking to a single batch or spiking in containers[10][11].

6.2.1.1 Spiking in batches to a batch

Spiking in batches consists of spiking a large volume of samples that will then be put in as many containers as is necessary.

6.2.1.2 Spiking in containers

Spiking in containers consists of pouring the total volume of the matrix required by the study into the number of containers specified in the study plan, then spiking each container.

Whatever the preparation methods chosen, before any sampling and sub-sampling, the material should be made homogeneous and it is necessary to wait until an equilibrium is reached between the matrix and the additive.

6.2.2 Characterization of the test materials

Each type of matrix in the scope of application of the method shall be studied. The influence parameters of each sample, as defined in ISO/TS 21231, shall be measured and recorded at least at T_0.

6.2.3 MQD Evaluation

MQDs is calculated using Formula (2):

$$MQD = 2 \times S_r \sqrt{\left(\frac{1}{r} + \frac{1}{q}\right)} \tag{2}$$

where:

S_r is the repeatability or the intermediate precision of the method;

q is the number of measurement/sample at D_i;

r is the number of measurement/sample at D_0.

S_r being equal to the repeatability standard deviation for isochronous or pseudo-isochronous type 1 studies (Annex B), or the intermediate precision standard deviation for type 2 pseudo-isochronous studies (Annex B) or chronological studies; the values of the standard deviation of repeatability and intermediate precision being the values defined during the characterization of the method.

MQDs shall be lower than MADs.

6.3 Definition of the test plan

6.3.1 Choice of the type of study

Isochronous studies are recommended. In view of the operating constraints of studies on aqueous matrices, and to minimize the effect of the intermediate precision on the results of the study, it is advisable to opt for pseudo-isochronous studies by default.

There are two types of pseudo-isochronous approaches, whose principles are described in detail in Annex B.

— The first is similar to the isochronous approach, from which it differs in terms of the preparation of the test materials, which are not prepared in a single batch at T_0, but at the different points of the stability study. The advantage of this approach is that the analyses (preparation of the sample and instrumental analysis) can take place under repeatability conditions;

— The second approach is similar to the chronological approach, from which it differs in terms of the steps of the analytical pre-treatment that the samples undergo at each stage. The analytical extracts are stored under reference conditions until the end of the study, before being analysed with measuring instruments under repeatability conditions.

In all instances, the choice of approach shall be substantiated.

6.3.2 Randomization

Irrespective of the test plan, it is advisable to apply random sampling of the test material prepared for the study in order to reduce or eliminate the influence of variables other than those being studied, and in particular:

— the preparation of the materials: spiking and sub-sampling;

— the sampling of sub-units after each time interval in the study;

— the instrumental analysis, in order to minimize the impact of drift effects.

7 Performance of the tests

7.1 General

The execution of the experiments in the study is determined by the choice of the type of stability study (Annex B). Irrespective of the type of study chosen, the temperature of the containers used to preserve the test materials shall be documented and recorded throughout the study in order to substantiate the stability of the conditions of preservation.

Irrespective of the type of approach chosen, the physico-chemical parameters of the test materials that influence the studied parameter shall be characterized on at least n study samples.

If the influence parameters of the study materials are likely to be modified by the concentration, they shall be characterized on $n \times p$ containers. When amines are the analytes, e.g., their concentration has an influence on the pH of the samples.

Irrespective of the type of approach chosen, and if the impact of adding stabilizers is being studied, whenever the stabilizers are likely to affect the influence parameters of the matrix, these parameters should be monitored on n x r samples at each stage of the study. For example, when studying how adding acid influences the stability of a parameter, measuring the pH of the test material at each stage can help with the interpretation of the study.

The complete analytical method is applied to all the test materials.

The value of the studied parameter should be checked before starting the study in order to decide whether spiking is necessary and/or to define the value of the latter.

7.2 Chronological stability study

A chronological stability study is illustrated in Annex B, Figure B.2.

The parameters influencing the tested samples should be physico-chemically characterized and recorded for a better understanding of the results of the study.

At T_0, for each sample:

— Prepare the entire batch of containers required for the complete stability study, as defined in 6.3.1;

— Randomly select r containers (at least three) from the batch;

— Keep all the other containers in the batch under the storage conditions specified for the stability study;

— Analyse the r containers on the same day using the selected measurement method.

After each time interval in the study, including at T_{max}, for each sample:

— Randomly select q containers from the batch, as described in 6.3.1;

— Analyse the q containers on the same day using the selected measurement method. Before this step, the q containers shall be returned to the analysis conditions (e.g., ambient temperature).

After each time interval, a given sample is analysed under repeatability conditions. The study data is acquired under intermediate precision conditions.

7.3 Type 1 pseudo-isochronous stability study

It is important to draw the user's attention to the experimental time intervals, which are reversed in comparison with the order in the chronological approach. (Figure B.4).

The parameters influencing the tested samples should be physico-chemically characterized and recorded for a better understanding of the results of the study.

On the first day of the study (T_0), for each sample:

— Prepare the *q* containers required by the stability study, as defined in 6.3.1;

— Keep all the containers under the storage conditions specified for the stability study.

After each time interval of the study (intermediate *T*), for each sample:

— Prepare *q* containers required by on the intermediate *T* of the stability study, as defined in 6.3.1;

— Keep all the containers under the storage conditions specified for the stability study.

At T_{max}, for each sample:

— Prepare the *r* containers required at T_0 of the stability study, as defined in 6.3.1;

— Take all the containers stored under the conditions specified for the stability study;

— Return all the containers to the laboratory conditions;

— Analyse all the containers using the selected measurement method.

All the containers in each sample of the study are randomly analysed under repeatability conditions.

7.4 Type 2 pseudo-isochronous stability study

A type 2 pseudo-isochronous stability study is illustrated in Figure B.4.

Type 2 pseudo-isochronous stability studies can only be chosen when the validation of the method has confirmed extracts or digestates preservation conditions.

For a better understanding of the results of the study, a characterization of the matrix (physico-chemical parameters such as suspended matter, organic carbon) should be carried out and recorded.

At T_0, for each sample:

— Prepare the *m* containers required by the stability study, as defined in 6.3.1;

— Randomly take *r* containers from the batch, as described in 6.3.1;

— Pre-treat the *r* containers using the selected measurement method. Stop the protocol before the instrumental step. Store the analytical extracts, digestates or samples under the specified conditions that guarantee their stability (e.g., by freezing);

— Keep all the other containers in the batch under the storage conditions specified for the stability study.

After each time interval of the study (intermediate *T*), for each sample:

— Randomly take *q* containers from the batch, as described in 6.3.1;

— Return the q containers to the laboratory conditions;

— Pre-treat the *q* containers using the selected measurement method. Stop the protocol before the instrumental analysis step. Store the analytical extracts under the specified conditions that guarantee the stability of the samples.

At T_{max}, for each sample:

— Randomly take *q* containers from the batch, as described in 6.3.1;

— Pre-treat the q containers using the selected measurement method. Stop the protocol before the instrumental analysis step;

— Continue the analysis on all the extracts, by including all the samples from T_0 and the intermediate Ts in the instrumental sequence (in a random order).

All the containers in each sample of the study are randomly analysed under repeatability conditions.

7.5 Isochronous stability study

An isochronous stability study is illustrated in Figure B.3.

For a better understanding of the results of the study, a characterization of the matrix (physico-chemical parameters such as suspended matter, organic carbon) should be carried out and recorded. At T0, for each sample:

— Prepare the m containers required by the stability study, as defined in 6.3.1;

— Randomly select the r containers required for the stability study at T_0, as defined in 6.1 and store them under conditions in which stability is proven, or that have instability kinetics different from those being studied (usually slower).

After each time interval, for each sample:

— Randomly select the q containers, as defined in 6.1 and store them under conditions in which stability is proven, or that have instability kinetics different from those being studied (usually slower).

At T_{max}, for each sample:

— Randomly select the q containers required at T_{max}, as defined in 6.1;

— Return all the containers ($r + q \times j = m$) stored under the reference conditions to the analysis conditions (e.g., ambient temperature);

— Take measurements on all the containers using the selected measurement method (in random order).

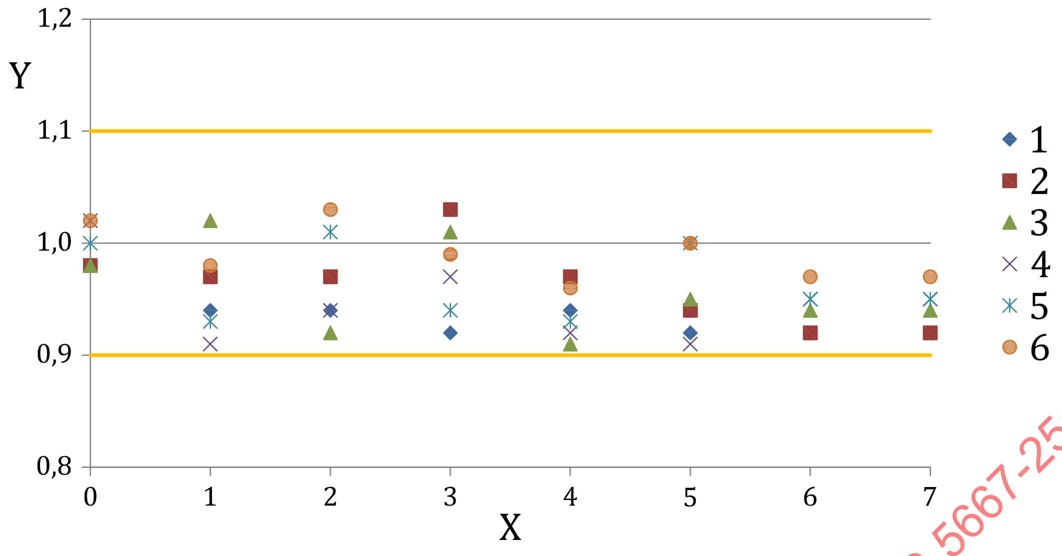
All the containers in each sample of the study are randomly analysed under repeatability conditions.

