
**Water quality — Multi-compound
class methods —**

Part 2:

**Criteria for the quantitative
determination of organic substances
using a multi-compound class
analytical method**

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Qualité de l'eau — Méthodes d'analyse de composés multi-classes —

*Partie 2: Critères pour la détermination quantitative de composés
organiques avec une méthode d'analyse de composés multi-classes*

ISO 21253-2:2019

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html. (standards.iteh.ai)

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A list of all parts in the ISO 21253 series can be found on the ISO website.

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.

Water quality — Multi-compound class methods —

Part 2:

Criteria for the quantitative determination of organic substances using a multi-compound class analytical method

1 Scope

This document specifies the criteria for developing an in-house mass spectrometry-based method for quantitative analysis of multiple subgroups of organic substances in the scope of physical-chemical analysis of water.

This document supplements ISO/TS 13530 which provides guidance on the initial characterization of the measurement performances, by providing details to select the test matrix and internal standards and criteria for analyte and internal standard recoveries.

This document is not intended as a substitute for the currently applicable analytical standards dedicated to organic compounds but as a resource bringing additional characterization elements.

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2 Normative references (standards.iteh.ai)

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 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

ISO 8466-2, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 2: Calibration strategy for non-linear second-order calibration functions*

ISO 11352, *Water quality — Estimation of measurement uncertainty based on validation and quality control data*

ISO 21253-1, *Water quality — Multi-compound class methods — Part 1: Criteria for the identification of target compounds by gas and liquid chromatography and mass spectrometry*

3 Terms and definitions

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

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

analyte

substance to be determined

[SOURCE: ISO/TS 28581:2012, 3.1]

3.2

blank

aliquot of reagent water (reagent blank) or of a matrix in which the *analyte* (3.1) is absent (matrix blank) that is treated exactly as a sample through the complete analytical procedure including extraction, clean-up, identification and quantification including all the relevant reagents and materials

Note 1 to entry: It is crucial that the laboratory specifies which blank is considered.

3.3

calibration curve

expression of the relation between indication and corresponding measured quantity value

[SOURCE: ISO/IEC Guide 99:2007, 4.31]

3.4

limit of quantification

LOQ

lowest value of a determinand that can be determined with an acceptable level of accuracy, which could be estimated by different means and shall be verified in the intended matrix

[SOURCE: ISO/TS 21231:2019, 3.2.5, modified — Note to entry has been excluded.]

3.5

analytical method

unambiguously written procedure describing all details required to carry out the analysis of the determinand or parameter, namely: scope and field of application, principle and/or reactions, definitions, reagents, apparatus, analytical procedures, calculations and presentation of results, performance data and test report

[SOURCE: ISO/TS 16489:2006, 3.3]

3.6

recovery

relative recovery

extent to which a known, added quantity of determinand in a sample can be measured by an analytical system

Note 1 to entry: Recovery is calculated from the difference between results obtained from a spiked and an unspiked aliquot of sample and is usually expressed as a percentage.

[SOURCE: ISO 5667-14:2014, 3.8]

3.7

relative retention time

ratio between the retention time of the target compound and the retention time of the calibration standard

[SOURCE: ISO 15680:2003, 3.5, modified — "retention-time standard" has been replaced by "calibration standard"]

3.8

injection standard

standard mixture added to a sample before injection into the GC-MS apparatus, to monitor variability of instrument response and to calculate internal standard recovery

Note 1 to entry: The same definition is applied for GC-MS/MS, LC-MS and LC-MS/MS.

[SOURCE: ISO 28540:2011, 3.4, modified — Note to entry has been replaced.]

3.9

subgroup of compounds

analytes (3.1) pre-classified into groups that share similar patterns of analytical behaviour in response to extraction mode and analysis

3.10

internal standard

compound added in a known amount to the sample from the beginning of the protocol and enabling analytical coverage throughout the procedure, and that is used to correct for losses during sample preparation and analysis by accounting for all-system matrix effects (recoveries, ionization effect, variability of the detector response of the instrument for example)

Note 1 to entry: Determining the ratio of characteristic molecule signal intensity to the internal standard makes it possible to calculate the quantitative ratio between molecule and internal standard and thereby to deduce molecule concentration in the sample. An identical amount is also added to the calibration standard solutions.

3.11

yield

absolute recovery

amount of *analyte* (3.1) added in the test sample corrected by the relative recovery of the internal standard (analyte-to-internal standard ratio)

Note 1 to entry: Yield is a value that accounts for both sample matrix effect and compound recovery.

4 Principle

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This document specifies the critical points to be considered when developing a mass spectrometry-based method for the analysis of multiple subgroups of organic substances in water samples. The critical points addressed here involve:

- selection of the matrix;
- internal standards and internal standard recoveries;
- analyte recovery.

This document proposes a rating-scale method for analytical protocols dedicated to huge numbers of compounds, making it possible to give the method a reliability score for each compound. When a review of these critical points concludes that the method can reliably quantify the analytes, then the initial characterization of the measurement performances of the method can be started.

This document concludes by proposing quality assurance criteria that should be made routine to ensure that performance levels do not degrade over time.

If there is a need to expand the initial list of reliably quantifiable analytes in a method, then verify the analogue transitions, retention times, LOQ performances and accuracy. Modification of method performances of previously characterized analytes to new performance levels requires an update of the method characterization in the way described before.

For each substance, the method can only be claimed as reliable for those matrices that were effectively tested during the characterization process.

If one or more purification steps need to be carried out, and if substances are present that are liable to create interferences in the chromatogram or contaminate the analytical system, then the measured yield for each substance shall account for the purification step(s).