8 Graphic representation of the data

All the sets of data (different levels of concentration, different samples, different matrices) and the performance (repeatability, precisions or uncertainty) shall be graphically represented, and each set shall be individually identified, for example by using different colours or symbols. The ratio of the values in relation to the average of each condition or each study at T_0 can be calculated in order to facilitate the graphic construction, especially when the stability of several analytes is studied simultaneously or by different operators/laboratories or at different levels of concentration.

These ratios shall not be used to calculate the standard deviation of values for a sample after each time interval.

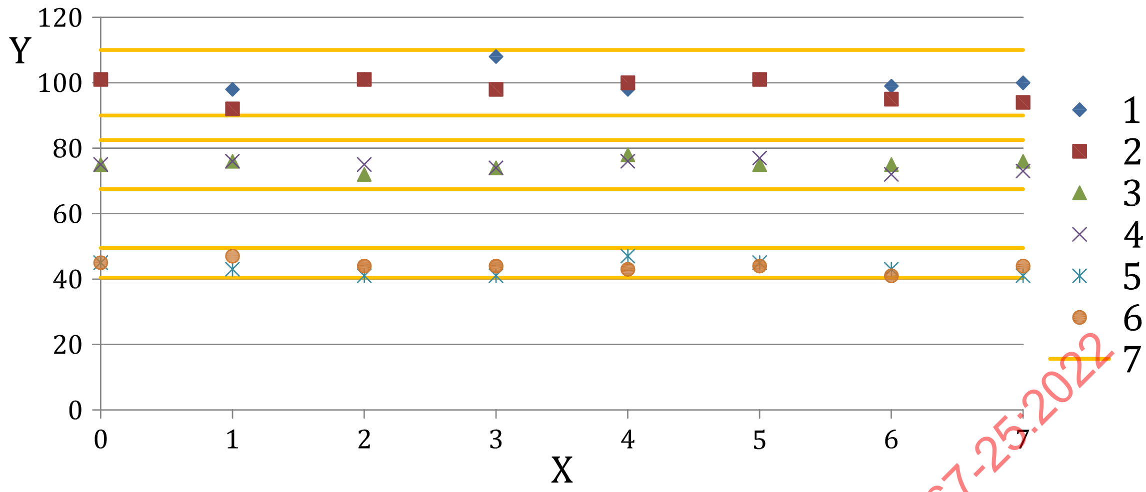
Figure 3 and Figure 4 show examples of graphical representations.



Key

X time (days)
Y ratio
1 sample 1
2 sample 2
3 sample 3
4 sample 4
5 sample 5
6 sample 6

Figure 3 — Initial representation of the data sets ratio vs. time



Key

X time (days)
Y ratio
1 sample 1
2 sample 2
3 sample 3
4 sample 4
5 sample 5
6 sample 6
7 repeatability

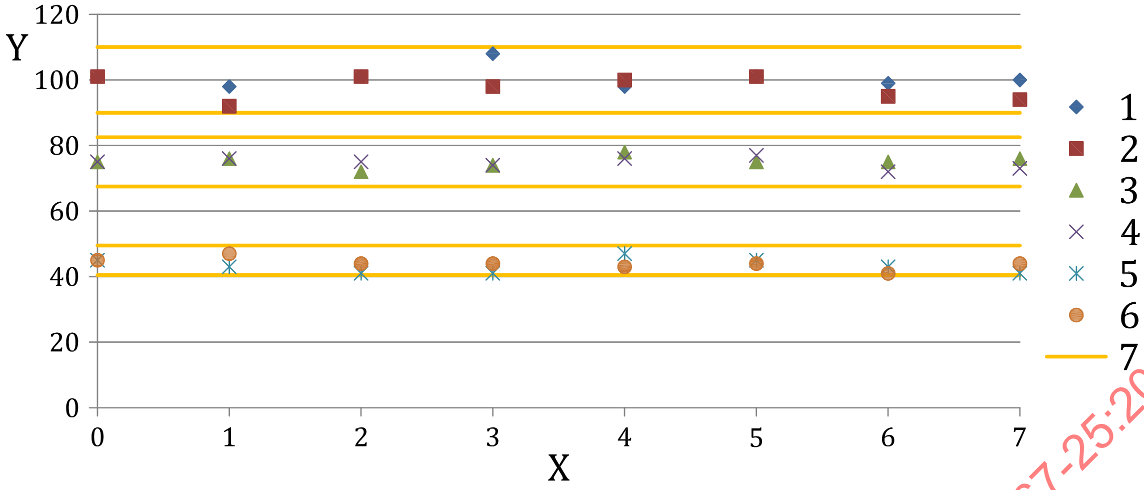
Figure 4 — Initial representation of the data sets concentration vs. time

It is necessary to compare the dispersion at each point in time with the performance of the method. The analyst shall question any dispersion that is greater than the performance of the method and its influence on the continuation of the analysis of the results.

If one or more data sets are visually different from the others, they can be processed separately, while remaining compatible with the goals of the study. A graphical representation of each of the datasets (different concentration levels, different samples, different matrices) each set being identified individually, for example with different colors or patterns, should be made.

This graphical representation will allow the analyst to visualize obvious problems of repeatability, inhomogeneity between samples, to re-evaluate the time steps if necessary or not to consider all the data.

An example of a graphical representation is shown in Figure 5.



Key

X time (days)

Y ratio

1 sample 1

2 sample 2

3 sample 3

4 sample 4

5 sample 5

6 sample 6

7 repeatability

Figure 5 — Initial representation of the data sets concentration vs. time

If the dispersion of the measurements on D_0 is greater than the dispersion of the measurement method (repeatability), the causes should be identified (points outside the repeatability interval). In any case, J_0 is not validated and the study stops.

If one or more datasets is (are) visually different from the others, they can be treated separately while remaining compatible with the objectives of the study.

9 Validation of the data

9.1 Initial validation of the study at T_0

The validation of the study data at T_0 is a critical step, because it determines the entire assessment of stability. It verifies the level of concentration and the dispersion of the measurements on the sample.

It also makes sure that the target value is reached on material that has been artificially contaminated by additives.

9.1.1 Calculation of the value assigned to T_0 (VA_{T0})

The analyst checks for any abnormal data in the data sets at T_0. If the numbers of accepted values for a sample is lower than 3 after this check, the sample is removed from the study plan.

The value assigned to T_0 for a sample is the average of the r_i results of the measurements given in Formula (3):

$$VA_{T0} = \bar{X}_{T0} \quad (3)$$

where $\bar{X}_{T0}$ is the average of the results at T_0 data set, or of all the data sets (ratio of the concentrations)

If the dispersion of the measurements at T_0 is greater than the dispersion of the measurement method (repeatability), the causes should be identified. In all instances, T_0 is not validated and the study is stopped.

9.1.1.1 Estimate of the uncertainty of the value assigned to T_0 ($u^2{}_{T0}$)

The main components of the uncertainty at T_0 are the dispersion and the trueness of the measurements. The measurement uncertainty of the value assigned to T_0 includes the standard deviation of the measurements at T_0 and the trueness component, are as given in Formula (4):

$$u^2(T_0) = u^2_{\text{repeatability}}(T_0) + u^2_{\text{trueness}}(T_0) \quad (4)$$

where

$u^2_{\text{repeatability}}$ is the standard deviation of the values at T_0;

u^2_{trueness} is the uncertainty of the trueness component of the experimental values at T_0 (systematic bias relative to the target value, derived from a yield study, for example).

9.1.2 Verification of the accuracy

The data set at T_0 is validated by verifying the accuracy of the value assigned to T_0 in relation to the target value. This verification is conducted in the same way on natural or spiked samples.

Beforehand, it is necessary to check that:

— minimum quantifiable deviation (MQDs) ≤ maximum acceptable deviation (MADs);

— the repeatability on the samples is not greater than the intermediate precision of the method.

$$|V_{T0\max} - V_{T0\min}| < k.s_r \quad (5)$$

where

k is a factor defined according to the risks;

s_r is the repeatability of the method;

$V_{T0\max}$ is the maximum value obtained at T_0;

$V_{T0\min}$ is the minimum value obtained at T_0.

If this criterion is verified, the analysis of the study results can continue. If not, the laboratory has two possible options:

— to invalidate the study and conduct a new study under different operating conditions;

— to continue to use the results in order to acquire knowledge. The laboratory will substantiate their subsequent use.

The dispersion of the measurements on D_0 also incorporates the heterogeneity of the batch of samples. If the criterion is not verified, the laboratory shall also question the homogeneity of the batch of samples.

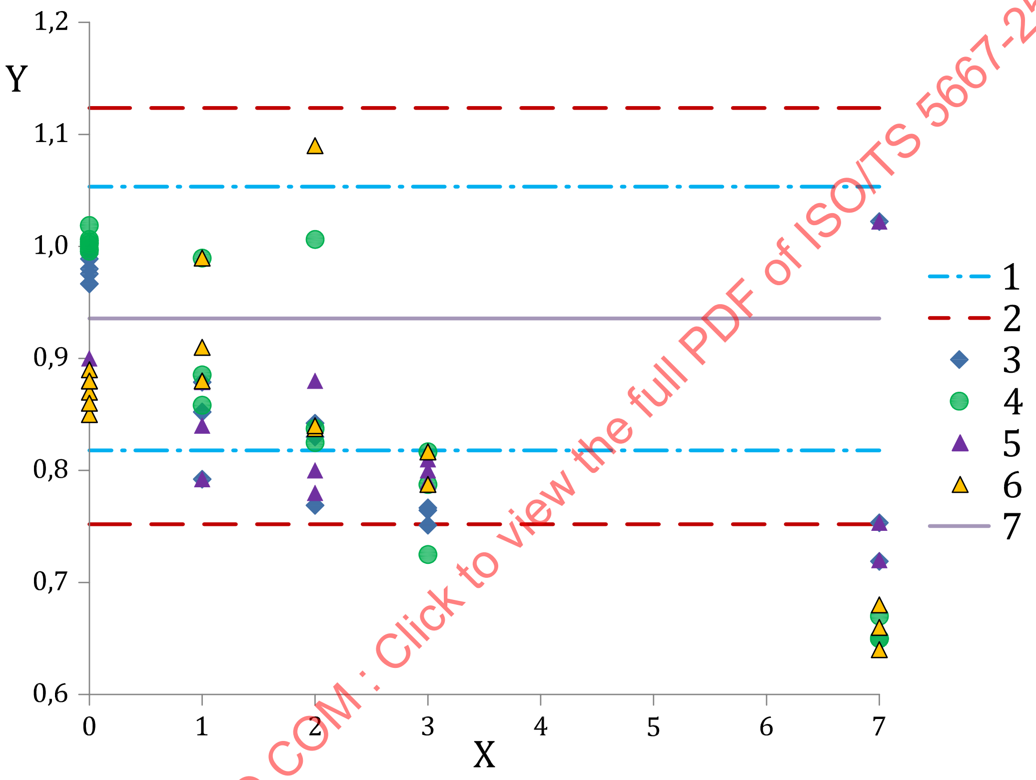
9.2 Validation at times different from T_0

It is necessary to check for any abnormal data in the data sets associated with the various points in time. The analyst can observe the data visually, compare the standard deviations at each point in time with that at T_0 or use statistical tools (Annex C).

10 Using the results

10.1 Graphic view of all the data

A graphic view of the complete data set, i.e.; all the conditions tested in the experiments plan (Figure 6), may illustrate changes in the dispersion of the data for each condition, or a tendency of the average.



Key

X time (days)
Y C normalised
1 MQDs
2 MADs
3 tests series 1
4 tests series 2
5 tests series 3
6 tests series 4
7 assigned value

Figure 6 — Example of a graphic illustration of the data of a stability study

10.2 Determination of the maximum acceptable delay before analysis by condition

The results are used as follows:

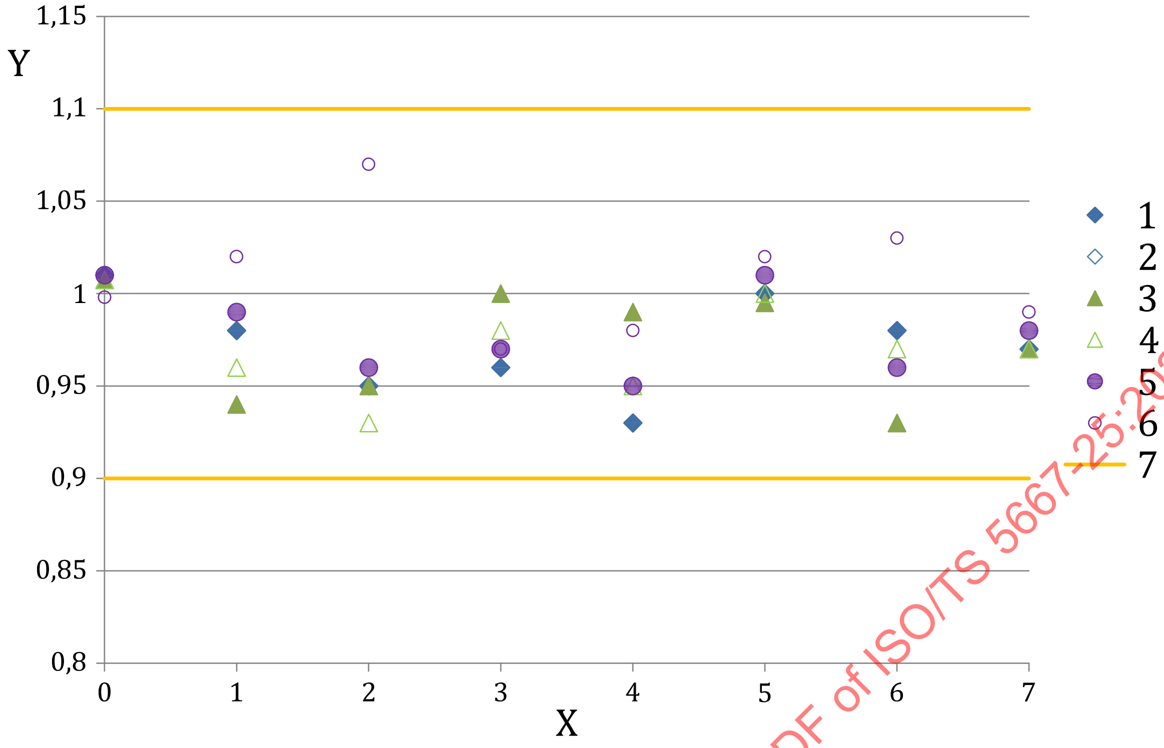
— The averages of r_i and q_i replicates by condition (sample and concentration) are calculated at each point in time T_i;

— If all the averages, irrespective of the condition and the point in time, are included in the stability interval (maximum acceptable deviation), the maximum acceptable delay before analysis of the study corresponds to the T_{max} of the study (Figures 7, 9, 13 and 15);

— If, for one condition, all the averages are included in the stability interval (maximum acceptable deviation), the maximum acceptable delay before analysis of this condition corresponds to the T_{max} of the study (Figure 8);

— If, for one condition, one average is not included in the stability interval (maximum acceptable deviation) at a point in time T_i, the maximum acceptable delay before analysis of this condition corresponds to the time interval T_{i-1} (Figure 10 and Figure 11). If, at T_{i+j} for this condition with $j \geq 2$, the averages are included in the stability interval (maximum acceptable deviation), the maximum acceptable delay before analysis corresponds to T_{i+j} (Figure 12 and Figure 14);

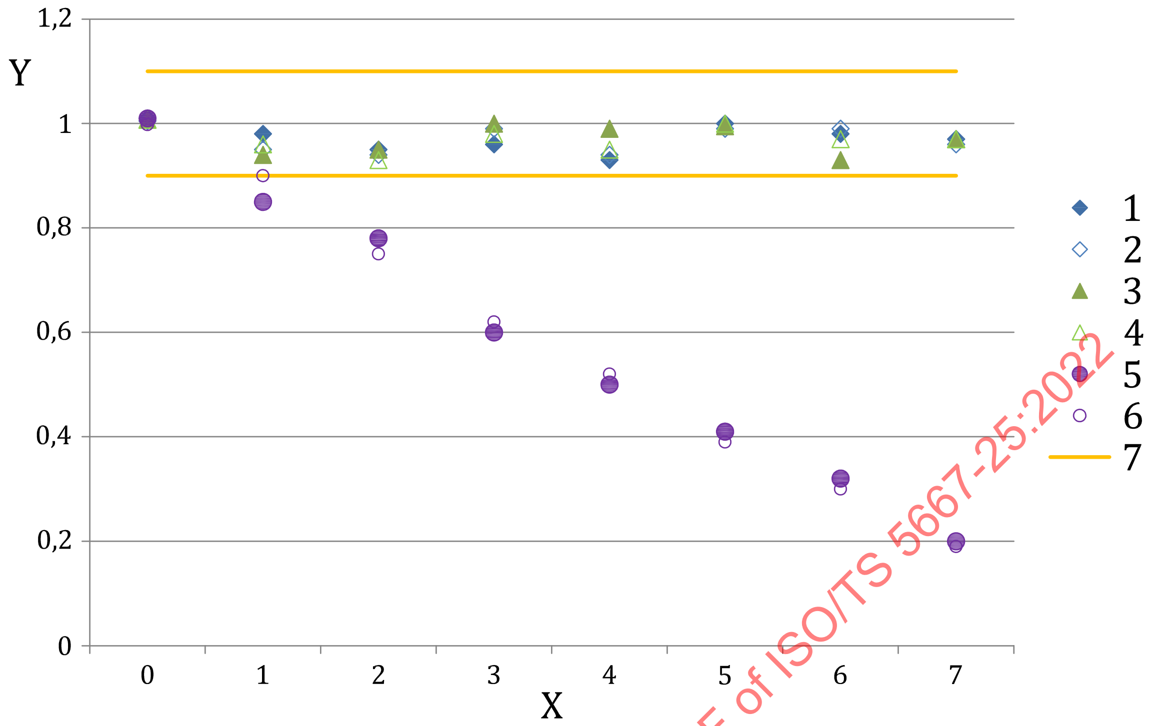
— If, for one condition, none of the averages are included in the stability interval (maximum acceptable deviation), the maximum acceptable delay before analysis of this condition is lower than the first-time interval of the study (Figure 16).