5 Selection of the matrix

The method shall be characterized on a matrix that is representative of the claimed scope. The water matrix is characterized by its physicochemical properties (SPM, TOC, conductivity, pH, major ions, etc.).

Additional information on the source of the sample (marine water, waste water, groundwater, drinking water, etc.) can inform the final user on the likelihood of certain physicochemical parameters. These parameters shall be identified at the start of the method development and recorded.

The representative matrix can be natural or synthetic. Laboratory-grade water (distilled water, deionized water) is not representative of any natural water.

In the absence of a suitable analyte-free real matrix blank (especially for the characterization of the LOQ), then the natural matrix can be diluted (with drinking water or laboratory-grade water, for example) in order to lower the concentration of organic compounds, taking care to appropriately adjust the modified properties (SPM, TOC, conductivity, pH, etc.) as specified in ISO/TS 21231:2019, 7.1.

6 Sample preservation prior to analysis

In the absence of normative requirements expressly covering the analytes, storage time and sample preservation mode shall be defined and verified from collection of the sample through completion of all analysis-related operations. The storage time defined commences at the date of sample collection.

Sample preservation integrity tests shall be conducted on the representative matrix (see [Clause 5](#)) and, where appropriate, in presence of the preservative. They shall be conducted at least one level at or near $10 \times$ LOQ or at the regulatory value, with field/laboratory duplicates; ISO 5667-3:2018, Annex C provides a more complete protocol, for example, where appropriate, these tests shall also extend to preservation time for sample extracts.

Special attention shall be paid to any breakdown products of analyte molecules (parent molecules and metabolites) if they are included in the scope of the method.

7 Internal standards and injection standards

ISO 21253-2:2019

7.1 General

<https://standards.iteh.ai/catalog/standards/sist/c6acdf3-e883-4a1b-8edd-1d3a878bde4a/iso-21253-2-2019>

One or more internal standards shall be used and introduced into the sample right at the start of the protocol. This is the most efficient way to screen for and correct any potential matrix-effect interferences at extraction and analysis of each sample.

The use of stable isotopically labelled internal standards is recommended to account for specific losses of individual analytes and for matrix effects.

The injection standards are added just before the analysis step. They provide additional information on the instrumental analysis, detector response for example, and are used to calculate internal standard recoveries.

Both these types of standards can be used for the quantification step, although the use of internal standards is preferred.

7.2 Selection of standards

The internal standard and the injection standard shall possess properties making them representative of the analytes. Suitable criteria are available in ISO/TS 13530:2009, 4.4.3.1. The near-identical retention time may also be a useful criterion.

For methods featuring a large number of compounds and in those cases where it is impossible to get an internal standard for each analyte, it is recommended to assign one internal standard or injection standard per subgroup of compounds, giving a suitable justification for the rationale involved.

For complex matrices liable to create strong interferences, for example signal enhancement, signal suppression, or altered retention time, it is recommended to have a labelled analogue for each substance subject to such interferences.

Every effort shall be made to ensure the internal or injection standards are well distributed along the full duration of the chromatogram, and if necessary, in each ionization mode.

Cumulating a series of internal standards and injection standards may help to overcome the analytical difficulties involved when implementing multi-compound class methods and to make the quantification reliable (see [11.2](#)).

For the mass spectrometric detection, it is recommended to use a stable isotopically labelled analogue as an internal standard; ^{13}C or ^{15}N -labelled compounds are preferred rather than deuterated compounds. There is generally an observable drift in retention time compared to the natural compound when working with isotopically labelled standards (isotope effect).

Only a stable part of the molecule shall be labelled, and degree of labelling shall not change over the course of analysis. Exchanges may take place, via tautomerization processes for example, and so this type of mechanism shall be experimentally verified in the event that the abundance of internal standard is shown to vary.

Spectral overlapping between labelled analogue and native substances shall be avoided. Consequently, for the majority of small-molecule applications, the labelled analogue should preferably have a monoisotopic mass greater than at least 3 Da.

The isotopic purity of the internal standards shall be checked. It is not a critical factor provided that its impurities, including unlabelled analogue, have preferably zero contribution on target compounds of the analytical method. In case it is not possible, the contribution of the labelled analogue to the unlabelled analogue should be negligible at LOQ level.

To confirm the accuracy of the method, it is necessary to confirm the right balance between labelled analogue and native substances. It may be necessary to run pre-investigative studies to check that:

- the internal standard has been fully extracted without degradation, or
- that internal standard is in equilibrium with the sample and bound to suspended particulate matter (SPM) in the same way as the native substance.

If a filtration is intended in the laboratory, the internal standards can be added before filtration, allowing delay for the equilibrium to take place, and the applicability of the internal standards for this step shall be verified (see [9.3](#)).

8 Calibration

In order to analyse and quantify a substance of interest, the analyst shall acquire the compound as a calibration standard of the highest purity possible. This analytical standard may be sourced either pure or in solution. Firstly, it will guarantee that instrumental analysis is both feasible and under control. It will also confirm that the substance is present in the sample and will serve to establish the calibration curve needed to quantify, and further to establish the performance of the extraction step.

Important information (whether for in-house prepared solutions or commercially-sourced solutions) is:

- the identity of the substances: special attention is needed when dealing with mixtures of substances (nonylphenols for example) and with separately distributed isomers or isomer mixtures (endosulfan for example);
- their purity;
- the uncertainty on their assessed concentrations;
- the behaviour patterns of the internal standards and injection standards, which is acquired by individually injecting them;
- the behaviour pattern of the compounds in the course of analysis, whether injected individually or in mixture of a small number of substances on one hand, and mixed with all the other intended substances,