Key

X time (days)
Y ratio
1 sample 1 – rep 1
2 sample 1 – rep 2
3 sample 2 – rep 1
4 sample 2 – rep 2
5 sample 3 – rep 1
6 sample 3 – rep 2
7 MADs

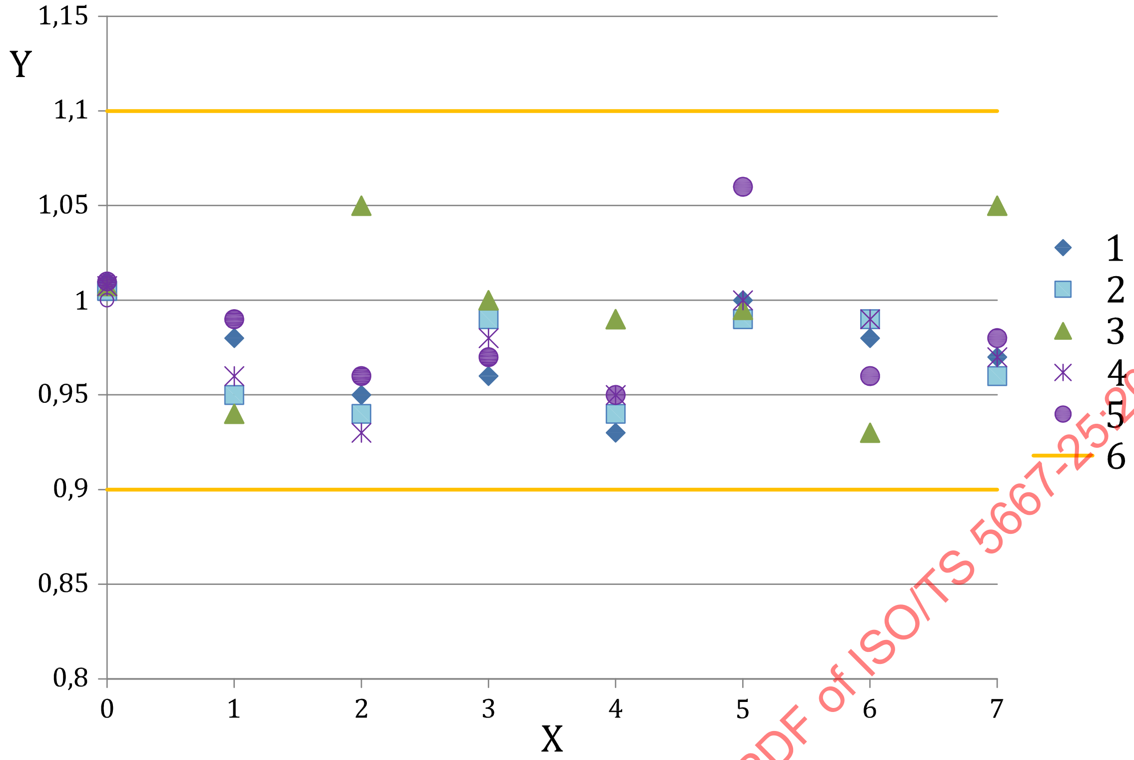
Figure 7 — Example of the stability of all the samples



Key

X time (days)
Y ratio
1 sample 1 – rep 1
2 sample 1 – rep 2
3 sample 2 – rep 1
4 sample 2 – rep 2
5 sample 3 – rep 1
6 sample 3 – rep 2
7 MADs

Figure 8 — Example of instability for a type of sample



Key

X time (days)

Y ratio

1 sample 1

2 sample 2

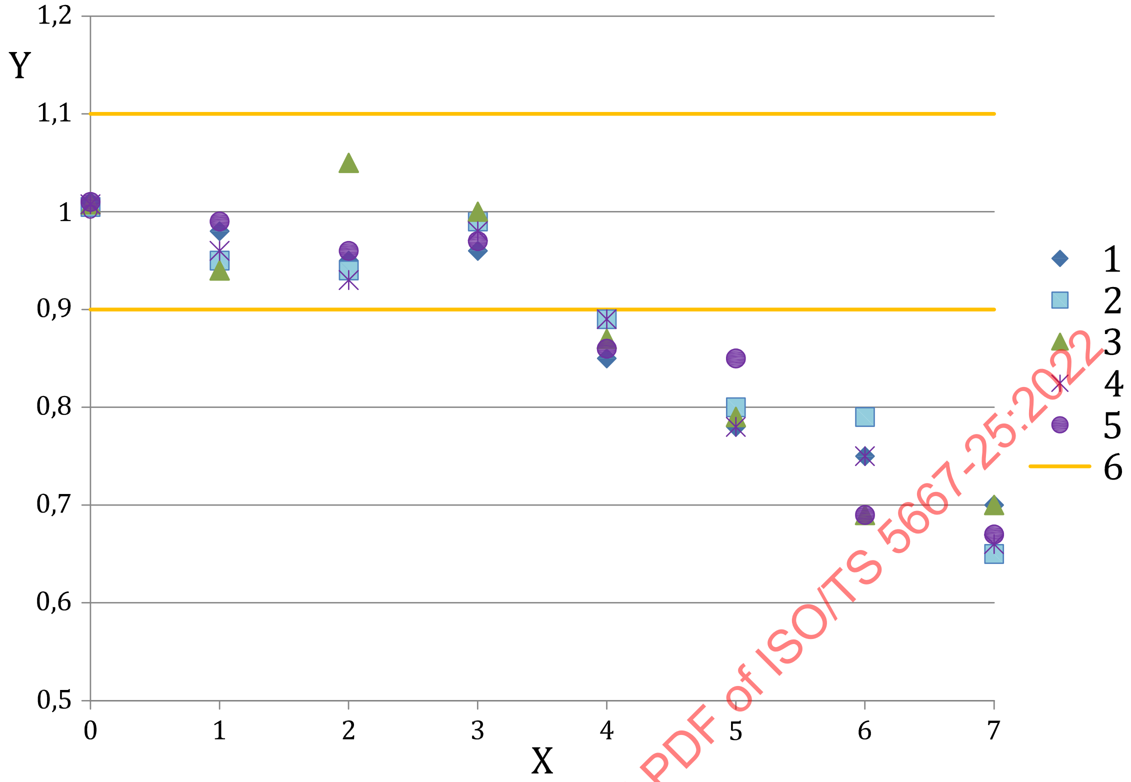
3 sample 3

4 sample 4

5 sample 5

6 MADs

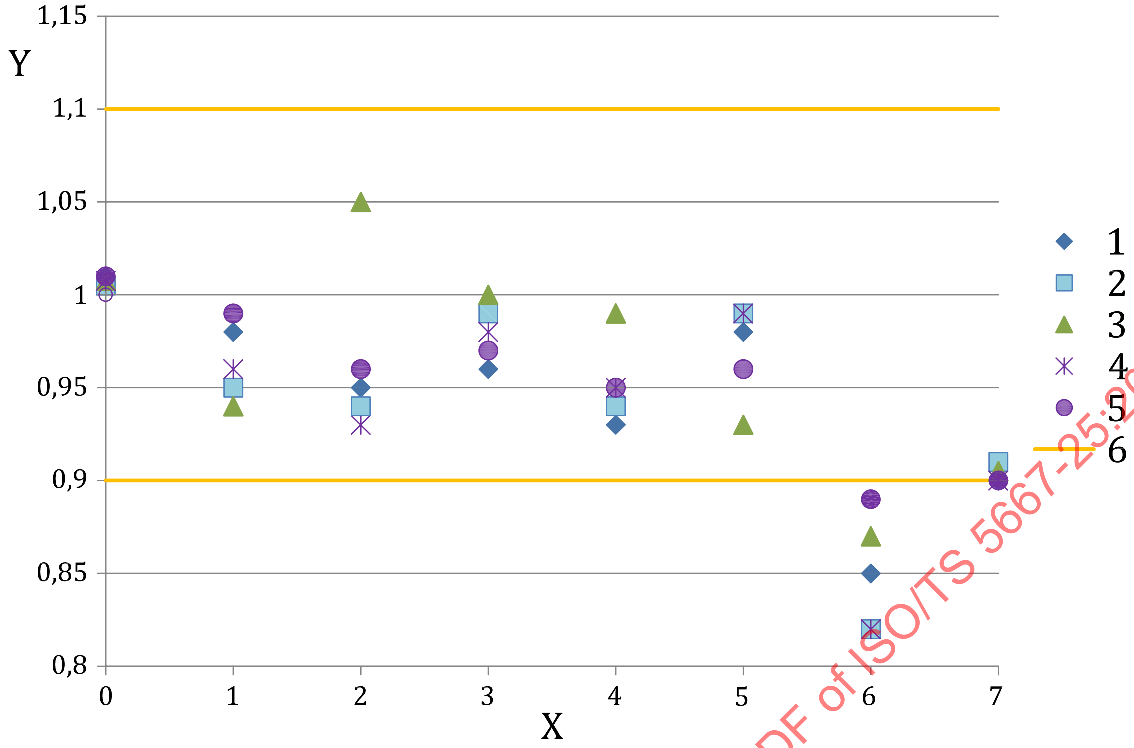
Figure 9 — Example of stability



Key

X time (days)
Y ratio
1 sample 1
2 sample 2
3 sample 3
4 sample 4
5 sample 5
6 MADs

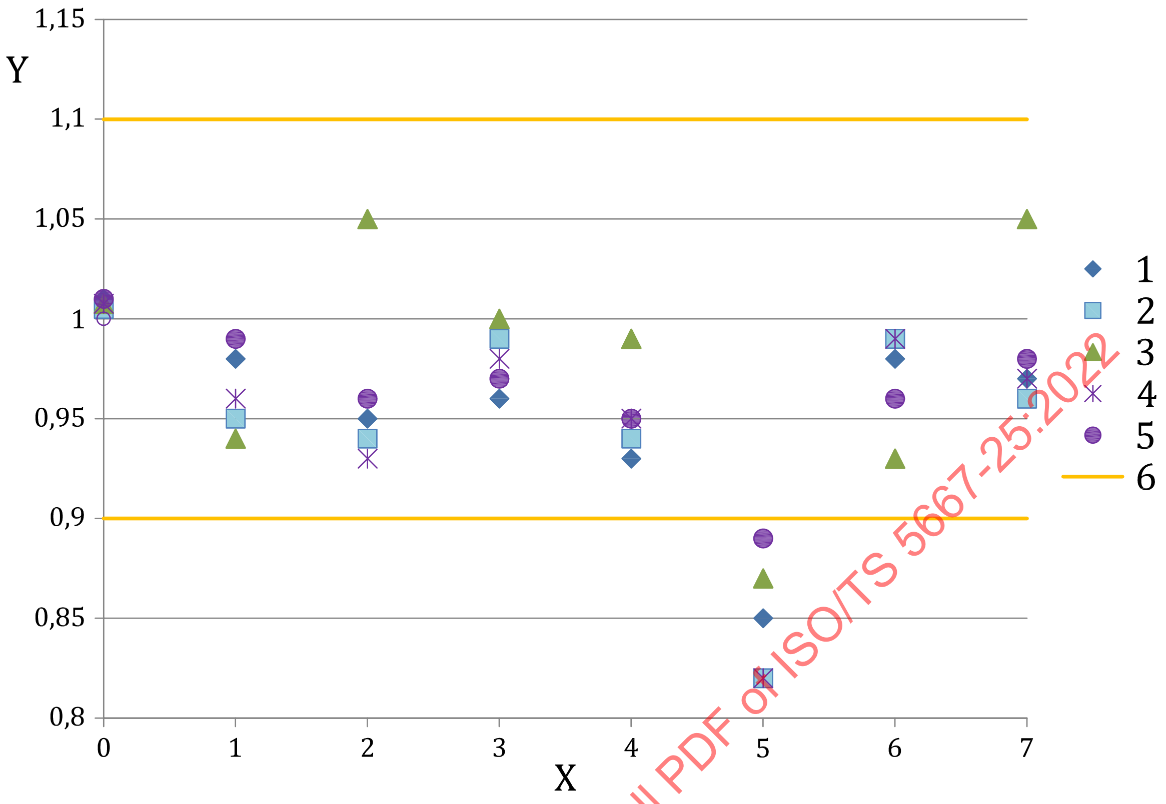
Figure 10 — Example of instability after a given time



Key

X time (days)
Y ratio
1 sample 1
2 sample 2
3 sample 3
4 sample 4
5 sample 5
6 MADs

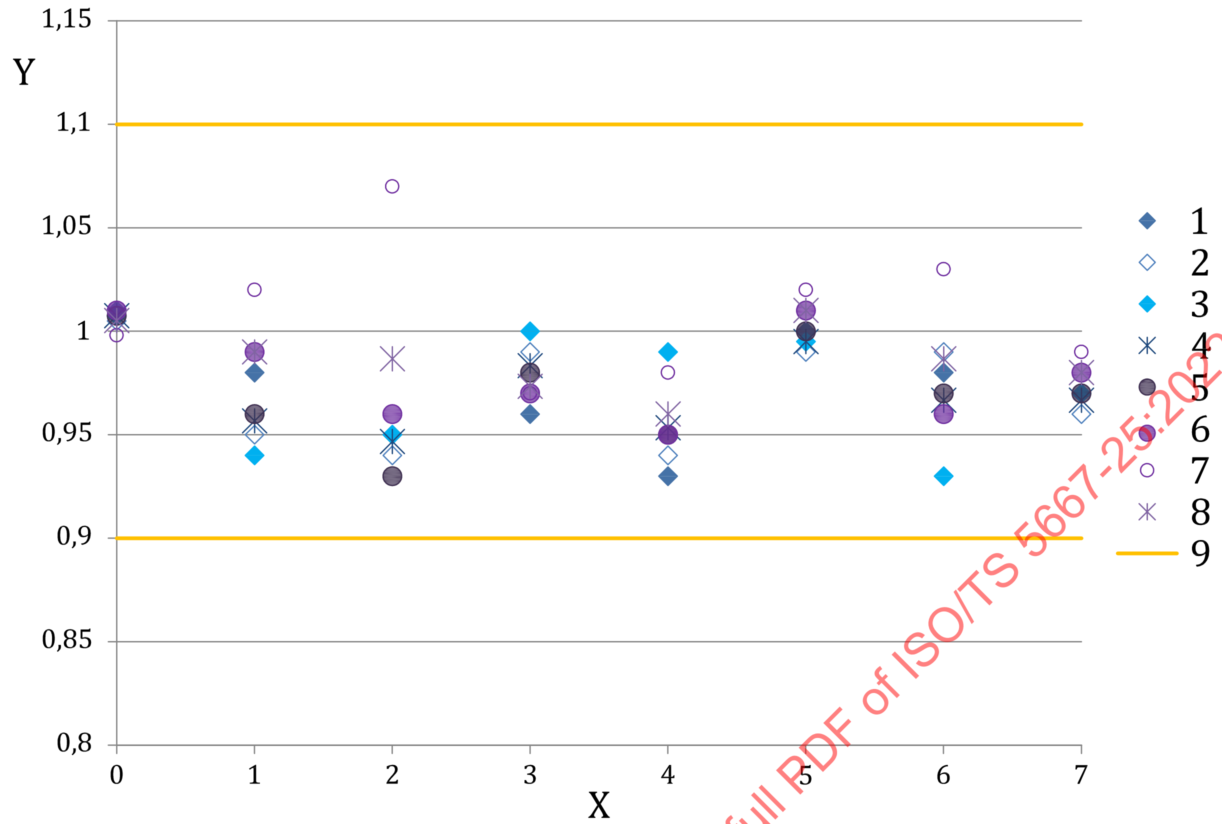
Figure 11 — Example of instability



Key

X time (days)
Y ratio
1 sample 1
2 sample 2
3 sample 3
4 sample 4
5 sample 5
6 MADs

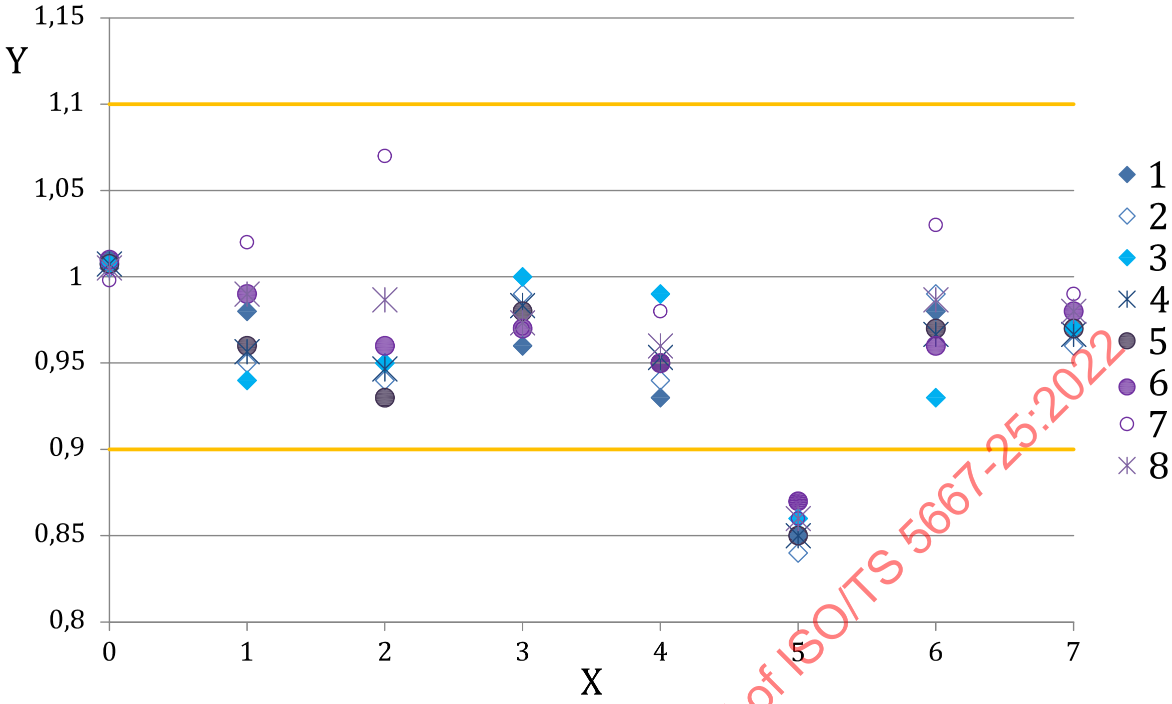
Figure 12 — Example of stability



Key

X time (days)
Y ratio
1 sample 1 – rep 1
2 sample 1 – rep 2
3 sample 1 – rep 3
4 mean – sample 1
5 sample 2 – rep 1
6 sample 2 – rep 2
7 sample 2 – rep 3
8 mean – sample 2
9 MADs

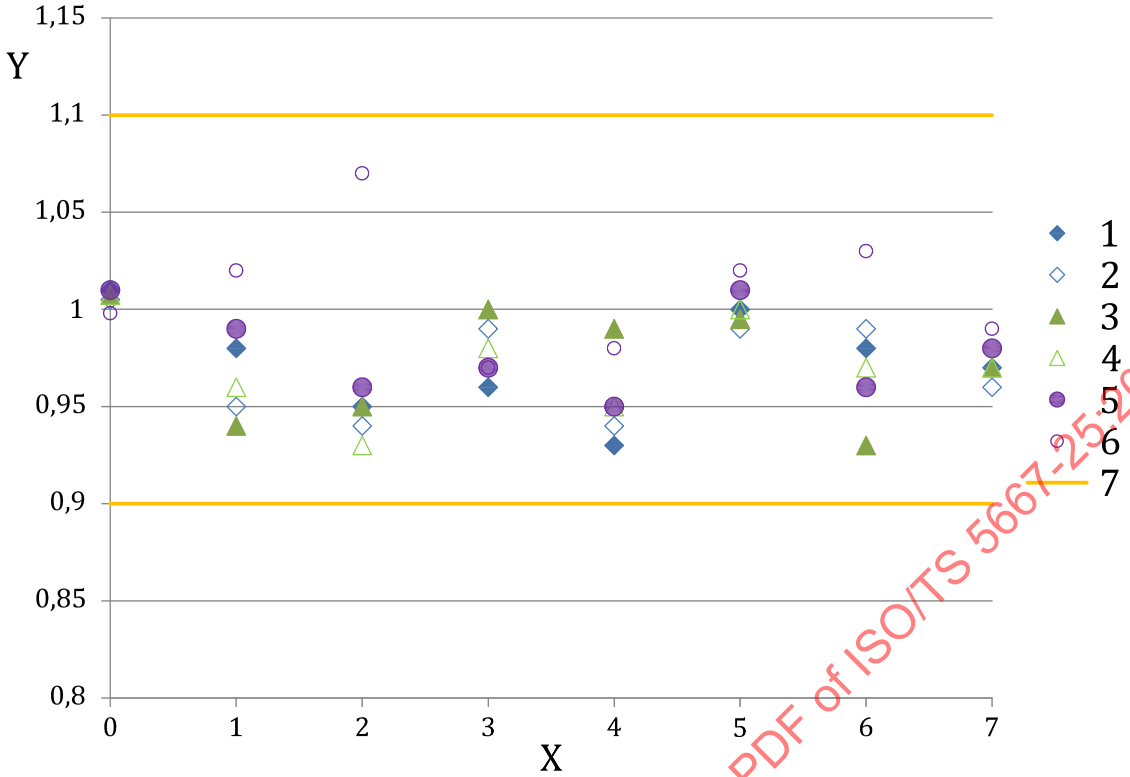
Figure 13 — Example of stability observed by comparing averages



Key

X time (days)
Y ratio
1 sample 1 – rep 1
2 sample 1 – rep 2
3 sample 1 – rep 3
4 mean – sample 1
5 sample 2 – rep 1
6 sample 2 – rep 2
7 sample 2 – rep 3
8 mean – sample 2

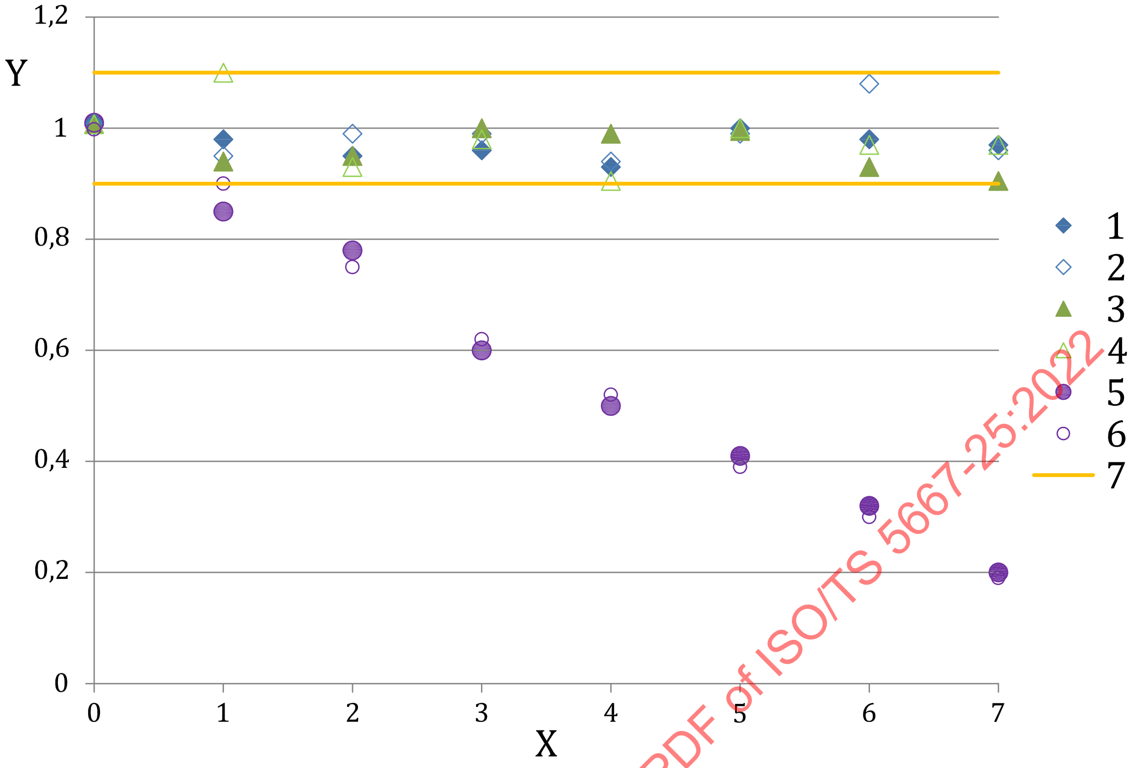
Figure 14 — Example of stability observed by comparing averages with an average not included in the stability interval



Key

X time (days)
Y ratio
1 sample 1 – rep 1
2 sample 1 – rep 2
3 sample 2 – rep 1
4 sample 2 – rep 2
5 sample 3 – rep 1
6 sample 3 – rep 2
7 MADs

Figure 15 — Example of stability of all the samples



Key

X time (days)
Y ratio
1 sample 1 – rep 1
2 sample 1 – rep 2
3 sample 2 – rep 1
4 sample 2 – rep 2
5 sample 3 – rep 1
6 sample 3 – rep 2
7 MADs

Figure 16 — Example of instability for a type of sample

10.3 Conclusion of the study

If the maximum acceptable delays before analysis are identical for all of the conditions, the person in charge of the study can apply this maximum acceptable delay before analysis to the entire scope of application. If not, they can:

— Apply a single maximum acceptable delay before analysis to the minimum value obtained for one condition to the entire scope of application

— Apply several maximum acceptable delays before analysis within the scope of application according to the conditions studied (concentration, type of sample), while making sure that the data sets meet the requirements in Table 2.

When instability is observed, it is recommended to look for the causes that could explain these observations. This can be done by adopting a statistical approach presented in Annex C, for example.

If the conclusions of the stability study are incompatible with the requirements, and the search for influencing factors that could explain the observations does not reveal any paths of improvement, then techniques that could improve the preservation of the samples should be found (for example, the addition of reagents, filtration, sterilization).

An example of a study stability of atrazine in water is given in Annex D.

11 Expression of stability

At the end of the stability study, at least the following shall be known and recorded:

a) Unequivocal identification of the tested analyte, including the reference of the measurement method for index;

b) The stability study conditions: containers, storage temperature, etc.

c) Tested matrix/matrices: type, fraction, number of samples and description of the characteristics demonstrating their representativeness, including the physico-chemical characterization of the sample at T_0, and in particular the influence parameters of the matrix;

d) Chemical analysis methods: principles and performances of the method justifying its use;

e) Choice of the type of execution of the stability study (see Clause 7) and justification;

f) Initial verification of the validity of the results of the stability study (see 9.1);

g) Use of the results of the stability study (see Clause 10);

h) The conclusion: stability (3.24) expressed in the form of a maximum acceptable delay before analysis (MaxADs, 3.11), plus: the maximum acceptable deviation criterion, whether the criterion is met or not, % of instability, influencing factors, operational conclusion.

Annex A
(informative)

Measurement process

The integrity of the sample shall guarantee that the measurement data properly reflects the initial value in the medium, matrix or material to be recorded. Integrity shall be guaranteed along the complete measurement chain. The duration of stability shall extend from the end of the sampling (F) to the start of the first operation of the analytical process (D). It determines the maximum acceptable delay before analysis. Several cases may occur, according to the diagram below that represents different types of organization of the measurement chain. These cases include three possible options.

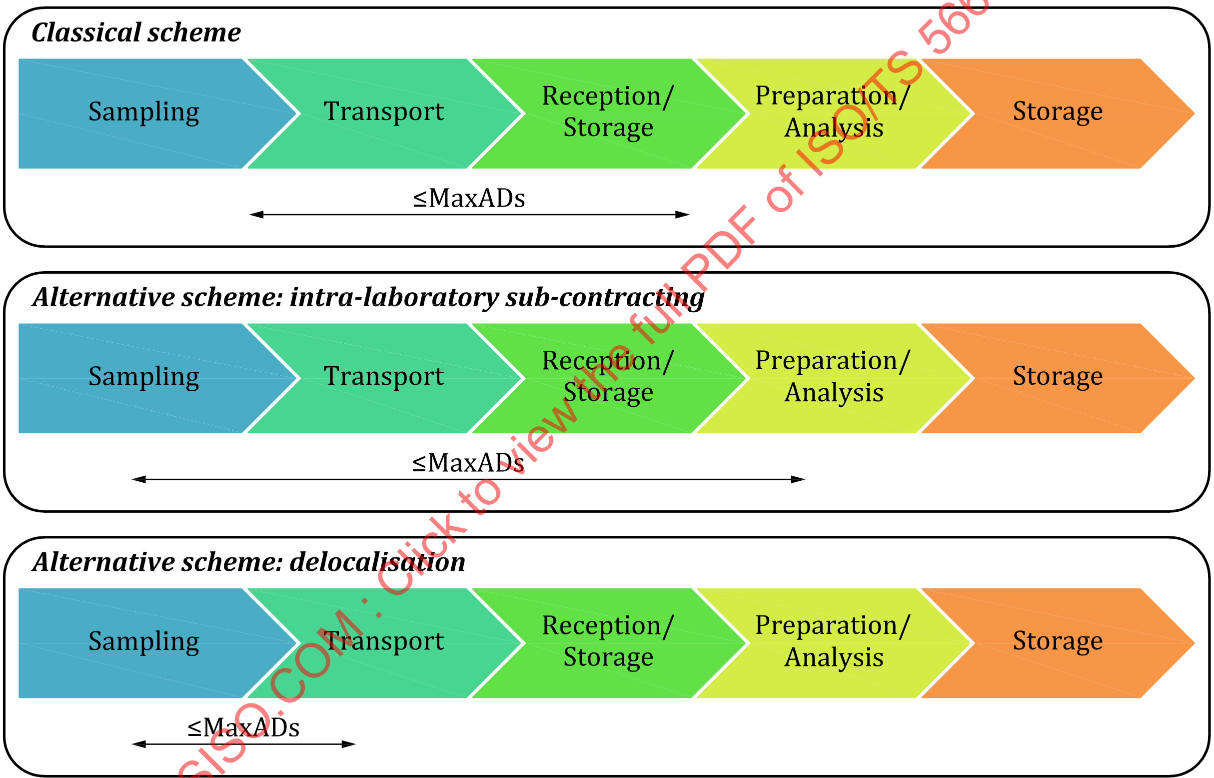


Figure A.1 — Different DMAA according the analytical process

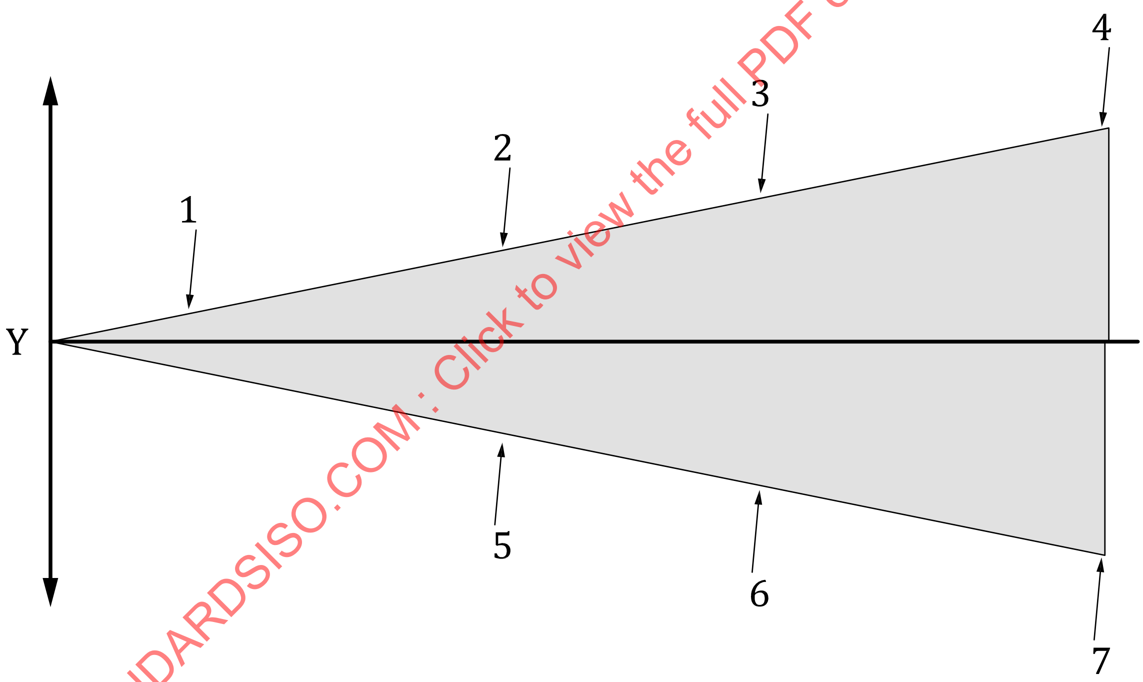
Annex B
(informative)

Fundamental notions

B.1 Measurement

An analytical method is basically characterized by its performances. These performances (specificity, limit of quantification, repeatability, intermediate precision and reproducibility) shall be known, controlled and have sufficient characteristics to meet the needs of the stability study. The use of analytical methods shall meet the requirements in terms of repeatability and intermediate precision (time effect).

Figure B.1 shows the theoretical relations between the estimates of measurement precision and the different measurement conditions. The closer the analysis comes to the repeatability conditions, the greater the measurement precision (reliable value) and, therefore, the greater the reliability and the relevance of the observations and conclusions.



Key

Y dispersion scale
1 between injections
2 within batch (replicates)
3 between batches
4 between laboratories
5 repeatability
6 intermediate precision
7 precision/reproducibility

Figure B.1 — Theoretical relation between the estimates of measurement precision and the different measurement conditions

In this case the illustration is presented in terms of the scale of variability in each condition 0.

B.2 Stability studies

B.2.1 Theoretical principles of homogeneity studies

Homogeneity studies address the demonstration of inhomogeneity. There is an experimental limit to the detection of inhomogeneity. It is strictly related to the repeatability and/or precision of the method. The performances of the analytical method used and the impact on the assessment of homogeneity should be challenged, especially when only methods with poor repeatability are available.

B.2.2 Theoretical principles of stability studies

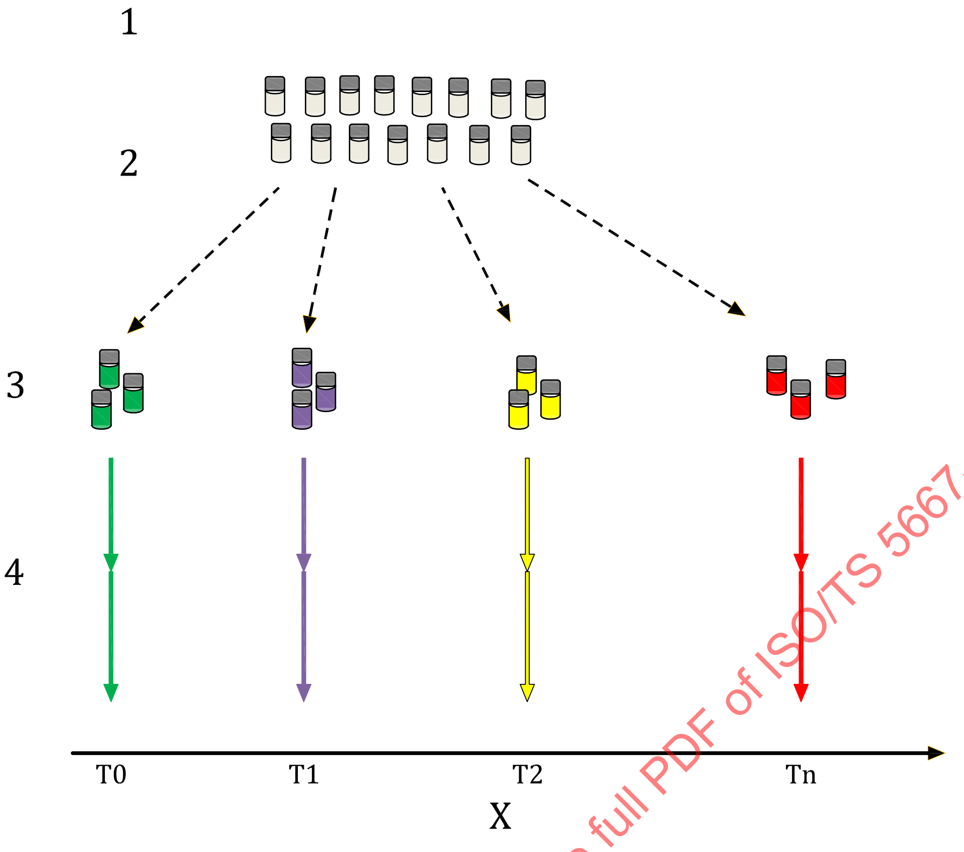
The basic goal of a stability study is to study the influence of the environmental conditions or of the matrix on the stability of the analytes, the matrix or the combination of the two.

The results of all stability studies are highly dependent on the repeatability and the intermediate precision of the analytical method used.

ISO Guide 35 on reference materials[2] describes two approaches to stability studies: The so-called chronological and isochronous approaches.

B.2.2.1 Chronological stability study

The samples are first exposed to different storage conditions, then the preparation and measurement steps take place in a laboratory under intermediate precision conditions (Figure B.2).



Key

X time

1 preparation of a single batch of samples

2 storage in the conditions of the stability study

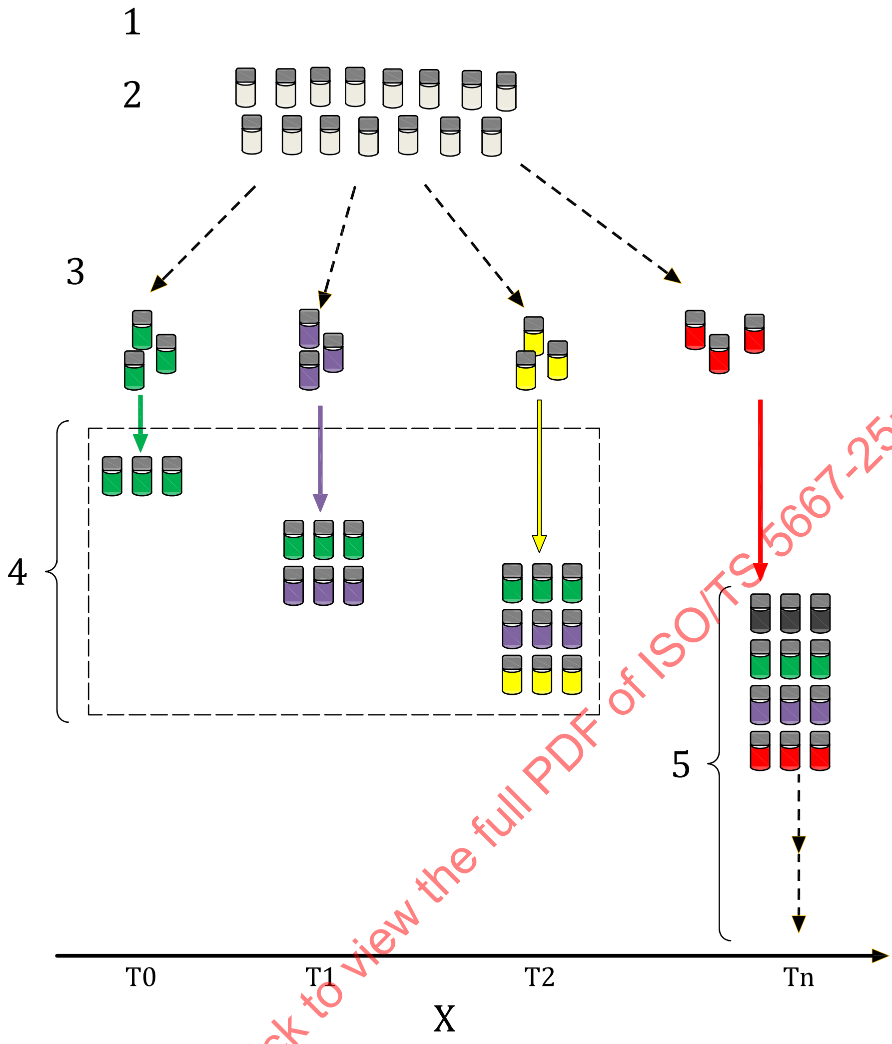
3 random sampling at each time interval

4 sample preparation and measurement at each time interval (under intermediate precision conditions)

Figure B.2 — Diagrammatic illustration of a chronological stability study

B.2.2.2 Isochronous stability study

To begin with (t = 0 or T_0), all the samples of the stability study are transferred under the reference conditions, in which degradation is considered to have very low probability. Samples are moved from the reference conditions at the test temperature at different times for each of the studied storage temperatures. At the defined time, the samples are prepared immediately up until the analysis stage or returned to the reference temperature before the analysis. The analyses are made under repeatability conditions (i.e., in a single series). Consequently, the absence of a precision component reduces the uncertainty of the assessment of the stability of the sample (Figure B.3).



Key

X time

1 preparation of a single batch of samples

2 storage in reference conditions

3 random sampling at each time interval

4 storage in the conditions of the instability study

5 sample preparation and measurement under repeatability conditions

Figure B.3 — Diagrammatic illustration of an isochronous stability study

B.2.2.3 Pseudo-isochronous stability study

Isochronous approaches are not widely adopted in environmental analyses, especially for aqueous matrices. The stability studies are usually conducted at a temperature of:

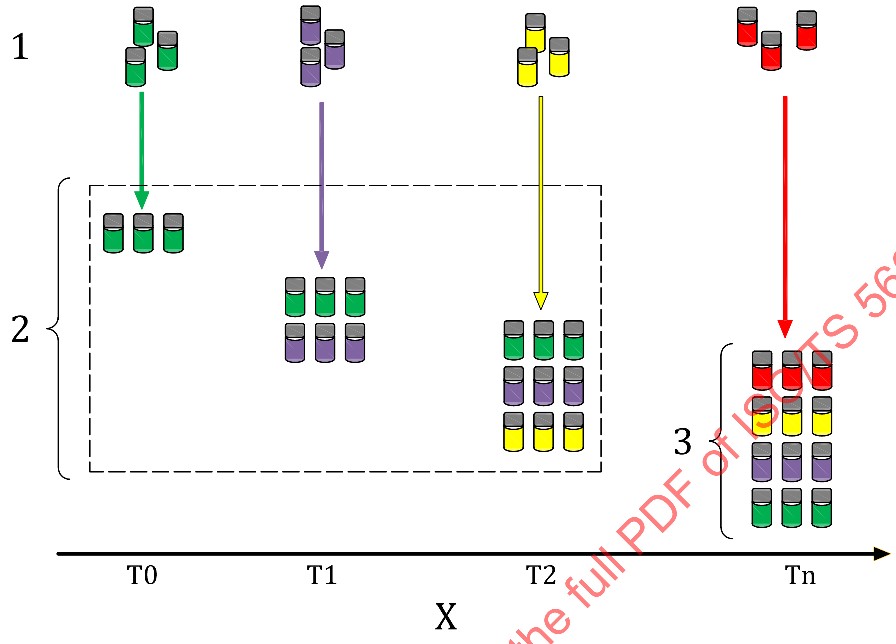
— 3 ± 2 °C, which corresponds to the reference preservation temperature of the samples after they arrive at the laboratory;

— 5 ± 3 °C, which corresponds to the reference preservation temperature of the samples during transportation.

Freezing shall be avoided.

Alternative compromises between the isochronous and the chronological approaches can be considered. They are illustrated in Figure B.4 and Figure B.5.

Type 1 pseudo-isochronous studies (Figure B.4) differ from the isochronous approach inasmuch as the test materials are not prepared in a single batch at $T_{initial}$ of the study, but after the different time intervals in the study. The advantage of this approach is that the analyses (preparation of the sample and instrumental analysis) can take place under repeatability conditions.



Key

X time

1 preparation of multiple batches of samples: one at each time interval

2 storage in the conditions of the stability study

3 sample preparation and measurement under repeatability conditions

Figure B.4 — Diagrammatic illustration of a type 1 pseudo-isochronous stability study

Type 2 pseudo-isochronous studies (Figure B.5) differ from the chronological approach inasmuch as the samples are prepared after each of the time intervals defined in the stability study. The analytical extracts are stored under reference conditions until the end of the study, before being analysed with measuring instruments under repeatability conditions.